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RESEARCH PAPER

Relative food limitation drives geographical clutch size variation in South African passerines: a large-scale test of Ashmole's seasonality hypothesis

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ABSTRACT

Aim To separate the effect of overall resource level from the effect of seasonality on avian clutch size to test Ashmole's hypothesis that birds have larger clutch sizes in seasonal environments due to high per capita food availability during the breeding season.

Location South Africa and Lesotho.

Methods We used a large-scale environmental gradient to test the effects of maximum resource availability and resource seasonality (estimated by the normalized difference vegetation index) on clutch size variation among local passerine assemblages (25 km × 25 km grid cells). The importance of maximum resource availability was distinguished from the importance of resource seasonality by using multivariate general additive models and by subsetting the data so that variation in one of these parameters was minimized. Spatial autocorrelation was controlled for by using spatial generalized least squares.

Results Assemblage mean clutch size showed a hump-shaped relationship with maximum resource availability but an increase with resource seasonality. When the variation in maximum resource availability was fixed, clutch size increased with increasing seasonality, but it decreased with increasing maximum resource availability when we fixed the variation in seasonality. These results hold for all feeding guilds except granivores, for which we found opposite patterns. The patterns were much less pronounced when family membership was controlled for, indicating that the overall trends are mostly driven by variation between families.

Main conclusions Although clutch size can be affected by many factors related to environmental productivity and its variation, Ashmole's hypothesis provides the most parsimonious explanation of the observed patterns: geographical patterns in mean clutch size across bird assemblages seem to be driven by variation of per capita food availability determined by seasonal variation of population density.

Keywords

Avian assemblages, David Lack, macroecological approach, normalized difference vegetation index, number of eggs, Philip Nelson Ashmole.

INTRODUCTION

Clutch size is a key demographic trait in avian biology. Its variation has attracted much scientific attention since the work of David Lack, who established an evolutionary perspective in ecology in the middle of the last century (Lack, 1947, 1948). The most striking geographical pattern in clutch size is observed along latitude: tropical birds tend to produce smaller clutches than their temperate zone counterparts (Moreau, 1944; Jetz *et al.*, 2008). Many hypotheses have been proposed to explain this observation. Smaller clutches in the tropics are frequently attributed to higher rates of nest predation (Skutch, 1949), lower

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food availability during breeding (Slagsvold, 1975), an adjustment of reproductive effort to the probability of future reproduction (Moreau, 1944; Williams, 1966; Martin, 2002) or even a lower availability of calcium necessary for the production of eggshells (Patten, 2007). The effect of resource availability on clutch size has been tested most often (e.g. Ricklefs, 1980; Koenig, 1984; Jetz *et al.*, 2008; Hořák *et al.*, 2011), and it has already been validated at a global scale (Jetz *et al.*, 2008). The popularity of the 'food limitation hypothesis' is not surprising, as it was first formulated by Lack (1947) himself who believed that the number of eggs within a clutch corresponds to the number of young that the parents can nourish, determined by the amount of food available. The hypothesis thus seems to imply that larger clutches are to be expected in food-rich environments. However, the situation is more complicated. Ashmole (1963) suggested that it is not the total amount of food available, but the amount relative to population density that affects clutch size. He proposed that population densities are determined during periods when food is scarce (i.e. during winter time in the temperate zone), while clutch size is determined by per capita food availability during the breeding season (i.e. spring time in the temperate zone). Clutch size is thus expected to be higher in places where food is scarce during the harsh period of the year (when population numbers decline) and abundant during the breeding season (low population densities lead to high food resources available per capita). Consequently, the larger the difference between maximum and minimum resource availability in the course of the year – a seasonality *sensu* Ashmole (1963) (see Fig. 1) – the larger clutches should be.

Although the seasonality of food availability has been linked to clutch size in a number of studies (Lack & Moreau, 1965; Ricklefs, 1980; Lepage & Lloyd, 2004; Jetz *et al.*, 2008; Hořák *et al.*, 2011), its effect is often interwoven with overall food levels (but see Ricklefs, 1980). This is mostly because the maximum amount of food resources and the seasonality of food availability are highly correlated in natural environments (see, e.g., the supplementary material in Jetz *et al.*, 2008), which hampers the attribution of clutch size variation to one of these factors. More importantly, the effect of the absolute difference between minimum and maximum food levels on clutch size should be considered in relation to overall levels of food abundance. This leads to an additional prediction that, besides increasing clutch size with seasonality, clutch size should decrease with increasing maximum amount of resources under constant seasonality (measured as absolute differences between resource levels in winter versus breeding season). A more food-rich environment should host more abundant populations at the same level of seasonality, which in turn lowers per capita food income during breeding, because the *relative* difference between resource levels in breeding and non-breeding seasons is lower in such a situation (Fig. 1). The effect of relative difference in resource availability could be potentially investigated by testing the effects of the minimum/maximum productivity ratio (cf. Ricklefs, 1980), but since it is not clear whether food availability relates linearly to productivity, the phenomenon is better investigated under fixed levels of environmental variables. This is the approach we take here.

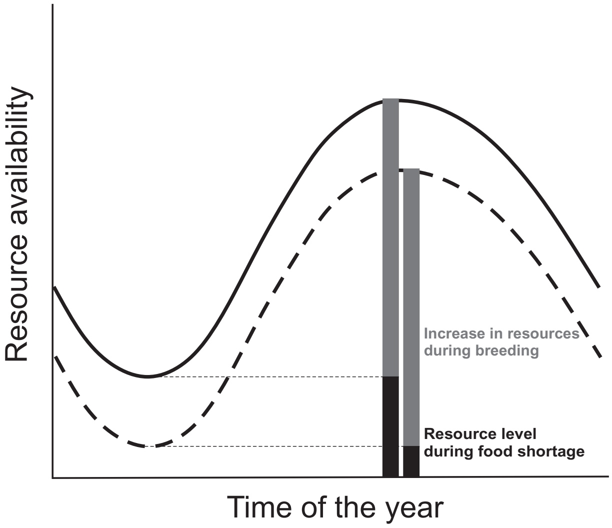

Figure 1 A graphical model of the variation in resource availability during the seasonal cycle between two imaginary locations (solid and dashed curves) that differ in maximum resource level but not in seasonality – an absolute increase in resources between periods of minimum and maximum resource availability (adjusted according to Ricklefs, 1980). Ashmole's hypothesis is based on the assumption that the adult population level is limited by resource availability during the stage of the seasonal cycle when resources are at their minimum. Therefore, per capita energy availability during breeding (resource maximum during the seasonal cycle) depends on its relative increase during the seasonal cycle. Ashmole's hypothesis thus suggests larger clutches at locations indicated by the dashed curve, as relative increment during the breeding season is higher there. Consequently, at fixed levels of seasonality, clutch size should decrease with increasing overall resource levels.

In this study, we focus on birds breeding in South Africa. We restricted the analyses to passerines, as they represent a phylogenetically homogeneous set of species with relatively similar life histories including the mode of energy allocation into reproduction. We used a large-scale gradient of environmental productivity in South Africa, which is convenient for the purpose of our study for two reasons: (1) both productivity and seasonality vary considerably in space, and (2) environmental variation is relatively gradual and situated mostly longitudinally, allowing the implicit filtering out of some biogeographical effects that may affect global-scale variation in avian clutch size.

The aim of this study is to describe geographical patterns in mean clutch size of species assemblages and relate them to variation in the surrogates of resource availability and seasonality. We make the following predictions. If Ashmole's density hypothesis holds, assemblage mean clutch size should (1) increase with seasonality when controlled for overall resource levels, and (2) decrease with maximum resource level during the breeding season when controlled for seasonality (see above; Fig. 1). Additionally, (3) these effects should be more prominent in birds that are dependent on seasonal food resources. In order to separate the effects of environmental variables we:

(1) investigated how residual variation of the maximum productivity–seasonality relationship affects assemblage mean clutch size, and (2) looked separately at selected parts of the productivity gradient within which the variation in one of the focal environmental variables was limited.

METHODS

Species distribution data

Data on the geographical distribution of birds were taken from information collected between 1987 and 1992 as part of the Southern African Bird Atlas Project (Harrison *et al.*, 1997) at 0.25° spatial resolution. All passerine species recorded within each grid cell (*c.* 25 km × 25 km) were considered a local assemblage. The whole territory of South Africa and Lesotho was covered by 1858 grid cells/assemblages.

Clutch size information

From Hockey *et al.* (2005) we collected information about mean clutch sizes for 277 passerine species which breed in South Africa and Lesotho, and for which we had information about their geographical distribution. For each local assemblage, we calculated mean clutch size. We excluded brood parasites, as their clutch sizes are difficult to define, and rarely occurring vagrants, marginal species (occurring in fewer than six grid cells) and species escaped from captivity. The situation might be further complicated by intra-African migrants and vagrants which could avoid seasonal food shortages by moving elsewhere. However, omitting intra-African migrants had no effect on mean assemblage clutch sizes (see Appendix S6 in Supporting Information), so we decided to keep them in the data set. To reveal whether the clutch size patterns were determined by different geographical distributions of the members of passerine families with conservative clutch sizes or by environmentally induced variation of clutch size within families, we tested for the effect of the taxonomic family membership of species as a part of the analyses. We performed ANOVA with family as an explanatory factor. Then we used residuals from this analysis as well as raw clutch size data for further calculations.

Different feeding guilds might utilize different food resources. Therefore, we also performed the analyses separately for three groups of passerines: (1) insectivorous species ($n = 140$), (2) species eating fruits and a combination of fruits and insects ($n = 37$), and (3) species eating grains and a combination of grains and insects ($n = 76$). We omitted nectarivorous ($n = 15$) and omnivorous birds ($n = 9$) from these analyses due to the small number of species within these groups. The information about dietary composition was compiled from Hockey *et al.* (2005).

Environmental productivity information

For each grid cell, we obtained the normalized difference vegetation index (hereafter NDVI), an estimate of the environmental productivity based on the spectral properties of vegetation. Average monthly maxima from the period 1982–2004 were obtained from the 'Africa Data Dissemination Service' data set provided by the United States Geological Survey (http://iridl.ldeo.columbia.edu/SOURCES/.USGS/.ADDS). For each grid cell, the month showing the highest NDVI value was used as an estimate of the maximum environmental productivity (hereafter $\mathrm{NDVI}_{\mathrm{maximum}}$). To estimate environmental seasonality we used the difference between the maximum and minimum NDVI values for each grid cell (hereafter $\mathrm{NDVI}_{\mathrm{seasonality}}$). As NDVI describes the 'greenness' of vegetation and has been shown to be closely correlated with rainfall, total green biomass and net primary productivity (Goward & Dye, 1987; Chong *et al.*, 1993; Paruelo *et al.*, 1997; Schmidt & Karnieli, 2002), we believe that its variation reflects the differences in the amount of food available to birds over large spatial scales. This assumes that the amount of food for breeding, such as arthropod abundance, correlates with overall plant productivity and biomass, as has been suggested, e.g. by Gordo (2007). A positive relationship between arthropod abundance and NDVI has been shown by Lassau & Hochuli (2008) and Lafage *et al.* (2014).

Data analyses

During exploratory analyses we found that $\mathrm{NDVI}_{\mathrm{maximum}}$ and $\mathrm{NDVI}_{\mathrm{seasonality}}$ were strongly related across grid cells in South Africa (Fig. 2). Their mutual relationship was positive and

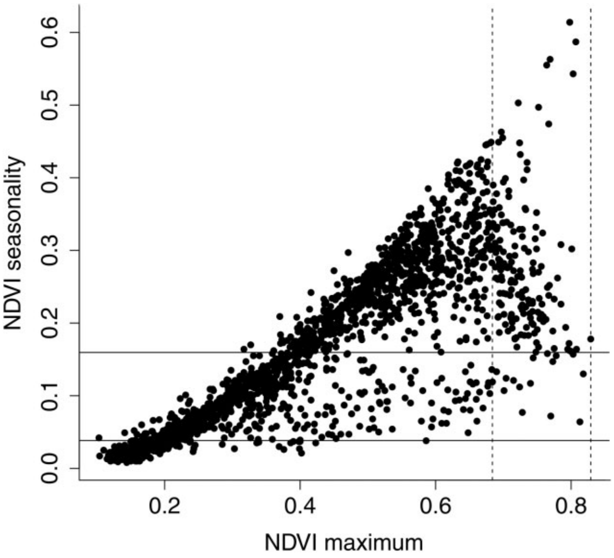

Figure 2 The relationship between maximum normalized difference vegetation index (NDVI) and seasonality in NDVI (the difference between minimum and maximum NDVI) in South Africa. To disentangle the effects of NDVI maximum and NDVI seasonality on avian clutch size, we selected blocks of data in which the variation in one of the environmental variables was limited while the second one varied normally. The blocks of data with limited variation in NDVI seasonality (solid line) and NDVI maximum (dashed line) are depicted.

highly significant ($r = 0.83$, $P < 0.001$). Given such collinearity between explanatory variables, it is difficult to distinguish between the effects of $\mathrm{NDVI}_{\mathrm{maximum}}$ and $\mathrm{NDVI}_{\mathrm{seasonality}}$ (Dormann *et al.*, 2013). We decided to cope with this difficulty using two approaches that at the same time test for the importance of relative values of $\mathrm{NDVI}_{\mathrm{maximum}}$ and $\mathrm{NDVI}_{\mathrm{seasonality}}$.

First, we investigated the effect of the residual variation of the relationship between $\mathrm{NDVI}_{\mathrm{maximum}}$ and $\mathrm{NDVI}_{\mathrm{seasonality}}$ on the variation in assemblage mean clutch size. In order to do this, we took residuals from a linear model [ordinary least squares (OLS) regression] where $\mathrm{NDVI}_{\mathrm{maximum}}$ figured as the response and $\mathrm{NDVI}_{\mathrm{seasonality}}$ as the explanatory variable (hereafter RES1). RES1 residuals highlight places where $\mathrm{NDVI}_{\mathrm{maximum}}$ is higher (positive residuals) or lower (negative residuals) than predicted by $\mathrm{NDVI}_{\mathrm{seasonality}}$. Then, we took residuals from the linear relationship where the environmental variables were reversed in comparison with the previous model, i.e. $\mathrm{NDVI}_{\mathrm{maximum}}$ was the explanatory variable and $\mathrm{NDVI}_{\mathrm{seasonality}}$ was the response variable (hereafter RES2). Thus, RES2 residuals highlight places where $\mathrm{NDVI}_{\mathrm{seasonality}}$ is higher or lower than predicted by $\mathrm{NDVI}_{\mathrm{maximum}}$.

Second, we created two subsets of data containing only ad hoc selected grid cells. In the first subset, we restricted the variation in $\mathrm{NDVI}_{\mathrm{maximum}}$ as much as possible (so that we still had a reasonable sample size) and kept the whole variation in $\mathrm{NDVI}_{\mathrm{seasonality}}$. For this purpose we selected only grid cells for which the $\mathrm{NDVI}_{\mathrm{maximum}}$ values were within the upper 20% of the range (i.e. between 0.6838 and 0.829, delimited by dashed lines in Fig. 2). This subset of data contained 182 grid cells and allowed us to test the effect of $\mathrm{NDVI}_{\mathrm{seasonality}}$ on clutch size at restricted and high levels of $\mathrm{NDVI}_{\mathrm{maximum}}$. In the second subset, we did the opposite and restricted the variation in $\mathrm{NDVI}_{\mathrm{seasonality}}$ in a similar way. However, the lowest values of $\mathrm{NDVI}_{\mathrm{seasonality}}$ are found only in grid cells with the lowest values of $\mathrm{NDVI}_{\mathrm{maximum}}$, which hampers the intention of our analysis. Therefore, to include the whole range of $\mathrm{NDVI}_{\mathrm{maximum}}$ values, we shifted the range of selected grid cells slightly (5%) towards the higher values of $\mathrm{NDVI}_{\mathrm{seasonality}}$ (delimited by solid lines in Fig. 2). As a result, the second subset of data contained grid cells having $\mathrm{NDVI}_{\mathrm{seasonality}}$ values between the lower 5% and 25% of the range (i.e. between 0.0383 and 0.1595). The second subset of data contained 718 grid cells and allowed us to test the effect of $\mathrm{NDVI}_{\mathrm{maximum}}$ on clutch size at restricted and low levels of $\mathrm{NDVI}_{\mathrm{seasonality}}$.

To investigate general patterns in the data we first decided to employ simple linear models. However, exploratory graphical analyses suggested that nonlinear patterns might be hidden within the huge number of data points. For that reason, we repeated the analyses using generalized additive models (hereafter GAM), a useful tool for this purpose as they allow the shape of a relationship to be captured without pre-judging the issue by choosing a particular parametric form (Crawley, 2007).

As our data have spatial structure, we run spatial generalized least squares (GLS) models as an alternative to OLS regression. However, apart from more similar species compositions (and therefore clutch sizes) between nearby grid cells, a strong spatial autocorrelation can be observed in environmental variables along our gradient. Consequently, it is difficult to distinguish between a proximity effect per se and similarities caused by environmental conditions. Thus, we decided to present the results of both non-spatial and spatial models.

Data processing and the statistical analyses were performed in R 3.0.2 (R Development Core Team, 2013) and the GAMs were done using the 'mgcv' R library. Spatial patterns in assemblage mean clutch size were visualized in SAM software (Rangel *et al.*, 2010).

RESULTS

Geographical patterns

Smaller clutches on average were found in arid areas of the transition zone between the arid savanna and Karoo, but also in productive places along the shore of the Indian Ocean and in tropical parts in the eastern part of the country (e.g. Kruger National Park). In contrast, larger clutches characterize central eastern parts of South Africa in the grassland region (see Fig. 3a).

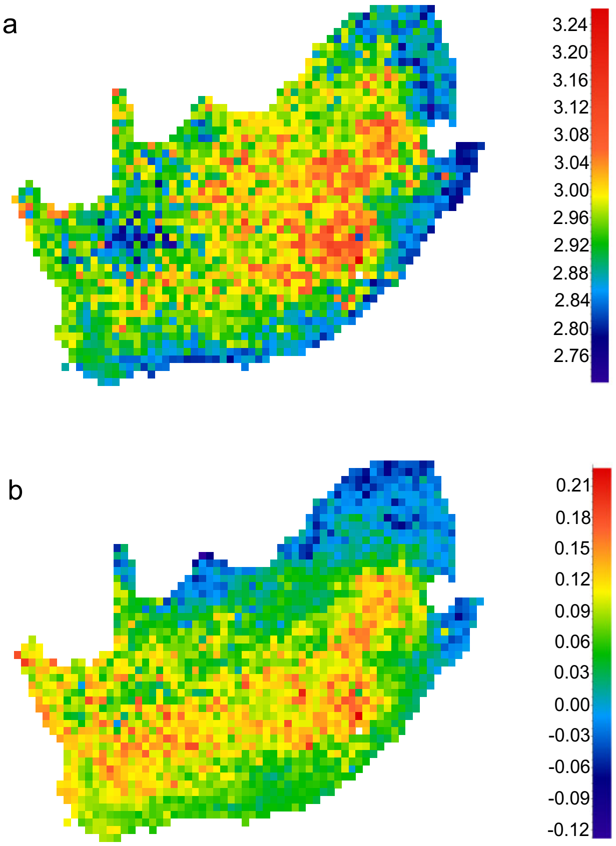

Figure 3 Geographical patterns of assemblage mean clutch size in South African passerines. Geographical variation in raw local assemblage (0.25° × 0.25° grid cell) means (a) and the values obtained after controlling for taxonomy, i.e. membership in passerine families (b).

Linear models

We performed linear models evaluating the effect of $\text{NDVI}_{\text{maximum}}$ and $\text{NDVI}_{\text{seasonality}}$ on assemblage mean clutch size. First, we built a multivariate model of both NDVI variables and their interaction, and subsequently univariate models for each variable separately. Then, we explained the variation in clutch size by RES1 and RES2, respectively. Finally, we tested the relationships using subsetted data. In all tests, we found a significant and negative effect of $\text{NDVI}_{\text{maximum}}$ and a significant and positive effect of $\text{NDVI}_{\text{seasonality}}$ on clutch size. The results were different after controlling for taxonomy. For detailed information see Appendix S1.

Spatial GLS models

The model that included the effects of $\text{NDVI}_{\text{maximum}}$, $\text{NDVI}_{\text{seasonality}}$ and their interaction on clutch size variation explained 18.4% of the total variance (predictors and space together explained 57.2%), and suggested a significant and negative effect of $\text{NDVI}_{\text{maximum}}$ (Std Coeff = –0.278, $t = -3.876$, $P < 0.001$) and a significant but positive effect of $\text{NDVI}_{\text{seasonality}}$ (Std Coeff = 0.675, $t = 4.601$, $P < 0.001$). However, the interaction term of the model was also significant and negative (Std Coeff = –0.444, $t = -2.953$, $P = 0.003$). Further, we ran the same model but with clutch size controlled for taxonomy. It explained 5.2% of the total variance (predictors and space together explained 84%) and revealed a significant and negative effect of $\text{NDVI}_{\text{maximum}}$ (Std Coeff = –0.154, $t = -2.361$, $P = 0.018$). However, neither $\text{NDVI}_{\text{seasonality}}$ (Std Coeff = –0.019, $t = -0.149$, $P = 0.881$) nor the interaction term (Std Coeff = 0.107, $t = 0.799$, $P = 0.424$) were statistically significant in this case.

Univariate analyses

Clutch size was significantly and negatively related to $\text{NDVI}_{\text{maximum}}$, RES1, as well as to $\text{NDVI}_{\text{maximum}}$ within the subset of grid cells with a fixed level of seasonality. However, it was significantly and positively related to $\text{NDVI}_{\text{seasonality}}$ and RES2. We found no significant relationship between clutch size and $\text{NDVI}_{\text{seasonality}}$ within the subset of grid cells with a fixed level of maximum productivity. All the spatial analyses became weaker or non-significant after controlling for taxonomy, which suggests that the majority of clutch size variation is among families (as in the OLS models above). Detailed results for the GLS models are provided in Appendix S2.

Generalized additive models (GAMs)

A model investigating the effect of $\text{NDVI}_{\text{maximum}}$, $\text{NDVI}_{\text{seasonality}}$ and their interaction on clutch size variation explained 36.7% of the variance (generalized cross-validation score = 0.0033). It suggested no significant effect of $\text{NDVI}_{\text{maximum}}$ ($F = 0.421$, $P = 0.765$, estimated d.f. = 3.1) but a significant effect of $\text{NDVI}_{\text{seasonality}}$ ($F = 9.18$, $P = 0.002$, estimated d.f. = 1). However, the interaction term was significant ($F = 5.28$, $P < 0.001$, estimated d.f. = 21.4). The same model with clutch size controlled for taxonomy explained a lower proportion of the total variance (21%, generalized cross-validation score = 0.0024) and none of the terms was statistically significant (all $P > 0.154$).

Univariate analyses

We fitted GAMs evaluating the effects of environmental variables on clutch size separately (Table 1). We found a significant effect of $\text{NDVI}_{\text{maximum}}$ on clutch size ($F = 58.54$, $P < 0.001$, estimated d.f. = 5.71, 18.1% deviance explained). Setting upper limit for d.f.s associated with the smoothing to three caused no significant change in the model deviance ($F = 1.98$, $P = 0.10$). This simplified model explained 17.7% of the deviance ($F = 199.8$, $P < 0.001$, estimated d.f. = 2) and revealed a hump-shaped relationship between clutch size and $\text{NDVI}_{\text{maximum}}$

Table 1 Results of univariate generalized additive models investigating the effects of environmental productivity (estimated by normalised difference vegetation index, NDVI) and its seasonal variation on geographical variation in assemblage mean clutch sizes of South African birds.

	Without taxonomy				Controlled for taxonomy			
Full dataset	Estimated d.f.	Deviance	F	P	Estimated d.f.	Deviance	F	P
$\text{NDVI}_{\text{maximum}}$*	2	17.7	199.8	< 0.001	6.2	11.1	31.1	< 0.001
$\text{NDVI}_{\text{seasonality}}$†	3.9	4.6	21.4	< 0.001	5.7	17.2	63.4	< 0.001
RES1‡	7.9	24.7	68.9	< 0.001	8.3	9.1	20.6	< 0.001
RES2§	8.2	20.6	54.1	< 0.001	6.8	11.4	33.4	< 0.001
Subset								
$\text{NDVI}_{\text{maximum}}$*	4.6	28.3	57.0	< 0.001	1	5.6	42.4	< 0.001
$\text{NDVI}_{\text{seasonality}}$†	3.9	26.8	12.9	< 0.001	2.9	31.6	26.5	< 0.001

*$\text{NDVI}_{\text{maximum}}$, average maximum monthly NDVI.

†$\text{NDVI}_{\text{seasonality}}$, seasonality in NDVI.

‡RES1, residuals from ordinary least square regression between $\text{NDVI}_{\text{maximum}}$ (response variable) and $\text{NDVI}_{\text{seasonality}}$ (explanatory variable).

§RES2, residuals from ordinary least square regression between $\text{NDVI}_{\text{seasonality}}$ (response variable) and $\text{NDVI}_{\text{maximum}}$ (explanatory variable).

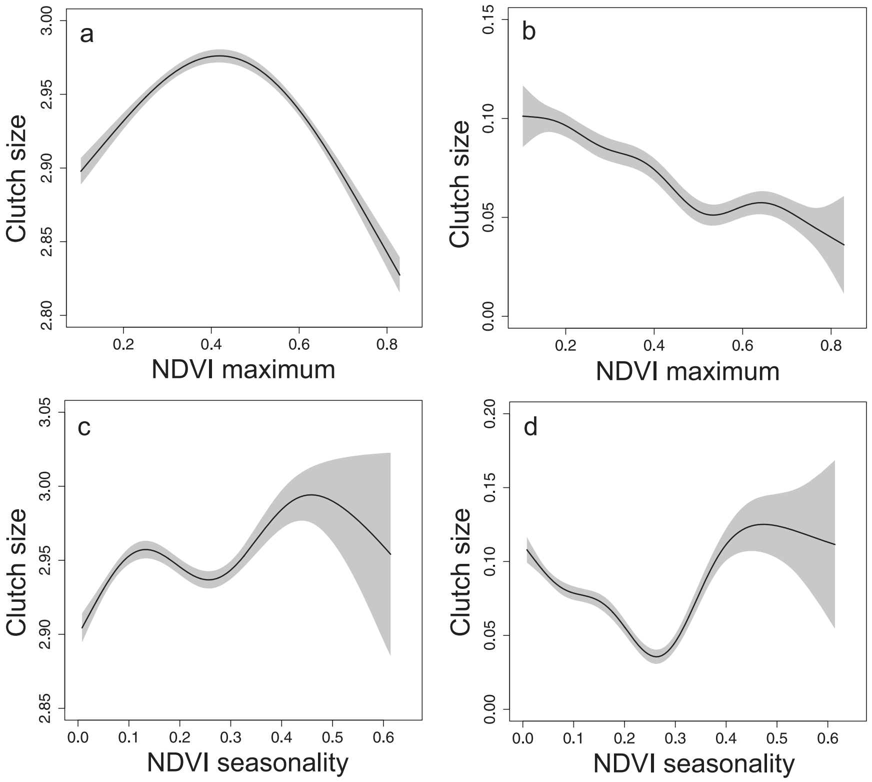

Figure 4 Nonlinear relationships (fitted by generalized additive models) between assemblage mean clutch size of South African passerines, maximum normalized difference vegetation index (NDVI) (a, b), and seasonality in NDVI (the difference between minimum and maximum NDVI (c, d). Raw clutch size data (a, c) and clutch size data controlled for taxonomy (b, d) are provided. Grey areas indicate confidence intervals.

(Fig. 4a). After controlling for taxonomy, the effect of $\text{NDVI}_{\text{maximum}}$ was significant ($F = 31.07$, $P < 0.001$, estimated d.f. = 6.18, 11.1% of variation explained). No model simplification was justified. Clutch size decreased nonlinearly with increasing $\text{NDVI}_{\text{maximum}}$ (Fig. 4b).

Further, we found a significant effect of $\text{NDVI}_{\text{seasonality}}$ on clutch size ($F = 12.14$, $P < 0.001$, estimated d.f. = 6.08, 4.8% of deviance explained). Simplification (upper limit of d.f.s set to five) caused no significant change in the model deviance ($F = 2.06$, $P = 0.12$). This simplified model explained 4.6% of the deviance ($F = 21.4$, $P < 0.001$, estimated d.f. = 3.94) and revealed a nonlinearly increasing relationship between clutch size and $\text{NDVI}_{\text{seasonality}}$ (Fig. 4c). After controlling for taxonomy, the effect of $\text{NDVI}_{\text{seasonality}}$ was still significant ($F = 47.18$, $P < 0.001$, estimated d.f. = 6.97, 17.2% of variation explained). Simplification (upper limit of d.f.s set to seven) caused no significant change in the model deviance ($F = 1.14$, $P = 0.30$). This simplified model explained 17.2% of the deviance ($F = 63.36$, $P < 0.001$, estimated d.f. = 5.7) and revealed a reverse hump-shaped relationship between clutch size and $\text{NDVI}_{\text{seasonality}}$ (Fig. 4d).

Residual analyses

We further investigated the relationships between clutch size and RES1 and RES2, respectively (Table 1). Clutch size decreased nonlinearly with increasing RES1 ($F = 68.89$, $P < 0.001$, estimated d.f. = 7.88; Fig. 5a) and explained 24.7% of the variance. No model reduction was justified. After controlling for taxonomy the model was still significant ($F = 20.56$, $P < 0.001$, estimated d.f. = 8.34) and explained 9.1% of the variance (Fig. 5b). No model reduction was justified. Then we investigated the relationship between clutch size and RES2. The model revealed a nonlinear and positive relationship ($F = 54.11$, $P < 0.001$, estimated d.f. = 8.18; Fig. 5c) and the model explained 20.6% of the total variance. No model reduction was justified. After controlling for taxonomy, RES2 explained less variance in clutch size (11.5%) but the model was still significant ($F = 26.41$, $P < 0.001$, estimated d.f. = 8.00). Simplification (upper limit of d.f.s set to eight) caused no significant change in the model deviance ($F = 2.10$, $P = 0.14$). The simplified model explained 11.4% of the variance ($F = 33.35$, $P < 0.001$, estimated d.f. = 6.78; Fig. 5d).

Subset analyses

Using subsetted dataset (see above), we limited variation in $\text{NDVI}_{\text{seasonality}}$ and tested the effect of $\text{NDVI}_{\text{maximum}}$ on variation in clutch size (Table 1). We found a unimodal but decreasing relationship ($F = 41.96$, $P < 0.001$, estimated d.f. = 5.46). Simplification (upper limit of d.f.s set to six) caused no significant change in the model deviance ($F = 2$, $P = 0.158$). The final model explained 28.3% of the total variation ($F = 57.03$, $P < 0.001$, estimated d.f. = 4.55; Fig. 6a). After we controlled for taxonomy, the relationship changed to linearly decreasing (Fig. 6b) and explained only 5.58% of the variance ($F = 42.35$, $P < 0.001$, estimated d.f. = 1). Using the reversely subsetted dataset (limited variation in $\text{NDVI}_{\text{maximum}}$), we tested the effect of $\text{NDVI}_{\text{seasonality}}$ on clutch size. We found a nonlinear and positive relationship

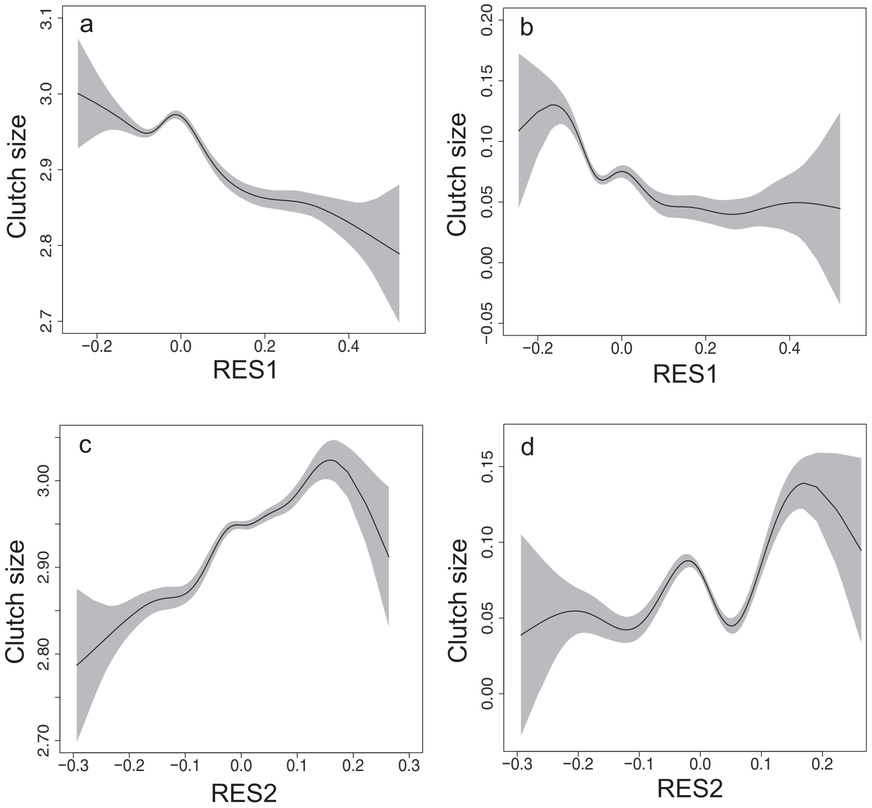

Figure 5 Nonlinear relationships (fitted by generalized additive models) between assemblage mean clutch size of South African passerines and residuals from ordinary least squares regressions between: (1) maximum normalized difference vegetation index (NDVI) as a response variable and the seasonality in NDVI (the difference between minimum and maximum NDVI) as an explanatory variable (RES1; a, b); (2) seasonality in NDVI as a response variable and maximum NDVI as an explanatory variable (RES2; c, d). Raw clutch size data (a, c) and clutch size data controlled for taxonomy (b, d) are provided. Grey areas indicate confidence intervals.

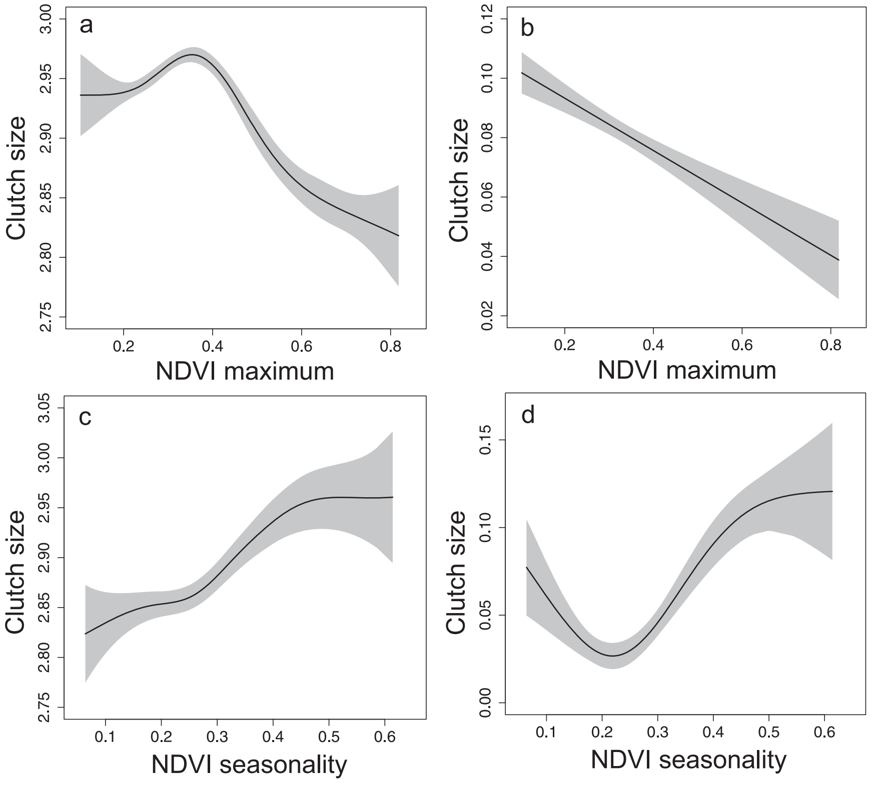

Figure 6 Nonlinear relationships (fitted by generalized additive models) between assemblage mean clutch size of South African passerines and maximum normalized difference vegetation index (NDVI) and NDVI seasonality within selected subsets of the whole dataset (see Methods and Figs 2 & S8). Relationships between assemblage mean clutch size and maximum NDVI for the dataset with restricted variation in NDVI seasonality (a, b). Relationships between assemblage mean clutch size and seasonality in NDVI for the dataset with restricted variation in maximum NDVI (c, d). Raw clutch size data (a, c) and clutch size data controlled for taxonomy (b, d) are provided. Grey areas indicate confidence intervals.

(Fig. 6c) that explained 26.8% of the total variance ($F = 12.86$, $P < 0.001$, estimated d.f. = 3.92). The pattern of the relationship changed to reverse hump-shaped after controlling for taxonomy ($F = 15.76$, $P < 0.001$, estimated d.f. = 4.03). The simplified model (upper limit of d.f.s set to four) did not differ significantly ($F = 1.117$, $P = 0.299$) and explained 31.6% of the variation ($F = 26.45$, $P < 0.001$, estimated d.f. = 2.92; Fig. 6d).

In summary, we found nonlinear patterns in the relationships between environmental variables and clutch size. However, the positive effect of seasonality on clutch size was still detectable in the data and the relationship between maximum productivity and clutch size seemed to be rather unimodal. The inclusion of taxonomy weakened the explanatory power of the environmental variables.

Differences among feeding guilds

The analyses performed within feeding guilds revealed that relationships between clutch size and environmental variables observed in insectivorous and fruit eating birds correspond to the situation observed in all passerines. In contrast, clutch size of granivorous species showed the opposite pattern. It decreased with increasing $\text{NDVI}_{\text{seasonality}}$ and increased with increasing $\text{NDVI}_{\text{maximum}}$. For results see Appendices S3–S5, S9 & S10.

DISCUSSION

Our results highlight the importance of Ashmole's specification (Ashmole, 1963) of the food limitation hypothesis originally proposed by Lack (1947). Assemblage mean clutch size reveals a significant spatial pattern across South African passerine assemblages (Fig. 3). Interestingly, two highly productive regions – the moist grasslands and savanna-woodlands in the eastern parts of South Africa – show consistent but different patterns. Moist grassland passerines lay larger clutches while moist savanna woodland birds lay smaller ones. Although both environments are productive, they differ in seasonality, which is higher in grasslands. This finding provides indirect support for Ashmole's hypothesis.

Using a more rigorous approach, we revealed that the relationship between clutch size and maximum NDVI is hump-shaped, whereas NDVI seasonality had a positive effect on clutch size. In other analyses, we fixed the variation in environmental variables statistically and found that relatively higher levels of maximum NDVI lead to smaller clutches, while relatively higher levels of seasonality in NDVI lead to larger clutches. We obtained similar results when the variation in environmental variables was fixed by subsetting the whole dataset. Seasonal variation in NDVI relative to its overall levels drives the clutch sizes of passerines across South Africa, thus providing a support for Ashmole's hypothesis.

We are obviously not the first to have corroborated the primary suggestion that resource seasonality increases bird clutch size. Lack & Moreau (1965) found smaller passerine clutches in tropical forests than in more seasonal savannas. Convincing evidence was also provided by Ricklefs (1980), who used information about 13 avian assemblages at different latitudes. Jetz *et al.* (2008) showed an important effect of environmental seasonality on avian clutch size at a global scale. However, their analysis revealed the effect of temperature seasonality (estimated as temperature range), not of the ratio characterizing seasonality in resource levels. Ashmole's hypothesis in combination with the cost of reproduction was also supported by simulations performed by Griebeler & Böhning-Gaese (2004). Finally, Lepage & Lloyd (2004) and Hořák *et al.* (2011) reported larger clutches in more seasonal environments for subsets of South African birds. However, here we were able to separate the effect of overall productivity level from seasonality itself, and show that seasonality determines clutch size variation not only between different latitudes but also along longitude and within one biogeographical unit. Moreover, our data confirmed a unique, so far overlooked, prediction of Ashmole's hypothesis: clutch sizes decrease with increasing maximum NDVI at fixed levels of NDVI difference (seasonality), because the same increment of productivity represents a smaller portion of total amount of resources in highly productive environment (see Fig. 1). This *relatively* small difference in resource availability is then reflected by only a low per capita increase in the amount of available food for nestlings.

The relationships described here frequently show nonlinear patterns. This might reflect real patterns (e.g. nonlinear variation in competition for food along an environmental productivity axis; Ballance *et al.*, 1997), but also some confounding effects due to the macroecological approach employed here. Nonlinear relationships could be attributed to other factors related differently to environmental productivity that also affect clutch size, such as nest predation (Skutch, 1949) – though this seems not to be the case here (Hořák *et al.*, 2011) – or life expectancy (Martin, 2002). Alternatively, they might be caused by factors which do not directly influence clutch size but are related to observed clutch size variation, such as geographical distributions of species. At least to some extent, distributional patterns seem to be a result of factors not directly linked to the environment, such as dispersal, stochasticity, evolutionary history or host–pathogen interactions (e.g. Ricklefs, 2013). Therefore, macroecological analyses based on species distributions may contain such kinds of noise.

The geographical pattern in assemblage mean clutch size we describe here seems to be partly caused by the different geographical distribution of passerine families, since it changed when we controlled for the taxonomic membership of birds (Fig. 3b). The relationships between the environment and clutch size thus seem to be driven by the differences in geographical distributions of avian families and phylogenetic conservatism in clutch size rather than by an adaptive variation in a response to the environment within families. However, using subsets of grid cells we found that taxonomical information did not change the observed relationships too much (compare Figs 4 and 5 with Fig. 6), supporting the idea of environmentally induced variation in clutch size even within families. This inconsistency might be related to the differences between the methodological approaches. The subsets of data include only parts of the focal geographical space, i.e. places with extremely high estimates of maximum NDVI and places with extremely low estimates of NDVI seasonality (see Fig. 2). These extreme conditions may provide strong selective pressure on avian variation of clutch size within families. The values of NDVI are highly spatially autocorrelated (see Appendix S7) and thus the selected grid cell subsets contain species sets that more or less belong to the same geographical regions (see Appendix S8). Consequently, our disparate results provide information for different spatial scales. For South Africa as a whole, spatial segregation among families with different clutch sizes plays a role. For the smaller spatial scales that include only smaller regions and therefore contain taxonomically more homogeneous assemblages, even slight differences driven by interspecific variation within families are pronounced.

These issues are related to the fact that our investigations of local assemblages are complicated by spatial autocorrelation (Dormann *et al.*, 2007). For that reason, we also performed analyses in which we controlled for this phenomenon. Especially for subsetted datasets, these analyses provided weaker support for the principal conclusions made here. This suggests that the spatial proximity of avian assemblages lies behind the similarity in clutch sizes of their members, and nearby locations are thus not independent. However, the interpretation of this fact is not straightforward. Since environmental variables reveal similarly strong spatial structure, we might speak rather about 'spatial dependence' *sensu* Legendre *et al.* (2002). Consequently, it is almost impossible to disentangle the effect of spatially structured environmental conditions on clutch size variation from spatial autocorrelation of assemblage compositions per se. We found that differential geographical distributions of avian taxa are likely to be responsible for the relationship between clutch size and environment, and it is reasonable to assume that geographical distribution reflects links between environmental conditions and avian adaptations. Therefore, spatial dependence among grid cells in the data does not negate our ecological explanations.

We acknowledge that other mechanisms besides density-dependent food limitation could play a role in explaining clutch size variation among bird assemblages. Specifically, the probability of future reproduction in terms of survival probability (Williams, 1966; Martin, 2002) and the number of reproductive attempts (Moreau, 1944) might be connected to resource levels in the environment and their changes (Erikstad *et al.*, 1998; Orzack & Tuljapurkar, 2001). Higher and/or stable levels of food resources might lower the probability of adult mortality (Oro & Furness, 2002; Harding *et al.*, 2011) and smaller clutches might be expected as a consequence. Also, seasonality influences the length of the bird breeding season, and more breeding attempts may be thus traded off for smaller clutches in less seasonal environments (Slagsvold, 1984; Farnsworth & Simons, 2001). However, in this case there would be no obvious reason for observing the decrease in assemblage mean clutch size with increasing maximum productivity at fixed levels of seasonality. Finally, abundant/diversified predator assemblages might increase nest predation pressure in more productive environments, in which in turn smaller clutches should be observed. This would fit with the patterns described here, although it would not explain the effect of seasonality on clutch size. Also, abundant bird populations and better concealed nests can in fact lower nest predation risk in more complex environments (Hořák *et al.*, 2011), which casts doubts on the generality of this explanation.

We found contrasting patterns for granivorous species (see Appendices S9 & S10). We suggest that this difference is due to the fact that granivore bird populations are not limited during the non-breeding season because the seed bank represents a stable food resource, so that the seasonality effect as described by Ashmole (1963) is not applicable. Granivorous species also feed their nestlings with insects, an additional source of food unrelated to the resource responsible for their population control. Therefore, their clutch size investment might not reflect availability of resources relatively to population sizes, and clutch size variation may rather mirror the absolute amount of available resources in the environment, leading to larger clutches at higher NDVI levels. In more seasonal locations, less food may be left for granivores due to higher breeding synchrony and stronger interspecific competition with insectivores which are better adapted for insect collection. Anyway, the finding that our predictions concerning NDVI and its seasonality do not work for the group whose food resource is apparently unrelated to NDVI is in accord with the other patterns reported here.

In sum, Ashmole's formulation has enabled application of Lack's original view to the comparison among different regions (differing in seasonality), thus explaining major geographical trends in assemblage mean clutch size across South Africa. The conclusions made here may be quite general and can potentially be applied to the tropical–extratropical comparison. Indeed, the 'seasonality hypothesis' predicts smaller clutches in highly productive but aseasonal tropical environments. We have also shown that geographical patterns in clutch size are most probably formed by both the different geographical distribution of higher taxa and the clutch size variation at the species level within passerine families. To completely understand the importance of seasonality for clutch size evolution, field data on local bird densities should be included to allow more mechanistic models of density-dependent food availability.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Appendix S1 Results of linear models.
Appendix S2 Results of generalized least squares models.
Appendix S3 Results of ordinary least squares models for particular feeding guilds.
Appendix S4 Results of spatial generalized least squares models for particular feeding guilds.
Appendix S5 Results of generalized additive models for particular feeding guilds.
Appendix S6 Effect of the presence of intra-African migrants in local assemblages on assemblage mean clutch size spatial patterns.
Appendix S7 Geographical variation in normalized difference vegetation index variables.
Appendix S8 Geographical locations of grid cell subsets used in the analyses.
Appendix S9 Differences in geographical patterns of assemblage mean clutch size among the three feeding guilds.
Appendix S10 Differences in nonlinear relationships between assemblage mean clutch size and normalized difference vegetation index variables among the three feeding guilds.

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