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 (Campos Uno, 1,801–1,865 m), and two high-elevation conifer-dominated sites (Campos 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, or degree of clumping, of speckles across the egg to small sample sizes at the level of the clutch (where 1 < n < 5). Means are given ± 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 ± 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 ± 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 ± 712 ppm, n = 21 and 2,488 ± 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...
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 = 0.33), we were therefore not excluded. Speckles ranged from being completely absent to covering nearly half of the shell’s surface (mean = 0.06 ± 0.09, range = 0 to 0.41). Speckle distribution ranged from being evenly distributed across the egg to highly clumped toward the crown of the egg, with the proportion of the egg’s speckles located on the crown ranging from 0 to 1 (mean = 0.43 ± 0.29). Our two continuous metrics of speckle clumping were highly correlated (Pearson r² = 0.94, P < 0.001, n = 167), and these were correlated with our categorical metric (Pearson r² = 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 = 68.80, df = 450, R² = 0.01, P = 0.001, n = 51). There was a negative relationship between soil calcium and elevation (regression: F = 0.50, df = 54, R² = 0.01, P = 0.48, n = 55; Fig. 1b), or soil calcium (regression: F = 49.01, df = 50, R² = 0.001, P = 0.49, n = 51).

Non-significant terms were removed in stepwise fashion starting with non-significant interaction terms: calcium × elevation (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, R² < 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 soil calcium (F = 0.07, df = 1.121, R² < 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 egg.
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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References


