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Divergence and ontogenetic coupling of larval behaviour and thermal reaction norms in three closely related butterflies

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Genetic trade-offs such as between generalist–specialist strategies can be masked by changes in compensatory processes involving energy allocation and acquisition which regulation depends on the state of the individual and its ecological surroundings. Failure to account for such state dependence may thus lead to misconceptions about the trade-off structure and nature of constraints governing reaction norm evolution. Using three closely related butterflies, we first show that foraging behaviours differ between species and change remarkably throughout ontogeny causing corresponding differences in the thermal niches experienced by the foraging larvae. We further predicted that thermal reaction norms for larval growth rate would show state-dependent variation throughout development as a result of selection for optimizing feeding strategies in the respective foraging niches of young and old larvae. We found substantial developmental plasticity in reaction norms that was species-specific and reflected the different ontogenetic niche shifts. Any conclusions regarding constraints on performance curves or species-differentiation in thermal physiology depend on when reaction norms were measured. This demonstrates that standardized estimates at single points in development, or in general, allow variation in only one ecological dimension, may sometimes provide incomplete information on reaction norm constraints.

Keywords: temperature; developmental plasticity; environmental tolerance; ontogeny; co-adaptation; performance curve

1. INTRODUCTION

The reaction norm, describing how trait expression varies along an environmental gradient, is a fundamental concept in evolutionary theory [1–4]. The theoretical framework for understanding the evolution of reaction norms for performance traits (e.g. performance curves) has a strong focus on trade-offs between generalist and specialist adaptations (e.g. [5–7]). However, performance curves are typically a result of multiple processes in addition to the directly underlying physiology and performance in sub-optimal environments may be compensated by changes in trade-offs involving behaviours or energy allocation [8–11]. The benefits of such regulation depend on the state of the organism and what other processes are prioritized by natural selection. Thus, not accounting for state-dependence may lead to misconceptions about the nature of evolutionary constraint on reaction norms and failure to predict how natural populations will respond to change in their environment [9,12].

Thermal performance is assumed to be constrained by generalist–specialist trade-offs, so that maximum performance is inversely related to the width of the thermal range in which the organism is still functional [13]. Thus, variable and unpredictable environments enforce selection for the evolution of broad thermal performance curves at the cost of a reduction in maximum performance [6,7]. Yet, generalist–specialist trade-offs and negative correlations between performance at warm and cold temperatures are often not evident when judging from empirical data [13–16]. Trade-offs between performance at high and low temperatures may originate from the conflicting demands of appropriate up- and downregulation of temperature-sensitive enzymes and cell membrane configuration, but such trade-offs could be masked by an overall increased allocation of energy devoted to enzyme production and membrane restructuring [17], or by an overall increase in energy acquisition mediated by foraging effort [9,18]. It is now recognized that allocation and acquisition trade-offs may play major roles for evolutionary responses to temperature constraints in seasonal environments when there is strong selection on fast growth [19–22].

While state dependence of reaction norms in terms of physiological acclimation—adaptive plasticity of reaction norms induced by direct environmental cues (most often acclimation temperature), has received considerable interest (e.g. [23–29]), less attention has been given to how allocation- and acquisition trade-offs may change with the general condition or state of individuals and thereby also affect organisms' thermal performance [30,31]. State dependence in allocation decisions and behaviours is one of the most essential concepts in behavioural ecology and life history theory. It is for example known that allocation to growth should be high at early ages and at one point be directed to reproduction [32,33], and that risk taking during foraging is highly dependent on the reproductive value of individuals and how predation risk varies with changes in body size and behaviour [34]. Often state-dependent decision rules materialize in ontogenetic niche

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shifts resulting in drastic changes in ecology [35,36]. When these shifts are part of the organism's developmental programme, as the case for many well-studied systems such as vertical migration in zooplankton and fishes or life-history transitions between water and land in insects and amphibians, intrinsic developmental cues may transfer valuable information about the forthcoming environmental change. Such cues may then be followed by developmental plasticity resulting in physiological changes allowing the individual to better cope with the new environment [23,37]. However, as ontogenetic niche shifts often entail myriad changes in both extrinsic biotic and abiotic conditions as well as in physiology, co-adaptation of several aspects of organism biology affecting performance is expected.

Previous studies on ontogenetic variation in thermal reaction norms have mostly focused on single-species comparisons between discrete life stages, such as between juveniles and adults in holometabolous insects. In addition, few attempts have been made to incorporate explicit testing of evolutionary theory, even though systems in which state-dependent trade-offs are to be expected offer ample opportunity to test adaptive hypotheses regarding environmental tolerance (reviewed in [31]; see Zani *et al.* [38] for an exception). We investigated the role of ontogeny and individual state on thermal reaction norm regulation within larval life in the three butterflies in the genus *Pararge* (Nymphalidae). These butterflies have speciated through independent long-distance dispersal events from northern Africa to the islands of Madeira (Portugal) and Tenerife (Spain). The larvae display size-dependent shifts in diurnal–nocturnal foraging activity, which seems species-specific and presumably represent adaptations for optimizing food intake rates under different predation regimes experienced by small and large individuals in their respective natural environments [39,40]. We quantify these ontogenetic shifts and further show that owing to the inherent differences between day (warm) and night (cold) temperatures, these behavioural shifts result in large species-specific differences in the thermal environments experienced during the feeding phase early and late in larval development. We hypothesized that the thermal reaction norms for larval growth rate measured at different points in development would show state-dependent variation reflecting each species' behavioural shift, and thus reveal a coupling of behavioural and physiological co-adaptation to the respective niches experienced by the foraging larvae. Results conformed to these predictions but also demonstrate constraints on reaction norm plasticity. Our study illustrates how standardized estimations of reaction norms performed at single points in an organism's life may, to a large extent, reflect state-dependent, and life stage-specific variation in performance as a result of adaptive developmental plasticity in response to various other ecological variables in addition to the environmental gradient of investigatory interest, complicating interpretation of evolutionary constraints on the reaction norm.

2. MATERIAL AND METHODS

(a) *Study species*
Pararge aegeria (Linnaeus) is a widespread satyrine butterfly that occurs throughout Europe and parts of northern

Africa [41]. Its closest relative *Pararge xiphoides* (Staudinger) is endemic to the Canary Islands that were colonized from northern Africa at a maximum of 3 Ma, when it separated from the common ancestor shared with *P. aegeria* [42]. Approximately 5 Ma, the third species of the genus, *P. xiphia* (Fabricius), split from its sister species when it colonized the island of Madeira to which it is endemic [42]. Some 40 years ago, *P. aegeria* colonized the Island of Madeira where it now co-occurs with *P. xiphia* [41]. Larvae of all species feed on grasses typically situated in open to semi-shaded areas and complete development through four instars. Diapause stages have not been recorded and all species seem to occur throughout the year. A previous study [39] and pilot experiments have implied a divergence in larval feeding behaviour between *P. aegeria* and *P. xiphia*; in early stages, feeding occurs around the 24 h period, whereas in the later stages of larval development, *P. xiphia* appears to almost exclusively feed during night-time and display anti-predatory behaviours during daytime [40], whereas *P. aegeria* remains active throughout the 24 h period. For both species, these behaviours seem canalized and do not change when reared in constant temperatures between 10 and 28°C, or by natural deviations in photoperiod (11 L : 13 D–15 L : 9 D; [40], D. Berger & K. Gotthard 2004–2006, unpublished data). Larval development typically lasts for 25–35 days in *P. aegeria* and *P. xiphoides*, and 40–50 days in *P. xiphia* when reared in temperatures between 17 and 24°C (warmer temperatures speed up development).

The laboratory population of *P. xiphoides* used in these experiments was founded in 2007 from a total 15 females caught from four locations on Tenerife, Spain: Aguamansa, La Perdoma, La Montaneta and Las Portelas. The laboratory populations of *P. xiphia* and *P. aegeria* were founded in 2006 from 12 and 8 females, respectively, caught at two sites on Madeira, Portugal: Levada Grande and 20 km northwest of Parque Natural do Ribeiro Frio. The laboratory populations have been maintained by letting approximately 100 adults reproduce during each generation and selectively harvesting eggs from each fecund female. Ontogenetic niche shifts were quantified in the F2-generation of all species. In the reaction norms-experiment of 2008, the *P. xiphia* larvae had been kept in the laboratory for six generations; *P. aegeria* for 10 generations; and *P. xiphoides* for four generations. All populations have been reared in constant temperatures varying between 17 and 22°C among generations, on one of their preferred host plants *Dactylis glomerata* (Linnaeus; [41]), which also was used in all the experiments described below.

(b) *Ontogenetic foraging shifts*
To quantify the niche shifts in foraging behaviour, the individual growth patterns of 20 larvae derived from four females of each species were monitored throughout development in a climate chamber set to a light regime of 15 L : 9 D and 17°C, which reflects average summer conditions (see Gotthard *et al.* [43]). In each instar, the growth rates during day and night time were obtained by measuring larval weight three times during a 24 h period: just before the onset of night, at the end of the night period and a final time at the end of the ensuing day period. In these and all later measures, larvae were weighed between days 2–4 in each instar. This ensured that the larvae had started feeding at maximal rates, while at the same time, measurements late in each instar when growth typically slows down, were avoided. We used the Cahn C-30

Microbalance that records weights with an error margin of 1 µg for first and second instar larvae and Precisa 205 ASCS that measures weight with 0.1 mg error margin for third and fourth instar larvae. Relative growth rates were calculated by the formula: [ln (end weight) − ln (start weight)]/ [growth period length]. Only larvae with a complete set of measurements (both day and night growth rates in all four instars) were chosen for analysis. This resulted in 13, 15 and 16 individuals for *P. aegeria*, *P. xiphoides* and *P. xiphia*, respectively. The proportion of weight increase taking place during the night, calculated as increase$_{night}$/increase$_{night+ day}$, was used to describe the foraging behaviour. Thus, a value close to 0.5 indicates constant feeding activity throughout the 24 h period; obligate day feeding generates values close to 0, and feeding exclusively at night renders values close to 1. The proportion of night feeding can take on values above 1 and below 0 as larvae sometimes dropped in weight over single periods due to defaecation; the variable was therefore approximately normally distributed.

Differences in foraging activity throughout ontogeny were analysed with a mixed linear model using the nlme-package in R v. 2.9.0 [44], with individual set as a random factor and species and larval instar as fixed effects. We also performed an additional analysis with individuals nested within family (i.e. considering family as the independent observation). In these as well as in all subsequent analyses, terms were dropped sequentially unless $p < 0.1$.

(c) *Thermal foraging niches*

To estimate the respective thermal foraging niches experienced by the larvae in early and late development, we calculated temperature frequency-distributions during foraging activity in the wild. We used hourly data on ambient temperature and sun radiation from the full years of 2006–2007 recorded on Tenerife and Madeira at stations on altitudes similar to where the butterflies were caught (660 and 617 m, respectively). Monthly averages (from 2006 to 2007) were compared with averages of a dataset covering 30 years to make sure that the chosen 2 years were representative (climate data obtained from the Spanish State Agency of Meteorology and the Institute of Meteorology of Portugal). We finally calibrated the climate data to microhabitat temperatures by comparing the metrological climate dataset and temperature data recorded by two TinyTag thermo-loggers placed in the microhabitat of the larvae on Madeira from May to September of 2006. The two loggers were placed in free air 20–30 cm above ground under light-coloured wooden boards and thus recorded shade-temperature. The loggers were placed approximately 50 m apart in the semi-enclosed grassland where we found females in flight. The difference between the loggers was below 1°C and we therefore calculated a logger-average before comparison with station data. Owing to the semi-enclosed habitat of the larvae, temperatures in the microhabitat lagged behind station recordings by 1 h. After adjusting for this lag, thermo-loggers and climate recordings showed a strong 1 : 1 linear relationship ($R^2 = 0.92$, microhabitat = 1.047 × climate data). As we did not record logger data from Tenerife, we have to assume that the correlation between microhabitat temperature and climate station data is similar to that estimated for Madeira. This seems to be a reasonable assumption given that the microhabitats where the butterflies are found on the Islands are very similar (if not the same). The light avoiding behaviour in fourth instar *P. xiphia* larvae is not

induced by temperature [40] and based on laboratory observations and outdoor rearing, *P. aegeria* and *P. xiphoides* do not seem to actively thermoregulate, but appear to be thermo-conformers that are most often found on sides, or underneath leaves, but never seem to be actively basking (D. Berger & K. Gotthard, personal observations). For temperature-conforming larvae of this size, internal body temperature is predicted to follow ambient temperature with next to no time delay and with a maximum deviation of 1−2°C based on both theoretical predictions [45] and empirical estimations [46,47]. Hence, we assume that the operative temperatures of the larvae in the field are equal to the ambient temperature. We cannot rule out that the larvae at temperature extremes do (i) thermoregulate (day-active *P. xiphoides* larvae may for example seek shade during extremely hot periods), or (ii) change feeding behaviour (night-active *P. xiphia* larvae may feed during daytime during extremely cold periods to avoid starvation). This should however not affect the qualitative differences in thermal niches experienced by the instars since our observations indicate that larval foraging behaviour is unaffected by temperature variation of the magnitude commonly experienced in nature (see above). Finally, we account for sun radiation by assuming that the incidence of sunlight causes a maximum (when irradiance = 100%) increase of 8.5° in body temperature. This is the most appropriate estimate available for insect larvae of this size (see Stevenson [45] and Gotthard *et al.* [48] for justification).

Kingsolver *et al.* [49] evaluated selection on thermal performance curves of larval growth rate in the butterfly *Pieris rapae*. Assuming that fitness is directly proportional to the sum of growth measured over an episode of selection, the authors showed that the strength of selection on the thermal reaction norm is proportional to the frequency at which specific temperatures are experienced:

$$\beta(T) = c(T)f(T),$$

where β is the selection gradient on thermal performance in temperature T, $c(T)$ is a weighting function giving relative importance of performance at specific temperatures and $f(T)$ is the density distribution of temperatures experienced during growth. If it is assumed that selection does not favour optimization of performance at any specific temperature (c constant across all temperatures), the strength of selection on performance is perfectly predicted by the density distribution f of operative temperatures T [5,50]. However, detailed analyses of the relative positioning of thermal performance curves to the thermal environment suggest that reaction norms are typically shifted towards warmer temperatures than the ones most frequently experienced in nature (reviewed in [16]) resulting in warm temperature contributing disproportionately to growth and suggesting that the strength of selection is not constant across temperatures. Several explanations have been given for this pattern involving disproportionately strong viability selection in warm temperatures owing to the inherent left-skew of thermal reaction norms (see Martin & Huey [51]) and the 'hotter is better' hypothesis (see Frazier *et al.* [52], Asbury & Angilletta [53] and Knies *et al.* [54]). Therefore, even though our calculations of thermal foraging niches are predicted to provide information on the strength of thermal selection experienced throughout development [6,55], it should not be expected that scored reaction norms will render perfect matches to our quantified thermal niches. Furthermore, selection on temperature-dependent growth performance in our *Pararge* larvae is also probably not independent of temperatures

experienced during the non-feeding phase since it seems likely that some part of the food digestion still takes place at this time. Thus, the estimates of thermal niches rather make the qualitative prediction that the directions of the shifts of growth reaction norms between instars should be consistent with the directions of change in the thermal environments associated with the ontogenetic foraging shifts. It does, for example, seem likely that the relatively cold temperature experienced by night-active last instar *P. xiphia* larvae imposes more severe constraints on growth than in the first instar, a condition that may select for either (i) developmental plasticity of the reaction norm towards colder temperatures in the last instar, (ii) increased compensatory foraging effort assuming selection on fast generation time, or (iii) reduced foraging effort as a result of orchestrated canalization of foraging effort to the expected slow metabolic rate experienced in the natural environment and the occurrence of natural enemies, more congruous with relaxed selection on development rate in a non-seasonal environment like Madeira [40].

Thus, by weighting the frequency distribution (f) of night-time (NT) and day-time (DT) temperature by the proportion of growth taking place during the night (qN) and day ($1 - qN$) in the instars of each species (figure 1), we can approximate the frequency distributions of operative temperatures $f(T)$, facing feeding larvae in the wild at larval stage z:

$$f_z(T) = qN_z * f(NT) + (1 - qN_z) * f(DT).$$

We present the estimated thermal foraging niches of first and fourth instar larvae based on year-round temperature variation (representing the total density distributions of temperatures experienced by genotypes) next to the respective empirically derived reaction norms (figure 2a). Since any single larva only experiences temperatures within the given season of its larval life, we also compared differences between first and fourth instar larvae in the coldest (January) and warmest (July) month of the year.

(d) *State-dependent thermal reaction norms*
For each species, four females were used to start the experimental populations. The mothers were kept at temperatures between 20 and 28°C during oviposition. Each female's eggs were put to 17°C but assigned among the four test temperatures: 10, 17, 24 or 31°C. For one 24 h period, the first and last instar larvae were moved from 17°C to the specific test temperature and growth rates were measured. Any single larva experienced only one and the same test temperature and was always kept at 17°C in between measurements in the first and last instar. Again, larvae were reared singly in 0.5 l plastic jars with access to *D. glomerata*, but this time the photoperiod was set to 12 L : 12 D, leaving strictly diurnal and nocturnal larvae with equal time budgets for feeding, enabling direct comparisons between all larval groups. Growth rates were calculated as before but this time by measuring individuals at the start and end of a full 24 h period. Thus, our measure of growth rate is the combined sum of both the period of feeding which thermal conditions are depicted in figure 2a, and the period of inactivity which might involve defaecation of digested food. Host plants were exchanged regularly to minimize indirect effects of temperature through host plant quality.

For *P. aegeria* and *P. xiphoides*, 48 individuals each were started in the experiment, whereas 45 individuals were started for *P. xiphia*. Again some individuals fell away from
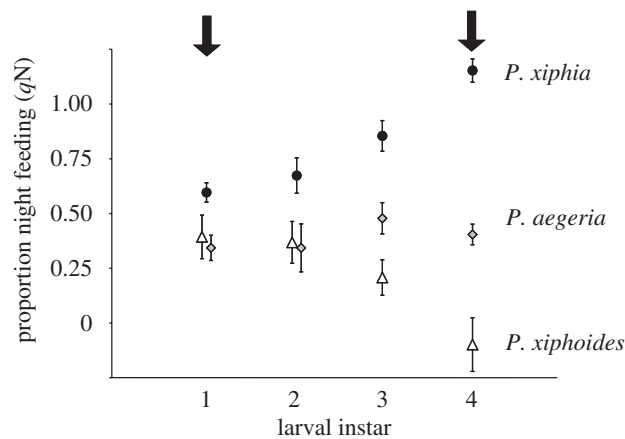

Figure 1. Ontogenetic niche shifts in foraging behaviour in *Pararge* larvae. The proportion of night feeding (mean $\pm$ 1 s.e.) throughout development. Black arrows indicate the stage of development when the thermal reaction norms for growth rate were measured.

the analysis owing to incomplete measurements. Growth rate differences were analysed with mixed linear models with individual set as random, and larval stage and test temperature as fixed effects in a full factorial design. We also analysed the effects of sex and family, but later dropped them from the analyses as the effects were non-significant.

3. RESULTS
(a) *Ontogenetic foraging shifts*
The species differed in their general night foraging activity with *P. xiphia* being most active and *P. xiphoides* the least active during dark ($F_{2,41} = 61.1$, $p < 0.001$). In the beginning of development, the species showed little differentiation in their foraging modes, but this changed markedly throughout development (species $\times$ stage: $F_{6,123} = 9.0$, $p < 0.001$; figure 1). Conducting the corresponding analysis considering family as the independent replicate generated highly similar results (species: $p < 0.001$, species $\times$ stage < 0.001).

(b) *Thermal foraging niches*
The calculations of the thermal environments suggest that the feeding larvae are exposed to very divergent thermal selection throughout development (figure 2a). The generalist forager *P. aegeria* does not change feeding behaviour throughout ontogeny and also does not experience any change in its thermal foraging environment. *Pararge xiphia* larvae that switch to strict night foraging late in development experience declining foraging temperatures throughout development, while *P. xiphoides* larvae that switch to strict day foraging behaviour instead experience an increase in temperature late in development. Comparison of the months January and July show that the *Pararge* larvae, lacking a mode of diapause, experience significant temperature variation between generations. At the altitude of the weather station on Tenerife, winter temperatures are 10°C below summer temperatures, whereas on Madeira they are 6.5°C below the summer average. However, within each season, the difference in thermal niches between instars was always large and of the same magnitude and direction as when considering
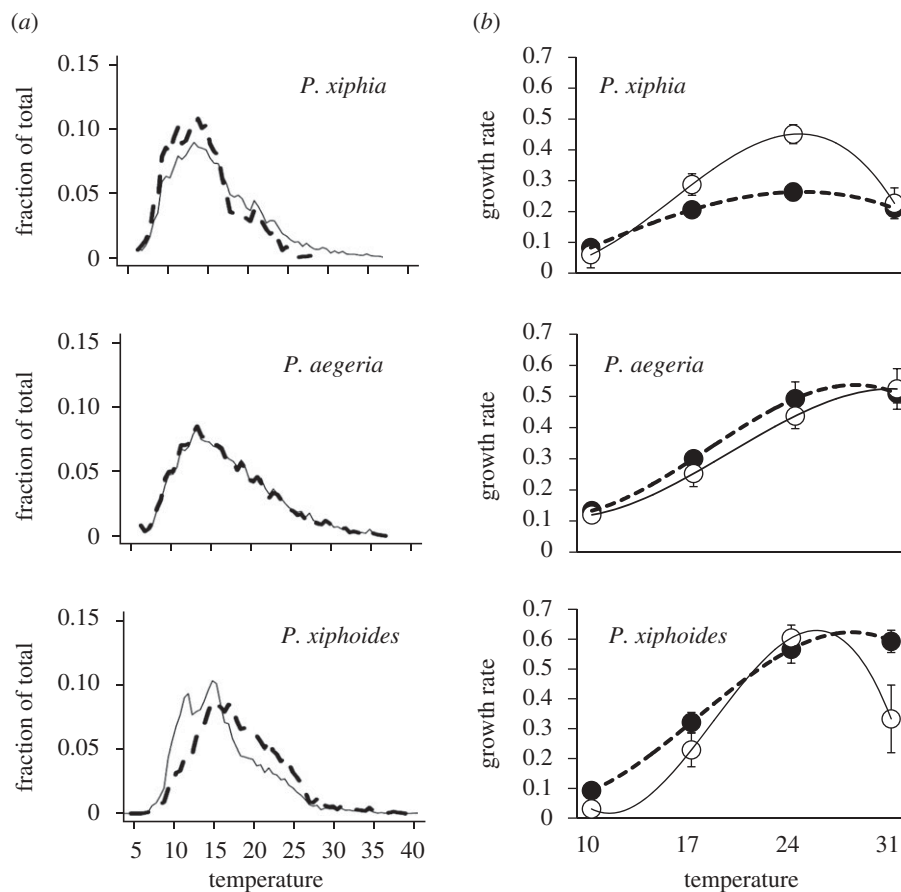
Figure 2. State-dependent reaction norms in *Pararge* larvae. (*a*) Estimated thermal foraging niches of first (full line) and fourth (dashed line) instar larvae. The relative incidence of specific temperatures is given as the fraction of feeding spent at the temperature in relation to total time. (*b*) Corresponding thermal reaction norms for relative growth rate (mean ± 1 s.e.) in the first instar (white points, full line) and fourth instar (black points, dashed line). Growth rate is given as the proportional increase in weight over 24 h in relation to the start weight of the larvae at the first measurement.

yearly variation. Thus, no matter the time of year, young and old larvae experience large predictable differences in their thermal foraging niches. Under the assumption that larval growth rate is the product of the joint optimization of several state-dependent behavioural and physiological processes (of which some are temperature-dependent), these estimates predict that the relationship between test temperature and growth rate should be dependent on which larval stage that is considered. Furthermore, the direction and magnitude of the differences in reaction norms between larval stages should depend on which species that is considered.

(c) State-dependent thermal reaction norms

We performed a detailed analysis on each species using mixed models to compare growth rates across temperatures in the first and fourth larval instar. As expected, for the generalist forager *P. aegeria*, thermal reaction norms did not differ between the beginning and the end of ontogeny (temp: $F_{3,33} = 35.4$, $p < 0.001$; stage: $F_{1,33} = 0.52$, $p = 0.48$; temp × stage: $F_{3,33} = 0.34$, $p = 0.76$). Further, for *P. xiphia* larvae that switch to strict night foraging prior to the fourth larval instar, the shape of the reaction norm differed between instars and the overall rate of growth decreased late in development (temp: $F_{3,33} = 21.0$, $p < 0.001$; stage: $F_{1,33} = 11.3$, $p = 0.002$; temp × stage: $F_{3,32} = 6.1$, $p < 0.001$). In contrast, for *P. xiphoides* larvae that make the

opposite switch to strict day foraging, overall growth rate instead increased late in development and again the shape of the reaction norm also changed as development progressed (temp: $F_{3,29} = 30.1$, $p < 0.001$; stage: $F_{1,29} = 5.6$, $p = 0.025$; temp × stage: $F_{3,29} = 2.5$, $p = 0.044$; figure 2*b*).

We also performed complementary analysis of variances and calculated effects sizes (eta-squares: η^2) in the two instars separately to compare how much of the variation in growth rate that was explained by the factors (species), (temperature) and their interaction, early and late in larval development, respectively. In the first instar, temperature explained 45.4 per cent of the variance in growth rate, whereas overall species-differences in growth rate only explained 2.8 per cent and the interaction 8.9 per cent (temp: $F_{3,95} = 33.5$, $p < 0.001$; species: $F_{2,95} = 3.1$, $p = 0.051$; temp × stage: $F_{6,95} = 3.3$, $p = 0.0056$; total model: $R^2 = 0.57$). In contrast, in the last instar when the species display pronounced differences in behaviour, temperature and the temperature–species interaction, similar to that in the first instar, explained 49.6 and 9.4 per cent of the variation in growth rate, respectively, but overall differences in growth rate between species accounted for as much as 20.9 per cent of the variation (temp: $F_{3,95} = 78.5$, $p < 0.001$; species: $F_{2,95} = 49.7$, $p < 0.001$, temp × stage: $F_{6,95} = 7.5$, $p < 0.001$; total model: $R^2 = 0.80$).

4. DISCUSSION

Our study illustrates how changes in individual state may contribute to substantial variation in thermal performance curves. We expected the behavioural shifts throughout ontogeny to be accompanied by developmental plasticity in the form of horizontal (warmer–colder) shifts of reaction norms towards warmer temperatures in *P. xiphoides* and colder temperatures in *P. xiphia*, to accommodate the changes in thermal environments in late development. This expectation was however only partly met in *P. xiphoides*, which increased performance in warm temperature but also slightly in cold temperatures, and in *P. xiphia*, no horizontal shift of the reaction norm was found. The incomplete responses suggest that reaction norm plasticity along the horizontal axis is constrained by direct costs of inducing or maintaining plasticity [23,28]. An additional, non-exclusive explanation for the lack of strong developmental shifts in reaction norms in the horizontal dimension may be that a fraction of the food consumed is digested at times of inactivity in *P. xiphia* and *P. xiphoides*. This would enforce selection on digestive capacity in the temperatures experienced during the non-feeding phase, reducing the benefits of plasticity. Our analysis of seasonal temperature variation implies that there also would be great benefits associated with physiological acclimation between generations because larvae growing in the summer and winter season experience large differences in temperature means. In preliminary experiments using acclimation temperatures of 17 and 24°C, we have however not been able to find support for beneficial temperature acclimation (cf. Leroi *et al.* [24]). Seasonal dispersal by adult butterflies up and down the mountain passes has been observed in these populations [41], which may reduce selection for an acclimation response. However, such behavioural compensation is also likely to bare costs [16,56], and it does not allow individuals to escape the within-generation temperature variation experienced throughout larval life. Thus, although tentative, our results on temperature acclimation further advocate the view that there are constraints associated with plasticity resulting in horizontal shifts of the reaction norm.

Most of the ontogenetic variation in growth rate instead seems to represent differences in resource acquisition rates resulting in vertical (faster–slower) shifts of the reaction norms and was thus not temperature-specific. This variation was predictable from the species-specific behavioural shifts, illustrated by the fact that overall species differences in growth rate accounted for less than 3 per cent of the variation in the first instar when behavioural differences between species are small, but for 21 per cent in the last instar when the species exhibit fundamentally different foraging strategies. Many organisms are forced to use suboptimal abiotic environments owing to biotic interactions such as competition or predation [7]. Huey & Bennett's [57] phylogenetic analysis of correlations between thermal preferences and thermal optima in lizard species which have evolved differences in diurnal–nocturnal activity suggested, similar to our results, that co-adaptation was incomplete, implying significant evolutionary constraints on reaction norms and trade-offs between thermal performance and reduced fitness owing to biotic interactions. The impact of predation risk on thermal- and nutritional biology has previously been discussed for lepidopteran larvae [58,59,60] and the growth strategies observed in *Pararge* butterfly larvae seem to have evolved and diversified under very different selection pressures of which one likely major force is predation risk. Ontogenetic changes in foraging behaviour often arise from natural selection owing to size-correlated changes in reproductive value or predation risk [34,35], and in *P. xiphia* feeding behaviour seems to be the result of a combination of both these factors. Together with seemingly weak selection for fast generation time, these conditions have given a selective advantage of a shift to low-risk night foraging in late development [39,40]. As follows, the moderate rates at which large *P. xiphia* larvae grow across the whole temperature range could be explained as adaptive canalization of foraging effort in response to high predation risk associated with elevated larval activity. Unlike in most caterpillars living directly on their food resource, digestion would in this case not limit growth rates except in very cold temperatures [58], and benefits of increasing digestion rates through thermal adaptation and developmental plasticity would be weak. The fast growth rates of large *P. xiphoides* larvae on the other hand, seem due to a growth maximization strategy, presumably via increased acquisition rates, resulting in a broader reaction norm and fast development and short generation time. Such a foraging strategy would only be at selective advantage given warm daytime temperatures allowing sufficient food digestion.

Thus, in summary our results show that, although adaptation to the thermal niches experienced in the respective larval stages seems incomplete, the growth patterns exhibited in each species and instar can be understood by accounting for the constraints on metabolism set by the specific thermal environments of the foraging larvae. This illustrates the importance of adopting a multivariate view of selection on physiology, behaviour and life history when studying physiological performance curves. Several theoretical models focusing on generalist–specialist trade-offs have been proposed to explain the evolution of reaction norms (e.g. [6,7,49]). Such models could easily be extended to involve trade-offs related to behavioural compensation, i.e. food acquisition or thermoregulation [56,61], or reproductive value and energy allocation [9,10]. In addition to the underlying trade-off structure, the evolutionary trajectory will also depend on the genetic architecture of the traits constituting the reaction norm [3,4,50]. Izem & Kingsolver [62] have developed statistical methodology that, given a rigorous quantitative genetics experimental design, allows partitioning of genetic variance constituting the reaction norm into such explained by allocation and acquisition-, hotter–colder- and generalist–specialist trade-offs. Such quantifications may better allow predictions of which out of several alternative routes evolution might take [9,12].

From an empirical perspective, our results demonstrate that reaction norms for performance traits will be hard to measure independently of behavioural and physiological compensation that varies with the state of the individual. The common practice in evolutionary physiology has been to standardize the point of measurement, knowing that individual condition may have large

effects on performance [31]. Chown *et al.* [63] showed that exposure times when scoring individuals of *Drosophila melanogaster* and the invasive ant *Linepithema humile* for temperature tolerance affected both phenotypic means and variances, not only changing the conclusions about the species' thermal sensitivities, but also estimates of genetic variance in tolerance thresholds. Thus, similar to the realization that estimates of genetic variance and heritabilities are specific to the environment they are measured in [64] and [65], estimates of reaction norms (and their heritability) will be context dependent [66]. Therefore, measuring reaction norms along a one-dimensional environmental gradient may not always be sufficient for predicting the evolutionary response even though effort is made to standardize the measurement. In this study, conclusions of how and between which species reaction norms have diverged would be different depending on the developmental stage in which they were measured, and conclusions on the nature of constraints temperature inflicts on growth would be flawed had reaction norms been measured in only one larval stage. The fundamental developmental shifts of reaction norms in *Pararge* larvae without any change in extrinsic conditions is perhaps an extreme example, however, state-dependent variation is present to some degree in most organisms and thus careful consideration of species-specific ecology is needed when assessing and evaluating evolutionary constraints on performance curves.

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