The impact of thermal fluctuations on reaction norms in specialist and generalist parasitic wasps

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Summary

1. Reaction norms depict the environmental effects on phenotypic traits and are used to predict the global change consequences on species distributions. However, studies performed at constant temperatures have limited ecological significance because expressed phenotypes depend on the range and frequency of environmental states.

2. Using Jensen's inequality (i.e. a mathematical property of nonlinear functions), we predicted that the effect of thermal fluctuations on the phenotype depends on the shape of the reaction norm. Thermal fluctuations around the optimal temperature are expected to reduce the phenotypic trait values, especially for specialists because of their narrower reaction norms.

3. This study measured the effects of diel fluctuations in developmental temperature on phenotypic expression of traits related to fitness and energetic resources in two strains of the parasitoid wasp *Venturia canescen*s from different habitats: a thermal generalist strain and a specialist one. In a first experiment, we compared the effect of constant thermal regimes versus fluctuating ones having the same means (20, 25 and 30 °C) on reaction norms of life-history traits and of energetic reserves. In a second experiment, we examined the effects of a natural thermoperiod in the field on these traits.

4. Our results show that the shape of the reaction norm defines the phenotypic changes induced by the development under fluctuating thermal conditions. These results match the predictions of the Jensen's inequality. Moreover, our results emphasize the significance of taking into account several phenotypic life-history traits to study the adaptive value of phenotypic plasticity. We also show that the level of energetic resources depends on the mean developmental temperature and not on the thermal regime. Finally, the field experiment confirms that the phenotype of these parasitoids depends on the temperature variation.

5. Our study highlights the relevance of the Jensen's inequality to predict the effect of thermal fluctuations on fitness of parasitoids with contrasted thermal sensitivities.

Key-words: environmental variability, Jensen's Inequality, parasitoid, performance curves, phenotypic plasticity, *Venturia canescens*

Introduction

Phenotypic plasticity, that is, capacity of an organism to react to an environmental input with a change in its phenotype (West-Eberhard 2003), is ubiquitous and well recognized as an adaptive response to environmental change (van Tienderen 1991; Moran 1992; Ernande & Dieckmann 2004). For instance, plastic responses are receiving increasing attention in the context of climate changes from both empirical (Chown *et al.* 2007; Hance *et al.* 2007; Brommer,

Rattiste & Wilson 2008; Charmantier *et al.* 2008) and theoretical points of view (Chevin, Lande & Mace 2010; Chown *et al.* 2010). The vast majority of studies focuses only on phenotypic responses to fixed environments, ignoring the growing evidence that phenotypes may vary relatively to the range and frequency of environmental variation (Miner & Vonesh 2004; Měráková & Goždík 2009; Clusella-Trullas, Blackburn & Chown 2011). Therefore, understanding the effects of fluctuating conditions on phenotypic plasticity remains a challenge to evolutionary biology. This is particularly so within the framework of global warming, because the ongoing climate change alters the variability as well as the mean of current thermal conditions (Easterling *et al.* 2000; Tebaldi *et al.* 2006; Zhou *et al.* 2009).

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In ectotherms, phenotypic responses to thermoperiod generally result from the interactions between the average and range of thermal regimes (Pétavy *et al.* 2001, 2004; Ragland & Kingsolver 2008; Kingsolver, Ragland & Diamond 2009; Terblanche, Nyamukondiwa & Kleynhans 2010; Bozinovic *et al.* 2011; Folguera *et al.* 2011; Niehaus *et al.* 2012). Larval growth rate in the butterfly *Bicyclus anynana* is illustrative; the growth rate under a variable thermal regime, with an amplitude of 8 °C around 19 °C, was higher than that observed in constant temperature, while the reverse holds for the same range of fluctuations around 25 °C (Brakefield & Kesbeke 1997). Thermal fluctuations can have complex effects on fitness-related traits. On the one hand, they can disrupt homeostasis, and extreme fluctuations can induce costly physiological responses to stressful temperatures (Podrabsky & Somero 2004; Marshall & Sinclair 2010; Folguera *et al.* 2011). On the other hand, thermal fluctuations can sometimes be beneficial, allowing recovery periods from stressful temperatures (Pétavy *et al.* 2001; Colinet *et al.* 2007; Koštál *et al.* 2007).

Costs or benefits of thermal fluctuations may strongly depend on the shape of the thermal reaction norm. The classical nonlinear shape for thermal sensitivity of traits related to fitness (Huey & Stevenson 1979; Martin & Huey 2008) provides an opportunity to make specific predictions about effects of thermal fluctuations on phenotypic expression using a mathematical property of nonlinear functions, Jensen's inequality (Jensen 1906; Ruel & Ayres 1999). It states that the mean of the output of a decelerating function (i.e. concave curve) will be less than the function of the mean of the inputs, and conversely that the mean of the output of an accelerating function (i.e. convex curve) will be more than the function of the mean of the inputs. Jensen's inequality shows a long history in the 'risk sensitivity' framework of optimal foraging (Caraco 1980; Smallwood 1996; Blackburn, Hobbs & Detling 2011), but has also a broad range of applications in ecology, for example in landscape ecology (Li & Reynolds 1993), plant pollination (Richards, Williams & Harder 2009), community ecology (Bolnick *et al.* 2011) and animal personality (Wolf & Weissing 2012). Applied to functional response of ectotherms to temperature, the Jensen's inequality predicts that when the reaction norm is concave, the organism will present a higher fitness under constant than fluctuating temperatures with the same mean. On the contrary, if the reaction norm is convex, the organism is expected to be prone under fluctuating conditions, as fitness will be higher under fluctuating than constant temperatures (Pasztor, Kisdi & Meszena 2000). The relevance of Jensen's inequality has been recently highlighted to understand the impact of thermal fluctuations on the energetic cost in an overwintering butterfly (Williams *et al.* 2012) and on wing traits and locomotor performance of *Drosophila melanogaster* (Kjærsgaard *et al.* 2012).

Thermal specialists and generalists have differently shaped reaction norms, as the specialists present reaction norms for fitness-related traits with a narrower thermal breadth and a higher maximal phenotypic value than generalists (Fig. 1). They are thus expected to differ in their responses to thermal fluctuations. For instance, in the pitcher plant mosquitoes (*Wyeomyia smithii*), populations differed in the shape of their reaction norms and in their responses to thermal fluctuations, as qualitatively predicted by the Jensen's inequality (Ragland & Kingsolver 2008). Determining benefits and costs of thermal fluctuations requires studying reaction norms of several traits. For an individual, the thermal sensitivity of each trait will vary according to its relationship with fitness. Traits related to survival are expected to have a more generalist-type of reaction norm shape than traits related to reproduction (Lynch & Gabriel 1987; Gilchrist 1995; Angilletta 2009). Despite this variability in response between traits, most of the studies on thermal fluctuations so far have focused on growth and size only (Brakefield & Kesbeke 1997; Pétavy *et al.* 2001, 2004; Ragland & Kingsolver 2008; Kingsolver, Ragland & Diamond 2009; but see Terblanche, Nyamukondiwa & Kleynhans 2010; Folguera *et al.* 2011).

In this study, we hypothesized that the fitness of a parasitoid wasp under natural thermal regime depends on the shape of its reaction norms, in accordance with Jensen's inequality. Therefore, we tested the effects of fluctuating thermal conditions on phenotype expression. We performed two experiments: one under controlled laboratory conditions and a second under field conditions. In the first experiment, we compared the effects of constant and fluctuating
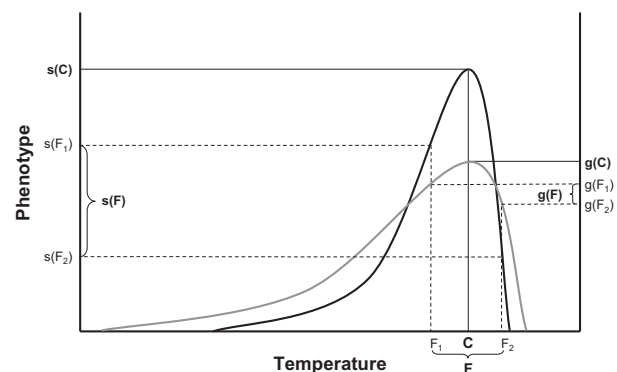
Fig. 1. Influence of fluctuating and constant temperatures on phenotypic trait values. Reaction norms (i.e. phenotypic values in y-axes) are described by classical asymmetric Gaussian-shaped thermal reaction norms of fitness-related traits relative to temperatures: *s*, reaction norm, for a specialist (solid black curve), and *g* for a generalist (solid grey curve). *C* denotes the optimal temperature, under constant and fluctuating thermal conditions, for the specialist and the generalist wasps. On the left *y*-axis and the right *y*-axis are represented the phenotypic values for the specialist and the generalist, respectively. *s(C)* and *g(C)* are the maximal phenotypic values under constant conditions for the specialist and the generalist, respectively. F_1 and F_2 represent the boundaries of the temperature range in the fluctuating conditions; *F* is the average value of temperature. *s(F)* and *g(F)* represent the mean phenotypic responses of the specialist and the generalist under fluctuating environment.

thermal regimes on the reaction norms of thermal specialist and generalist strains of the parasitoid wasp *Venturia canescens* Gravenhorst (Hymenoptera: Ichneumonidae) (Foray, Gibert & Desouhant 2011). We measured both physiological and life-history traits to investigate whether the latter depended on acquisition and allocation of energy reserves (Boggs 1992). In the second experiment, we investigated the effect of the natural thermal regime in the field with typically larger and more stochastic thermal variations. We tested whether field outcomes could be predicted from our laboratory results.

Because thermal reaction norms have usually an asymmetric Gaussian shape (Fig. 1, Huey & Stevenson 1979; Martin & Huey 2008), we used Jensen's inequality to predict that (i) fluctuations of temperature around the thermal optimum should result in decreased phenotypic trait values relative to development under fixed optimal temperature; (ii) this decrease should be more important for thermal specialists (Fig. 1); (iii) assuming that the reaction norms are convex at the boundaries of the thermal range, the effect of thermal fluctuations around suboptimal temperatures should be positive and greater for the specialist than for generalist reaction norm.

Venturia canescens represents a relevant biological model to test these predictions as it shows singular intraspecific variation. This species has strains with different reproductive modes in distinct habitats (Beukeboom et al. 1999). Parthenogenetic arrhenotokous wasps (called 'sexual') live exclusively in forests and orchards with clear seasonal and daily thermal fluctuations. In contrast, parthenogenetic thelytokous wasps ('asexual') thrive in anthropogenic environments (e.g. granaries and mills) with much more buffered thermal variations (Amat et al. 2006). Moreover, arrhenotokous wasps are able to reproduce over a wider range of developmental temperatures (and are thus more generalized) than their asexual counterparts (Foray, Gibert & Desouhant 2011). In the same way, arrhenotokous females show adaptive plasticity in their foraging behaviour in response to sudden shifts in temperatures; thelytokous wasps are unable to show similar behavioural plasticity (Amat et al. 2006).

Materials and methods

BIOLOGICAL MODEL AND CULTURES

Venturia canescens is an endoparasitoid of lepidopteran larvae (mainly Pyralidae; Salt 1976) occurring in dried fruits, like figs. This species has a Mediterranean distribution range and produces several generations a year from May to October in southeast France (Amat 2004). In arrhenotokous wasps, haploid males are produced from unfertilized eggs whereas thelytokous wasps produce diploid females from unfertilized eggs, leading to a lack of males (Beukeboom et al. 1999). Thelytokous parthenogenesis is not induced by endosymbiotic bacteria (Mateo-Leach et al. 2009; Foray et al. 2013a). Female parasitoids localize hosts by responding to mandibular gland secretions deposited by larvae while feeding in their medium (Castelo, Corley & Desouhant 2003).

We conducted experiments on individuals from thelytokous and arrhenotokous populations established from large samples trapped in orchards and in the vicinity of granaries near Valence (France, North: 44°58′34″, East: 4°55′66″, Gotheron INRA station) during the summer of 2008. *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) larvae, reared on wheat semolina, were used as hosts for arrhenotokous and thelytokous wasps. Adult wasps had food *ad libitum* (water-diluted honey 1:2) and were maintained in the laboratory under controlled conditions (25 ± 1 °C, 70 ± 5% RH and 12:12 L:D). Both strains had been kept in the laboratory for *c*. 10 generations before testing (*c*. 6 months) to prevent potential maternal effects.

EXPERIMENT 1: PLASTIC RESPONSES TO THERMAL FLUCTUATIONS UNDER CONTROLLED LABORATORY CONDITIONS

We reared wasps at three mean developmental temperatures. For each temperature, we used the two thermal regimes (i.e. constant vs. fluctuating) with a full factorial design. To obtain a sufficient and roughly the same number of daughters from females from the two reproductive modes, and because in the arrhenotokous population the sex ratio is nearly 1:2 (Metzger, Bernstein & Desouhant 2008), twice more arrhenotokous females were used. Thus, 72 arrhenotokous and 36 thelytokous females were randomly chosen from rearing boxes at emergence and transferred into plastic boxes (300 × 300 × 300 mm) with food (water-diluted honey 1:2). Each box contained 10 females of the same reproductive mode; the boxes with arrhenotokous females also contained 20 virgin males to induce mating. After 48 h, each female was individually placed for 72 h in a box (170 × 160 × 60 mm) containing two host patches and food. The patches consisted of Petri dishes (Ø: 52 mm, height: 3 mm) filled with semolina and containing 25 third-instar *E. kuehniella* larvae. Each female was free to lay in the patches. The patches were prepared one week before being offered to the parasitoids so that the medium would be soaked by the host kairomones. Nine females died (3 thelytokous and 6 arrhenotokous) and were excluded from the analysis.

For each wasp, the two patches with parasitized hosts were randomly assigned to the constant or the fluctuating thermal regime. Both regimes had the same average temperature. Identical incubators (MLR-352H SANYO) maintained average temperature at 20, 25 or 30 °C, respectively. Temperature of the fluctuating thermal regime followed a nycthemeral rhythm, ranging from ± 4 °C around the mean and changing of 2 °C (decrease or increase) every 3 h. Higher and lower temperatures than the average occurred during photophase and scotophase, respectively (see Figure S1 in Supporting information). Under the constant thermal regime, the temperature was regulated at the average temperature. The photoperiod (12:12 L:D) was identical in the incubators, as was relative humidity (70%). Due to logistic limits, developmental treatments with mean temperature of 25 and 30 °C were carried out in one experimental block, and those at 20 °C followed one month later. Host patches were inspected every day at 30-min intervals between 8:00 p.m. and 11:30 p.m. to collect the emerged wasps.

PHENOTYPIC MEASURES AND QUANTIFICATION OF ENERGETIC RESERVES

We measured the impact of the thermal treatments on phenotypic traits of daughters of both the reproductive modes. Each day after emergence, daughters were assigned randomly to two groups (see Table S1). For group 1, we measured egg load at emergence and energetic reserves (see below). For group 2, we measured longevity

and egg load at death (called 'maximal egg load'). For both groups, we used left hind tibia length as an index of body mass as this measure is positively correlated with dry body mass ($r^2 = 0.84$, $n = 78$, $P < 0.0001$, *data not shown*). After removing it from the thorax, the left hind tibia was photographed with a binocular microscope (Zeiss stemi 2000-C, Carl Zeiss S.A.S., France, magnification: $2.5\times$) coupled with a Motic® M100 Digital Camera (Motic China Group Co., Ltd, Hong Kong, China). Tibia length was measured from the pictures using the software Motic Images Plus® 2.0 ML (Motic China Group Co., Ltd, Hong Kong, China). The ratio between body size at emergence and development time was then used as an estimate of developmental growth rate.

The wasps assigned to group 1 were individually frozen at −20 °C after their emergence. To quantify energetic reserves for each individual, we used biochemical analyses modified from van Handel's methods (van Handel 1985a,b; Foray *et al.* 2012). We quantified for a single insect total protein, carbohydrate (free sugars and glycogen) and lipid contents. First, ovaries and oviducts of the wasps were gently removed in 10 μL of buffer solution (100 mM KH_2PO_4, 1 mM DTT and 1 mM EDTA, pH = 7·4). To quantify egg loads at emergence, we photographed the whole egg complements with a binocular microscope system (see above) and used an image analyser (see above) to count the eggs. Eggs were placed back into the buffer solution and added with the remaining wasp body in a 2 mL Eppendorf vial. This step was performed quickly (5 min max), and bodies were kept on ice to prevent enzymatic degradation. In each vial, 170 μL of buffer solution was added. The sample was then frozen at −20 °C until energetic assays. Wasps were crushed with stainless steel beads, and their total protein content was quantified with the Bradford assay. Afterwards, a chloroform/methanol (1: 2 v/v) solution was added to extract total lipids and carbohydrates (free sugars and glycogen), which were further assayed with vanillin and anthrone reagents, respectively (Foray *et al.* 2012).

Newly emerged daughters assigned to the second group were placed individually in plastic vials (Ø: 30 mm, h: 70 mm) with a piece of cotton wool soaked with 2 mL of water. They were maintained under controlled conditions (25 ± 1 °C, 70 ± 5% RH, 12:12 L:D) and kept without host stimulus until death. The tubes were inspected at 2-h intervals from 8:00 a.m. to 6:00 p.m. to record the time of death. When it occurred at night, 1:00 a.m. (the median time between 6:00 p.m. and 8:00 a.m.) was considered as the time of death (Desouhant *et al.* 2005). The time lapse between emergence and death was used as a measurement of longevity. When dead, wasps were dissected, and mature eggs from the whole egg complement were estimated (see above). Mature egg load at death was considered as a good proxy of maximum egg load because there is no egg re-absorption (Eliopoulos *et al.* 2003).

EXPERIMENT 2: PHENOTYPIC EXPRESSION UNDER FIELD CONDITIONS

The aim of the second experiment was to compare the traits of females developed under controlled and constant thermal conditions (identical to those of experiment 1) with those of females that developed in field conditions. Twenty arrhenotokous and 10 thelytokous female parasitoids were randomly chosen from rearing boxes at their emergence and then placed in cages (300 × 300 × 300 mm) with food (water-diluted honey 1:2). Each cage contained 10 females. Twenty virgin males were added to cages containing arrhenotokous females to allow mating. After 48 h, males were removed and boxes (265 × 135 × 75 mm) containing around 500 *E. kuehniella* larvae of third-instar in semolina were introduced in each cage. Females were allowed to lay eggs during 72 h and had free access to food.

For each box, 200 potentially parasitized hosts were chosen randomly. Next, they were placed individually in dried figs from organic culture (Rapunzel®). Most of the larvae remained inside the figs (V. Foray Pers. Obs.). Six batches of 100 parasitized figs were prepared: 3 were placed in the field and 3 were used as control in the laboratory. The 3 batches were placed in cages (300 × 300 × 300 mm) in an experimental field site (Villeurbanne, France, North: 45° 46′ 00″, East: 4° 52′ 49″). The netting of the cages consisted in fine mesh nylon to prevent the escape of host larvae, and the cages were protected against rain and predators (such as small mammals and carnivorous invertebrates) by a large wired net box with a Plexiglas lid. Cages were surrounded with a veil to prevent entrance of wild competitors for hosts (e.g. the parasitoid *Habrobracon spp.*). Throughout the experiment, the temperatures inside a fig was recorded by using a Temperature Recorder (LogTag® Trex-8) with a tipped probe (ST100T-15); the temperature and the relative humidity outside of the figs were recorded with an EasyLog USB recorder (Figure S2, Supporting information). The three batches of figs assigned to the control treatment were maintained under laboratory conditions in the climate chambers (incubators MLR-352H SANYO; 25 ± 1 °C, 70 ± 10% RH and 12 D: 12L). For both field and control cages, we daily checked for emergences of hosts and parasitoids at 11:00, and removed them from the boxes. Female parasitoids were then individually frozen at −20 °C to perform phenotypic measures (developmental time, body size, fecundity at emergence) following the same protocols as in the first experiment.

DATA ANALYSIS

Statistical analyses were performed with generalized linear models (GLM; Nelder & Weddenburn 1972). A Gamma error distribution (inverse link function) was used to analyse longevity, body size, developmental growth rate and energetic reserves. Egg loads were analysed by means of a Poisson error distribution (log link function).

For experiment 1, the three explanatory variables were reproductive mode (categorical variable, two classes), mean developmental temperature (continuous variable) and thermal regime (categorical variable, two classes). We also integrated a quadratic effect of mean developmental temperature to better fit the shape of the reaction norms. These explanatory variables were integrated multiplicatively, except for the combination of the linear and quadratic effects of mean developmental temperature. Among these interactions, three were of particular relevance to test our predictions: (i) the interaction between the reproductive mode and the quadratic effect of the mean developmental temperature was used to assess the difference of the shape of the reaction norms of thelytokous and arrhenotokous wasps; (ii) the interaction between the thermal regime and the mean developmental temperature (linear and quadratic effect) tested whether the fluctuation effect depended on mean developmental temperature and modified the shape of the reaction norm; (iii) the three-way interaction tested whether the effect induced by thermal fluctuations on the shape of the reaction norm differed between arrhenotokous and thelytokous wasps. In the second experiment, the two qualitative explanatory variables were the developmental environment (field vs. laboratory) and the reproductive mode.

To remove any confounding effect of body size on egg loads, longevity and energetic reserve measurements, tibia length was always introduced as the first explanatory variable (Pelosse *et al.* 2011). Selection of the best model was based on backward procedure with classical chi-square tests of likelihood ratios (LR) for nested models. All data analyses and graphics were carried out using R 2.12.1 (R Development Core Team 2010).

Results

EXPERIMENT 1: REACTION NORMS OF LIFE-HISTORY TRAITS

Wasp size

Adult size of the wasps differed significantly with the reproductive mode, the developmental temperature and the interaction effect (Table 1; Fig. 2a). The reaction norm of this trait was concave with a significant negative quadratic effect of the developmental temperature. Wasp size of both reproductive modes was maximal at intermediate temperature and decreased after development at 20 and 30 °C. The interaction e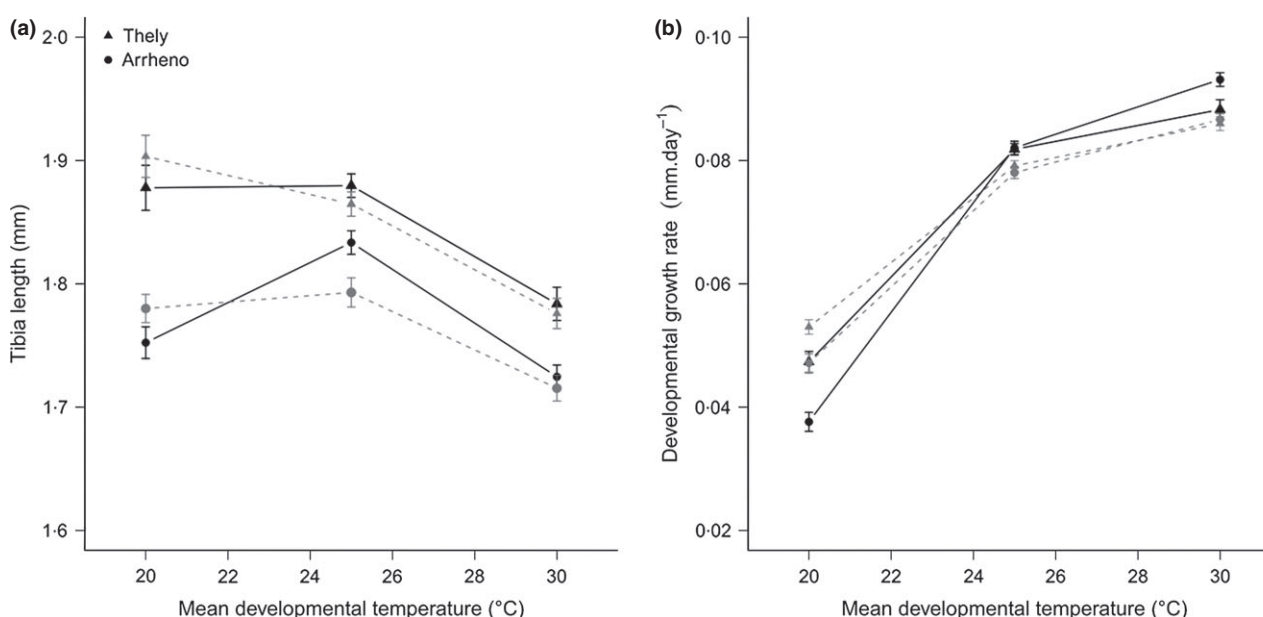ffect between the quadratic effect of the developmental temperature and the reproductive mode was significant: thelytokous and arrhenotokous wasps differed in the shape of their reaction norm for size. Further, a significant interaction between the thermal regime and the quadratic effect of the developmental temperature was found (Table 1; Fig. 2a). In accordance with our prediction (Fig. 1), the maximal body size (observed at 25 °C) was lower under fluctuating thermal conditions.

Developmental rate

The significant interaction between the reproductive mode and the quadratic effect of the developmental temperature indicated that the concave shape of the reaction norm differed with the reproductive mode (Table 1; Fig. 2b). Arrhenotokous females reached higher maximal growth rate than the thelytokous females did (Fig. 2b). As expected, the shape of the reaction norm was also affected by the thermal regime, with a decrease of the developmental growth rate at 25 and 30 °C under the fluctuating regime.

Egg load at emergence

After removing the body size effect, egg load at emergence depended on the interaction between the reproductive mode, the mean temperature and the thermal regime (Table 2). Egg load at emergence presented a concave reaction norm, denoted by the significant quadratic effect of the developmental temperature (Table 2; Fig. 3a). Thermal regime had also a significant effect in interaction with the quadratic effect of the developmental temperature (Fig. 3a). For thelytokous wasps, the fluctuating thermal regime resulted into a vertical shift of the reaction norm with a reduced egg load at emergence, irrespective of the

Table 1. Summary of results for generalized linear models for tibia length and developmental growth rate. d.f. = degree of freedom; dev = deviance; $P > |\chi^2|$ = P-value

Effect	Tibia length			Developmental growth rate		
	d.f.	Dev.	$P > |\chi^2|$	d.f.	Dev.	$P > |\chi^2|$
Reproductive mode (RM)	1	0·33	<0·0001	1	0·072	0·04
Temperature (T)	1	0·21	<0·0001	1	26·69	<0·0001
Temperature ^{2} (T^2)	1	0·15	<0·0001	1	11·61	<0·0001
Regime (R)	1	0·004	0·19	1	0·057	0·07
RM × T	1	0·02	0·005	1	0·67	<0·0001
RM × T^2	1	0·02	0·005	1	0·48	<0·0001
RM × R	1	0·00	0·54	1	0·006	0·56
T × R	1	0·005	0·13	1	0·56	<0·0001
T^2 × R	1	0·016	0·008	1	0·77	<0·0001
RM × T × R	1	0·00	0·97	1	0·11	0·013
RM × T^2 × R	1	0·002	0·31	1	0·09	0·021

Fig. 2. Mean (±SE) tibia length (a) and developmental growth rate (b) of thelytokous (triangle) and arrhenotokous (circle) *Venturia canescens* under constant (black solid lines) and fluctuating (grey dashed lines) thermal regimes for three mean developmental temperatures.

Table 2. Summary of results for generalized linear models for fecundity at emergence, maximal egg load, ovigeny index and adult longevity. For details, see legend of Table 1

Effect	Fecundity at emergence			Maximal egg load			Adult longevity		
	d.f.	Dev.	$P > |\chi^2|$	d.f.	Dev.	$P > |\chi^2|$	d.f.	Dev.	$P > |\chi^2|$
Size	1	1592·37	<0·0001	1	1021·22	<0·0001	1	0·46	0·001
RM	1	102·02	<0·0001	1	10·57	0·001	1	0·002	0·82
T	1	117·67	<0·0001	1	67·33	<0·0001	1	6·01	<0·0001
T²	1	375·68	<0·0001	1	126·97	<0·0001	1	0·0005	0·92
R	1	38·38	<0·0001	1	0·46	0·49	1	0·05	0·30
RM × T	1	27·33	<0·0001	1	123·73	<0·0001	1	0·01	0·60
RM × T²	1	5·62	0·018	1	16·45	<0·0001	1	0·34	0·005
RM × R	1	26·44	<0·0001	1	6·83	0·009	1	0·0002	0·94
T × R	1	12·56	0·0003	1	5·05	0·02	1	0·53	0·0005
T² × R	1	0·10	0·749	1	30·08	<0·0001	1	0·11	0·11
RM × T × R	1	52·97	<0·0001	1	5·20	0·02	1	0·0054	0·73
RM × T² × R	1	8·67	0·003	1	1·22	0·27	1	0·0028	0·80

temperature. For arrhenotokous, thermal fluctuations decreased egg load at emergence only at low temperature, while the fluctuations had either no effect at 25 °C or a positive effect at 30 °C (Fig. 3a).

Maximum egg load

This trait depended on the three explanatory factors (Table 2; Fig. 3b). As for the egg load at emergence, we observed a significant quadratic effect of the developmental temperature. This highlighted that reaction norms showed a maximum at 25 °C, mainly for thelytokous wasps under a constant thermal regime (Fig. 3b). The quadratic effect of the temperature interacted with the reproductive mode and was more pronounced for maximal egg load of thelytokous wasps than arrhenotokous wasps (Fig. 3b). The quadratic effect of the developmental temperature interacted also with the thermal regime, leading to a more linear reaction norms for both reproductive modes under thermal fluctuations. At optimal temperature (25 °C), maximal egg load of thelytokous wasps was more affected by thermal fluctuations than in arrhenotokous females.

Longevity

Adult longevity was positively related to the developmental temperature, and the shape of the reaction norms was nearly linear and depended on the reproductive mode (Table 2; Fig. 3c). Quadratic effect of the developmental temperature interacted with the reproductive mode, resulting in a convex curve for arrhenotokous wasps and a concave curve for thelytokous wasps. Thermal regime affected significantly adult longevity relatively to the developmental temperature (Table 2; Fig. 3c). On the one hand, we observed a positive effect of thermal fluctuations, relatively to the constant thermal regime, on adult longevity at 20 °C, and on the other hand, we observed a negative effect at 30 °C. After development at 25 °C, thermal

fluctuations had no effect on longevity, in accordance with the prediction expected with a more generalist-like shape of the reaction norm under constant regime.

FITTING OF THE PREDICTIONS OF JENSEN'S INEQUALITY WITH PHENOTYPIC RESPONSES UNDER FLUCTUATING REGIME

We tested whether the predictions of Jensen's inequality matched the effect of thermal fluctuations around 25 °C on the phenotypic trait values. These quantitative predictions were generated for the different traits by (i) determining the best-fitting models of reaction norms under constant regime, (ii) predicting the phenotypic values by the models at the different temperatures of the fluctuating thermal regime around 25 °C (i.e. at 21, 23, 25, 27 and 29 °C, see above), and (iii) calculating the average of the predicted values across 24 h. Both predicted and observed values under fluctuating regime around 25 °C were normalized (mean centred and divided by the standard deviation) to remove the effects of unit of measure and variance. The correlation between observed and predicted values was significant ($r = 0.79$; $P = 0.006$) and both distributions did not significantly differ (Kolmogorov–Smirnov test: $D = 0.3$; $P = 0.76$) (Fig. 4). Consequently, the observed values under thermal fluctuations around 25 °C did not deviate significantly from the first bissectrice, they thus matched the predictions of Jensen's inequality.

REACTION NORMS OF ENERGETIC RESOURCES

Larger wasps had larger energetic reserves (Table 3). The reproductive mode had a significant effect on the four energetic components: arrhenotokous wasp contained generally more proteins, lipids and glycogen but less free sugars than thelytokous wasps (Table 3; Fig. 5). There was a significant interaction effect between the reproductive mode and the quadratic effect of mean temperature for protein, glycogen and lipid contents, revealing that the

(a)

(b)

(c)
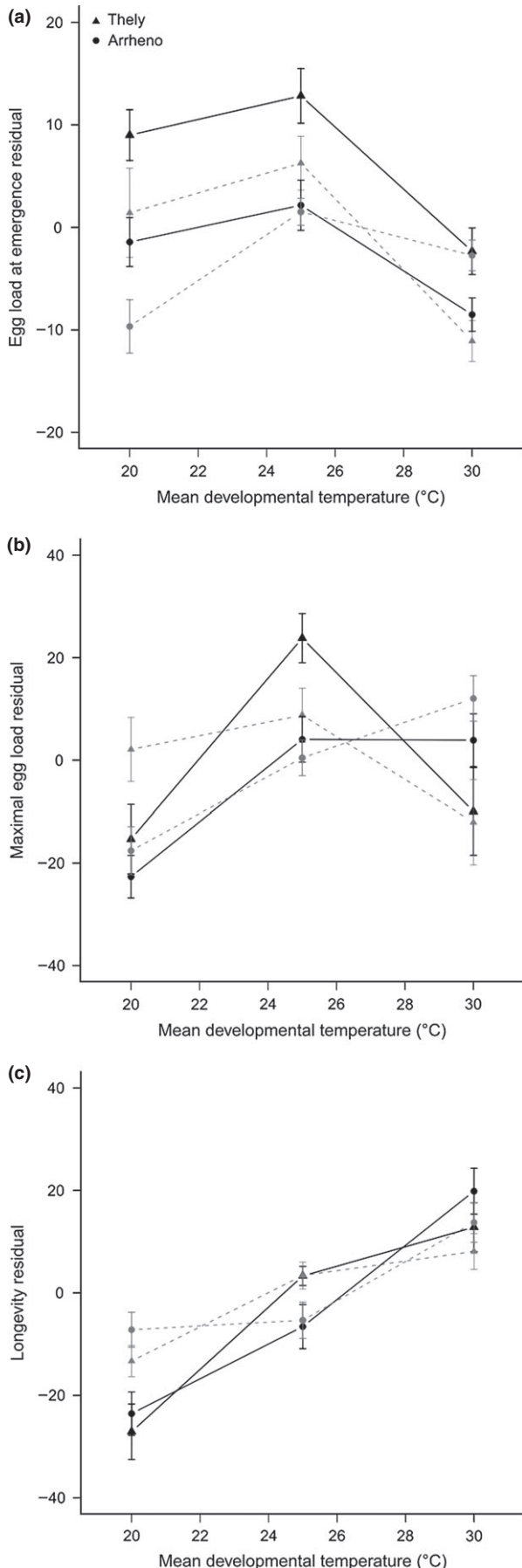
acquisition of energetic resources differed between thelytokous and arrhenotokous wasps relatively to the mean temperature (Table 3; Fig. 5). Thelytokous wasps had the highest content of lipids and glycogen after development at 25 °C, whereas arrhenotokous wasps kept a stable glycogen content and their lipid content increased with developmental temperature (Fig. 5c,d). Thelytokous wasps maintained a stable protein content relative to the developmental temperature, whereas it was highest at the lower developmental temperature. Thermal regime affected only the free sugar content in interaction with the reproductive mode, mainly because at 30 °C the free sugar content decreased under fluctuating temperatures, compared with constant temperature, for thelytokous wasps and, conversely, it increased for arrhenotokous wasps (Table 3; Fig. 5b).

EXPERIMENT 2: PHENOTYPIC EXPRESSION UNDER FIELD CONDITIONS

Laboratory and field environments presented different thermal parameters: under laboratory conditions, the temperature was constant at 25 °C, whereas under field conditions, temperature followed a natural regime (ranging from 10 °C to 35 °C; average: 22 °C) (see Figure S2).

Developmental growth rate was lower under field conditions than under laboratory conditions for females of both the reproductive modes ($\chi^2 = 0.82$, d.f. = 103, $P = 0.002$) (Fig. 6a). For this trait, there was no difference between thelytokous and arrhenotokous wasps, or interaction effect between the reproductive mode and the developmental environments.

After controlling for the positive effect of body size ($\chi^2 = 207.54$, d.f. = 103, $P < 0.0001$), egg load at emergence was significantly affected by the interaction between the reproductive mode and the developmental conditions ($\chi^2 = 18.45$, d.f. = 100, $P < 0.0001$). Thelytokous wasps emerged with more eggs than arrhenotokous wasps, but the difference between reproductive modes was smaller after development in the field than in the laboratory (Fig. 6b).

Discussion

Our study shows that the phenotypic responses to temperature of the parasitoid wasp *Venturia canescens* are not only affected by the mean but also by the variability of thermal conditions. For the first time in an experimental study, we evaluate Jensen's inequality to predict qualitatively and

Fig. 3. Mean (±SE) fecundity at emergence (a), maximal egg load (b), adult longevity (c) of thelytokous (triangle) and arrhenotokous (circle) *Venturia canescens* under constant (black solid line) and fluctuating (grey dashed line) thermal regimes for three mean developmental temperatures. Fecundity at emergence, maximal egg load and longevity are size corrected.
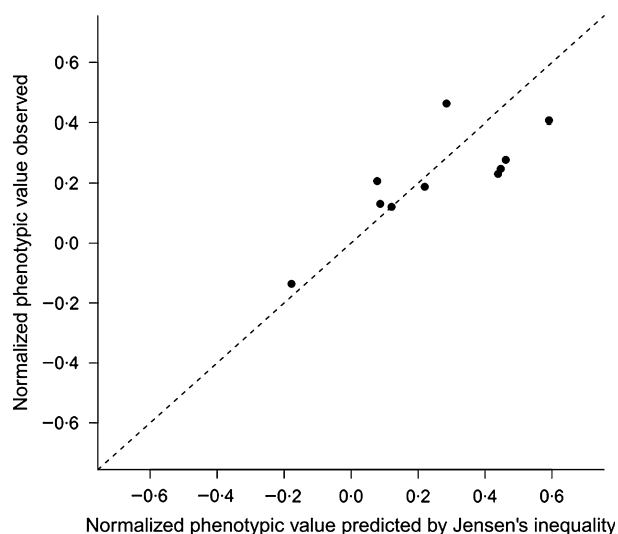
Fig. 4. Correlation between phenotypic values observed under fluctuating thermal regime at 25 °C and the predicted values by Jensen's inequality. Dots correspond to the normalized life-history traits observed for thelytokous and arrhenotokous wasps. Dotted line represents the first bissectrice along with the points are expected to fit when observed values perfectly match predictions.

quantitatively the effect of thermal fluctuations on several life-history and physiological traits of parasitoids with contrasted thermal sensitivity. Our results highlight that the effect of thermal fluctuations strongly depends on the reaction norm shape.

DOES THE SHAPE OF THE REACTION NORMS ALLOW PREDICTING THE EFFECT OF FLUCTUATIONS?

Reaction norms of ectotherms generally increase with temperature to reach maximal performance, beyond which performance decreases abruptly (see Fig. 1); this results in a typical concave curve (Huey & Stevenson 1979). The pattern is observed for many fitness-related traits, such as survival, reproduction, growth and locomotion (Angilletta

2009). *Venturia canescens* is not an exception to this general rule: reaction norms are concave, independently to the thermal regime. Our results confirm that arrhenotokous wasps of variable climates show a generalist-type of thermal reaction norm shape, while thelytokous wasps of more stable thermal conditions adopt a specialist-type of strategy in response to temperature, particularly for fecundity-related traits (Foray, Gibert & Desouhant 2011).

Applying Jensen's inequality and knowing shape of the reaction norms around 25 °C under constant regime allows to predict smaller phenotypic trait values under fluctuating than under constant thermal regime. Among the life-history traits investigated in our study, only the reaction norm for adult longevity does not show a pronounced concave shape; it tends to increase linearly with developmental temperature for both reproductive modes. Reaction norm of a survival-related trait (e.g. longevity) is expected to be broader, due to its multiplicative effect on fitness, compared with traits having an additive effect on fitness (e.g. fecundity) (Lynch & Gabriel 1987; Gilchrist 1995). So, we cannot rule out that the thermal range in our study is not wide enough to observe the expected decrease in longevity for extreme temperatures. Moreover, the observed reaction norm of longevity is in line with the Metabolic Cold Adaptation concept (Clarke 1993; Addo-Bediako, Chown & Gaston 2002) that predicts a high metabolic rate and so a low longevity of individuals from cold environments at warmer temperatures. All the other traits in *V. canescens* show concave reaction norms and, as predicted, thermal fluctuations around 25 °C result in a decrease in phenotypic trait values compared with the situation under constant environment. Furthermore, the observed phenotypic values under fluctuating conditions match quantitatively with the predicted values from the Jensen's inequality (Fig. 4). For a specialist-like reaction norm, negative effects of thermal fluctuations are, as expected (Fig. 1), harsher than for a generalist-like reaction norm. This is all the more true for maximal egg load in thelytokous wasps under fluctuating conditions. Under

Table 3. Summary of results for generalized linear models for protein, free sugar, glycogen and lipid contents. For details, see legend Table 1

| Effect	Protein content			Free sugar content			Glycogen content			Lipid content		
	d.f.	Dev.	$P > |\chi^2|$	d.f.	Dev.	$P > |\chi^2|$	d.f.	Dev.	$P > |\chi^2|$	d.f.	Dev.	$P > |\chi^2|$
Size	1	2·48	<0·0001	1	9·50	<0·0001	1	7·30	<0·0001	1	3·73	<0·0001
RM	1	1·64	<0·0001	1	0·29	0·05	1	1·21	0·02	1	1·20	<0·0001
T	1	0·14	0·038	1	0·02	0·65	1	0·55	0·11	1	1·49	<0·0001
T²	1	0·37	0·0005	1	0·16	0·15	1	1·57	0·006	1	0·05	0·32
R	1	0·0003	0·92	1	0·006	0·78	1	0·06	0·60	1	0·05	0·29
RM × T	1	0·02	0·40	1	0·14	0·18	1	0·05	0·62	1	0·0004	0·93
RM × T²	1	0·23	0·008	1	0·18	0·13	1	7·52	<0·0001	1	0·38	0·005
RM × R	1	0·02	0·41	1	0·71	0·003	1	0·03	0·70	1	0·05	0·29
T × R	1	0·004	0·71	1	0·002	0·86	1	0·0001	0·98	1	0·08	0·19
T² × R	1	0·004	0·72	1	0·07	0·34	1	0·06	0·58	1	0·05	0·32
RM × T × R	1	0·009	0·60	1	0·04	0·50	1	0·35	0·20	1	0·09	0·17
RM × T² × R	1	0·00	0·999	1	0·04	0·50	1	0·08	0·52	1	0·01	0·62
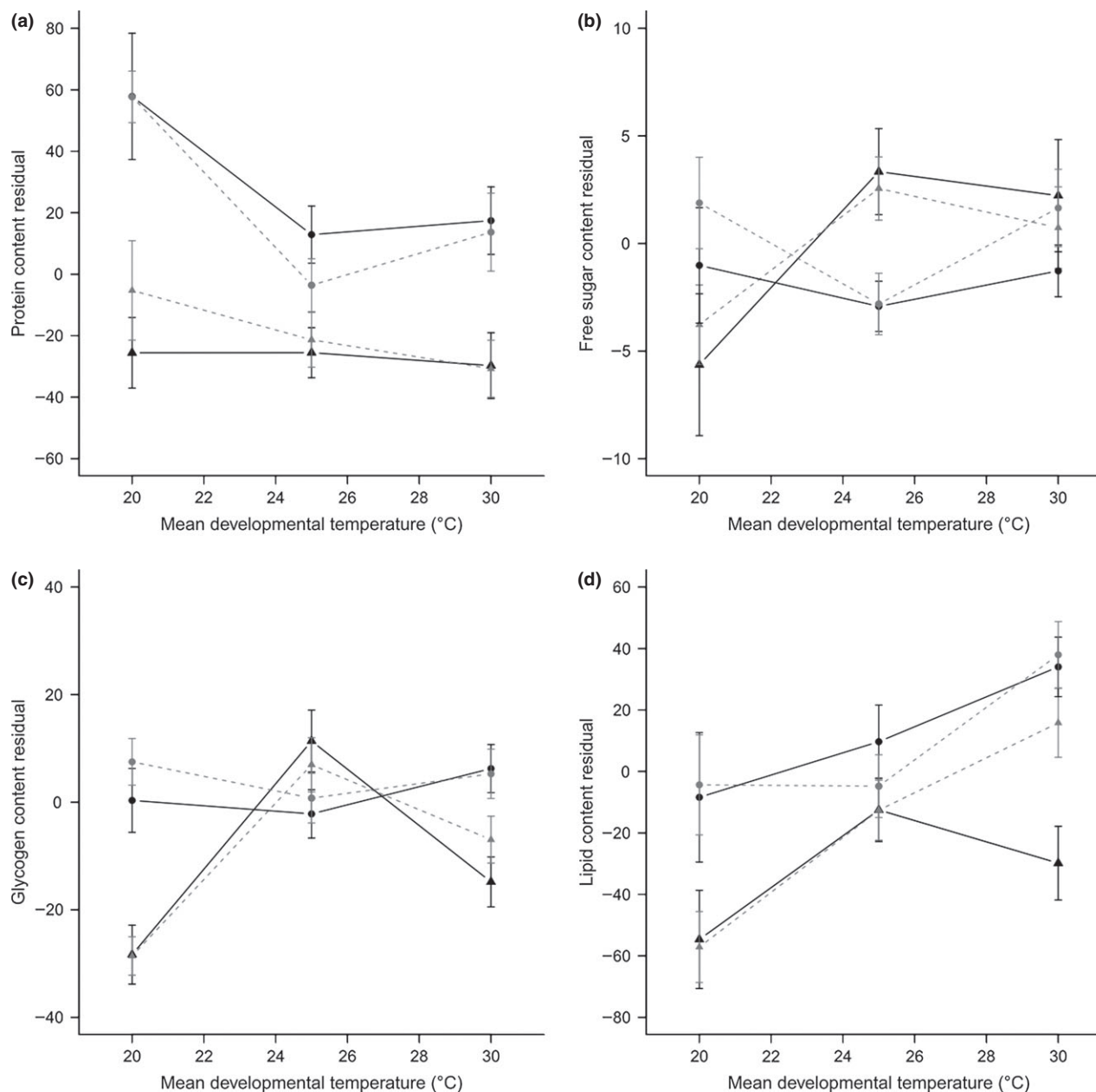
Fig. 5. Mean (±SE) protein (a), free sugar (b), glycogen (c) and lipid (d) contents of thelytokous (triangle) and arrhenotokous (circle) *Venturia canescens* under constant (black solid line) and fluctuating (grey dashed line) thermal regimes and at three mean developmental temperatures. Residuals are always against body size.

these conditions, while the mean developmental temperature is at the optimum for thelytokous wasps (25 °C), there is no more difference for this trait with arrhenotokous females. The selective advantage of thelytokous wasps seems to be restricted to a small range of thermal conditions, out of which arrhenotokous wasps perform better. However, given the fixed relative humidity in our experimental design, the temperature and the saturation deficit (i.e. the difference between saturation water vapour pressure and the water vapour pressure of ambient air) are confounded. We cannot be certain that the observed effects rely only on temperature and future experiments

should test the impact of temperature and humidity, respectively.

Results from the experiment under natural conditions reinforce the conclusions drawn from laboratory results. Reaction norms of egg load at emergence show as predicted a greater decrease in average phenotypic values in thelytokous than in arrhenotokous wasps, even if the decrease should also result from the lower mean temperature in the field (22 °C vs. 25 °C in the laboratory). Under natural conditions, egg loads of arrhenotokous and thelytokous females at emergence are similar. In our first experiment, we showed that arrhenotokous wasps tend to
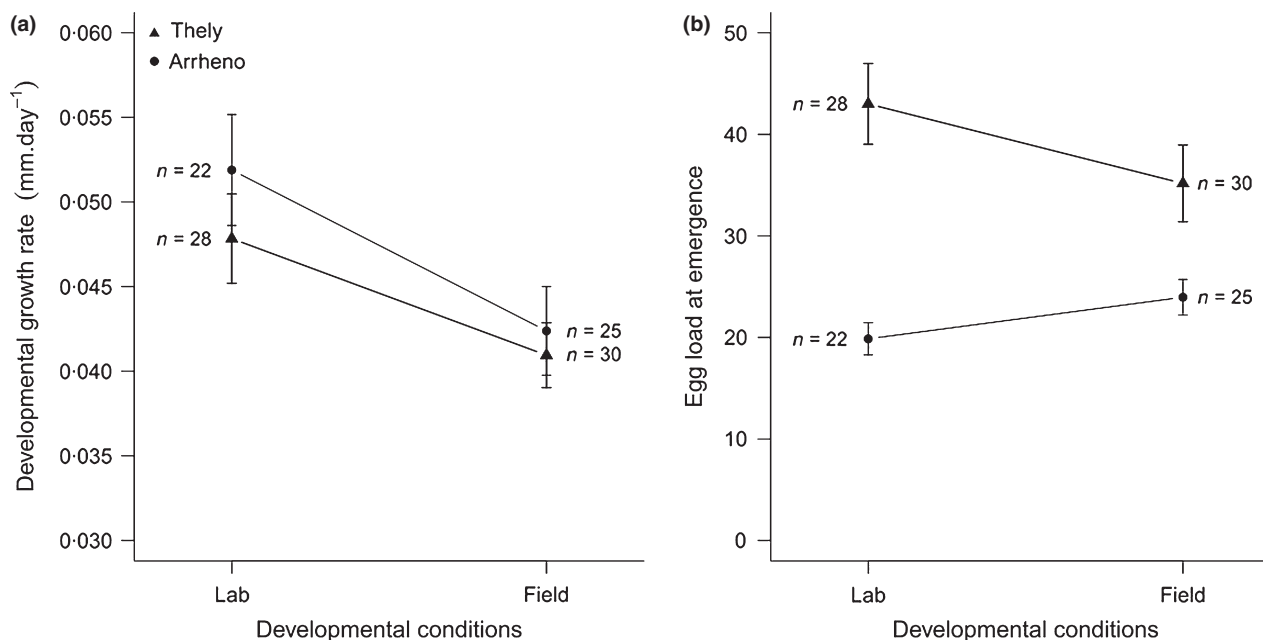
Fig. 6. Mean (±SE) developmental growth rate (a) and egg load at the emergence (b) of thelytokous (triangle) and arrhenotokous (circle) *Venturia canescens* under laboratory and field developmental conditions.

mature more eggs than thelytokous wasps during adult life, suggesting that lifetime fecundity of arrhenotokous wasps would be higher than for thelytokous wasps in the field. These results corroborate previous studies highlighting local adaptation of the two reproductive modes to different local environmental conditions (Schneider 2003; Amat *et al.* 2006; Thiel, Driessen & Hoffmeister 2006; Lucchetta *et al.* 2007; Pelosse, Bernstein & Desouhant 2007; Amat, Desouhant & Bernstein 2009; Liu, Bernstein & Thiel 2009b; Liu, Thiel & Hoffmeister 2009a; Pelosse *et al.* 2011; Foray *et al.* 2013b) and suggest that arrhenotokous wasps are better adapted to natural conditions than thelytokous wasps. The developmental growth rate decreases under field conditions, probably due to the lower mean temperature, but the amplitude of the decrease was equivalent in both reproductive modes.

Jensen's inequality is a relevant mathematical tool to predict the effect of thermal fluctuations on phenotype. The mathematical formula of the Jensen's inequality, based on an arithmetic mean, has two implications. First, the phenotypic expression does not depend on a single temperature but on the set of temperatures belonging to the thermal regime. Secondly, the chronology of temperatures undergone by the animal is not implied in the phenotypic expression. As a consequence, the Jensen's inequality should be efficient to predict effects of rapid and moderate fluctuations around the thermal optimum, but not relevant for studying impact of extreme temperatures inducing harsh effects. Finally, future studies should test the sensibility of the predictions by the Jensen's inequality to the number of mean temperature tested as the predictions are based on the estimated shape of the reaction norms.

PHENOTYPIC EXPRESSION AT THE BOUNDARIES OF THE THERMAL RANGE

Thermal fluctuations at 20 and 30 °C imply thermal ranges across which we do not know the shape of the reaction norms. Therefore, it would prevent applying Jensen's inequality at the boundaries of the thermal range. However, we can discuss our results on the basis of the assumed stereotypic shape of thermal reaction norms and of previous studies (e.g. Foray, Gibert & Desouhant 2011).

At low temperatures, fluctuations are known to have a positive effect on insect fitness, either at very cold temperatures (Colinet *et al.* 2007; Koštál *et al.* 2007; but see Marshall & Sinclair 2010) or at milder temperatures (Brakefield & Kesbeke 1997; Ragland & Kingsolver 2008; Kingsolver, Ragland & Diamond 2009). In our results, fluctuating temperatures result in a larger size, a higher growth rate, egg load and adult longevity compared with constant temperature for both thelytokous and arrhenotokous wasps, confirming this tendency. This effect is in accordance with the application of the Jensen's inequality to the classical slowly accelerating relationship between the phenotype and temperature across the range of low temperatures (left side of the curve; Fig. 1). However, the egg load at emergence exhibits the opposite pattern with higher values at constant than at fluctuating regimes for the two reproductive modes. This suggests that both thelytokous and arrhenotokous females invest less in current reproduction by producing fewer eggs at emergence when the temperature fluctuates. This is in agreement with the life-history theory (Stearns 1976, 1989) that predicts a lower investment in current than future reproduction in changing environments (Fischer, Taborsky & Dieckmann 2009;

Fischer, Dieckmann & Taborsky 2011); it has already been demonstrated for the Drosophila parasitoid *Asobara tabida* (Ellers & van Alphen 1997).

For most traits, fluctuations at high temperature result in a non-significant difference compared to the value expressed under constant thermal regime. This is not incompatible with the Jensen's inequality if reaction norms decrease rapidly after the optimal temperature, leading to a linear relationship. For the developmental growth rate, we observe a negative effect of fluctuations around 30 °C. This could result from the fact that wasps and/or their hosts meet temperatures closed to their upper developmental threshold (36 °C, Eliopoulos & Stathas 2003), leading to costly stress. For instance, in the woodlouse (*Porcellio laevis*), thermal fluctuations ranging from 16 to 32 °C up-regulated the expression of heat-shock protein HSP 70 and reduced longevity (Folguera *et al.* 2011). Interestingly, at high temperature, egg load at emergence shows an opposite pattern for the generalist (arrhenotokous) and the specialist (thelytokous) wasps: fluctuating temperatures have a negative effect on fecundity for thelytokous wasps and a positive effect for arrhenotokous females. Jensen's inequality could explain the positive effect on these latter if the decrease after the thermal optimum is slowing down, as it can be expected for reaction norms of generalist individuals.

LIFE-HISTORY TRAITS AND ENERGETIC RESERVES

Our results show that fluctuating temperatures can have a strong and different effect on fitness-related traits in generalist and specialist wasps. Surprisingly, we do not find any effect of thermal regime on the level of energetic resources. Energy acquisition during development is, however, largely affected by the average temperature during development and differs between the two modes of reproduction. The differences between thelytokous and arrhenotokous females are consistent with previous studies: arrhenotokous females emerge with larger energy reserves than thelytokous females; the difference is particularly true for lipid and glycogen (Pelosse, Bernstein & Desouhant 2007; Pelosse *et al.* 2010). This is thought to explain the greater longevity of arrhenotokous females (Pelosse, Bernstein & Desouhant 2007; Foray, Gibert & Desouhant 2011; Pelosse *et al.* 2011), although this trend does not appear in our results. The effect of the average temperature on some life-history traits (e.g. the number of mature eggs) appears to be correlated with energy reserves. Indeed, at the mean developmental temperature of 25 °C, thelytokous females maximize both their mature egg loads and fat and glycogen reserves. Similarly, in arrhenotokous females, there should be a correlation between homeostasis of both the lifetime egg load and the level of glycogen.

The lack of correlation between energy reserves and life-history traits under fluctuating thermal conditions suggests a complex allocation of energy to the different functions.

Both the amount of available energy, depending on the host, and the dynamic consumption of the reserves could be affected by the thermal regime. To test these hypotheses, future experiments should account for the host–parasitoid interaction and analyse the dynamic of energy reserves across the lifetime of the parasitoid.

Conclusion

Our study highlights several key-points. First, studying phenotypic plasticity requires taking environmental fluctuations into account and not only constant regimes, as phenotype of insects depends on the interaction between the mean temperature and the thermal regime (but see Fischer *et al.* 2011). Secondly, because all the traits will not react in the same manner to environmental variation, only an approach in which several traits are studied simultaneously will give relevant information about the adaptive value of different phenotypes. Thirdly, the use of Jensen's inequality is a promising approach to predict phenotypic responses to fluctuating environments, and the dynamic of some diseases in the context of climate changes (Rohr *et al.* 2011).

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Graphical representation of constant (solid lines) and fluctuating (dotted lines) thermal regimes set in the incubators at the three mean temperatures (20, 25 and 30 °C) for a 72-h period.

Figure S2. Distribution of temperatures in the field site during the development of wasps, that is, from June 15 to July 10, 2010. Mean temperature was 21.6 °C and is indicated by the red line.

Table S1. Sample sizes of thelytokous (T) and arrhenotokous (A) female wasps assigned randomly to two groups, three mean developmental temperatures (20, 25 and 30 °C) and the two thermal regimes (fluctuating and constant), with about twice more females assigned to the first group than to the second one.