



## Test of an adaptive hypothesis for egg speckling along an elevational gradient in a population of Mexican jays *Aphelocoma ultramarina*

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The adaptive significance of avian egg speckling patterns has been a subject of ongoing debate. We examined speckling in a population of Mexican jays *Aphelocoma ultramarina* exhibiting extreme eggshell variability. We sampled 167 eggs at 55 nests from sites ranging across a steep elevation gradient within the Sierra del Carmen mountain range in Coahuila, Mexico, in order to test the recent hypothesis that egg speckling lends structural support to eggs and should therefore be more prevalent in females subject to reduced environmental calcium. Although we documented high variation in the amount and distribution of eggshell speckling within the Sierra del Carmen jays, we found no relationship between local soil calcium levels and the pattern of speckling. Our results indicate that explanations in addition to soil calcium levels are necessary to explain extreme variation in eggshell speckling in birds.

The wide variety of colours and patterning of the avian eggshell both among and within species has invited considerable speculation as to its potential adaptive significance. While many hypotheses exist to explain differences in egg appearance, speckling, in particular, is puzzling from an adaptive perspective because it occurs in a wide variety of species in many ecological contexts – open-cup nesters, hole-nesters, and species with and without brood parasites (Kilner 2006). In some ground-nesting birds, eggshell speckling may camouflage the egg and decrease predation risk (e.g. blackheaded gulls *Larus ridibundus*, Tinbergen et al. 1962). However, this hypothesis is less compelling for cup-nesting and hole-nesting species, which are unlikely to receive the same benefits from egg camouflage (Underwood and Sealy 2002).

Gosler et al. (2005) proposed a novel hypothesis for egg speckling, suggesting that brown spots, which are produced by the pigment protoporphyrin and have qualities similar to solid-state lubricants used in engineering (Solomon 1997), might provide structural support in areas of the shell that are thin or weak. Egg breakage caused by thin or weak eggshells is exacerbated by a deficiency of calcium, which is a limiting resource for breeding female birds (Dhondt and Hochachka 2001). The calcium content of soil is highly correlated with the abundance of calcium-rich invertebrates such as snails, and calcium-specific foraging is a common way for many bird species to supplement calcium levels during the egg-laying period (Graveland and van der Wal 1996).

One prediction following from Gosler et al.'s (2005) hypothesis is that when calcium is limited, speckling should be densest around the crown of the egg, where the shells are thinnest ("calcium deficiency hypothesis"). Gosler et al. (2005) found a strong relationship between local soil calcium availability and both eggshell mass and the distribution of speckling on the egg of the great tit *Parus major*. Eggshells from low calcium areas were lighter and thinner, and speckles were more densely distributed at the crown of the egg. Thus, while speckling appears to act as structural support for eggs of great tits, we do not know if the calcium deficiency hypothesis for speckling holds generally for other bird species, and in particular for medium and large birds, where calcium reserves in larger bones could mitigate environmental calcium deficiencies (Dacke et al. 1993).

Here, we test the hypothesis that egg speckling patterns are related to environmental calcium available to breeding females of the Mexican jay *Aphelocoma ultramarina*, a medium-sized, cooperatively-breeding passerine bird that shows extreme intrapopulation variation in eggshell speckling. In the Sierra del Carmen of Coahuila, Mexico, where we conducted our study, Mexican jays inhabit a steep elevation gradient. They are found in highly-divergent habitats ranging from arid woodland in the low canyons (1,400 m) to mixed conifer forest at the highest peaks (2,750 m) (Miller 1955), making small-scale variation in soil chemistry likely.

## Methods

Our study was conducted on a population of the *couchii* subspecies of Mexican jays, which ranges from southwestern Texas to northern Nuevo León, Mexico, and is known to have speckled eggs (Ligon and Husar 1974). We collected data on egg speckling and soil calcium from sites comprising the full elevational range of Mexican jays in the Sierra del Carmen of Coahuila, Mexico, mainly focusing on one low-elevation oak woodland site (San Isidro Canyon, 1,458–1,614 m), one mid-elevation pine-oak-juniper woodland site (Campo Uno, 1,801–1,865 m), and two high-elevation conifer-dominated sites (Campo Dos and Tres, 2,221–2,585 m). GPS data including location and elevation were taken for each nest. Distances between nests ranged from 3–10 km.

Using a scalpel, we first confirmed that the speckles on Mexican jay eggs are not superficial markings (as in many raptor and Charadriiforme eggs; Mikhailov 1997), but that they penetrate into the shell. Thus, speckles form part of the ground colour and might conceivably lend structural support to the shells. We assume that speckles occur at thin parts of the eggshell, as has been shown in other species (Gosler et al. 2005, Jagannath et al. 2008). Speckling percentage and degree of clumping on the crown of the egg were quantified from digital photographs taken using a Canon PowerShot A40 fitted with a macro lens to prevent parallax. We used the Java image processing program ImageJ (version 1.36b, Abramoff et al. 2004) to quantify the area and percentage coverage of speckling on each egg and on each third of each egg: crown, middle, and foot. To avoid problems associated with inter-observer variability, only one of us (ECB) conducted all speckling analysis.

We quantified speckling in two ways. First, we calculated the proportion of the entire (two-dimensional) egg area that was covered in speckles by dividing the total speckle area by the total surface area of the egg. Second, we measured the distribution, or degree of clumping, of speckles across the egg's surface, per Gosler et al. (2005), using three methods. First, we calculated the proportion of the total speckle area that was contained within the crown. Second, to control for the relative differences in total speckle area across eggs, we ran a regression of the crown value against total speckle area and calculated the residuals. Third, we assigned each egg to one of five categories, ranging from an even speckle distribution (score of 0) to a highly clumped distribution (score of 4). For all analyses of speckling distribution we excluded eggs with no speckles (i.e. speckle area of zero).

Soil samples of 1 L were collected to a depth of approximately six inches from the base of each nest tree. To determine whether soil calcium values varied significantly within the nest area (where the nesting female was most likely to forage), we collected three additional soil samples from randomly-selected locations within a 50-meter radius of the nest tree at one low-elevation and one high-elevation nest site. Samples were analyzed for extractable cations, including calcium, at A and L Western Agricultural Laboratories in Modesto, USA. Other minerals are known to be important to eggshell formation, especially in their ratios with calcium (e.g. phosphorus and magnesium; Fox 1976). However, as these other minerals occur in trace amounts in the eggshell and we approach the question

of eggshell quality from a limiting-resource perspective, we have focused exclusively on calcium. For the two nests where we collected four samples instead of just one, we also conducted an analysis of alkaline earth carbonates (percent limestone). The first test provides a measure of the amount of calcium found in organic matter (in parts per million, ppm), while the second test accounts for inorganic sources of calcium, such as calcium carbonate, that females might be incorporating into their diet.

All statistical tests were carried out using the statistical software package Stata Intercooled (version 10.0, StataCorp 2003). We tested continuous data for normality and normalized data when necessary using standard transformations. We assessed relationships among variables with simple regression and multiple regression, using speckling or speckle clumping as the dependent variable and soil calcium, elevation, year, clutch size, and interaction terms as independent variables, removing non-significant terms from multiple regression analyses with backward selection. We tested for relationships among variables in two ways. First we assessed the intraclass correlation coefficient for the dependent variables to determine if these variables were highly correlated among eggs within nests. If so, we used nest averages in regressions. Otherwise, we controlled for correlations among eggs within clutches with the cluster (*variable*) command in Stata, which calculates p-values using robust standard errors (Williams 2000). We also analyzed the data with mixed-model ANOVA. Results for both methods were similar, but we preferred the regression model because mixed-model ANOVAs can be sensitive to small sample sizes at the level of the clutch (where  $1 < n < 5$ ). Means are given  $\pm$  one standard deviation.

## Results

We collected representative soil samples from 51 of the 55 nest sites spanning a broad elevation gradient (1,421–2,585 m). Within-nest calcium values were highly correlated (intraclass correlation coefficient = 0.97), indicating that a single sample is probably sufficient to represent soil calcium levels in the broader nest area. The two calcium measures – extractable calcium and calcium carbonates – were also highly correlated (Pearson  $R^2 = 0.72$ ,  $P = 0.044$ ,  $n = 8$ ); thus, our analyses focus only on extractable calcium.

Across the study site, soil calcium ranged from 783 to 6,138 ppm. There was a highly significant negative relationship between soil calcium and elevation (regression:  $F = 68.80$ ,  $df = 1,49$ ,  $P < 0.001$ ,  $R^2 = 0.58$ ,  $n = 51$ ; Fig. 1a). For example, the lowest-elevation site, San Isidro Canyon (1,458–1,614 m), had relatively high soil calcium levels ( $4,519 \pm 1,024$  ppm,  $n = 20$ ), whereas soil calcium at the highest-elevation site, Campo Tres (2,476–2,585 m), was relatively low ( $1,198 \pm 410$  ppm,  $n = 5$ ). Campo Uno (1,801–1,865 m) and Campo Dos (2,221–2,400 m) were similar to each other with intermediate calcium levels ( $2,610 \pm 712$  ppm,  $n = 21$  and  $2,488 \pm 944$  ppm,  $n = 5$ , respectively).

From 2003–2007, we measured 167 eggs from 55 nests along a steep elevation gradient in the Sierra del Carmen. From knowledge of flock location, we were reasonably certain that 42 of these nests were from different flocks and

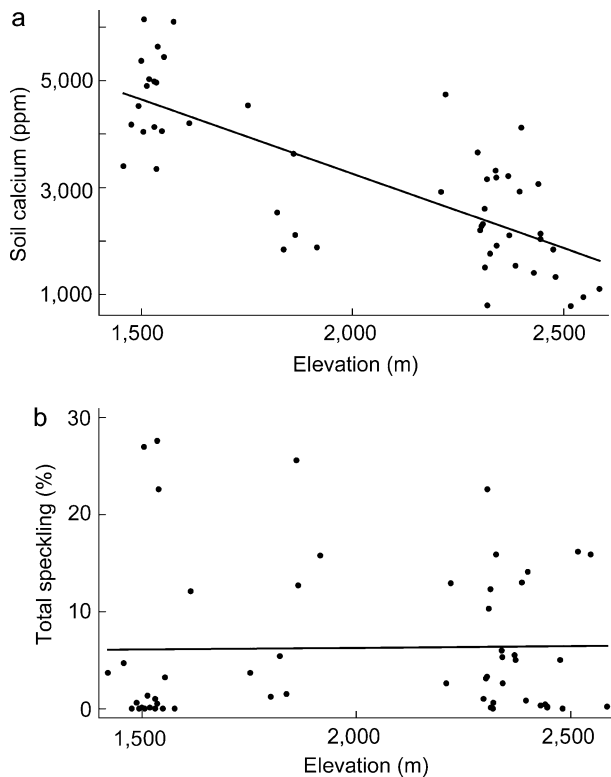


Figure 1. a: Negative relationship between soil calcium and elevation at nest sites in the Sierra del Carmen (regression:  $F = 68.80$ ,  $df = 1,49$ ,  $R^2 = 0.58$ ,  $P < 0.001$ ,  $n = 51$ ). b: No relationship between average speckling of clutches and elevation (regression:  $F = 0.50$ ,  $df = 54$ ,  $R^2 < 0.01$ ,  $P = 0.48$ ,  $n = 55$ ).

therefore highly likely to be from different females. The remaining 13 nests were from 10 flocks that were already represented by one nest. Because dominant females often remain as breeders within flocks over multiple years (McCormack and Brown 2008), we were therefore not certain if these 13 nests represented different females. However, because our dependent variables were not highly correlated among the nests within a flock (intraflock correlation coefficient for speckle clumping = 0.36 and for percent speckling = 0.45), we included these nests in our analyses. The results did not change when these data were excluded.

Speckles ranged from being completely absent to covering nearly half of the shell's surface (mean =  $0.06 \pm 0.09$ , range = 0 to 0.41). Speckle distribution ranged from being evenly distributed across the egg to highly clumped toward the crown, with the proportion of the egg's speckles located on the crown ranging from 0 to 1 (mean =  $0.43 \pm 0.29$ ). Our two continuous metrics of speckle clumping were highly correlated (Pearson  $r^2 = 0.94$ ,  $P < 0.001$ ,  $n = 167$ ), and these were correlated with our categorical metric (Pearson  $r^2 = 0.58$ ,  $P < 0.001$ ,  $n = 73$ ). Thus, we report only the results of analyses using the residuals of crown speckle area against total speckle area.

Because percentage of the egg's surface covered with speckles (hereafter 'speckling') was highly correlated within nests (intraclass correlation coefficient = 0.73), we calculated nest averages for this variable (see Methods). There

was no relationship between average speckling per nest (arcsine-square-root transformed to normalize) and elevation (regression:  $F = 0.50$ ,  $df = 54$ ,  $R^2 < 0.01$ ,  $P = 0.48$ ,  $n = 55$ ; Fig. 1b), or soil calcium (regression:  $F = 0.49$ ,  $df = 50$ ,  $R^2 = 0.01$ ,  $P = 0.49$ ,  $n = 51$ ).

There was relatively low correlation for speckle clumping within nests (intraclass correlation coefficient = 0.33), so we treated eggs as individual data points while controlling for correlations among eggs within nests (see Methods). Results from a multiple regression analysis indicated no significant relationship between speckle clumping and a model including calcium, elevation, year, and calcium  $\times$  elevation, year  $\times$  elevation, and year  $\times$  calcium interactions (regression:  $F = 0.96$ ,  $df = 8,37$ ,  $R^2 = 0.05$ ,  $P = 0.46$ ,  $n = 123$ ). Non-significant terms were removed in stepwise fashion starting with non-significant interaction terms: calcium  $\times$  elevation (marginal  $t = -1.72$ ;  $P = 0.09$ ) and year  $\times$  elevation (marginal  $t = 1.93$ ;  $P = 0.06$ ), year  $\times$  calcium (marginal  $t = 1.42$ ;  $P = 0.16$ ), and year (marginal  $t = -0.91$ ;  $P = 0.37$ ). The resulting simple regression between speckle clumping and calcium was not significant ( $F = 0.08$ ,  $df = 1,42$ ,  $R^2 < 0.001$ ,  $P = 0.78$ ,  $n = 123$ ; Fig. 2). Even when these data were analyzed without adjusting degrees of freedom to account for non-independence of eggs within nests, there was no significant relationship between speckle clumping and calcium ( $F = 0.07$ ,  $df = 1,121$ ,  $R^2 < 0.001$ ,  $P = 0.79$ ,  $n = 123$ ).

## Discussion

The population of Mexican jays we studied was particularly well-suited to a test of the calcium-deficiency hypothesis because we found high variation in egg speckling (0–41% surface area speckled) and soil calcium, the latter varying along an elevation gradient. However, we found that eggs from nests at low-calcium sites were not more speckled and speckles were not more clumped toward the crown of the

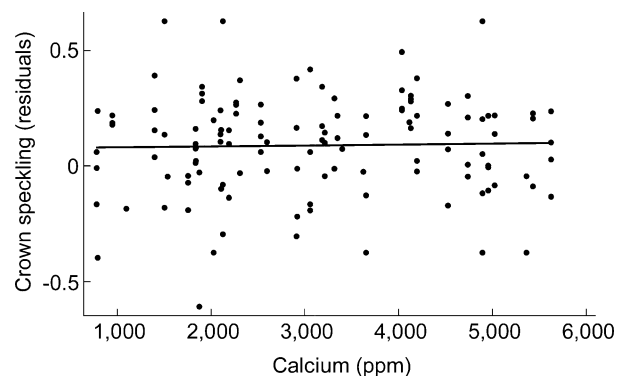


Figure 2. No relationship between soil calcium and clumping of speckles on the crown of the egg (crown speckling) (regression:  $F = 0.08$ ,  $df = 1,42$ ,  $R^2 < 0.001$ ,  $P = 0.78$ ,  $n = 123$ ). Values for crown speckling were normalized with arcsine-square-root transformation. Eggs are represented here as independent data points, but correlations within nests were taken into account when calculating the significance of the relationship (see Methods).

egg than at high-calcium sites. Likewise, elevation itself was not correlated with either speckling or speckle clumping. Thus, our results do not support the calcium-deficiency hypothesis for egg speckling.

There are two possible explanations for why we might have failed to detect a relationship between soil calcium and speckling. One is that soil calcium levels in the Sierra del Carmen might not be low enough to limit eggshell formation in Mexican jays. In our study, extractable calcium ranged from 783 to 6,138 ppm. In Oxford's Wytham Woods where Gosler et al.'s (2005) study took place, extractable calcium ranged from 410 to 230,000 ppm (Farmer 1995). While the range of values was more extreme in Gosler et al.'s (2005) study, the low-calcium values were similar. Thus, all else being equal, female Mexican jays from low-calcium nest sites in the Sierra del Carmen were expected to experience similar levels of calcium deficiency as the great tits in Gosler et al.'s (2005) study. It is important to note that the range of calcium values that we recorded is similar to the variation found in other studies that demonstrated a strong relationship between soil calcium and eggshell characteristics in birds (e.g. Graveland et al. 1994), suggesting that if soil calcium does indeed influence speckling in this species we would have been able to detect this effect.

Alternatively, Gosler et al.'s (2005) hypothesis might not be generally applicable to all bird species. Kilner (2006) reasons that a "hierarchy of selective forces" has probably influenced the evolution of eggshell polymorphism, with crypsis providing the most general explanation for variation in eggshell colour and patterning, brood parasitism playing a secondary role, and finally, the need for structural support driving variation in just a few species. Medium to large-bodied birds might not be as dependent on environmental calcium during the egg formation stage. For instance, Pahl et al. (1997) showed that great tits cannot store calcium in their bones, whereas a study of Japanese quail *Coturnix japonica* suggested that larger species do draw on medullary bone as a source of calcium during egg-laying (Dacke et al. 1993). Mexican jays are much larger than great tits (mean of 120 g versus 19 g), and female jays may thus be able to supplement dietary calcium with bone calcium, which can be stored throughout the year in anticipation of egg laying. A study on a larger-bodied species, the sparrowhawk *Accipiter nisus*, showed that speckling was indeed related to eggshell thinning, but that the effect was mediated through environmental DDT, which blocks uptake of calcium by the shell gland (Jagannath et al. 2008). While this study supports the idea that speckling can provide structural support to calcium-deficient eggs even in a raptor, it did not test whether environmental calcium variation was an important factor or if large-bodied birds instead draw on medullary sources of calcium during egg-laying.

Considering that nearly all New World corvids have speckled eggs (Madge and Burn 1994), including congeners of the scrub-jay complex, speckled eggs in the *couchii* subspecies of Mexican jays could represent the retention of an ancestral state that currently has no adaptive value. Before accepting this explanation, however, the adaptive value of speckling in this species should probably now be

tackled by returning to and rigorously testing hypotheses previously considered unlikely in this species, such as egg crypsis and brood parasitism.

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