BEHAVIORAL ECOLOGY

Prenatal acoustic communication programs offspring for high posthatching temperatures in a songbird

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In many species, embryos can perceive and learn external sounds. Yet, the possibility that parents may use these embryonic capacities to alter their offspring's developmental trajectories has not been considered. Here, we demonstrate that zebra finch parents acoustically signal high ambient temperatures (above 26°C) to their embryos. We show that exposure of embryos to these acoustic cues alone adaptively alters subsequent nestling begging and growth in response to nest temperature and influences individuals' reproductive success and thermal preferences as adults. These findings have implications for our understanding of maternal effects, phenotypic plasticity, developmental programming, and the adaptation of endothermic species to a warming world.

By shaping offspring phenotype to the environment experienced by the mother, maternal effects have the capacity to alter population dynamics in fluctuating environments (*1*–*3*). Maternal effects, as other forms of phenotypic plasticity, may therefore provide a mechanism for species' persistence under global warming (*4*, *5*). In particular, parental effects may improve offspring thermal acclimation (*6*) and optimize offspring growth in current temperature regimes, as demonstrated in invertebrates (*7*) and recently in ectothermic vertebrates (*8*). Whether parents in endothermic species can program their offspring for current climatic conditions is currently unknown.

Here, we investigated whether avian parents can signal environmental conditions to their embryos, allowing them to adaptively modulate their growth in response to temperature. Because, in birds, mothers can no longer affect the biochemical environment of their embryos after laying, we tested the possibility that parents signal current climatic conditions via acoustic cues. Indeed, parents call to their eggs during incubation in several bird species (*9*–*11*), and late-stage avian embryos can perceive, and even produce, sound (*12*–*15*). Prenatal acoustic communication, mostly in precocial birds, has been found to synchronize hatching (*14*), allow embryos to solicit parental incubation (*12*, *15*), and improve imprinting, perceptual learning, and brain functions (*16*–*18*), although the direct fitness impact of these effects has not been quantified. Recent studies in two species of fairy wrens (*Malurus* spp.) showed that nestlings imitate the call of their foster incubating mother, and provisioning mothers tend to discriminate against foreign-sounding nestlings (*10*, *11*). Therefore, by altering nestling begging, incubation calling may have the potential to function similarly to other avian maternal effects, such as yolk hormone content that modifies nestling begging and growth (*3*, *19*, *20*). Yet, whether parents signal environmental conditions and alter the developmental trajectory of their offspring using prenatal acoustic communication is unknown.

The Australian zebra finch *(Taeniopygia guttata)* is an arid-adapted songbird that breeds opportunistically in fluctuating temperature regimes (*21*) and in which nestling mass before fledging varies by up to 30%, depending on nesting density (*22*) and parental care coordination (*23*). We identified that, in outdoor aviaries, wild-derived zebra finches produced an "incubation call" while alone with their eggs (real or dummy) with their partner away from the nest (*24*) (fig. S1). This call was exclusively uttered toward the end of the incubation period, within 5 days of hatching (Fig. 1A) (time to expected hatch date: linear: Z = 2.19, P = 0.03; quadratic: Z = −2.38, P = 0.02, n = 266 recordings) (table S1), and calling rate per hour increased closer to expected hatch date (table S1). In addition, incubation calling was very clearly associated with elevated ambient temperatures, occurring only when maximal daily temperature rose above 26°C (Z = 2.81, P = 0.005, n = 266) (Fig. 1B), regardless of seasonal variation (fig. S2 and table S1). Therefore, as suggested in fairy wrens (*10*, *11*, *25*), zebra finch parents appear to control the production of incubation calls to signal environmental conditions to their embryos, because calling only occurred close to hatching, rather than as a spontaneous reaction to heat.

To investigate experimentally whether this calling behavior may prepare offspring for high ambient temperatures, eggs were artificially incubated at a standard temperature (37.7°C) and exposed to acoustic playback of either incubation calls ("treatment") or control contact calls in the last 5 days of incubation (fig. S1) (*24*). Nestlings hatched in the incubators were then returned to the aviary to be raised in nest boxes with naturally contrasting temperature profiles, depending on sun exposure throughout the day. Nestlings exposed to incubation calls as embryos followed a different growth pattern in response to nest temperature than did control nestlings: Treatment nestling mass (and to a lesser extent tarsus length) on day 13 decreased with nest temperature, whereas it increased in control nestlings (Fig. 2) (mass: t = −3.30, P = 0.001, n = 130 nestlings from 45 broods) (tables S2 and S3). This effect at the end of the nestling period (day 13) was already arising just 1 day

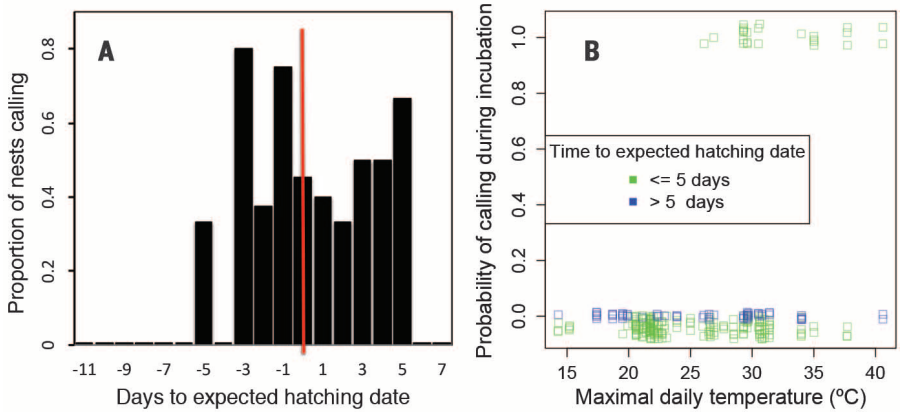

Fig. 1. Incubation calling behavior in relation to incubation stage and ambient temperatures. (**A**) Proportion of nests producing incubation calls on hot days (maximal daily temperature ≥26°C) in relation to the number of days before or after expected hatching date (vertical red line). (**B**) Probability of pairs emitting incubation calls in relation to maximal daily temperature on the day of recording for nests within 5 days of hatching (green) or either before or after this period (blue). Each data point represents one nest in a recording session.

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after hatching [linear mixed model (LMM) on mass at day 1: t = −2.18, P = 0.032, n = 125 nestlings from 45 broods] and was observed throughout nestling development (same interaction on two random subsamples: 2 days post-hatching: t = −2.20, P = 0.0037, n = 61 nestlings from 30 broods; and 10 days: t = −2.91, P = 0.0094, n = 44 nestlings from 22 broods). This interaction was, however, not present at hatching (LMM: playback by future nest temperature: t = −1.03, P = 0.31; playback alone: t = −0.13, P = 0.89, n = 130 nestlings from 45 broods) and was not due to differential nestling mortality (Cox proportional hazard regression: playback by nest temperature: Z = 0.25, P = 0.80, n = 166 nestlings in 52 broods; nest temperature in treatment: Z = −0.43, P = 0.67, n = 79 nestlings in 35 broods; nest temperature in control: Z = −0.38, P = 0.70, n = 87 nestlings in 37 broods).

In birds, maternal effects on nestling growth induced by differential hormone concentrations in the egg are partly mediated posthatching by their effects on nestling begging (*19*, *20*). Therefore, and because incubation calling altered nestling begging in fairy wrens (*10*, *11*), we investigated whether differences in nestling begging could underlie the differential growth patterns followed by experimental nestlings in response to nest temperatures (*24*). Accordingly, in the first 3 days after hatching, treatment nestlings (i.e., incubation call playback) were more likely to call while begging when they had experienced high temperatures in the nest since hatching, whereas control nestlings called less, independently of temperature (Fig. 3) (generalized LMM: playback by temperature in nest: Z = 2.23, P = 0.026, n = 77 recordings for 59 chicks from 19 broods). Nestling mass and satiation at the time of recording had no effect on the probability of calling while begging (mass: Z = −0.71, P = 0.48; presence of seeds in crop: Z = 0.86, P = 0.39), which suggests that this call might signal thermal state (*12*, *15*) rather than hunger level or body condition.

The decrease in mass with increasing nest temperatures that we found in treatment nestlings (compared with control nestlings) (Fig. 2) is consistent with the current pattern observed in many wild bird species worldwide (*26*). Although this environmentally driven size reduction is generally expected to have negative effects on offspring fitness [e.g., (*27*, *28*)], reducing mass gain at high temperature might be beneficial if it reduces oxidative damage associated with growth in such an environment or if small body size facilitates heat loss (*26*, *29*, *30*). Therefore, to determine whether such a pattern is adaptive, we measured the reproductive success of our experimental birds at adulthood (*24*). Resembling the pattern in treatment nestlings, females that as nestlings had low body mass under hot conditions during development or greater body mass in cold rearing conditions produced more fledglings in their first breeding season (Fig. 4A) [generalized linear model (GLM): Z = −2.66, P = 0.0078, n = 39 females]. This effect applied to both treatment and control

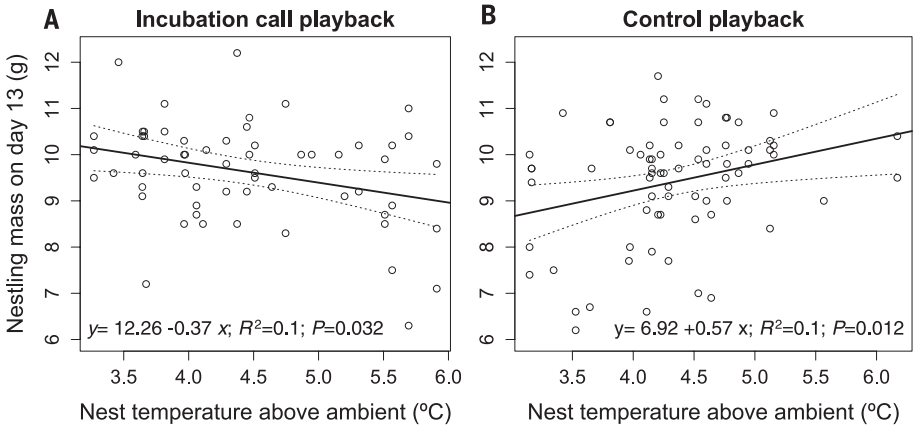

Fig. 2. Nestling mass on day 13 (i.e., at the end of the nestling phase) in relation to nest temperature above ambient. (**A**) Treatment nestlings exposed to incubation calls. (**B**) Control nestlings exposed to parental contact calls. Raw data are shown, with one data point per nestling. The lines depict the regression line (full) and 95% confidence intervals (dotted).

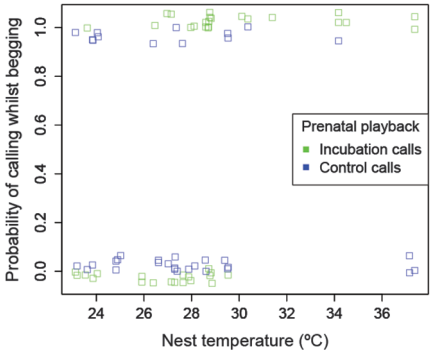

Fig. 3. Probability of calling while begging in relation to nest temperature experienced since hatching (nest temperature above ambient + mean daily maximum temperature) for nestlings that heard incubation calls (green) or control calls (blue) as embryos. Each data point represents one nestling.

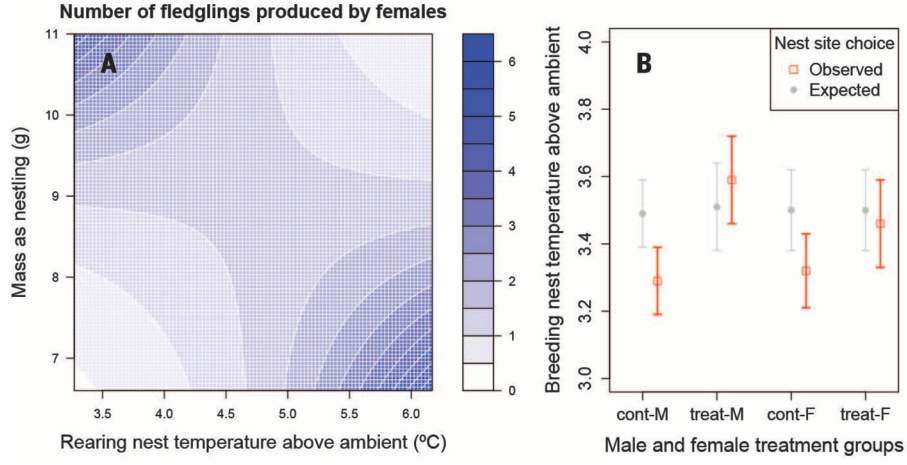

Fig. 4. Effect of incubation calling as late-stage embryos on individuals' reproductive success and thermal preferences in their first year. (**A**) The number of fledglings produced by females (predicted values from GLM) in relation to the nest temperature (above ambient) that they experienced as nestlings and their mass as 13-day-old nestlings. (**B**) Observed (red) mean (±SE) temperature above ambient of breeding nest boxes used at adulthood by treatment ("treat") and control ("cont") males and females, compared with values expected by chance (gray) given nest box availability and pair composition (two treatment, two control, or mixed partners).

females (no additional effect of playback treatment: $Z = 0.39$, $P = 0.69$) after controlling for female mass as adult ($Z = 2.83$, $P = 0.005$). Furthermore, this fitness effect of early mass and thermal conditions persisted in females' second year of life despite repairing (GLM: $Z = -2.31$, $P = 0.021$; repairing: $Z = 1.17$, $P = 0.24$; adult mass: $Z = 2.82$, $P = 0.005$, $n = 36$ females, including 25 with a new partner). Males followed a similar trend to females in their first year [GLM with temperature in nest (i.e., ambient temperature + nest differential): $Z = -1.97$, $P = 0.049$, $n = 36$ males] but not their second year (GLM: $Z = 1.65$, $P = 0.10$, $n = 38$ males).

Last, the evolutionary advantage of maternal effects has been questioned in unpredictable environments, where environmental conditions during development do not predict those encountered later in life (*31*). However, individuals may partly compensate for this by seeking microhabitats that best suit their phenotype. Accordingly, treatment individuals exposed to incubation calls in the egg went on to consistently breed in hotter nest boxes than control birds, because control males used cooler boxes and treatment males used warmer boxes than expected by chance in the first and second years, respectively (Fig. 4B) (Monte-Carlo simulations: first-year control males: $P = 0.024$, $n = 22$, and females: $P = 0.075$, $n = 15$; second-year treatment males: $P = 0.046$, $n = 10$; all others: $P > 0.05$) (*24*).

Overall, we have demonstrated experimentally that by acoustically signaling high ambient temperatures to their embryos before hatching, zebra finch parents can program the developmental trajectories of their offspring in response to this key environmental variable. Our findings therefore provide both an adaptive function for prenatal communication and a type of maternal effect where parental control over signal production can be unambiguously tested. By uncovering a mechanism for a transgenerational effect of temperature on development in endotherms, our study also advances our understanding of the acclimatization capacities of organisms to rising temperatures.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/353/6301/812/suppl/DC1
Materials and Methods
Figs. S1 and S2
Tables S1 to S3
References (*32*–*40*)

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GENE REGULATION

Integration of omic networks in a developmental atlas of maize

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Coexpression networks and gene regulatory networks (GRNs) are emerging as important tools for predicting functional roles of individual genes at a system-wide scale. To enable network reconstructions, we built a large-scale gene expression atlas composed of 62,547 messenger RNAs (mRNAs), 17,862 nonmodified proteins, and 6227 phosphoproteins harboring 31,595 phosphorylation sites quantified across maize development. Networks in which nodes are genes connected on the basis of highly correlated expression patterns of mRNAs were very different from networks that were based on coexpression of proteins. Roughly 85% of highly interconnected hubs were not conserved in expression between RNA and protein networks. However, networks from either data type were enriched in similar ontological categories and were effective in predicting known regulatory relationships. Integration of mRNA, protein, and phosphoprotein data sets greatly improved the predictive power of GRNs.

Predicting the functional roles of individual genes at a system-wide scale is a complex challenge in biology. Transcriptome data have been used to generate genome-wide gene regulatory networks (GRNs) (*1*–*4*) and coexpression networks (*5*–*7*), the design of which was based on the presumption that mRNA measurements are a proxy for protein abundance measurements. However, genome-wide correlations between the levels of proteins and mRNAs are weakly positive (*8*–*15*), which indicates that cellular networks built solely on transcriptome data may be enhanced by

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