A trade-off between embryonic development rate and immune function of avian offspring is revealed by considering embryonic temperature

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Long embryonic periods are assumed to reflect slower intrinsic development that are thought to trade off to allow enhanced physiological systems, such as immune function. Yet, the relatively rare studies of this trade-off in avian offspring have not found the expected trade-off. Theory and tests have not taken into account the strong extrinsic effects of temperature on embryonic periods of birds. Here, we show that length of the embryonic period did not explain variation in two measures of immune function when temperature was ignored, based on studies of 34 Passerine species in tropical Venezuela (23 species) and north temperate Arizona (11 species). Variation in immune function was explained when embryonic periods were corrected for average embryonic temperature, in order to better estimate intrinsic rates of development. Immune function of offspring trades off with intrinsic rates of embryonic development once the extrinsic effects of embryonic temperatures are taken into account.

Keywords: embryonic temperature; embryonic period; immune function; trade-offs

1. INTRODUCTION

Slower embryonic development, reflected by longer embryonic periods, is thought to allow enhanced development of physiological systems, like the immune system, that increase survival and longevity [1–3]. Such trade-offs are thought to underlie the well-known difference between tropical and north temperate birds in length of embryonic periods and adult survival [2–4]. Yet, the only two tests of a trade-off between length of the incubation period and immune function of nestling birds found no support for a trade-off [5,6] and no study has included offspring in tropical and temperate sites. Thus, a trade-off between embryonic development rate and offspring immune function remains unclear, especially across latitudes, but is critical to furthering our understanding of trade-offs in the evolution of offspring quality and life-history strategies.

Previous tests relied on the assumption that embryonic periods reflect the inverse of intrinsic embryonic development rate, yielding a predicted increase in strength of immune defence with length of the embryonic period (figure 1a). This assumption may be problematic for ectothermic embryos, like birds, because of temperature effects. Temperatures experienced by ectothermic embryos influence both embryonic development rate and the amount of energy converted into tissue [7,8]. Variation in parental incubation behaviour determines temperatures experienced by avian embryos, and experimental swapping of eggs showed that temperature affects length of the embryonic period [9]. Differences in embryonic period owing to such extrinsic effects of temperature do not reflect differences in intrinsic rates of development [10]. Instead, intrinsic rates of embryonic development should be better estimated by deviations from period lengths predicted by embryonic temperature while controlling for any allometric effects of size (figure 1b). In particular, species with embryonic periods that are long for their temperature (points above the line in figure 1b) reflect slower intrinsic development, controlling for extrinsic effects of temperature. Conversely, species with embryonic periods that are short for their temperature (points below the line in figure 1b) reflect faster intrinsic development, controlling for temperature. If this is true, species differences in immune function should be best predicted by the temperature-corrected embryonic period as an estimate of intrinsic development rates (figure 1c). Here, we test these alternatives.

2. MATERIAL AND METHODS

Study sites were high-elevation (2300 m elevation) mixed forest in Arizona (34° N, 111° W) and cloud forest (1350–2000 m elevation) in the Andes of Venezuela (9° N, 69° W). We intensively measured embryonic temperatures, embryonic periods and immune function for 11 Passerine species in north temperate Arizona, and 23 species in tropical Venezuela (appendix A), which comprise the set of species used for analyses here. Study sites were searched for nests during breeding seasons of 1988–2009 in Arizona and 2002–2008 in Venezuela. Embryonic periods were quantified as the difference in days between last egg laid and last egg hatched [9] for nests found prior to laying of the last egg and for which hatch day was observed.

Embryonic temperatures were measured by placing a thermistor in the centre of one egg and measuring temperature experienced by the embryo every 12 or 24 s in three to nine nests per species over 5 days per nest, on average [9]. We used two common assays of immune function [5,11,12] that have been related to offspring survival and disease resistance (e.g. [13]). In particular, we measured the inflammatory response to injection of phytohaemagglutinin (PHA, Sigma L-8754) at a standardized developmental stage (the day nesting primary pin-feathers broke their sheaths) and averaged the response for each nest. Nestlings were injected in the patagium with 0.04 ml of a solution of 5 mg ml⁻¹ of PHA in phosphate-buffered saline (PBS). The inflammatory response at the point of injection was measured after 24 h with a pressure-sensitive micrometer to the nearest 0.01 mm [5]. This inflammatory response reflects an induced innate and adaptive immune response [11,14]. We also measured the activity of natural antibodies that circulate in normal individuals in the absence of exogenous antigenic stimulation as a measure of constitutive innate immune defences [14]. We followed the protocol by Matson et al. [12] scaled to our sample sizes of plasma (between 15 and 25 µl). Plasma samples were serially diluted twofold with 0.01 M PBS (Sigma P3813) and incubated at 37°C for 90 min with a 1 per cent rabbit blood cell suspension (Colorado Serum, CO, USA). We collected blood samples from nestlings when the primary feathers broke their sheaths in order to standardize stage of development. Blood samples were kept on ice in the field and centrifuged within 8 h to separate the plasma. Plasma samples were stored frozen at −20°C until analyses were performed.
Figure 1. Immune function is predicted to trade off with intrinsic embryonic development rate. (a) Length of the embryonic period is assumed to reflect the inverse of intrinsic development rate and, so immune defence is predicted to increase with embryonic period length. (b) Embryonic temperature is an extrinsic effect on embryonic period, where cooler temperatures cause longer embryonic periods. Points above \((w,x)\) and below \((y,z)\) the line reflect species with relatively longer versus shorter periods and slower versus faster intrinsic development (shown in grey), respectively, corrected for temperature. Species at points \(x\) and \(y\) have the same absolute embryonic period but differ in their intrinsic development rates. (c) Immune function is expected to increase with embryonic period corrected for possible extrinsic temperature effects to provide a more accurate index of intrinsic embryonic development rate.

Figure 2. Measures of immune function based on the inflammatory response to phytohemagglutinin (PHA) injection and natural antibody activity relative to lengths of embryonic periods. Plots reflect the relationships of residuals from analyses in table 1. (a) Inflammatory response and natural antibody activity residuals correct for body mass and site and are not related to log-transformed length of embryonic periods for Passerine species in tropical Venezuela and north temperate Arizona (table 1). (b) The immune measure residuals correct for embryonic temperature, body mass, and site and are related to temperature-corrected embryonic periods that correct for embryonic temperature, body mass and site (table 1). Note that shorter temperature-corrected periods (left side of \(x\)-axis) reflect faster embryonic development for a given temperature and longer temperature-corrected periods (right side) reflect slower development for a given temperature (from figure 1c). Solid lines reflect the regression line for Arizona and the dashed line is Venezuela.
Table 1. Results of analyses of covariance to explain interspecific variation in immune function for raw data and phylogenetically independent contrasts. Interactions were dropped from the models when \( p > 0.10 \), but main effects were always retained.

<table>
<thead>
<tr>
<th>Variables</th>
<th>D.f.</th>
<th>F-value</th>
<th>p-value</th>
<th>D.f.</th>
<th>F-value</th>
<th>p-value</th>
</tr>
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<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>inflammatory immune response</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>2.99</td>
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<td>1.72</td>
<td>0.20</td>
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<td>1</td>
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<tr>
<td>natural antibody activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.52</td>
<td>1</td>
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(b) accounting for embryonic temperature effects (figure 2b)

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<tr>
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<td>4.18</td>
<td>0.052</td>
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3. RESULTS

The expected positive relationship between length of the embryonic period and immune function (i.e. figure 1a) was not observed for either inflammatory or natural antibody measures of immune function (figure 2a and table 1). Indeed, if anything, the relationships tended towards negative patterns (figure 2a), rather than the expected positive (i.e. figure 1a) patterns.

When embryonic temperature was included in models, the embryonic period became highly significant and positive in direction (table 1). However, interactions with sites were also highly significant (table 1), so we analysed each site separately. Tests of both measures of immune function against the temperature-corrected embryonic period (i.e. figure 1c) were positive and significant in both sites (figure 2b).

4. DISCUSSION

A trade-off between intrinsic embryonic development rate and offspring quality traits, like immune function, is fundamental to life-history theory [17]. Yet, two broad measures of immune function showed no relationship with the absolute length of the embryonic period in bird species from both tropical and temperate sites (i.e. figure 2a). Once embryonic temperature effects on length of embryonic periods are incorporated, the trade-off is clear (figure 2b).

These interspecific results mesh well with a recent intraspecific experimental study where nestlings from eggs that were cooled during the embryonic period had lower immune function than control nestlings [7]. Cooler embryonic temperatures can cause longer embryonic periods and decreased developmental efficiency from increased metabolic costs, and thereby compromise postnatal offspring quality [8,18]. The assumption that the absolute length of the embryonic period is an estimate of intrinsic embryonic development rate may be misplaced when embryonic temperature varies extensively, as commonly seen among passerines [9]. Embryonic development period became a significant positive predictor of nestling immune function once the extrinsic effects of temperature on embryonic development were considered.

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APPENDIX A

Species included in the sampling and analyses by location

Cardellina rubrifrons          Arizona
Catharus guttatus             Arizona
Empidonax occidentalis       Arizona
Junco hyemalis              Arizona
Pipilo chlorus              Arizona
Poeole gambeli             Arizona
Sialia mexicana             Arizona
Troglydtes aedon           Arizona
Turdus migratorius         Arizona
Vermivora celata          Arizona
Vermivora virginiernae   Arizona
Atlapetes semirufus        Venezuela
Basilnoterus rufifrons     Venezuela
Buurremon bruneinucha    Venezuela
Catharus aurantirostris   Venezuela
Catharus fuscater         Venezuela
Dyssithamnus mentalis     Venezuela
Grallaricula ferrugineiceps Venezuela
Henchorhina leucophrys     Venezuela
Masius chryosperus        Venezuela
Mionectes olivaceus       Venezuela
Myadestes ralloides       Venezuela
Myioborus miniatus        Venezuela
Myrmotherula chrichticlor Arizona
Phrycichla flaviceps      Venezuela
Phrycichla leucops        Venezuela
Pronopel brunneinucha     Venezuela
Saltator maximus          Venezuela
Tachypelus rufus           Venezuela
Thraupis episcopus        Venezuela
Thraupis palmarum        Venezuela
Troglydtes aedon           Venezuela
Turdus olivater           Venezuela
Zimmerius chrysops       Venezuela