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E-Article

Ecdysteroid Hormones Link the Juvenile Environment to Alternative Adult Life Histories in a Seasonal Insect

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abstract: The conditional expression of alternative life strategies is a widespread feature of animal life and a pivotal adaptation to life in seasonal environments. To optimally match suites of traits to seasonally changing ecological opportunities, animals living in seasonal environments need mechanisms linking information on environmental quality to resource allocation decisions. The butterfly *Bicyclus anynana* expresses alternative adult life histories in the alternating wet and dry seasons of its habitat as endpoints of divergent developmental pathways triggered by seasonal variation in preadult temperature. Pupal ecdysteroid hormone titers are correlated with the seasonal environment, but whether they play a functional role in coordinating the coupling of adult traits in the alternative life histories is unknown. Here, we show that manipulating pupal ecdysteroid levels is sufficient to mimic in direction and magnitude the shifts in adult reproductive resource allocation normally induced by seasonal temperature. Crucially, this allocation shift is accompanied by changes in ecologically relevant traits, including timing of reproduction, life span, and starvation resistance. Together, our results support a functional role for ecdysteroids during development in mediating strategic reproductive investment decisions in response to predictive indicators of environmental quality. This study provides a physiological mechanism for adaptive developmental plasticity, allowing organisms to cope with variable environments.

Keywords: developmental plasticity, seasonal adaptation, life history, resource allocation, diapause, polyphenism, 20-hydroxyecdysone.

Introduction

Understanding how animals cope with the seasonal fluctuations in environmental quality that characterize many temperate and tropical habitats is a key challenge in evolutionary ecology and an important requirement if we want to predict ecological responses to climate change (Hofmann and Todgham 2010; Visser et al. 2010; Meylan et al. 2012). To optimally match suites of traits—that is, alternative life histories—to seasonally changing ecological opportunities, animals living in seasonal environments need mechanisms linking information on environmental quality to resource-allocation decisions. In many animals, hormones provide such mechanisms (Nijhout 2003; Beldade et al. 2011; Simpson et al. 2011). They play crucial regulatory roles in transducing indicators of seasonal progression—for instance, temperature or photoperiod—into adaptive alterations of the phenotype, such as timing of reproduction or preparation for diapause (e.g., Denlinger 2002; Dawson 2008; Brakefield and Zwaan 2011). These same hormonal mechanisms are also involved in the regulation of phenotypic plasticity when the environmental stimulus is not (directly) related to seasonality, such as crowding (e.g., in crickets and locusts; Simpson and Sword 2009; Zera 2009), nutrition (e.g., in nematodes, social insects, and beetles; Smith et al. 2008; Sommer and Ogawa 2011; Emlen et al. 2012), or a combination of stimuli (e.g., in aphids; Brisson 2010). Understanding seasonal adaptations from an evolutionary perspective will require combining a detailed dissection of hormonal mechanisms of plasticity with ecological experiments aimed at establishing the relationships between these mechanisms and fitness in the field (Zera et al. 2007; Visser et al. 2010; Beldade et al. 2011; Braendle et al. 2011; Gilbert 2012). However, addressing seasonal plasticity in an integrative way—from the environmental sensitivity, the hormonal changes, and the sensitivity of the target phenotype to the hormone to the ecological relevance of the altered phenotype—is not possible in many systems. Here, we take such an approach and study seasonal adaptation in the butterfly *Bicyclus anynana* from the developmental and hormonal mechanisms

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to the alternative life-history strategies relevant for natural populations.

The East African butterfly *B. anynana* expresses distinct life strategies in each season. During the warm, wet season, larval and adult food is plentiful, larvae develop fast, and adults live active lives with rapid reproduction and relatively short life spans. In contrast, during the cool, dry season characterized by no larval resources and adult food scarcity, adults display a higher investment in body reserves, have longer life spans, and postpone reproduction (Brakefield and Reitsma 1991; Brakefield and Zwaan 2011). These phenotypic differences are determined by the seasonal temperatures that larvae and pupae experience during development, with a high temperature signaling the wet season and a decline to lower temperatures predicting the approaching dry season (Brakefield and Reitsma 1991). In the laboratory, several aspects of these alternate life histories can be induced by development at different temperatures (Fischer et al. 2003; Pijpe et al. 2007; Steigenga and Fischer 2007; de Jong et al. 2010). Recently, we showed that females reared at high temperatures (wet-season conditions) develop a relatively larger abdomen compared to those reared at low temperatures (dry-season conditions). This response is discontinuous, with a threshold at an intermediate temperature (Oostra et al. 2011). Resting metabolic rate (RMR) in young adults is also affected by developmental temperature: butterflies developed at low temperatures have a higher RMR as adults, irrespective of adult temperatures (Pijpe et al. 2007; Oostra et al. 2011). The proximate mechanisms linking preadult temperatures to adult phenotypes are unknown, but previous observations suggest an involvement of ecdysteroid hormones during the pupal stage. Seasonal temperatures experienced during larval development drive dynamics of pupal ecdysteroids, with an earlier peak in hormone concentration in pupae reared at high versus low temperatures (Koch et al. 1996; Brakefield et al. 1998). A detailed characterization of hormonal reaction norms showed that the shift in hormone dynamics is discontinuous, with a similar shape and identical threshold temperature as the phenotypic reaction norm for female abdomen size (Oostra et al. 2011). Together, these correlative studies suggest that ecdysteroid signaling is a regulator of the developmental plasticity in life history.

The first aim of this study was to establish the extent to which pupal ecdysteroids play a functional role in fully inducing alternative life histories in response to developmental temperature. We approached this question by manipulating ecdysteroids in pupae reared at three different temperatures spanning the range of natural seasonal environments (Brakefield and Reitsma 1991) and then monitoring the phenotypic effects for a suite of seasonally plastic traits: (1) pupal development time, (2) adult RMR, (3) allocation of adult body mass to abdomen, and (4) adult fat content.

The second aim of this study was to assess windows of hormone sensitivity during the pupal stage. In our previous experiments, we observed differences in thermal responses among traits putatively regulated by the same hormone and suggested that these could arise as a result of differences among traits in their windows of sensitivity to that hormone (Oostra et al. 2011). To assess hormone sensitivity across time, a pupa was injected at one of four separate time points, representing different stages of the natural dynamics in ecdysteroid concentrations during the pupal stage (Brakefield et al. 1998; Zijlstra et al. 2004; Oostra et al. 2011).

Our third goal was to test, in an independent follow-up experiment, the ecological consequences of any hormone-induced changes in morphology and physiology observed in the initial experiment. We again manipulated ecdysteroids, focusing on a single temperature and injection time point, and monitored effects on multiple aspects of adult fitness: (1) onset of oviposition, (2) early life fecundity, (3) egg size, (4) life span, and (5) starvation resistance.

In this study, we show that ecdysteroids are responsible for temperature-induced seasonal developmental plasticity of allocation of body resources to the abdomen in *B. anynana* females. In addition, we demonstrate that ecdysteroid-induced allocation changes between thorax and abdomen have consequences for fitness: pupal hormone injections accelerate onset of oviposition and increase egg size but reduce fecundity later in life as well as life span. These results support a functional role for ecdysteroids in reproductive investment decisions during development in response to variation in environmental quality and provide insight into mechanisms enabling organisms to persist in fluctuating environments.

Methods

Experimental Design

We first performed a full factorial experiment with three developmental temperatures and four injection time points. Immediately after hatching, larvae were divided into three temperature treatments: 19°, 23°, and 27°C. We recorded pupations to the nearest 15 min using time-lapse photography, excluded male pupae, and assigned female pupae to one of four injection time points: 3%, 16%, 29%, or 34% of total pupal development time (DT). Total pupal DT in absence of hormone manipulation was strongly affected by temperature, with pupae reared at 19°, 23°, and 27°C developing on average in 356, 193, and 158 h, respectively. For pupae reared at 19°C, the four injection time points thus correspond to 10 h 41 s, 56 h 58 s, 103 h 14 s, and

121 h 02 s after pupation; for pupae at 23°C, they correspond to 5 h 47 s, 30 h 53 s, 55 h 58 s, and 65 h 37 s after pupation; and for pupae at 27°C, they correspond to 4 h 44 s, 25 h 17 s, 45 h 49 s, and 53 h 43 s after pupation. Previous data on natural ecdysone titers in absence of manipulations for the three temperatures allowed us to identify four time points representing relevant stages of the pupal ecdysteroid pulse: (i) overall low titers (3% DT), (ii) titers ascending for wet season but not for dry season (16%), (iii) titers descending for wet season but not for dry season (29%), and (iv) titers descending for dry season and low for wet season (34%). At the latter three time points, the natural titers differ between wet- and dry-season pupae (Oostra et al. 2011). Pupae were injected with either 20-hydroxyecdysone (20E) or control solutions, after which they were allowed to continue development and eclose individually at their respective larval temperatures. After eclosion, we measured resting metabolic rate and abdominal dry weight and fat content in $N = 15$–45 per temperature per injection time point per injection treatment.

In the follow-up experiment, we reared larvae at 23°C, injected the pupae at 16% DT, and measured fecundity, life span, and starvation resistance in the adult females ($N =$ 50–80 per injection treatment). In both experiments, all larvae were derived from the same outbred *Bicyclus anynana* captive population and reared on young maize plants sprayed with an antifungal agent (see Brakefield et al. 2009 for rearing protocols).

Hormone Injections

Fresh injection solutions were prepared daily by combining 107 μL of × 1 Ringer's physiological solution with 3 μL of Vital Red dye (Fluka, Buchs, Switzerland) and either 10 μL of 100% ethanol (control treatments) or 10 μL of 1 mg/mL 20E (Sigma-Aldrich, St. Louis) in 100% EtOH (hormone treatments). Using a 10-μL Hamilton (Bonaduz, Switzerland) microsyringe with a 0.3-mm needle, we injected pupae laterally between the fourth and fifth abdominal segments with 3 μL of injection solution (0 or 0.25 μg 20E for the control and hormone treatments, respectively), injecting each female only once. To avoid easily induced pharmacological effects of exogenous hormone applications, it is critical that titers of injected hormones are well within physiological ranges, and this can be established only by knowledge on natural hormone concentrations (Zera 2007). Therefore, we based the amount of hormone to inject on previous studies on pupal ecdysteroids in *B. anynana*, which yielded detailed knowledge on natural 20E concentrations throughout the pupal stage as well as dose-response curves for mortality (Koch et al. 1996; Brakefield et al. 1998; Zijlstra et al. 2004; Oostra et al. 2011). These data also allowed us to inject at biologically relevant time points, when ecdysteroids are active and their titers differ between seasonal morphs (see above). In addition, we quantified how 20E hormone injections affect internal 20E titers and found that these levels are similar to the natural 20E concentrations during the early pupal stage and much lower than peak concentrations (R. Mateus and P. Beldade, unpublished data). Thus, our hormone manipulations did not raise 20E titers to unnatural levels.

Measurements of Phenotypic Responses

First Experiment: Pupal Development Time, RMR, Abdominal Dry Weight, and Fat Content. All pupae were weighed to the nearest 0.1 mg within 36 h of pupation. In the first experiment, a subset of pupae (~20%) was kept separately to measure pupal development time with 15-min precision. We monitored these pupae toward the end of the pupal period and recorded new eclosions every 15 min by time-lapse photography. One day after eclosion, we measured RMR for each female as the individual rate of CO_2 respiration (mL per hour) over a period of 20 min, following Pijpe et al. (2007). All RMR measurements were done at 27°C during the dark phase of the diurnal cycle. Next, abdomens were cut off to measure their dry weight, extract total fat (triglyceride and free fatty acids), and measure fat-free dry weight following Oostra et al. (2011). Fat content was calculated by subtracting the fat-free dry weight from the initial dry mass.

Second Experiment: Fecundity, Life Span, and Starvation Resistance. One day after eclosion, we weighed each adult female to the nearest 0.1 mg and introduced her into a mating cage with 10–30 virgin males (3–10 days old), keeping the ratio of females to males in these cages below 1. We inspected the cages every 15 min and separated mating pairs into cylindrical oviposition pots. After each mating had finished, we removed the male and provided the female with ad lib. food and a fresh cutting of *Oplismenus* sp. grass for oviposition. After 72 h, we moved the female to a new pot. This was repeated three times, yielding a total of four consecutive egg measurement periods with age classes of 2–4, 5–7, 8–10, and 11–13 days. After each period, we counted the total number of eggs in the oviposition pot. To estimate egg size, we photographed the spherical eggs (in three batches of ~10 eggs each) against a black background using a Leica DC200 digital still camera connected to a Leica (Wetzlar, Germany) MZ12 stereo microscope (× 3.2 magnification). On every image, we measured egg area as a measure of egg size (following Fischer et al. 2003), using an automated macro in ImageJ software. After four egg measurement periods covering the 12 days after mating, we transferred females to larger cages, with a maximum of 10 females per cage, provided oviposition plants and food ad

lib., and monitored survival daily. Females that laid only unfertilized eggs were excluded from the analysis.

Each day, we separated a fraction of newly eclosed females and excluded them from the fecundity assay. Instead, we kept them virgin, introduced them into larger cages with a maximum of 15 females per cage, and provided them with ad lib. access to water (wet cotton) but not food to record starvation resistance (SR). We scored and removed dead females twice a day.

Statistical Analyses

In the first experiment, we initially analyzed the data using a three-way analysis of variance (ANOVA) for each phenotypic trait, with rearing temperature, injection time point, and hormone treatment as fixed variables (see table A1; tables A1–A4 and figs. A1 and A2 are available in the online appendix). To identify time point-specific treatment effects, in cases where injection time point interacted significantly with hormone treatment, we subsequently analyzed each time point separately using two-way ANOVAs, with rearing temperature and hormone treatment as fixed effects (see table A3). Prior to the ANOVAs, pupal development time was natural log transformed. We analyzed RMR, abdomen dry weight, abdomen fat content, and abdomen fat-free dry weight first in separate linear regressions models, with pupal mass as the only predictor variable (see table A2), and subsequently used the residuals of these regressions as dependent variables in the ANOVAs. Post hoc comparisons between 20E- and control-treated females at specific temperatures were performed with Tukey's honest significant differences (HSD) tests.

In the second experiment, fecundity was strongly nonnormally distributed during the first egg measurement period (age 2–4 days), as a large fraction of females had not yet laid any eggs in this period. Therefore, we chose to analyze this first period separately, treating fecundity as a categorical variable: females either had or had not started to lay eggs in this period. Numbers of females in each category were compared between injection treatments using a χ^2 test. For the three subsequent egg-laying periods (ages 5–13 days), we analyzed fecundity using a repeated measures general linear model (GLM) with injection treatment and age as fixed variables and individual as a random variable. In order to obtain P values for each main effect, we constructed a model without the main effect and compared it to the full model with a likelihood-ratio test. For specific comparisons at each age class between 20E- and control-treated females, we obtained P values using a Markov chain Monte Carlo method (MCMC; Baayen 2011). We also analyzed egg size using a repeated measures GLM with injection treatment and age as fixed variables and individual and egg measurement batch as random variables. We analyzed life span and starvation resistance using a Cox proportional hazard model, with adult mass as a covariate and injection treatment as a fixed variable; age at death was used as the dependent variable. All analyses were performed in R (R Development Core Team 2010) with the survival (Therneau 2012), lme4 (Bates et al. 2011), and languageR (Baayen 2011) packages. All data are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.b42s4 (Oostra et al. 2014).

Results

Ecdysteroids Accelerate Pupal Development and Increase Adult Mass Allocation to Abdomen

The 20E treatment affected pupal development time differently depending on the time of injection, as indicated by a significant interaction between injection time point and treatment in the three-way ANOVA (table A1). When pupae were injected at 3% and 16% DT, 20E treatment induced a substantial acceleration of pupal development, while this was not the case at either 29% or 34% DT (fig. 1; table A3). Pupae reared at 27°C showed the weakest response to early 20E treatment compared to pupae reared at the other temperatures. At this same temperature, 20E treatment at 34% DT had the reverse effect on these pupae: development was slowed rather than accelerated (Tukey's HSD $P < .0005$). No such effect was observed at the other temperatures. The overall acceleration in development on injections earlier in development was due to a higher proportion of butterflies eclosing a full day or more earlier and was not accompanied by a change in time of day at which they eclosed.

Relative abdomen mass was substantially increased after pupal 20E injection at 3% or 16% but not at 29% or 34% DT (fig. 2; tables A1, A3). This reveals a period of ecdysteroid sensitivity during development of the abdomen. The effect of 20E treatment on relative abdomen mass was similar in magnitude and direction to the effect of developmental temperature. In particular, 20E-injected pupae reared at 19°C have abdomens similarly sized to those of control-injected pupae reared at 23°C, and 20E-injected pupae reared at 23°C have abdomens similarly sized to those of control-injected pupae reared at 27°C (fig. 2; table A3). Thus, exogenous ecdysteroids phenocopy the temperature-induced seasonal differences in abdomen size.

We then asked whether this hormone-induced increase in abdomen mass was due to an increase in fat content, fat-free dry weight, or both. As was the case for total abdomen mass, the effect of 20E treatment on abdominal fat content depended on the timing of injections: fat content was higher in females injected as pupae with 20E compared to controls for manipulations at 3% and 16%

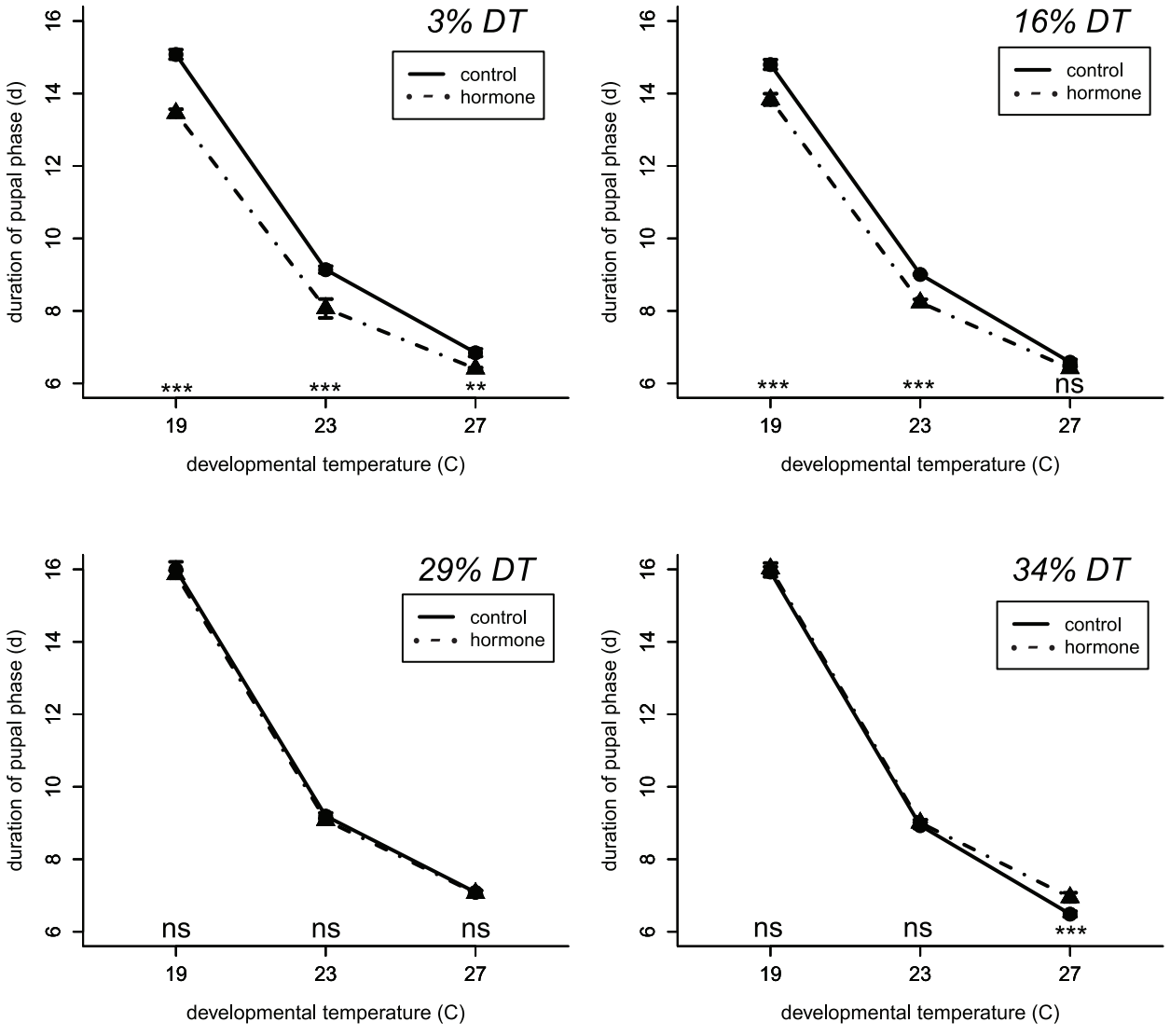

Figure 1: Early but not late 20-hydroxyecdysone (20E) treatment accelerates pupal development. Duration of pupal stage (days ± SEM) is strongly affected by developmental temperature, as indicated by the shape of reaction norms and large differences between extreme temperatures. In addition, pupae injected with 20E (triangles and dot-dashed line) at 3% or 16% of pupal development time (DT) show significant acceleration of development in comparison with controls (circles and solid line; two-way ANOVA $P < .00001$), while those injected at 29% or 34% DT show no such effect. Late injections (34% DT) decelerate development, but only at 27°C (Tukey's honest significant differences [HSD] $P < .001$). See tables A1 and A3, available online. Asterisks indicate significant differences between control- and 20E-treated animals; two asterisks = $P < .01$; three asterisks = $P < .001$; ns = not significant, $P > .05$); in the case of significant temperature by treatment interaction in two-way ANOVAs, P values from post hoc Tukey's HSD are reported; when this interaction was not significant, the overall treatment effect of the two-way ANOVA is given. For pupae reared at 19°C, the four injection time points correspond to 10 h 41 s, 56 h 58 s, 103 h 14 s, and 121 h 02 s after pupation; for pupae reared at 23°C, they correspond to 5 h 47 s, 30 h 53 s, 55 h 58 s, and 65 h 37 s after pupation; and for pupae reared at 27°C, they correspond to 4 h 44 s, 25 h 17 s, 45 h 49 s, and 53 h 43 s after pupation.

DT but not at 29% or 34% DT (fig. A1; tables A1, A3). In addition, at 3% DT, we observed a significant interaction between treatment and temperature (table A3); pupae reared at 19° and 23°C showed a response to 20E (Tukey's HSD $P < 0.001$), whereas those reared at 27°C did not. Likewise, abdominal fat-free dry weight increased in response to pupal 20E injections, but, again, only when injected at 3% and 16% and not at 29% or 34% (fig. A2; tables A1, A3). Considered together, we conclude that the increase in abdomen mass in the females injected with 20E

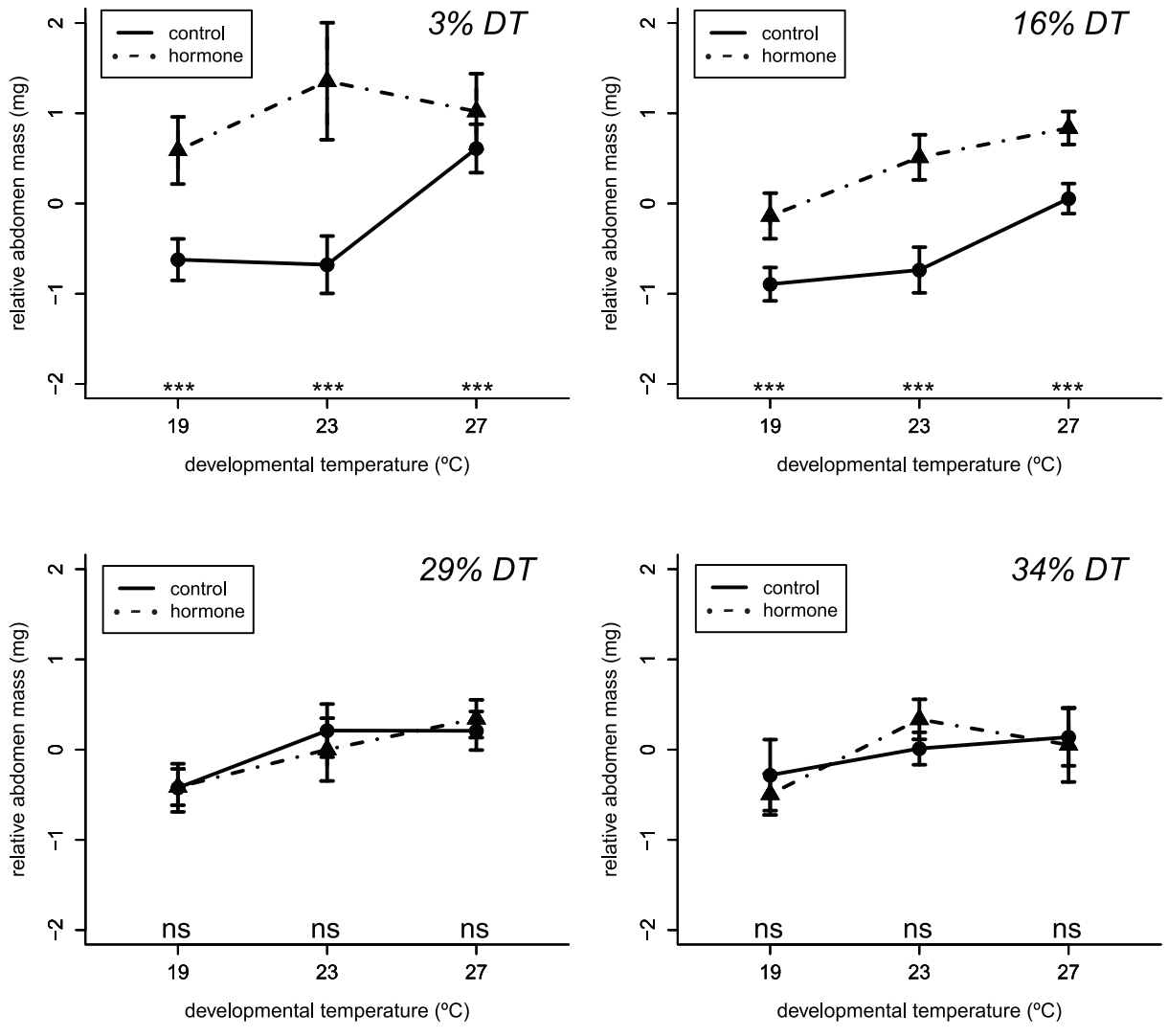

Figure 2: Pupal ecdysteroids induce high, wet-season-like allocation to abdomen mass. Mass-corrected abdomen dry weight (mg; see table A2; tables A1–A4 and figs. A1 and A2 are available online) is significantly affected by developmental temperature with females reared at high temperatures (wet-season conditions) having a larger abdomen. In addition, pupae injected with 20E (dot-dashed line) at 3% or 16% development time (DT), but not at 29% or 34% DT, show a substantial increase in abdomen mass compared to controls (solid line), similar in magnitude and direction to the temperature effect (two-way ANOVA $P < .0005$). See tables A1 and A3 and figures A1 and A2. Significance is as in figure 1.

as pupae in the earlier time points was due to an increase in both fat and nonfat mass, with both traits showing an identical window of sensitivity to the 20E injections.

Developmental Signature on Adult RMR Is Not Mediated by Ecdysteroids

We found no evidence for a role for ecdysteroids in mediating the preadult temperature effect on adult RMR. As observed previously (Pijpe et al. 2007; Oostra et al. 2011), RMR corrected for body size (see table A2) was higher in females developed at lower (dry season) temperatures. However, we observed no significant effect of 20E treatment on size-corrected RMR for any of the four injection time points at any of the three temperatures (fig. 3; tables A1, A3).

Pupae Show a Limited Window of Sensitivity to Ecdysteroid Manipulation

Sensitivity of pupal development rate, abdomen dry weight, and fat content to 20E treatment was not constant

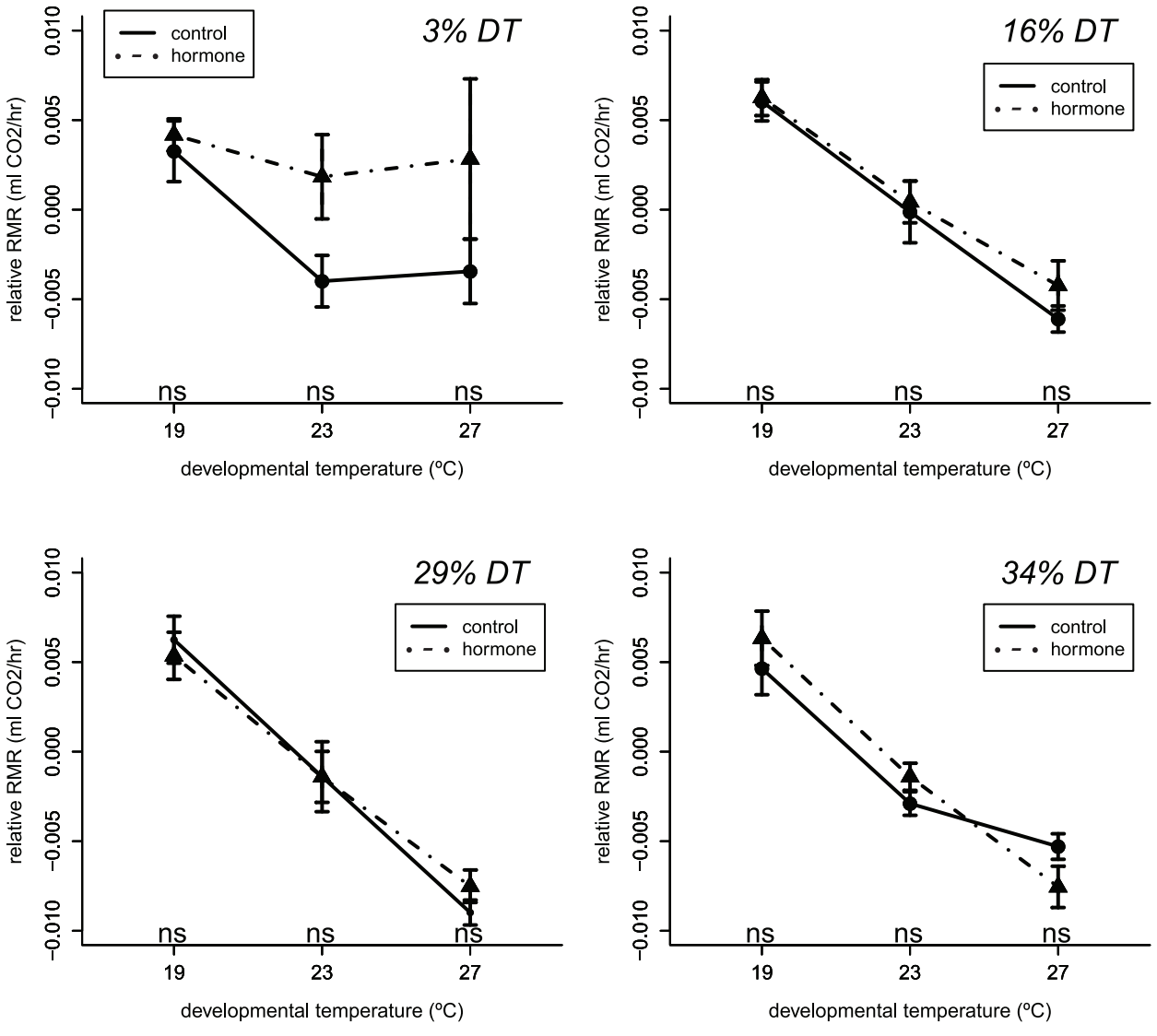

Figure 3: Developmental temperature signature on adult resting metabolic rate (RMR) is not mediated by pupal ecdysteroids. Mass-corrected RMR (mL CO_2 h^{-1}; see table A1; tables A1–A4 are available online) is significantly affected by developmental temperature, with individuals reared at lower temperature having higher RMR (two-way ANOVA $P < .005$). However, 20E treatment in the pupal stage has no significant effect on RMR at any of the four injection time points (compare solid line and dot-dashed line reaction norms). See tables A1 and A3.

in time, as indicated by a significant effect on all of these traits of the interaction between treatment and injection time point (table A1). In particular, the traits were most strongly affected by injections at the two earlier time points (3% and 16% DT; figs. 1, 3, A1, and A2; table A3), when natural ecdysone titers are rising (Oostra et al. 2011; see also "Methods"). In contrast, later in the pupal stage (29% and 34% DT), when natural ecdysone titers are decreasing (Oostra et al. 2011), these traits showed little if any response to injections. Furthermore, this window of hormone sensitivity was affected by the temperature at which the pupae had developed. Pupae from 19°C or 23°C developed an enlarged abdomen with increased fat content and accelerated pupal development rate in response to 20E injections at both 3% and 16% DT (figs.1, 2, A1). However, those reared at the wet season temperature of 27°C only showed increased abdominal fat content when injected at 16%, not 3%, DT (fig. A1) and accelerated development when injected at 3%, not 16%, DT (fig. 1). In the same 27°C cohort (and not at 19° or 23°C), late injections at 34% DT had the reverse effect on rate of development compared to injections at

3% and 16% DT; development was slowed rather than accelerated.

Pupal Ecdysteroids Affect Reproductive Schedule, Life Span, and Starvation Resistance

To assess whether the observed induction of relatively larger, wet-season-like abdomens by pupal ecdysteroid levels has fitness consequences for the adult life history, we reared an independent cohort of larvae at 23°C, injected females at 16% of pupal development time, and measured effects on adult performance. We focused on this temperature and time point because they revealed the largest effects of ecdysteroids on abdomen size in the first set of experiments (fig. 2).

After eclosion, females were mated and allowed to oviposit. In the first period of oviposition (age 2–4 days), not all females had started laying eggs. Among the control-treated females, 35% had not laid their first egg during this period, while this percentage was less than half (17%) among the 20E-treated individuals (fig. 4*B*). Thus, 20E treatment during the early pupal stage significantly accelerated the onset of first egg laying (χ^2 $P < .05$; table A4), resulting in a ~31% increase in mean number of eggs produced in this period (fig. 4*A*). Among those females that laid eggs in this period, there was no significant difference in mean number of eggs between the 20E- and control-treated group (table A4). This indicates that ecdysteroids probably do not increase the rate of egg production once it has started but instead bring forward the onset of oviposition.

Later in life, after the peak in egg laying, the 20E-treated females laid fewer eggs compared to the control females (fig. 4*A*; table A4); at age 8–10 days, the reduction was 9% (MCMC $P = .19$; see "Methods"), but in the final oviposition period that was monitored (age 11–13 days), the difference was more substantial (23%, MCMC $P < .005$). Although the total number of eggs produced in all four oviposition periods combined was 7% lower in the 20E-treated females compared to the controls, this effect was not significant (table A4). Thus, it appears that pupal 20E treatment, while accelerating the onset of oviposition, inflicts a fecundity cost later in life by accelerating the normal age-related decline in fecundity.

Since females can alter their egg size and number (Fischer et al. 2003), we wanted to know whether the decrease in later-life fecundity was offset by an increase in egg size. This was indeed the case: eggs of the 20E-treated females were larger compared to those of the control-treated females (fig. 4*C*; table A4). However, this was only observed at age 8–10 days (MCMC $P < .05$) and, to a lesser extent, at age 11–13 days (MCMC $P = .07$).

After the final fecundity measurements, we monitored individual daily survival. Females treated with 20E as pupae lived, on average, 12% fewer days than control females (fig. 4*D*; Cox proportional hazard $P = .06$, hazard ratio $= 1.38$; table A4). Splitting the females into two groups according to early reproductive status revealed that the negative effect of 20E treatment on life span was significant only for those females that had reproduced before the age of 4 days; the females that showed accelerated egg laying in response to 20E showed reduced life span (Cox proportional hazard $P < .05$, hazard ratio $= 1.58$; table A4), while those that did not lay eggs in that period showed the same life span as control females. It appears that, in addition to reducing fecundity later in life (fig. 4*A*), ecdysteroid-induced acceleration in onset of oviposition (fig. 4*B*) inflicts a fitness cost on life span (fig. 4*D*).

The increased allocation to abdomen mass in the ecdysteroid-injected females observed in the first experiment (fig. 2) could also have been related to aspects of adult performance other than fecundity. In particular, both nonfat and fat mass were increased in these females (figs. A1 and A2), which could contribute to survival under starvation (Zwaan et al. 1991). To test this hypothesis, we measured starvation resistance in adult females from the cohort of larvae reared at 23°C and injected at 16% pupal DT. We found that 20E-treated females survived, on average, ~1 day (8%) longer without food compared to the control-treated females (fig. 4*E*; Cox proportional hazard $P < .01$, hazard ratio $= 0.68$). In addition, smaller females showed the largest increase in adult SR when injected with 20E (Cox proportional hazard $P < .05$ for mass × treatment interaction; table A4). This suggests that virgin females with an ecdysteroid-induced increased abdomen mass are able to use the increased abdominal resources to live longer when confronted with food stress.

Discussion

Seasonal developmental plasticity in *Bicyclus anynana* involves a suite of morphological, physiological, and life-history traits covarying across the seasons in response to developmental temperature. Previously, we observed a correlation between expression of some of these adult traits and ecdysteroid dynamics during the pupal stage. Here, we functionally test the involvement of these hormones in the developmental regulation of the alternative adult life histories. We manipulate ecdysteroids during pupal development and observe significant shifts in adult reproductive resource allocation, mimicking in direction and magnitude the seasonal phenotypic changes normally induced by temperature experienced during development. This reveals that pupal ecdysteroid hormone titers provide the causal link between the seasonal environment during development and allocation of adult mass to reproductive

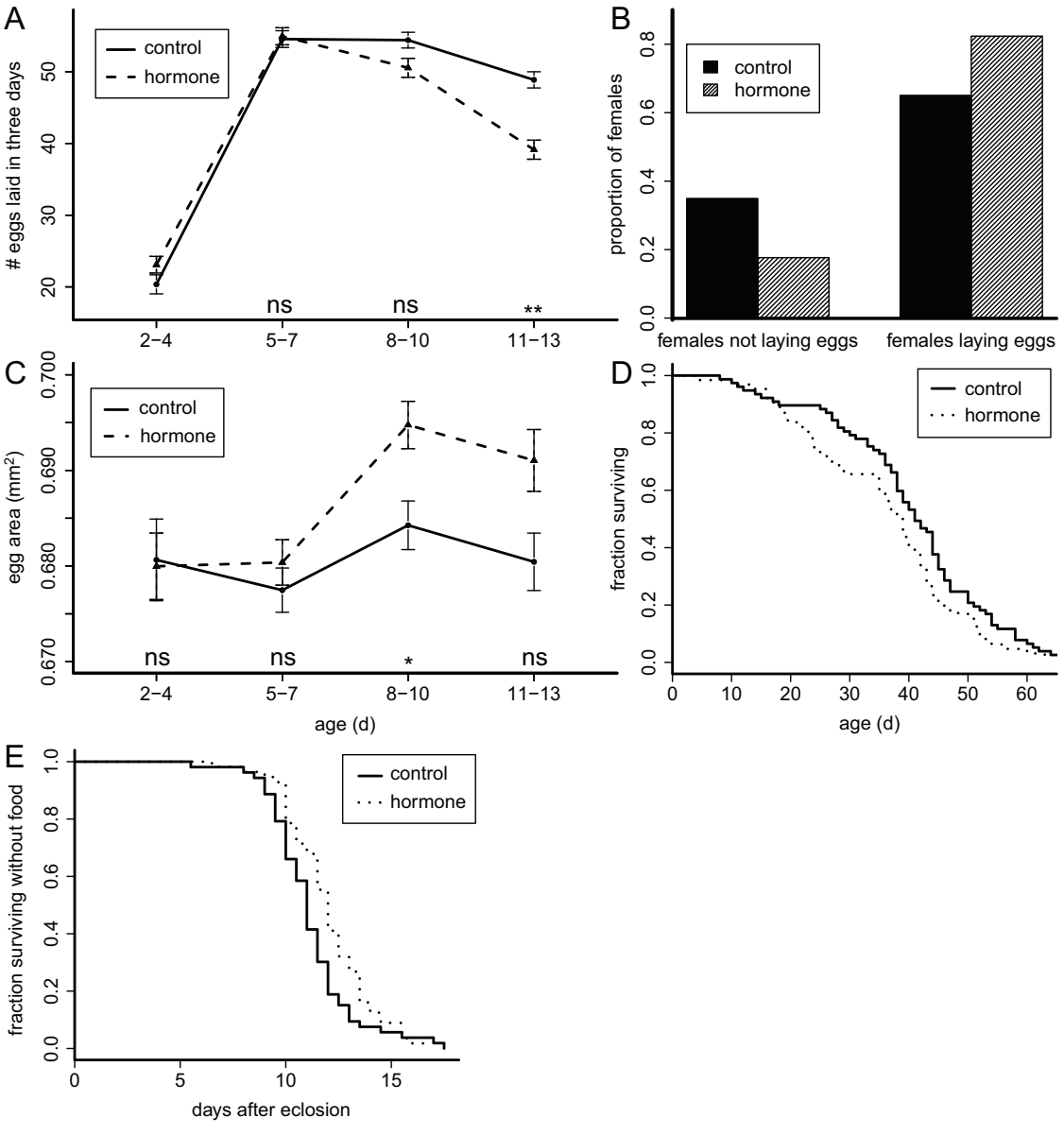

Figure 4: Pupal ecdysteroids affect reproductive schedule, life span, and starvation resistance. *A*, Female fecundity (number of eggs laid) is highly affected by female age ($P < .00001$ for likelihood ratio test [LRT] between model with and without age). In addition, adult females injected as pupa with 20-hydroxyecdysone (20E; dashed line) had lower fecundity compared to controls (solid line), but only later in life ($P < .001$ for LRT with and without treatment × age interaction). *B*, Pupal ecdysteroids accelerate onset of oviposition. Proportion of females that have already started laying eggs at age 4 days is significantly higher when injected as pupa with 20E (hatched bars) than when injected with control solution (solid bars; χ^2 $P < .05$). All females not laying eggs at age 4 days did lay eggs later in life. *C*, Pupal ecdysteroids induce increased egg size. Egg area (mm^2) is significantly affected by female age ($P < .00001$ for LRT between model with and without age), and females injected as pupa with 20E (dashed line) lay larger eggs than control females (solid line), but only at age 8–10 days ($P < .05$ for LRT with and without treatment × age interaction). *D*, Pupal ecdysteroids reduce adult life span of mated females. Daily adult survival under ad lib. food is reduced in mated females injected as pupa with 20E (dotted line) compared to controls (solid line; Cox proportional hazard $P = .06$, hazard ratio = 1.38). Life-span reduction was stronger for females that had started laying eggs before age 4 days (Cox proportional hazard $P < .05$, hazard ratio = 1.58) than for those that did not lay eggs before age 4 days (see table A4 in the online appendix). *E*, Pupal ecdysteroids enhance adult starvation resistance in virgin females. Daily adult survival without food is increased in virgin females injected as pupa with 20E (dotted line) compared to controls (solid line; Cox proportional hazard $P < .01$, hazard ratio = 0.68). See also table A4.

function. Crucially, these allocation changes are accompanied by changes in ecologically relevant adult performance traits, including timing of reproduction, egg size, life span, and survival under starvation. Thus, ecdysteroids after pupation mediate strategic adult reproductive investment decisions in response to variation in the quality of the environment.

As reported previously for *B. anynana* (Koch et al. 1996; Zijlstra et al. 2004), exogenous ecdysteroids applied early in the pupal stage accelerate pupal development. In the current study, we included two additional, later injection time points and found no such hormone-induced acceleration later in the pupal stage (fig. 1; tables A1, A3). Thus, as was the case for abdomen size (fig. 2), we observed a restricted window of sensitivity to hormone manipulations. In both cases, sensitivity was limited to the earliest 16% of the pupal stage. We have thus identified a critical period during which ecdysteroids are able to alter the developmental trajectory and, ultimately, the adult phenotype. This critical hormone-sensitive period is transient and occurs early in the pupal stage, when wet-season pupae already have increasing natural ecdysteroid titers, while those of dry-season pupae are still lower (Oostra et al. 2011). We chose our injection time points precisely because they represent the main stages in natural ecdysteroid dynamics (low, ascending, or descending titers), and at the three latest time points, the seasonal morphs differ most in their ecdysone titers. Thus, in the wet season, increasing natural ecdysteroid titers coincide with the hormone-sensitive period, whereas in the dry season, the hormone-sensitive period passes with low ecdysteroid titers. In *B. anynana*, ecdysteroids can thus be considered to act as a developmental switch sensu Nijhout (2003). Such developmental switches have been identified for numerous other animals displaying alternative phenotypes (discussed in Hartfelder and Emlen 2012). For example, in *Araschnia levana* pupae destined to develop directly, an ecdysteroid-sensitive period coincides with a pulse of high ecdysteroid titers during early development. This same sensitive period occurs in pupae destined to go into diapause, but the ecdysteroid pulse occurs much later, after the critical period (Koch and Bückmann 1987). A similar temporal match or mismatch between ecdysteroid titers and ecdysteroid sensitivity determines development of *Junonia* (*Precis*) *coenia* pupae into summer and autumn adult forms, respectively. With 25–56 h after pupation, the hormone-sensitive period in *B. anynana* is similar to that of *J. coenia* (28–48 h; Rountree and Nijhout 1995) but shorter than that of *A. levana* (3–9 days after pupation; Koch and Bückmann 1987). As *B. anynana* belongs to a group of Lepidoptera in which oocytes mature after eclosion (Ramaswamy et al. 1997) and no vitellogenins are yet detectable in pupae or freshly eclosed females (Geister et al. 2008), the much earlier occurring ecdysteroid signaling is unlikely to directly affect adult reproductive function. Instead, the early developmental switch in *B. anynana* probably acts as a cascade switch (West-Eberhard 2003), in which the initial ecdysteroid-mediated decision sets in motion downstream alternative developmental pathways that ultimately produce the seasonal morphs. Such a scenario explains the lack of phenotypic response to our late injections (fig. 2). After the hormone-sensitive period, the downstream developmental pathways have already been initiated and can no longer be modified by ecdysteroids.

It is likely that these downstream pathways involve other hormones, as studies in other insects show myriad interactions at a variety of life stages between ecdysteroids and other hormones (e.g., Shingleton et al. 2007). In particular, insulin-like peptides are expected to play an important role in the developmental pathways that regulate abdomen size. Thus, early ecdysteroid manipulations likely assert their ultimate phenotypic effects indirectly, by initiating alternative developmental pathways whose downstream mechanisms are unknown but likely involve other hormones.

Another mechanism by which ecdysteroids induce the alternate seasonal morphs in *B. anynana* may be changes in timing of developmental events. Both pupal development time and abdomen size showed the same window of hormone sensitivity. Furthermore, pupal development time and timing of ecdysteroid pulses in the pupal stage are genetically correlated (Zijlstra et al. 2004), and discrete variation in timing of ecdysteroid pulses in the pupal stage is phenotypically correlated with adult reproductive allocation (Oostra et al. 2011). In the wet season, an early ecdysteroid pulse coinciding with the sensitive period would accelerate development, resulting in an increased abdomen size and accelerated onset of oviposition. This is consistent with the well-known function of ecdysteroids as a developmental timer during the larval stage (Klowden 2007). In our experiment, pupal development time was more strongly affected by the seasonal environment than by the hormonal manipulations (fig. 1), that is, ecdysteroids did not fully phenocopy the temperature response. Temperature is known to have a major impact on rates of growth and development in ectotherms, independent of any adaptive plasticity and likely as a result of the direct effect of temperature on metabolic rate (Nylin and Gotthard 1998).

Developmental plasticity in *B. anynana* might also share components of its regulatory mechanisms with larval and pupal diapause expression in other insects, which has been linked to ecdysteroids (Denlinger 2002). In some cases, ecdysteroid titers are lower in diapausing larvae or pupae (e.g., Koch 1996; Munyiri and Ishikawa 2004), and in other cases, exogenous ecdysteroid applications terminate dia-

pause and induce the continuation of normal development (Arpagaus et al. 1986; Singtripop et al. 1999). In adult insects, ecdysteroids interplay with other hormones (in particular, juvenile hormones) to regulate several aspects of female reproduction (Klowden 2007). For example, ovarian growth in young *Gryllus firmus* adults is positively correlated with ecdysteroid titers (Zera 2009). Mutant *Drosophila melanogaster* females with reduced ecdysteroid signaling show reduced rates of oocyte maturation or oviposition, as well as increased life span (reviewed in Schwedes and Carney 2012). Adult reproductive diapause in *D. melanogaster* females, characterized by arrested reproductive development and increased life span (see Schmidt 2011), can be terminated by ecdysteroid injection (Richard et al. 2001). Such a reproductive function of ecdysteroids in adult females is consistent with the increased abdomen size and accelerated onset of oviposition we observed in ecdysteroid-injected *B. anynana* females, suggesting some overlap in function between ecdysteroid signaling in the pupal and adult stages.

The environmental induction of alternative phenotypes consists not only of the developmental switch and subsequent cascade but is preceded by a period of environmental sensitivity. During this period, the developing organism senses and processes environmental cues that then yield the hormone-mediated decision between alternative pathways, as discussed above. The environmental sensitive period generally occurs much earlier in development, and it is well known that, in seasonally plastic insects, this period almost always occurs during the larval stage (Danks 1987; Nijhout 2003). Indeed, for *B. anynana*, it has been shown for a long time that the environmental induction of the seasonal adult wing patterns occurs mainly during the late larval stage (Kooi and Brakefield 1999).

In the ecdysteroid-treated females, the onset of oviposition was accelerated, similar to the naturally induced wet-season morph (Brakefield and Zwaan 2011). However, fecundity after peak egg laying was reduced, while the natural wet-season morph shows generally higher fecundity throughout adult life. This was contrary to our initial expectation that the ecdysteroid-treated females would be more wet-season-like in all aspects of adult life history. Previously, it was shown that fecundity after peak egg laying is mainly determined by temperature during oviposition and, to a lesser extent, by developmental temperature (e.g., Fischer et al. 2003). It thus seems likely that, unlike the onset of oviposition, later-life fecundity differences between the naturally occurring wet- and dry-season morphs are not under control of ecdysteroid-mediated developmental plasticity but instead are determined by adult acclimation (Brakefield et al. 2007). The reduction in late-life fecundity observed in our experiments likely reflects a fitness cost of the accelerated early oviposition, which in the natural wet-season morph would be masked by adult conditions. It remains to be tested whether other traits that commonly trade off with reproductive investment, such as flight ability (cf. Zera 2009), are also integrated into the hormone-mediated adult life history. One indication that this might indeed be the case is the observation that larval food stress-induced allocation to the thorax at the expense of the abdomen increases flight endurance in adults (Saastamoinen et al. 2010), which a modeling approach showed to be a potentially adaptive response (van den Heuvel et al. 2013).

In contrast to their effects on abdomen size, development time, and adult reproductive strategy, exogenously applied ecdysteroids did not affect adult RMR. Previous studies in *B. anynana* and other insects reported a negative effect of developmental temperature on adult RMR (Berrigan 1997; Pijpe et al. 2007; Le Lann et al. 2011) and, in the opposite direction, to the positive effect of adult acclimation temperature (Oostra et al. 2011). We confirmed the developmental imprint of temperature on adult RMR but showed that hormone manipulations did not, at any of the tested time points or rearing temperatures, induce significant changes in RMR (fig. 2; tables A1, A3). This result is unlikely to be due to lack of statistical power, as smaller sample sizes than the ones used in our study have been used previously in this species to statistically detect effects of sex, developmental and adult temperature, genetic background, and age on adult RMR (e.g., Pijpe et al. 2007). Indeed, in the present study, the increasing effect on adult RMR of lower (dry season) developmental temperatures was clearly detectable, but we observed no pattern in our data even weakly suggesting that pupal ecdysteroids decrease adult RMR, as one would expect if these hormones would mediate the natural seasonal plasticity of RMR. The most parsimonious explanation for our results is that, despite a correlated response with developmental temperature, RMR and pupal ecdysteroid signaling are not functionally linked. Thus, the developmental signature is independent of pupal ecdysteroid signaling and probably originates during the larval stage (cf. Pijpe et al. 2007). Clearly, the RMR reaction norm deserves follow-up studies to uncover what mechanisms underpin the differences in metabolic rate between the seasonal forms and at which stage during development these differences are set.

Adult RMR and SR show a negative phenotypic correlation in *B. anynana*, responding in opposite directions to developmental temperature (Pijpe et al. 2007). Nevertheless, here we uncovered independent variation between RMR and SR; virgin females injected with ecdysteroids live longer under starvation despite having unchanged RMR (figs. 3, 4*E*). The proximate cause of the increased SR probably lies in the observed increase in abdominal fat content in re-

sponse to pupal ecdysteroids injections (fig. A1). This strongly suggests that under stressful conditions, females can reallocate these abdominal resources—and, in particular, fat (cf. Zwaan et al. 1991)—to survival rather than reproduction. Our findings reveal that not all traits involved in the alternative adult life histories (and responding to developmental temperature) are regulated by pupal ecdysteroids. This underscores the idea that, even when traits are correlated and covary with hormonal patterns, a functional study is needed to ascertain whether a particular hormone is indeed mediating these relationships, including potential trade-offs (see Zera and Harshman 2001).

In conclusion, our results support a functional role for ecdysteroids during *B. anynana* development in translating information on environmental quality into adaptive alterations in the adult. In particular, we show that these hormones act as a switch between developmental pathways that culminate in alternative adult life histories. Although such developmentally restricted hormonal switches have been found in many insects that display phenotypic plasticity, seasonal or otherwise (Hartfelder and Emlen 2012; Simpson et al. 2011), they likely occur in all animals that display condition-dependent alternative life histories or behaviors. Vertebrates show a wide diversity of reproductive traits that can be coupled to alternative reproductive tactics (Oliveira et al. 2008). In birds and lizards, among others, it has been shown that hormones are involved in morphological and neuro-organizational changes during development that underpin these alternative tactics (reviewed in Oliveira et al. 2008). A more dramatic example of a condition-dependent developmental switch between alternative developmental pathways is environmental sex determination, such as occurs in many reptile species, where the sex of the developing embryo is determined by the temperature at which the egg is incubated (Sarre et al. 2004). More generally, hormone-mediated developmental switches allow organisms to mount a systemic, integrated, and coordinated response to environmental variation, as systemic hormone titers are centrally regulated from the central nervous system in response to signals sensed from the environment. At the same time, how the tissues and cells that ultimately bring about the phenotypic changes respond to the hormone is a local property of those tissues, which can be regulated via a myriad of mechanisms, including variation in expression, intracellular activity, or localization of hormone receptors. Such local hormone sensitivity allows for a cell-, tissue-, or trait-dependent differentiated response to the circulating hormone. Our results illustrate how organisms can use systemic hormones and their time- and tissue-specific sensitivity to respond to predictive indicators of environmental quality and to make strategic life-history decisions that enable them to cope with fluctuating environments.

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Literature Cited

Arpagaus, M., P. Beydon, and R. Lafont. 1986. Effects of ecdysone injection on diapause termination in *Pieris brassicae* pupae: evidence for a stimulatory action on endogenous ecdysone production. Reproduction Nutrition Development 26:1305–1317.

Baayen, R. H. 2011. languageR: data sets and functions with "analyzing linguistic data: a practical introduction to statistics." R package. Version 1.4. http://CRAN.R-project.org/package=languageR.

Bates, D. M., M. Maechler, and B. Bolker. 2011. lme4: linear mixed-effects models using S4 classes. R package. Version 0.999375–39. http://CRAN.R-project.org/package=lme4.

Beldade, P., A. R. A. Mateus, and R. A. Keller. 2011. Evolution and molecular mechanisms of adaptive developmental plasticity. Molecular Ecology 20:1347–1363.

Berrigan, D. 1997. Acclimation of metabolic rate in response to developmental temperature in *Drosophila melanogaster*. Journal of Thermal Biology 22:213–218.

Braendle, C., A. Heyland, and T. Flatt. 2011. Integrating mechanistic and evolutionary analysis of life history variation. Pages 3–10 *in* T. Flatt and A. Heyland, eds. Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs. Oxford University Press, Oxford.

Brakefield, P. M., P. Beldade, and B. J. Zwaan. 2009. The African butterfly *Bicyclus anynana*: a model for evolutionary genetics and evolutionary developmental biology. Cold Spring Harbor Protocols, doi:10.1101/pdb.emo122.

Brakefield, P. M., F. Kesbeke, and P. B. Koch. 1998. The regulation of phenotypic plasticity of eyespots in the butterfly *Bicyclus anynana*. American Naturalist 152:853–860.

Brakefield, P. M., J. Pijpe, and B. J. Zwaan. 2007. Developmental plasticity and acclimation both contribute to adaptive responses to alternating seasons of plenty and of stress in *Bicyclus* butterflies. Journal of Biosciences 32:465–475.

Brakefield, P. M., and N. Reitsma. 1991. Phenotypic plasticity, seasonal climate and the population biology of *Bicyclus* butterflies (Satyridae) in Malawi. Ecological Entomology 16:291–303.

Brakefield, P. M., and B. J. Zwaan. 2011. Seasonal polyphenisms and environmentally-induced plasticity in the Lepidoptera: the coor-

dinated evolution of many traits on multiple levels. Pages 243–252 *in* T. Flatt and A. Heyland, eds. Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs. Oxford University Press, Oxford.

Brisson, J. A. 2010. Aphid wing dimorphisms: linking environmental and genetic control of trait variation. Philosophical Transactions of the Royal Society B: Biological Sciences 365:605–616.

Danks, H. V. 1987. Insect dormancy: an ecological perspective. Biological Survey of Canada, Ottawa.

Dawson, A. 2008. Control of the annual cycle in birds: endocrine constraints and plasticity in response to ecological variability. Philosophical Transactions of the Royal Society B: Biological Sciences 363:1621–1633.

de Jong, M. A., F. M. N. H. Kesbeke, P. M. Brakefield, and B. J. Zwaan. 2010. Geographic variation in thermal plasticity of life history and wing pattern in *Bicyclus anynana*. Climate Research 43:91–102.

Denlinger, D. L. 2002. Regulation of diapause. Annual Review of Entomology 47:93–122.

Emlen, D. J., I. A. Warren, A. Johns, I. Dworkin, and L. C. Lavine. 2012. A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. Science 337:860–864.

Fischer, K., E. Eenhoorn, A. N. Bot, P. M. Brakefield, and B. J. Zwaan. 2003. Cooler butterflies lay larger eggs: developmental plasticity versus acclimation. Proceedings of the Royal Society B: Biological Sciences 270:2051–2056.

Geister, T. L., M. W. Lorenz, M. Meyering-Vos, K. H. Hoffmann, and K. Fischer. 2008. Effects of temperature on reproductive output, egg provisioning, juvenile hormone and vitellogenin titers in the butterfly *Bicyclus anynana*. Journal of Insect Physiology 54: 1253–1260.

Gilbert, S. F. 2012. Ecological developmental biology: environmental signals for normal animal development. Evolution and Development 14:20–28.

Hartfelder, K., and D. J. Emlen. 2012. Endocrine control of insect polyphenism. Pages 464–522 *in* L. Gilbert, ed. Insect endocrinology. Academic Press, London.

Hofmann, G. E., and A. E. Todgham. 2010. Living in the now: physiological mechanisms to tolerate a rapidly changing environment. Annual Review of Physiology 72:127–145.

Klowden, M. J. 2007. Physiological systems in insects. Elsevier Science/Academic Press, London.

Koch, P. B. 1996. Preadult changes of ecdysteroid and juvenile hormone titers in relation to diapause and pigmental variations in two lepidopteran species, *Cerura vinula* and *Araschnia levana* (Lepidoptera: Notodontidae Nymphalidae). Entomologia Generalis 20: 143–155.

Koch, P. B., P. M. Brakefield, and F. Kesbeke. 1996. Ecdysteroids control eyespot size and wing color pattern in the polyphenic butterfly *Bicyclus anynana* (Lepidoptera: Satyridae). Journal of Insect Physiology 42:223–230.

Koch, P. B., and D. Bückmann. 1987. Hormonal control of seasonal morphs by the timing of ecdysteroid release in *Araschnia levana* L. (Nymphalidae: Lepidoptera). Journal of Insect Physiology 33: 823–829.

Kooi, R. E., and P. M. Brakefield. 1999. The critical period for wing pattern induction in the polyphenic tropical butterfly *Bicyclus anynana* (Satyrinae). Journal of Insect Physiology 45:201–212.

Le Lann, C., T. Wardziak, J. van Baaren, and J. J. M. van Alphen. 2011. Thermal plasticity of metabolic rates linked to life-history traits and foraging behaviour in a parasitic wasp. Functional Ecology 25:641–651.

Meylan, S., D. B. Miles, and J. Clobert. 2012. Hormonally mediated maternal effects, individual strategy and global change. Philosophical Transactions of the Royal Society B: Biological Sciences 367:1647–1664.

Munyiri, F. N., and Y. Ishikawa. 2004. Endocrine changes associated with metamorphosis and diapause induction in the yellow-spotted longicorn beetle, *Psacothea hilaris*. Journal of Insect Physiology 50: 1075–1081.

Nijhout, H. F. 2003. Development and evolution of adaptive polyphenisms. Evolution and Development 5:9–18.

Nylin, S., and K. Gotthard. 1998. Plasticity in life-history traits. Annual Review of Entomology 43:63–83.

Oliveira, R. F., A. V. M. Canário, and A. F. H. Ros. 2008. Hormones and alternative reproductive tactics in vertebrates. Pages 132–173 *in* R. F. Oliveira, M. Taborsky, and H. J. Brockmann, eds. Alternative reproductive tactics. Cambridge University Press, Cambridge.

Oostra, V., M. A. de Jong, B. M. Invergo, F. Kesbeke, F. Wende, P. M. Brakefield, and B. J. Zwaan. 2011. Translating environmental gradients into discontinuous reaction norms via hormone signalling in a polyphenic butterfly. Proceedings of the Royal Society B: Biological Sciences 278:789–797.

Oostra, V., A. R. A. Mateus, K. R. L. van der Burg, T. Piessens, M. van Eijk, P. M. Brakefield, P. Beldade, and B. J. Zwaan. 2014. Data from: Ecdysteroid hormones link the juvenile environment to alternative adult life histories in a seasonal insect. American Naturalist, Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.b42s4.

Pijpe, J., P. M. Brakefield, and B. J. Zwaan. 2007. Phenotypic plasticity of starvation resistance in the butterfly *Bicyclus anynana*. Evolutionary Ecology 21:589–600.

R Development Core Team. 2010. R: a language and environment for statistical computing (v. 2.11.1). R Foundation for Statistical Computing, Vienna.

Ramaswamy, S. B., S. Shu, Y. I. Park, and F. Zeng. 1997. Dynamics of juvenile hormone-mediated gonadotropism in the Lepidoptera. Archives of Insect Biochemistry and Physiology 35:539–558.

Richard, D. S., J. M. Jones, M. R. Barbarito, C. Stacy, J. P. Detweiler, S. J. Fisher, D. M. Brannigan, and D. M. Scheswohl. 2001. Vitellogenesis in diapausing and mutant *Drosophila melanogaster*: further evidence for the relative roles of ecdysteroids and juvenile hormones. Journal of Insect Physiology 47:905–913.

Rountree, D. B., and H. F. Nijhout. 1995. Hormonal control of a seasonal polyphenism in *Precis coenia* (Lepidoptera: Nymphalidae). Journal of Insect Physiology 41:987–992.

Saastamoinen, M., D. van der Sterren, N. Vastenhout, B. J. Zwaan, and P. M. Brakefield. 2010. Predictive adaptive responses: condition-dependent impact of adult nutrition and flight in the tropical butterfly *Bicyclus anynana*. American Naturalist 176:686–698.

Sarre, S. D., A. Georges, and A. Quinn. 2004. The ends of a continuum: genetic and temperature-dependent sex determination in reptiles. BioEssays 26:639–645.

Schmidt, P. S. 2011. Evolution and mechanisms of insect reproductive diapause: a plastic and pleiotropic life history syndrome. Pages 230–242 *in* T. Flatt and A. Heyland, eds. Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs. Oxford University Press, Oxford.

Schwedes, C. C., and G. E. Carney. 2012. Ecdysone signaling in adult *Drosophila melanogaster*. Journal of Insect Physiology 58:293–302.

Shingleton, A. W., W. A. Frankino, T. Flatt, H. F. Nijhout, and D. J.

Emlen. 2007. Size and shape: the developmental regulation of static allometry in insects. BioEssays 29:1–13.
Simpson, S. J., and G. A. Sword. 2009. Phase polyphenism in locusts: mechanisms, population, consequences, adaptive significance and evolution. Pages 147–189 *in* T. N. Ananthakrishnan and D. W. Whitman, eds. Phenotypic plasticity of insects: mechanisms and consequences. Science, Plymouth, UK.
Simpson, S. J., G. A. Sword, and N. Lo. 2011. Polyphenism in insects. Current Biology 21:R738–R749.
Singtripop, T., S. Wanichacheewa, S. Tsuzuki, and S. Sakurai. 1999. Larval growth and diapause in a tropical moth, *Omphisa fuscidentalis* Hampson. Zoological Science 16:725–733.
Smith, C. R., A. L. Toth, A. V. Suarez, and G. E. Robinson. 2008. Genetic and genomic analyses of the division of labour in insect societies. Nature Reviews Genetics 9:735–748.
Sommer, R. J., and A. Ogawa. 2011. Hormone signaling and phenotypic plasticity in nematode development and evolution. Current Biology 21:R758–R766.
Steigenga, M. J., and K. Fischer. 2007. Within- and between-generation effects of temperature on life-history traits in a butterfly. Journal of Thermal Biology 32:396–405.
Therneau, T. 2012. A package for survival analysis in S. R package. Version 2.36–14. http://CRAN.R-project.org/package = survival.
van den Heuvel, J., M. Saastamoinen, P. M. Brakefield, T. B. L. Kirkwood, B. J. Zwaan, and D. P. Shanley. 2013. The predictive adaptive response: modeling the life-history evolution of the butterfly *Bicyclus anynana* in seasonal environments. American Naturalist 181:E28–E42.
Visser, M. E., S. P. Caro, K. van Oers, S. V. Schaper, and B. Helm. 2010. Phenology, seasonal timing and circannual rhythms: towards a unified framework. Philosophical Transactions of the Royal Society B: Biological Sciences 365:3113–3127.
West-Eberhard, M. J. 2003. Developmental plasticity and evolution. Oxford University Press, New York.
Zera, A. J. 2007. Endocrine analysis in evolutionary-developmental studies of insect polymorphism: hormone manipulation versus direct measurement of hormonal regulators. Evolution and Development 9:499–513.
———. 2009. Wing polymorphism in *Gryllus* (Orthoptera: Gryllidae): proximate endocrine, energetic and biochemical bases underlying morph specializations for flight vs. reproduction. Pages 609–653 *in* T. N. Ananthakrishnan and D. W. Whitman, eds. Phenotypic plasticity of insects: mechanisms and consequences. Science, Plymouth, UK.
Zera, A. J., and L. G. Harshman. 2001. The physiology of life history trade-offs in animals. Annual Review of Ecology and Systematics 32:95–126.
Zera, A. J., L. G. Harshman, and T. D. Williams. 2007. Evolutionary endocrinology: the developing synthesis between endocrinology and evolutionary genetics. Annual Review of Ecology and Systematics 38:793–817.
Zijlstra, W. G., M. J. Steigenga, P. B. Koch, B. J. Zwaan, and P. M. Brakefield. 2004. Butterfly selected lines explore the hormonal basis of interactions between life histories and morphology. American Naturalist 163:E76–E87.
Zwaan, B. J., R. Bijlsma, and R. F. Hoekstra. 1991. On the developmental theory of ageing. I. Starvation resistance and longevity in *Drosophila melanogaster* in relation to preadult breeding conditions. Heredity 68:123–130.

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A *Bicyclus anynana* butterfly expressing the wet season form in South Africa. Photo by Andre Coetzer.