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Evolutionary ecology of insect growth: from geographic patterns to biochemical trade-offs

UNIVERSITY OF TARTU
Press

Department of Zoology, Institute of Ecology and Earth Sciences, Faculty of Science and Technology, University of Tartu, Estonia

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Supervisor: Prof. Toomas Tammaru, University of Tartu, Estonia

Opponent: Dr. Sarah E. Diamond, Case Western Reserve University, U.S.A.

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LIST OF ORIGINAL PUBLICATIONS

This thesis consists of the following articles which are referred by their Roman numerals.

I **Meister**, **H.**, Esperk, T., Välimäki, P. and Tammaru, T. 2017. Evaluating the role and measures of juvenile growth rate: latitudinal variation in insect life histories. Oikos 126: 1726–1737.

II **Meister, H.**, Hämäläinen, R., Valdma, D., Martverk, M. and Tammaru, T. 2017. How to become larger: ontogenetic basis of among-population size differences in a moth. Entomologia Experimentalis et Applicata, in press. Doi 10.1111/eea.12634.

III Tammaru, T., **Meister, H.**, Välimäki, P. and Teder, T. Thermal reaction norms of larval growth in insects: physiological rather than ecological determinants. *Submitted manuscript*.

IV **Meister**, **H.**, Tammaru, T., Sandre, S.-L. and Freitak, D. 2017. Sources of variance in immunological traits: evidence of congruent latitudinal trends across species. Journal of Experimental Biology 220: 2606–2615.

The participation of the author in preparing the listed publications (* denotes a moderate contribution, ** a high contribution, *** the leading role):

	I	**II**	**III**	**IV**
Original idea	**	**	*	***
Experiment design	**	***	**	***
Data collection	***	**	***	***
Data analyses	***	***	*	***
Writing the paper	**	***	*	***

1. INTRODUCTION

The question about selective pressures which shape animals' body sizes and related traits has turned out not to be a simple one (Blanckenhorn 2000; Berger *et al.* 2006, 2012; Ringsby *et al.* 2015; Rollinson and Rowe 2015). In particular, while benefits of e.g. large size and fast growth are easy to see, forces which create balancing selective pressures often remain elusive. To understand natural selection, one has to associate phenotypes with environmental conditions. One may, for example, manipulate the environment of the populations, and to record corresponding genetic changes applying the tools of quantitative genetics. However, one may also compare populations inhabiting different environmental conditions (Blanckenhorn and Demont 2004; De Frenne *et al.* 2013; Horne *et al.* 2015). Latitudinal clines representing systematic changes in the environment have proved to be especially well applicable in this context (Angilletta and Dunham 2003; Blanckenhorn and Demont 2004; De Frenne *et al*. 2013). A wide geographical coverage is of special value as it minimizes the confounding effect of microclimatic variation (Shelomi 2012). Furthermore, to be able to reach generalisations, it is advisable to rely on multiple species in studies on latitudinal gradients (e.g. Nilsson-Örtman *et al*. 2012; Horne *et al.* 2015) which is, however, not frequently done in respective case studies.

Body size often varies systematically with latitude in insects. In addition to specialised studies on latitudinal gradients (Pincheira-Donoso 2010; Huston and Wolverton 2011; Shelomi 2012), respective information can often be found in taxonomic handbooks (e.g. Mikkola and Jalas 1977, 1979). Nevertheless, trends are not consistent among insect species with both positive (Bergmann's clines) and negative correlations between body size and latitude having frequently been reported (converse Bergmann's clines). Positive phenotypic clines can often be explained through plastic responses to the concomitant change in ambient temperature: ectothermic organisms grow usually larger in colder conditions (Atkinson and Sibly 1997; Chown and Gaston 1999). Negative clines are primarily believed to represent a by-product of selection towards shorter development times at higher latitudes (Blanckenhorn and Demont 2004; Chown and Gaston 2010). Indeed, it seems natural to assume that shorter summers leave the insects less time to complete their developmental cycles, as well as it is natural to expect a positive genetic correlation between development time and final size. However, also the number of generations may differ between populations. As a result, time stress may be also higher in regions where longer summers facilitate the development of a higher number generations (Roff 1980; Pöykkö 2005).

To understand the costs and benefits of (large) body size, one also needs to consider the mechanisms how size differences are attained in the course of early development. Even though there naturally are studies reporting among-population differences in various parameters of the immature stages (e.g. Tikkanen *et al*. 2000; Armbruster and Conn 2006; Nilsson-Örtman *et al*. 2015),

there is shortage of works systematically scrutinizing the ontogenetic patterns behind among-population size differences in a detailed manner. There are just three basic ways how size differences can be attained: the ultimately larger individuals can grow faster (Blanckenhorn *et al*. 2007), they can be larger from the beginning (eggs, newly hatched larva) or develop for a longer time (Blanckenhorn *et al*. 2007; Tammaru *et al*. 2010). Similar questions have most frequently been asked in the context of the ontogenetic basis of sexual size dimorphism (SSD). Females, usually the larger sex in insects (Fairbairn 1997; Teder and Tammaru 2005; Stillwell *et al*. 2010), have mostly found to have a longer development times (Fischer and Fiedler 2001; Teder 2014), sometimes accompanied by an increase in the number of larval instars (Gomi 2005; Etilé and Despland 2008; Barraclough *et al*. 2014; Montezano *et al*. 2014; see Esperk *et al*. 2007 for a review). 'Being larger from the beginning' hypothesis appears to have received little attention: the question whether sex-related differences are present already in the size of eggs or newly hatched larvae is not frequently asked. There are some studies on insects with a haplodiploid sex determination system (Macke *et al*. 2011; Budrienė *et al*. 2013; Walzer and Schausberger 2015) which report sex-specific egg sizes, whereas this possibility appears to be unattested for other insects (Kim 1999; Yasuda and Dixon 2002; Schenk and Söndegerath 2005).

In recent decades, juvenile growth rate has increasingly been considered as a life history trait on its own right (Arendt 1997; Gotthard 2008; Dmitriew 2011). Growth rates have been suggested (Abrams *et al.* 1996) and reported (Gotthard *et al.* 1994; Bronikowski 2000; Gotthard 2004; Berger *et al.* 2011; Nilsson-Örtman *et al*. 2015) to be subjected to adaptive evolution. This implies that growth rates are not always maximised within physiological limits but are rather determined by a balance between costs and benefits of fast/slow growth (Abrams and Rowe 1996; Tammaru *et al.* 2004). The obvious benefits of fast growth are in allowing the juveniles to pass the vulnerable (Cornell and Hawkins 1996; Remmel *et al*. 2011) larval stage quickly, and in ensuring that a certain developmental stage will be reached within a restricted time (Tauber *et al*. 1986). The costs of high growth rate are more difficult to understand. One option is that faster growth is associated with higher extrinsic juvenile mortality (Munch and Conover 2003; Stoks *et al.* 2005; Careau *et al.* 2013), because actively feeding insects are more vulnerable to predators due to increased exposure time (Gotthard 2000; Stoks *et al.* 2005; Laurila *et al*. 2008). Alternatively, the costs may be related to various physiological effects (Mangel and Munch 2005; Campero *et al.* 2008; Scharf *et al*. 2009; Stoks and De Block 2011; Lee *et al.* 2013). For example, due to increased consumption of oxygen (Mangel and Munch 2005) and increased metabolic rate (Finkel and Holbrook 2000), the fast growing larvae may be exposed to oxidative stress (De Block and Stoks 2008; Harrison 2013; Smith *et al.* 2016). Damage to DNA and the increase in the rate of transcription errors (Mangel and Munch 2005; De Block and Stoks 2008) have also been reported, in addition to decreased starvation resistance (Stoks *et al.* 2006; Scharf *et al.* 2009).

Quite obviously, geographical gradients can also be used to study the evolutionary ecology of growth rate. Latitudinal trends in growth rate have been primarily addressed in amphibians and fish while insects have received some attention as well (Blanckenhorn and Demont 2004). Overall, the findings appear inconsistent: growth rates have reported to either increase (Pöykkö and Tammaru 2010; Kivelä *et al*. 2011; Välimäki *et al*. 2013) or decrease (Nygren *et al*. 2008; Stoks *et al*. 2012) with increasing latitude in the Northern Hemisphere. Curiously, invariability of growth rates across latitudinal gradients seems not to have been reported before.

Studies on evolutionary ecology of growth rate have, however, suffered from conceptual and methodological problems related to defining and measuring growth rate, particularly in organisms with discontinuous growth. Previous research on insect growth rates has mostly operated with **integral measures** of the variable (IGR, e.g. Gotthard *et al*. 1994; Karl *et al.* 2008; Välimäki *et al*. 2013). This has implied dividing the final size (usually pupal weight) by full development time from hatching to pupation, without considering the complexity of insect growth curve. The complexity is primarily due to the growing period being divided to discrete larval instars (Nijhout 1981; Sehnal 1985) which constrains the realizable shapes of larval growth curve (Sehnal 1985; Ayres and MacLean 1987; Higgins and Rankin 1996; Esperk and Tammaru 2004; Maino and Kearney 2015; Tammaru *et al*. 2015). As a result, growth rate (mass increment per unit time) differs considerably between different stages of larval development, it drops to negative values prior to moult and pupation when the larvae do not actively feed (Esperk and Tammaru 2004). Moreover, growth rates may show variation for other reasons like filling the gut after moulting, during food depletion and searching for food. Therefore, any integral measure of growth rate necessarily includes periods when the larvae do not actually grow. Using integral growth rates may be problematic also for 'simpler reasons'. For example, if two groups have identical but non-linear growth functions but one stops growing earlier at a smaller size, an integral measure may erroneously result in dimorphic estimates of growth rate (Figure 1; Tammaru *et al*. 2010).

Using approximations of **differential** (instantaneous) growth rate (DGR) offers an alternative (Esperk and Tammaru 2004; Tammaru and Esperk 2007; Tammaru *et al*. 2010; Figure 2). Short-term measurements allow one to measure growth at specific points of the growth curve, making it possible to select periods when the growth is continuous. Using differential growth rate has several benefits compared to integral measures. The use of DGR will allow one to circumvent problems like those described in the previous paragraph. Furthermore, importantly, the costs of fast growth (see above) are primarily manifested as costs of DGR (rather than IGR), and focussing on DGR may be necessary in order to understand respective trade-offs. Specific attention to DGR is therefore advisable in life-history studies, including those focussing on latitudinal gradients. This appears not to have been done so far.

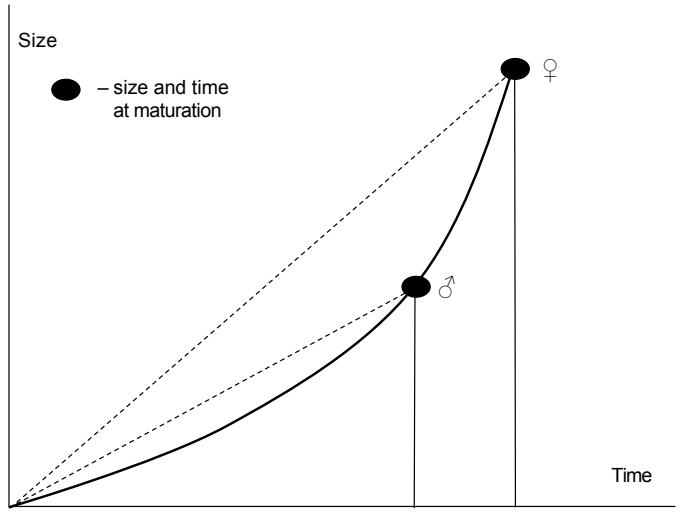


Figure 1. The figure presents the case in which males and females have identical non-linear growth functions but males stop earlier to mature at a smaller size. In this case, calculating growth rate as the ratio of final weight over total development time (integral growth rate) results in sexually dimorphic estimates of growth rate (slopes of the dashed lines) also in the absence of a sex-related difference in differential growth rate (derivative of size with respect to time). Text and graph Tammaru *et al*. (2010).

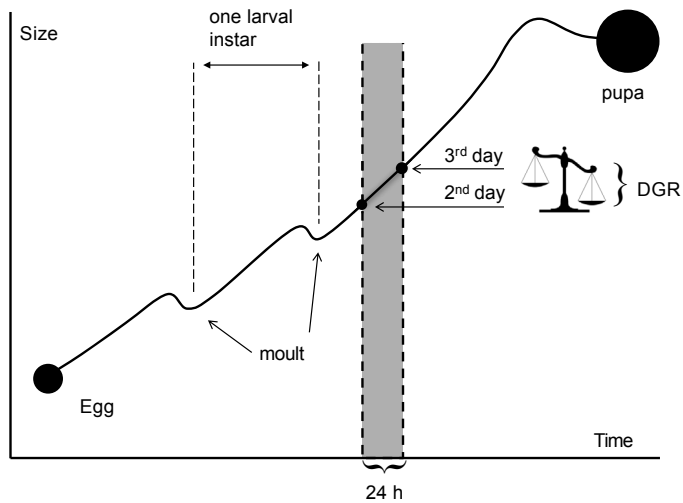


Figure 2. The differential (instantaneous) growth rate (DGR), as used in this study, is based on 24-hour mass increment measured on the 2nd 24-hour period within the final larval instar (Esperk and Tammaru 2004; Tammaru and Esperk 2007; Tammaru *et al*. 2010), a point in the development which is not affected by neither the preceding larval moult nor preparations for pupation (Esperk and Tammaru 2004). The present schematic presentation shows larval development consisting of three instars while the typical number of larval instars in Lepidoptera is five. Text and graph from article **I**.

Latitudinal gradients allow us to find and, hopefully, explain differences in life-history traits, but they also show how phenotypic plasticity varies at the geographic scale. For an ectotherm, temperature is perhaps the most important parameter of its environment, and studying plasticity in relation to temperature cannot therefore be less important (Angilletta 2009; Dell *et al.* 2013; Schulte 2015). Adaptionistic interpretation of thermal reaction norms is, however, complicated because non-adaptive (constraint-based) explanations should always be considered in such cases (Angilletta *et al*. 2003; Arendt 2011). This is just because ambient temperature has, for obvious reasons, clear physical and physiological effects on ectothermic organisms (Cossins and Bowler 1987; Clarke 2006; Dell *et al*. 2013). It is, however, an open question to which extent can ecologically-based selective pressures interfere with the universal positive relationship between the rate of (bio)chemical reactions and temperature (Irlich *et al*. 2009; Kutcherov 2016).

An estimate of relative importance of physiological vs. ecological factors as ultimate determinants of thermal reaction norms may be obtained examining the patterns of among- and within-species variability in respective responses. An eventual high within species genetic variance (among- and within-populations) in thermal reaction norms would emphasize the dominance of ecologically based determinants, simultaneously indicating the potential of a population to adapt to changes in its thermal environment (Hoffmann and Sgro 2011; Diamond 2017). Limited variability in reaction norms should be more consistent with physiological explanations. Indeed, it is hard to see why should the physiological determinants differ among species within a particular insect order, considering the invariability of basic anatomy and physiology at the taxonomic level (Chapman *et al*. 2013).

With respect to the question about costs of body size and related traits, the role of potential trade-offs between immune function and life-history traits is still insufficiently known. Immune parameters of insects vary: populations have evolved genetic differences in immune responses but also plastic responses are common (e.g. temperature, and host plant effects) (Schmid-Hempel 2005, 2011). The effect of temperature on immune traits appear to be inconsistent, however. For example, temperature has been found to inconsistently affect resistance to viral infections (Samuel *et al*. 2016): both no effect (Gherlenda *et al*. 2016) and decrease with temperature (Salehipour-shirazi *et al*. 2017) have been reported. In addition to temperature, host plant is another environmental factor involved in respective trade-offs: certain host plants can make the larvae better defended (Bukovinzky *et al*. 2009; Sandre *et al*. 2011; Lampert 2012) and can be used as 'medication' (Chapuisat *et al*. 2007; Bos *et al*. 2015).

Immunity bears costs (Luong and Polak 2007; Iserbyt *et al*. 2012; Prokkola *et al*. 2013) and, therefore, trade-offs are expected between immunological and life-history traits. It is unclear, however, how general such trade-offs are across various species, environmental conditions, and immunological indices (for case studies, see Diamond and Kingsolver 2011; Vogelweith *et al.* 2013b). Once again, among-population studies can shed light on selective forces determining

the levels of immunological traits, and those shaping plastic responses in such variables to environmental parameters. Geographical trends in immunity have been studied mostly in birds, crustaceans and fish (Conover *et al*. 2009). Insects have been considered to some but to a lesser extent, there are studies primarily on Lepidoptera, Odonata and Diptera. The results appear to be inconsistent, however, as both the increase and decrease of immune response have been shown with increasing latitude in the Northern Hemisphere.

The aim of this thesis is primarily explorative: to detect any consistent latitudinal trends across species. Life-history traits and immune indices are investigated comparatively in a number of insect species. First, growth parameters (body size, development time, growth rate) are analysed using six moth species reared under *common garden* design. Latitudinal differences in differential (instantaneous) growth rate of the larvae are studied applying an original approach (**I**). Thereafter, ontogenetic determinants of among-population differences in body size are investigated in one of the species in more detail, monitoring the immature development from the egg to the adult stage. The species chosen for that study was the one with the highest among-population difference in body size (**II**). Next, among-population differences in thermal plasticity of growth-related traits are considered (**III**). Possible trade-offs between life-history traits and immune indices are subsequently studied to determine if immune capacity is involved in latitudinal variation in life-history traits and respective reaction norms (**IV**). Three species out of six (large enough for haemolymph sampling) are used in this study. The empirical results obtained are thereafter discussed in the framework of general questions of evolutionary ecology, such as the usefulness of the concept of growth rate, and the opposition of adaptive and constraint-based explanations. Moreover, various methodological suggestions applicable to analogous studies with insects are made.

2. MATERIAL AND METHODS

2.1. Study insects and regions

To study latitudinal gradients in insect life-history traits, we established laboratory populations of species whose geographic ranges span from northern to southern Europe. The list of countries included was formed on the basis of collaboration possibilities and field trips organized to respective locations (see also Figure 3 and Table 1). The list includes northern and southern Finland, southern U.K., Estonia, eastern Germany, southern Czech Republic, and Georgia. The studied populations thus form a gradient from 41°N to 65°N, with the extreme points being separated by about 2700 km.

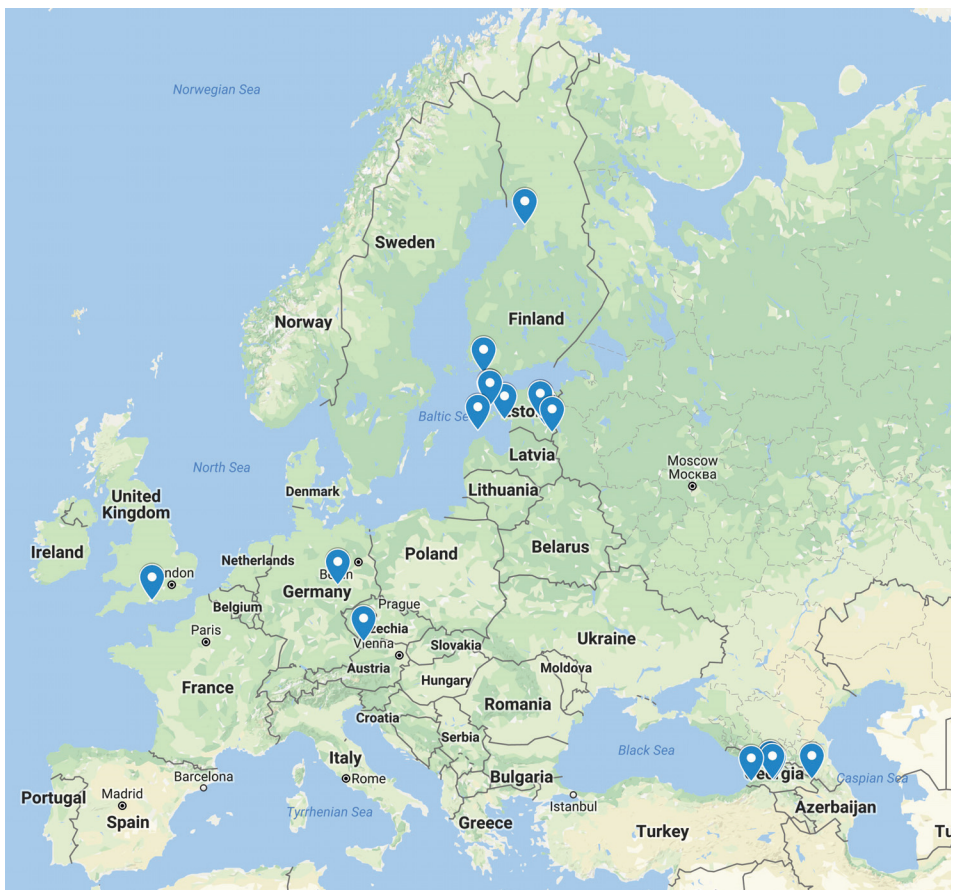
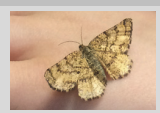
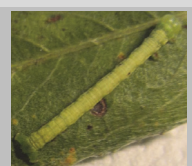
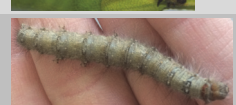
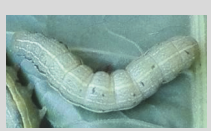


Figure 3. The origin of the moths used in the experiments (blue stickers; Google Maps goo.gl/e54HK6).

The six species studied represented three distantly related (Mutanen *et al*. 2010) macrolepidopteran families: Lasiocampidae, Noctuidae and Geometridae (Table 1).

Table 1. Species used in the studies.

Species, family	Generations per year	Average pupal weight, mg	Host plants used	Population	Study	Larvae/ adult photos
Acronicta rumicis (Noctuidae)	2–3	219	*Cirsium arvense* *Melilotus albus*	Estonia (EST) Georgia (GEO)	**I**, **III**, **IV**	
Ematurga atomaria (Geometridae)	1–2	74	*Salix alba* *Trifolium repens* *Vaccinium myrtillus*	Estonia (EST) Georgia (GEO)	**I**, **II**, **III**	 
Cabera pusaria (Geometridae)	1–2	50	*Alnus glutinosa*	Finland (FIN) Estonia (EST) Germany (DEU) Georgia (GEO)	**I**, **III**	
Hypomecis punctinalis (Lasiocampidae)	1–2	155	*Betula pendula* *Tilia cordata*	Estonia (EST) Georgia (GEO)	**I**, **III**, **IV**	
Poecilocampa populi (Lasiocampidae)	1	368	*Alnus incana* *Betula pendula* *Populus tremula*	Finland (FIN) Estonia (EST) Czech Republic (CZE)	**I**, **III**, **IV**	
Orthosia gothica (Noctuidae)	1	160	*Betula pendula*	Finland (FIN) Estonia (EST) United Kingdom (U.K.)	**I**, **III**	

The primary criteria of selecting the species were practical ones: the species had to be abundant enough throughout the range to allow us to obtain the founder females with reasonable effort, as well as we preferred species which are reasonably easy to rear in the laboratory (species overwintering in the larval stage were avoided). All six species were used in studies in which growth-related life-history traits (body size, development time, growth rate) (**I**) and thermal plasticity in those (**III**) were analysed. Latitudinal differences in immune response (**IV**) were restricted to three species out of six: only the species which were large enough for haemolymph extraction could be included in that substudy.

Detailed monitoring of ontogenetic determination of among-population size differences was performed on a species with the highest among-population difference in body size, the geometrid *Ematurga atomaria* (**II**). Hybrids of the moths from the latitudinally-extreme (Estonian vs. Georgian) populations were also included. Additionally, both lowland (400 m asl) and alpine populations (1500 m asl) from Georgia were incorporated.

Progeny of wild-caught females formed the laboratory stock (in study **I** and **III** $\geq$ 10 founding females; **II** $\geq$ 4; **IV** $\geq$ 11). First generation offspring were used or, in some cases, laboratory stocks had been maintained at the University of Tartu for 1–4 generations before the experiments. The moths were mated within populations so that inbreeding was avoided. Each larva was housed individually in a plastic jar (50 or 100 ml dependent on larval body size, see Table 1), kept at 20 °C, with fresh leaves of a host plant provided every second day. As some of the used species (*A. rumicis*, *C. pusaria* and *E. atomaria*) are multivoltine (= having several generations per year), during both stock maintenance and experiments, L10:D14 h photoperiodic regime was used to induce diapause development and to make sure that all the individuals in the laboratory rearings had the same number of generations per year.

2.2. Methodology

To study latitudinal genetic differences in life-history traits (differential growth rate, instar-specific mass increment, development time, pupal weight and survival) and immune indices (phenoloxidase and total lytic activity), a *common garden* design was applied. For this purpose, final instar larvae representing different populations and broods (= offspring of one individual female) were divided equally between three rearing temperatures (16 °C, 20 °C and 24 °C) and multiple host plants (if applicable, see Table 1 for details) within species (**I**, **II**, **III**, **IV**). Rearing the larvae over a range of environmental conditions allowed us to record among-population differences in parameters as such (consistent differences across all experimental environments), and to untangle them from population-specific adaptations to different temperatures or host plants (environment-specific among-population differences). For example, had the southern moths been larger under higher temperatures only, this could have

indicated an adaptation of these populations to warmer conditions, and not that the southern moths are larger in general compared to their northern conspecifics.

Synchronization of larvae for the *common garden* experiments was achieved by adjusting temperatures individually during the younger instars. Exact synchronisation was considered necessary to standardize environmental conditions across populations being compared, facilitating isolation of genetic (as opposed to plastic, i.e. environmental) differences. The larvae which were ahead of the others in development, were kept at +4 °C for one or two days before they moulted into the final instar (Tammaru and Esperk 2007; Tammaru *et al.* 2010). Also those larvae which did not need any treatments to be synchronised were kept at +4 °C for at least 12 h before the experiment, to eliminate differences related to physiological effects of low-temperature treatments. The larvae were simultaneously brought to room temperature and were let to moult into final instar without access to food, moulting happened within an about 12 h period. Subsequently, every individual was provided with host plant at exactly the same time. Positions of the vials in rearing trays were randomised with respect to population and brood. For the purpose of calculating instantaneous growth rate (see below), the larvae were weighed on the second and third day of their development in the final instar. All larvae were weighed individually in about 24 h after they resumed feeding in the beginning of the last instar, and for the second time, exactly 24 h after the first measurement. This was achieved by weighing the larvae in the same order on the two subsequent days. First day of the final instar was not used, because immediately after moulting, the increase in larval weights reflects filling guts rather than actual somatic growth. We did not take further measurements on the last days of the final instar because our intention was to focus on the 'free growth' period, i.e. the period not affected by preparations to pupation. Indeed, from our experience, we know that, slowing down the growth may already begin from 4th day onwards (Esperk and Tammaru 2004). Moreover, considering the work load based trade-off between the number of measurements and sample size, we decided to prioritise the number of species, and individuals studied.

Subsequently, in an auxiliary experiment (**I**), the larvae of five species were reared from hatching to pupation at 20 °C on their optimal host plants. This allowed us to obtain population-specific estimates of total developmental time and pupal weights, unconfounded by procedures specific to the experiments described above.

In an additional paper (**II**), we expanded the time span of the study of ontogenetic determinants of among-population size differences. In particular, we monitored the development throughout the entire larval period, from eggs to pupae (the Monitoring experiment). We reared the larvae representing different populations of the species with the highest differences in body size (*E. atomaria*) on its optimal host plant (*Vaccinium myrtillus*) at 20 °C. The larvae were inspected daily and the weight at each developmental stage was determined: in addition to eggs and pupae, the larvae were weighed when newly hatched, and at each moult.

Do determine fitness benefits of large body size, adult longevity and fecundity were measured for the representatives of lowland Georgian (large) and Estonian (small) populations of *E. atomaria* (**II**). The moths were mated in 100 ml plastic jars (see Javoiš *et al*. 2011 for details of the method). Longevities of both the male and female partner were determined by daily inspection. The eggs which had been laid were counted, as well as the dead females were dissected and the number of eggs remaining in their abdomens was determined.

For the purposes of the immunological study (**III**), extraction of haemolymph took place following growth rate measurements. On the 3rd day of the final instar, a haemolymph sample was extracted from *A. rumicis*, *H. punctinalis* and *P. populi* (**IV**). Thereafter, the larvae were reared until pupation in their native jars. Haemolymph was extracted from the larvae in randomized order with respect to rearing chamber, population and brood in order to exclude confounding effects of absolute time and ontogenetic stage of the larvae. This was considered important because immunological indices are known to be highly condition dependent, being affected by, for example, developmental phase, day length and various other environmental parameters (e.g. Ueda *et al*. 2002; Lazzaro *et al*. 2008; Stoepler *et al*. 2013). Samples were taken by puncturing larvae with an insulin syringe to antepenultimate dorsal segment above the stigma. Samples of 3 µl for *H. punctinalis*, 10 µl for *A. rumicis* and 20 µl for *P. populi* were extracted, diluted in potassium phosphate buffer (PBS) solution and stored at –80 °C.

Do determine among-population differences in immune responses, the levels of phenoloxidase (PO) and lytic activity were measured. PO activity is induced to fight with bacteria, fungi and viruses (Cerenius *et al*. 2008; González-Santoyo and Córdoba-Aguilar 2012), it is effective also towards multicellular parasite eggs and nematodes (Nappi and Ottaviani 2000; Marmaras and Lampropoulou 2009). Total lytic activity on the other hand indicates the ability to cope with microorganisms (Bulet *et al*. 2004), especially bacteria (McNamara *et al*. 2013a; Graham *et al*. 2015). PO assay was performed based on the methodology of Laughton and Siva-Jothy (2011) with some modifications described below. To obtain supernatant, fixed samples were thawed and centrifuged (9000 g) at 4 °C for 10 min. Supernatant levels of 3 µl for *H. punctinalis*, 10 µl for *A. rumicis*, 15 µl for *P. populi* was added to respective amounts of PBS. Thereafter, reaction was initiated by pipetting 200 µl of 3 mmol l^{-1} L-Dopa (L-3,4-dihydroxyphenylalanine; 333786, Sigma, St Louis, MO, U.S.A.) to each well and measuring the absorbance curve with a spectrophotometer (Enspire, Perkin-Elmer, Waltham, MA, U.S.A.). The slope of time vs. absorbance from 10 to 60 min in *H. punctinalis* and *P. populi* and 10 to 60 min in *H. punctinalis* and *P. populi* and 10 to 40 min in *A. rumicis* was used to estimate PO activity.

Another parameter reflecting immune response was total lytic activity of the haemolymph (the ability to degrade bacterial cell wells of Gram-positive bacteria). 9 cm in diameter Petri dishes were filled with 10 ml of sterilised 1X PBS buffer containing 1000 µg ml^{-1} *Micrococcus luteus* freeze-dried and

lyophilised cells (Sigma). In the agar, 2 mm diameter wells were created by puncturing it with a plastic pipette and removing the pieces of agar. For each species, haemolymph samples of 4 µl (one per individual moth) were pipetted directly into the wells on the plates and were incubated for 38 h at 30 °C. Standard curve was based on dilution series of chicken egg white lysozyme located on two separate plates (Sigma: 2000, 1000, 750, 500, 250, 125, 620 and 310 µg ml^{-1}). To control between-plate variation, lysozyme controls of 63 and 250 µg ml^{-1} were added to each plate. Ideally, we would have placed all the control series on one Petri dish next to the haemolymph samples, but the space for samples would then have been limited on one Petri dish, resulting us increasing the number of Petri dishes used drastically. Lytic activity was determined as the radius of the clear zone around the sample. This indicates the equivalent lytic enzymes concentration (µg ml^{-1}), consisting of cocktail of small lytic enzymes, lysozyme being only one of them. When no bacteria free zone was formed, respective wells were scored as 'no lytic activity present'.

2.3. Recorded variables

Differential growth rate was expressed as $\mathrm{DGR} = \frac{\text{final mass}^{\frac{1}{3}} - \text{initial mass}^{\frac{1}{3}}}{\text{time (days)}}$. DGR (**I**) was based on mass increment of the second day in the beginning of the final (5^{th}, or 6^{th} for *O. gothica* and *A. rumicis*) instar. To test for robustness of the results, DGR was alternatively expressed in a more traditional way as $\frac{\log\frac{\text{final mass}}{\text{initial mass}}}{\text{time (days)}}$ (**I**) (Scriber and Slansky 1981). To compare conclusions made for DGR with those based on integral growth rates (IGR), growth rate was also expressed as $\mathrm{IGR} = \frac{\log(\text{pupal mass})}{\text{larval period (days)}}$ (**I**). Instar-specific mass increment was calculated by dividing final (pupal) mass and initial mass of the instar (**II**, **III**). Larval development time was recorded in three different ways: either development time in each instar (**II**), development time in the final instar (**I**, **II**, **III**, **IV**), or total development time from hatching to formation of pre-pupae (**I**, **II**). Exact date of pupation could not be determined as, in most of the species, the larvae burrow into the soil (sphagnum moss was provided) for pupation. The pupae were weighed no earlier than 5 days from estimated pupation, when also sex was determined based on genital scars on the pupal cuticle. Survival was measured in two ways: by determining survival to pupation (**I**, **II**, **IV**) and overwintering survival to eclosion (**IV**). Immune response was measured as enzyme activity of phenoloxidase (PO) and total lytic activity (lysozyme concentration) (**IV**). PO (enzyme activity determined under spectrophotometer) and lytic activity (concentration of lytic enzymes determined by bacteria free zone) were the two primary parameters used for characterising constitutive immune response.

2.4. Data analysis

To explore the among-population differences in growth parameters (pupal mass, development time in the final instar, DGR) and immunological traits (PO, lytic activity) general linear mixed models (GLMMs, SAS procedure MIXED; Littell *et al*. 1996) were constructed with population, temperature treatment, host plant and sex as categorical fixed factors, whereas brood (= offspring of a particular female) was added as a random factor (**I**, **II**, **III**, **IV**). We first fitted models including all the independent variables (population, initial weight, sex, temperature, host plant and brood as a random factor), as well as population × temperature, population × host plant interactions (**I**, **II**, **IV**) and sometimes also population × sex (**II**) interactions. Initial mass of the final instar was included as a covariate to eliminate the purely mechanistic effect of body size on growth rate (relative instantaneous growth rate is a decreasing function of body size; see also Tammaru and Esperk 2007) (**I**), as well as to correct for size-dependence of immunological indices (Vogelweith *et al*. 2013a) (**IV**). The effect of initial mass on differential growth rates was, however, weak to non-existent, and exclusion or inclusion of this variable to statistical models had no influence on qualitative results. This should indicate that the cube-root transformation of mass was efficient in linearizing growth trajectories (Tammaru and Esperk 2007). For this reason, we also decided not to correct growth rates for body size in the detailed analysis of *E. atomaria* growth patterns (**II**).

Initially "full" models were fitted meaning that all measured and possibly meaningful main effects and interactions were included. Thereafter backward eliminations were carried out to improve the interpretation of the models by eliminating non-significant parameters (α= 0.05) (**I**, **II**, **IV**). Generalized linear mixed models (PROC GLIMMIX; Littell *et al.* 1996) with logit link function, but otherwise analogous to those for the continuous traits were fitted (**I**, **II**, **IV**) for binary response traits. Furthermore, Spearman correlations or Chi-Square tests were applied to determine relationships between life-history traits and immune parameters (**IV**).

In addition to the analyses performed separately by individual species, in some cases, alternative analyses were made with the data for all species combined (**I**, **III**, **IV**). This allowed us to increase power of the analyses, as well as facilitated a quantitative comparison across species. First, to test for an overall latitudinal trend in growth rates, we performed such an analysis of the combined data set of DGR (**I**). For DGR as a response variable, we fitted a GLMM in which the independent variables were species, sex, host and temperature treatment (the latter three nested within species) as categorical factors, and latitude of origin, as a continuous variable. Additionally, brood (nested within species) was included as a random factor. Second, incidence of lytic activity as a binary trait (presence= '1' or absence= '0'), was also analysed (PROC GLIMMIX) with brood nested within species as a random variable (**IV**). Third, to determine cross-species similarity in thermal reaction norms for

mass increment within final larval instar (pupal weight/final instar body size), and for development time in the final instar (**III**), the effects of population, sex, host and brood were nested within the effects of species. In addition to allowing us to test for similarity in the responses among species (species × temperature interactions), such models produced an estimate the components of variance attributable to different effects.

In study **II (the Monitoring experiment)**, focal traits (egg size, larval body mass in the beginning of each instar, instar-specific mass increment, development time by instar, total development time, pupal mass) were analysed as dependent on two fixed factors (population and sex) using SAS PROC MIXED. Brood was included as a random factor.

When assessing fitness consequences of body size (**II**), we ran a two-way ANOVA for adult longevity population and sex as independent variables. Egg production was thereafter analysed first by comparing the populations with a one-way ANOVA. Further, pupal weight was added to the resulting ACOVA model with the aim to test for possible among-populations differences in the number of eggs produced per unit body mass.

When analysing thermal reaction norms (**III**), species-specific analyses were conducted with the following independent variables: temperature treatment (16 °C, 20 °C and 24 °C), population (= country of origin), host plant (if applicable) and sex as categorical fixed factors, whereas brood (= offspring of an individual female) was included as a random factor in the GLMMs. To test for the effect of temperature treatment on respective growth parameters (relative increment or development time), we first fitted models including main effects of all the independent variables. Thereafter, to test for similarity of the responses across subsets of the data, we added population × temperature and sex × temperature interactions into the model, one interaction at a time. To analyse among-brood differences in the reaction norms, in alternative models, brood and brood × temperature interactions were added to the models as fixed factors; these models included no random effects. In all models, we assumed multiplicative (rather than additive) character of the effects. In the multiplicative model, the absence of an (population × temperature, for example) interaction (= the null hypothesis) should be interpreted as an equal *relative* effect of temperature in the populations being compared.

A few outlier observations (less than one per analysis, on average) were excluded by visual inspection of model residuals (**I**, **II**, **III**) or by using the method of modified z-scores (**IV**) (Iglewicz and Hoaglin 1993). Outliers needed special attention in the immunological study (**IV**) due to the high variability in the immunological variables studied. In the GLM models, denominator degrees of freedom (d.d.f.) were estimated using the Kenward–Roger method (Littell *et al*. 1996) (**I**, **II**, **III**, **IV**) to account for the non-independence of observations on siblings. Accordingly, when testing for the effect of population, the number of d.d.f. were derived from the number of broods. All statistical analyses were conducted in SAS 9.4 (SAS Institute Inc., Cary, North Carolina).

3. RESULTS

Differential growth rates

Our rearing experiment with a *common garden* design found invariability in differential growth rates across a latitudinal gradient (**I**, **II**). All six moth species, sampled from northern to southern Europe, consistently lacked genetically based among-population differences in DGR (**I**): proportion of variance accounted by population, expressed as semi-partial ω^2 (SAS PROC GLM) was estimated to be equal to zero in four species out of six, with two exceptions: in *E. atomaria* and in *C. pusaria*. This was also the case when, in alternative analysis, all six species were combined: the effect of latitude on DGR was estimated to be non-significant, with a semi-partial ω^2 equal to zero.

The main effects of temperature and host plant on DGR were invariably strong and statistically significant. In contrast, sex attained a significant effect only once, in *H. punctinalis* (DGR 1.33 times higher in females). Qualitative results did not differ when growth rate was alternatively expressed as a logarithm of the relative mass increase per 24 h. In contrast, a measure of integral growth rate (IGR: body mass divided with full development time) showed a different pattern: it correlated positively with latitude (**I**) in a consistent way across the species with one exception (a comparison between Finnish and Estonian *P. populi*). The positive trend attained statistical significance in *A. rumicis* and *H. punctinalis*.

Initial mass at the beginning of last instar had an effect on DGR in only one species out of six (*H. punctinalis*), indicating that cube-root transformation mostly removed size-dependence of daily increments on body size. Size differences did not therefore complicate among-population comparisons of DGR. In *H. punctinalis,* we repeated the analyses with initial mass removed and found that the qualitative results remained unchanged.

Body size and development time

Body size showed latitudinal differences in both the *common garden* (Figure 4) and in the auxiliary experiment: the moths originating from southern populations were consistently larger, as much as twice in the extreme case, in terms of pupal weight (**I**, **II**). These results were consistent between, and significant in at least one of the experiments: either in the *common garden* (final instar development time) or auxiliary experiment (total development time). When in *E. atomaria,* alpine Georgian populations and hybrids (Estonia vs. Georgian lowland) were included in the *common garden* analyses, hybrids attained intermediate values in both pupal mass and development time, compared to their parent populations. No population × temperature nor population × host plant interactions were significant, indicating that the differences in body size were consistent across the environments (i.e. temperatures and host plants).

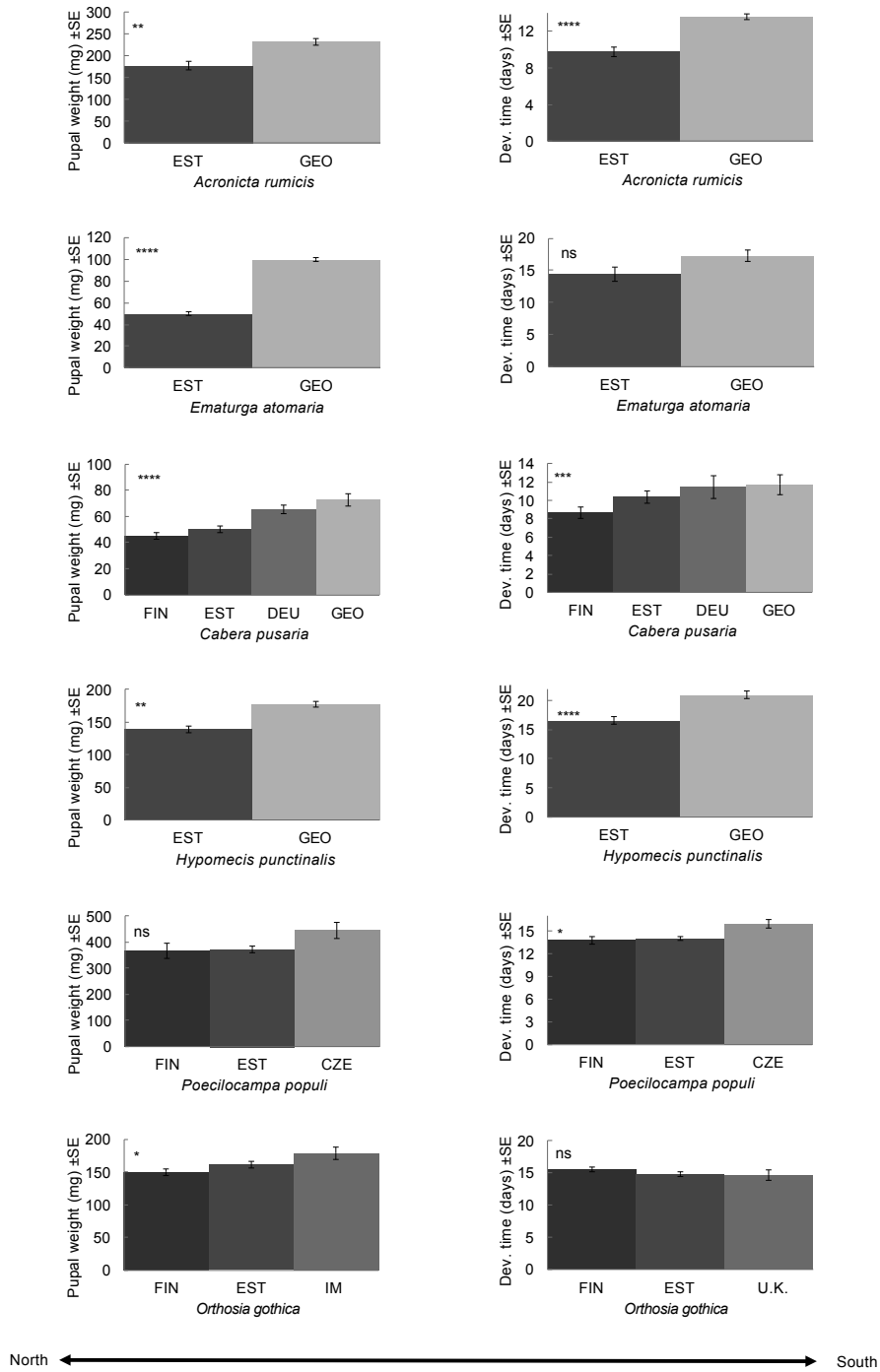


Figure 4. Among-population differences in pupal masses and final instar development times in the *common garden* experiment. The significance of the main effect of population is noted as follows: ns – $p>0.05$; * – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$; **** – $p<0.0001$. Text and graph from article **I**.

Development time (Figure 4) was found to be mostly longer in southern populations compared to northern ones: in *common garden* comparisons, *A. rumicis* had the highest (1.4-fold) difference and the lowest difference was found in *P. populi* (1.1-fold) for the final instar development times. Some exceptions from the southward increasing trend in developmental times were detected. One exception was detected in the *common garden* rearing: in *O. gothica*, the individuals from northern populations developed longer (1.07-fold), though this difference remained statistically non-significant. Surprisingly, when hybrids were included in the *E. atomaria common garden* analysis (**II**), among-group differences in the final instar development time lost its statistical significance, possibly due to the low number of hybrid broods (2).

To subject the ontogeny of the among-population differences to a closer analysis, in the Monitoring experiment, the immature development was scrutinized in detail in the species with the highest difference in body size: *E. atomaria* (Figure 5). We compared two most distant populations, Estonia and lowland Georgia, which also had the largest difference in the body size of the moths: the ratio of pupal weights ranged from 1.32 to 2.02 in different experiments, in favour of the Georgian individuals. We found eggs to be 1.14 times heavier in lowland Georgia compared to Estonia. This was also the case for newly hatched larvae (1.21-fold difference in body mass). Curiously, the among-population size difference was lost from the first instar onwards and only reappeared after the larvae moulted into the 4th instar, being thereafter present until the larvae pupated.

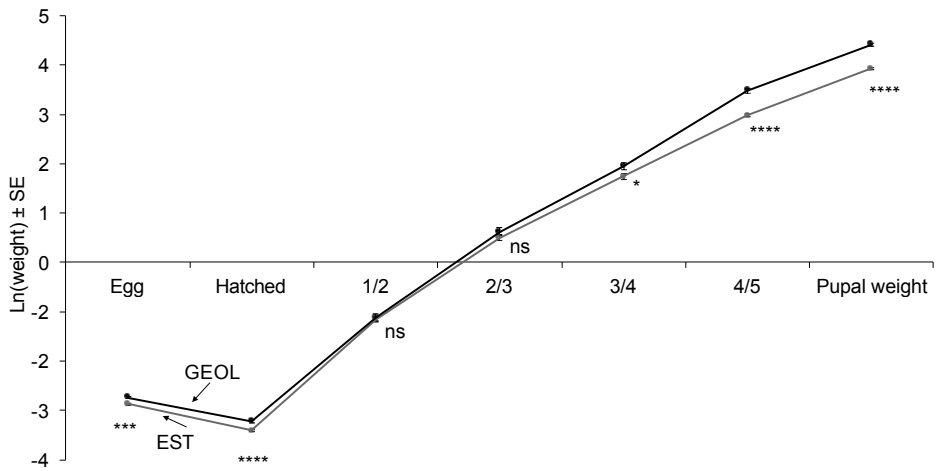


Figure 5. Longitudinal changes in body mass [natural logarithm of mean (± SEM) body mass (mg)] throughout the immature development of *E. atomaria* of Estonian (EST) and lowland Georgian (GEOL) origin. For example, '1/2' stands for mass recorded during larval moult from instar 1 to 2. Asterisks indicate the significance of differences between the two populations: *0.01<P<0.05, ***0.0001<P<0.001, ****P<0.0001; ns, P>0.05. Text and graph from article **II**.

Similarly to the *common garden* and auxiliary experiments, the larger body size was acquired through longer instar specific development times from 3rd instar onwards (1.22, 1.63 and 1.36 times, respectively). The opposite was true for the first two instars, though the difference remained statistically non-significant. Relative mass increase within an instar was 1.26-fold in Estonian conspecifics compared to Georgian ones during the first instar, though the opposite trend was present in the older instars (1.09 to 1.17-fold difference in body size).

Adult longevity and egg numbers in two distant populations

Adults of the lowland Georgian population had 1.16 times higher longevities (6.4 vs. 5.5 days) (**II**). Total egg production per females was 1.36 times higher in the Georgian lowland population, though the oviposition success (eggs laid/eggs produced) did not differ between the populations. When the body size of females was included into the model as a covariate, Georgian moths were found to lay 1.22 times less eggs per unit mass than the females from the northern population (least square means 319 vs. 389, respectively). In respective ANCOVA model, the effect of population on the number of eggs laid remained, however, non-significant, as was the case for population × pupal mass interaction.

Thermal reaction norms

Temperature had an effect on last instar mass gain in five species out of six (**III**). The highest, an almost 1.5-fold difference in pupal mass was recorded between the individuals of *A. rumicis* reared at 16 °C and 24 °C. In four species, weight gain was the highest at the lowest temperature, whereas the opposite was true in one species (*C. pusaria*), consistent with the significant species × temperature interaction. At the within-species level, however, weight gain showed little evidence of differences in its responses to temperature: population × temperature and brood × temperature interactions never attained significance, sex × temperature interaction did so twice (*O. gothica* and *P. populi*). The results of the analysis with the data on all species combined did not differ qualitatively from the patterns revealed by species-specific analyses.

As expected, the larvae grew for a longer time at lower temperatures. There was an about two-fold difference in final instar development times between the larvae growing at 16 °C with those growing at 24 °C. Despite a qualitatively consistent pattern across species, statistically significant species × temperature interaction was found. In particular, in *P. populi*, development time appeared to be less temperature-sensitive compared to the other species. Similarly to mass increment, no population × temperature, brood × temperature and sex × temperature interactions were found. When combining all the six study species in one analysis, the results on the temperature-dependence of development time generally remained the same, with the exception of a weak but significant sex × temperature interaction.

Immune indices

The immunological traits studied showed no consistent across-species latitudinal trends, with however some weak indication of higher immune response at higher latitudes (**IV**) (Figure 6). No significant among-population differences in PO were detected in either *H. punctinalis* or *P. populi*, though *A. rumicis* showed a statistically significant, 4.6 times higher PO activity in the north. Lytic activity as a continuous trait did not show any variation with latitude.

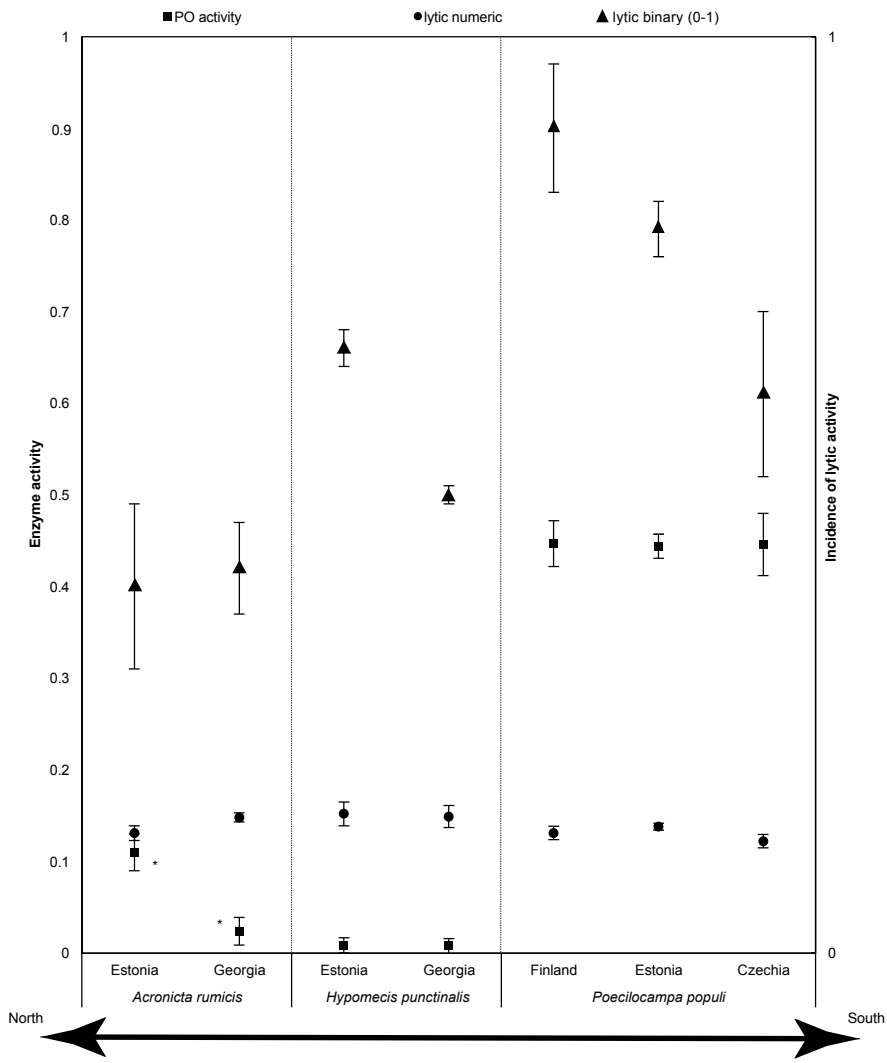


Figure 6. Enzyme activities by species and populations. PO activity (absorbance V_{max}) and lytic activity (lysozyme equivalent, μg ml^{-1}) are shown as continuous variables on the left vertical axis; the incidence of lytic activity as a binary trait on the right vertical axis. *Significant pair-wise comparison. Text and graph from article **IV**.

In contrast, when lytic activity was scored as a binary trait (present or absent, the incidence of lytic activity, hereafter), both the data on *H. punctinalis* and *P. populi* let us to anticipate an increasing trend towards northern populations, though the differences remained statistically non-significant. However, in an analysis combining the data on these two species, northern populations were shown to have a higher incidence of lytic activity.

The main effects of temperature, host plant and sex on immune traits were species-specific. In *A. rumicis,* PO activity increased with increasing temperature and was higher on *Cirsium arvese* (the better host plant in terms of larval survival) compared to *Melilotus albus*, in addition to attaining higher PO in females compared to males. Increase in temperature led to increased incidence of lytic activity in *H. punctinalis*. Neither host plant, sex nor temperature had an effect on lytic activity in any of the species studied. No interactions between the main effects were found either, apart from *A. rumicis* in which species population × host plant interaction was present for the incidence of lytic activity.

The two immunological indices, PO and lytic activity did not correlate with one another in any of the three study species. There were some correlations between these immunological indices and life-history traits of the insects but there was little consistency among the species. Negative correlations between DGR and lytic activity were found in *H. punctinalis*, between pupal mass and lytic activity in *P. populi*, between development time and PO activity in *A. rumicis* and between development time and incidence of lytic activity in *P. populi*. Positive correlations were found between development time and lytic activity in *P. populi*, between pupal mass and lytic activity in *A. rumicis* and between development time and lytic activity in *H. punctinalis*. Additionally, survival to pupation increased with PO activity in *P. populi*, as well with the increase in lytic activity in *A. rumicis* and *H. punctinalis*. In contrast, survival to adult eclosion was not affected by either PO or lytic activity.

4. DISCUSSION

Latitudinal variation in growth parameters

Our results revealed the absence of latitudinal variation in differential growth rate (DGR) of lepidopteran larvae. The qualitative results were not affected by the way how exactly DRG was calculated. Strictly standardized conditions, achieved through synchronizing the development of the larvae enabled us to successfully separate genetic differences from environmental (plastic) responses. Moreover, we are confident that the differences were in growth parameters per se, rather than representing population-specific adaptations to different environmental conditions. This is because all the responses were consistent across the treatments (three different temperatures, and several host plants) applied in our *common garden* design. This excludes the alternative interpretation that 'better' values of traits (e.g. larger size) were occasionally attained simply because some larvae were reared on temperatures and hosts plants that were closer to their population-specific optima.

The absence of latitudinal variation in differential growth rate allows us to challenge a view which has gained popularity during recent decades: the view that growth rate is a trait rapidly responding to ecologically based selective pressures (Abrams and Rowe 1996; Arendt 1997; Kivelä *et al*. 2013; Nilsson-Örtman *et al*. 2015). Rather than ascribing this discrepancy to the methodological details of our *common garden* approach or the set of species studied, we suggest that the difference is primarily due to the way how growth rate was defined in this study. So far, insect growth rates have been mostly studied as based on integral measures of this variable (IGR), usually just by dividing final mass by total development time (e.g. Gotthard *et al*. 1994; Karl *et al*. 2008; Kivelä *et al*. 2011; Välimäki *et al*. 2013). Indeed, also in our study, the larvae representing north European moth populations were found to grow faster in terms of IGR (in consistence with Kivelä *et al*. 2011; Välimäki *et al*. 2013). This shows that the way of calculating growth rate can make a difference. Even if, naturally, final instar DGR does not provide an exhaustive description of larval growth we still believe that this variable has an ecological significance of its own.

Growth rate has been quantified in different ways, and different approaches may indeed be preferred dependent on the question asked. From the perspective of evolutionary-ecological studies on insects, there are several advantages of DGR (as compared to IGR). Primarily, due to the complex shape of larval growth curve, integral measures of growth rate may easily miss the biological nature of the differences. An example is provided by an analysis of size differences among seasonal generations of a butterfly (Esperk *et al*. 2013): body size differences between directly developing and diapausing pupae were not caused by differences in DGR during the time when the larvae were actually growing. Instead, the differences appeared during the physiological preparations to pupation. Any analysis of body size determination based solely on IGR

would have missed this, failing to provide sufficient information for an adaptationistic interpretation of the phenomenon.

Clearly, we do not claim that adaptive variation in insect differential growth rates does not exist. A well-known example is provided by seasonal differences in larval growth rate in satyrine butterflies (Gotthard 2004, 2008). In this case, however, the long larval period as such appears to be the target of natural selection. Such cases should result from selective pressures favouring synchronisation of the development with some crucial aspect of the environment (Tammaru *et al*. 2001; Bentz *et al*. 2014), such as seasonally variable predator pressures or onset of winter conditions. Another example comes from sexual size dimorphism: Wiklund *et al*. (1991) described a case when large size and early emergence of males are simultaneously favoured by sexual selection, resulting in higher growth rates of males, as compared to conspecific females.

In contrast to DGR, our studies found a consistent pattern of genetically based latitudinal differences in adult body size. We found body size to correlate negatively with latitude: southern moths appear to be larger. Also at the altitudinal scale, examined in one species, insects representing a lowland population attained higher body size compared to an alpine population. The most popular explanation for such converse Bergmann's latitudinal clines (Blanckenhorn and Demont 2004) in body sizes relies on the idea that there should be selection in favour of short development time at higher latitudes. Nevertheless, three species out of the studied six are strictly univoltine in Europe and the length of Estonian summer cannot be limiting in terms of successfully completing their single generation (cf. Tammaru *et al*. 2001). Moreover, the broadly polyphagous larvae of some of the species cannot be time-limited by the availability of host plants (see Vellau and Tammaru 2012, for more discussion; cf. van Asch *et al*. 2007).

When interpreting the negative dependence of body size on latitude, geographically variable mortality should be considered as a possible explanation. Indeed, even moderate differences in larval mortality can cause substantial variations in optimal body sizes (Teder *et al*. 2010; Remmel *et al*. 2011). Accordingly, natural selection might favour small body sizes in the north/mountains, if predation risk in such habitats is systematically higher than in lowland habitats of southern Europe, though this needs experimental verification. This hypothesis is, however, challenged by recent work by Roslin *et al*. (2017) reporting higher predation risk at lower latitudes. In addition, the same pattern is supported by the works on latitudinal variation in population densities of passerine birds (Orell 1989; Blondel and Pradel 1992; Sanz *et al*. 2010). In addition to predators, parasitoids have the potential to inflict selection on growth parameters of their hosts (Solbreck *et al*. 1989; Teder *et al*. 1999; Kingsolver *et al*. 2012). Unfortunately, at this time there is no substantially complete information on geographic differences in parasitoid complexes of our study species.

Alternatively, the genetically larger adult sizes in the south may have evolved to compensate for the cost of high temperatures typically experienced

in their native environment. In consistence with the 'temperature-size rule' (TSR) ectotherms developing under higher temperatures tend to attain smaller adult sizes (Atkinson 1994; Angilletta *et al*. 2004; Horne *et al*. 2015). Given that the optimal adult size does not differ between latitudes, the larvae growing at higher temperatures may need to compensate for such an environmentally induced reduction in size. Being larger genetically would then allow the low-latitude insects to attain the same adult size as do their high-latitude conspecifics. Despite the rather intuitive character of this scenario, we are not aware of attempts to discuss it as an alternative explanation for converse Bergmann's clines.

Body size is hardly selectively neutral: in capital breeding insects in particular, the fecundity of females strongly correlates with the number of eggs laid (Honěk 1993; Tammaru *et al*. 1996ab, 2002; Fischer and Fiedler 2001). Our study shows the fecundity advantage of size to be valid also when populations differing in body size are compared. We studied this question in one of our study species, the capital breeding (Javoiš *et al*. 2011) geometrid *E. atomaria*. We found that the large Georgian moths were clearly more fecund compared to their Estonian conspecifics. The counteracting effect of geographically variable egg size proved to be too weak to substantially affect this relationship. The among-population difference in fecundity should translate to a corresponding difference in adult fitness as there was equal oviposition success (eggs laid/eggs produced) of the females from the two populations (Estonian and lowland Georgian), and just some minor differences were recorded in longevity.

Origin of size differences: the ontogeny of becoming larger

With no evidence of geographic differences in growth rate (see above), we should expect that the ultimately larger southern moths should grow for a longer time. This was, indeed, the case: our *common garden* rearings showed that the larger body size in the south is acquired through increased development time. Both in terms of total and final-instar development time, southern individuals had consistently longer development times in southern areas. This is consistent with previous work on SSD which has shown that the higher body size of the females is acquired by means of longer, not faster growth (Fischer and Fiedler 2001; Esperk *et al*. 2007; Tammaru *et al*. 2010; Teder 2014).

To understand the ontogenetic origin of size differences, one needs to monitor the full developmental cycle of the insects (Grunert *et al*. 2015). In a detailed study on the ontogeny of among-population size differences in *E. atomaria* (the species with the largest among-population difference in size), we found that the larger size of the Georgian moths is attained through longer development times in several larval instars, and the correspondingly higher within-instar relative mass increments. In this species, the among-population difference in development time was not accompanied by a difference in the number of larval instars. This is not always the case, however: for examples of a

variable instar number, see Etilé and Despland (2008), Barraclough *et al*. (2014) and Montezano *et al*. (2014). The pattern of 'accumulating' size difference in the course of several instars indicates some constraint on a large evolutionary (or plastic) increase in weight gain within one larval instar (Tammaru *et al*. 2010). This constraint can be related to oxygen limitation: the tracheal respiratory system grows only at moults but within an instar the oxygen demand of growing tissues gradually becomes too large for the amount of oxygen available in the larval respiratory system (Greenlee and Harrison 2004; Callier and Nijhout 2011; Kivelä *et al*. 2016ab).

Larger sizes appear thus to be associated with longer growth periods. This is, however, not necessarily the whole story: the ultimately larger insects can also be larger from the beginning. When monitoring size throughout the whole immature development of *E. atomaria,* we found that the among-population size differences were present as early as at the egg stage. The difference in egg size was carried over to newly hatched larvae. Geographic differences in insect egg size have been interpreted as adaptations to different levels of predation or starvation risk of the neonates (Solbreck *et al*. 1989), or extreme temperatures during development (Brittain *et al*. 1984; Azevedo *et al*. 1996). Alternatively, benefits of larger egg size have been seen in 'being large from the beginning' (Parry *et al*. 2001; Berthiaume *et al*. 2009): a large initial size may contribute to attaining the larger adult size in due time, especially when growing period is limited. Surprisingly, larger size of Georgian larvae transiently disappeared in the course of larval development. This leads us to reject the 'being larger from the beginning' hypothesis, as initial and final sizes of an individual appear to be decoupled.

Alternatively, the larger egg size can be hypothesized to be needed to compensate for negative consequences of growing at higher temperatures in the south. Larger sizes of eggs and larvae in the Georgian lowland habitat are expected: in the south, larvae need to cope with higher temperatures, more xerophytic vegetation and tougher plant leaves. We may even speculate that egg size has been the primary target of natural selection, and the large adult size of the females has evolved to facilitate laying larger eggs, which give a good start for the newborn larvae in tough conditions.

Egg size has been found to have a negative correlation with latitude in Lepidoptera (Garcia-Barros 1992) and Heteroptera (Blanckenhorn and Fairbairn 1995). In contrast, positive correlations of egg size with latitude have been recorded more often (Blau 1981; Harvey 1983; Solbreck *et al*. 1989; Azevedo *et al*. 1996; Parry *et al*. 2001; Berthiaume *et al*. 2009). In our study, egg mass had a 1.12-fold difference between the distant populations (Estonia vs. Georgian lowland). These results seem to fit well within the range of values reported in other studies (up to 1.5-fold; Solbreck *et al*. 1989). Furthermore, in addition to positive correlations across species (Berrigan 1991; Davis *et al*. 2012), positive among-population correlations between body size and egg number have also been found within insect species (e.g. Blau 1981; García-Barros 1992; Czesak and Fox 2003).

Thermal reaction norms at among- and within-species level

The standardized conditions of our *common garden* experiment enabled us to compare thermal reaction norms both between and within species: among populations, sexes, and offspring of individual females. As a general finding, temperature had a considerable effect on weight gain (mass increment during the final instar) and development periods of larvae, with substantial differences among, and just limited differences within species.

The direction and strength of the effect of temperature on within-instar mass gain varied among species. Consistency with the temperature–size rule (TSR) was found in most but not all species. In *C. pusaria*, for example, largest body sizes were attained at the highest temperatures. Deviations from TSR are not exceptional among insects, as larger sizes at higher temperatures have been found in Ephemeroptera (Atkinson 1995) and Orthoptera (Willott and Hassall 1998; Walters and Hassall 2006) as well as in some Lepidoptera (Kingsolver *et al*. 2007; Fu *et al*. 2016; Xiao *et al*. 2016). The detected among-species variation in thermal reaction norms demonstrates that reaction norms can evolve substantially at the time scale of some tens of millions of years, which is the approximate time span which separates the species in our sample from their common ancestors (e.g. Wahlberg *et al*. 2010; Zahiri *et al*. 2013).

Deviations from TSR were found in two smallest-bodied species (*C. pusaria* and *E. atomaria*) in our sample. These results may support the idea that oxygen limitation is the proximate reason for TSR: the smaller final size at higher temperatures may be seen as escape from oxygen shortage (Greenlee and Harrison 2004; Callier and Nijhout 2011; Kivelä *et al*. 2016ab). This hypothesis predicts that larger-bodied individuals should be more inclined to obey TSR (Forster *et al*. 2012), though there is some counterevidence (Klok and Harrison 2013).

We have to conclude that thermal reaction norms of body size may have limited evolvability at short time scales. Indeed, we found no differences in reaction norms among the geographically-differing populations studied. Additionally, no sex-related differences were detected (consistent with Hirst *et al*. 2015, but see Fischer and Fiedler 2000, 2001). As it is reasonable to assume that selection pressures on body sizes do differ between regions (e.g. Blanckenhorn and Demont 2004; Nygren *et al*. 2008; Välimäki *et al*. 2013), the hypothesis of no variation in selection pressures appears unlikely: it is more plausible that the invariability of reaction norms across the geographic gradient reflects organisms' inability to respond to selection. Furthermore, most Lepidoptera express sexual size dimorphism (Teder and Tammaru 2005; Stillwell *et al*. 2010), likely as the consequence of selection pressures on male and female body sizes which differ drastically (Tammaru *et al*. 1996ab). Therefore, we could hypothesize that the pattern of no sex-related differences in reaction norms indicates limited power of natural selection to modify the responses of body size to temperature. This idea is further supported by consistent absence of differences between broods.

Development time in the last instar was strongly affected by temperature. Similarly with body size, the principal differences in thermal reaction norms were among species but not among the populations nor sexes. In concert with the results on predictable among-population differences in developmental periods, the revealed pattern suggests that, at the microevolutionary level, differences can easily evolve in the length of larval period but not in the responses to larval periods to different temperatures.

The conclusion about limited evolvability of temporal reaction norms of both size and development time may been as conflicting to the reports that artificial selection (cf. Scheiner and Lyman 1991) is able to change the shape of reaction norms, as well as with some field studies (Kingsolver *et al*. 2007) which have reported rapid evolution of such reaction norms. Nevertheless, these pieces of evidence do not necessarily contradict to each other. One should perhaps assume that while (even rapid) evolution of thermal reaction norms is possible, such changes are opposed by some strong, most likely physiologically based selective pressures. The evidence suggests that physiological factors tend to outweigh ecological adaptations as the drivers of the shape of thermal reaction norms.

Sources of variance in immune response

Our results indicate that there is no easy way to interpret among-population differences in immunological traits by the latitude of origin of the populations. Neither could the patterns be attributed to trade-offs between two immunological traits, nor convincingly explained by trade-offs with size-related life-history traits. In the broader context of the present work, this should indicate that the detected latitudinal differences in the size-related traits are not likely mediated by immunological traits in the insects studied. In the more specific immunoecological context, the patterns discussed below may deserve attention.

To our knowledge we are the first to document higher incidence of lytic activity with increasing latitude, though requiring combining data on two of the species. We are, nevertheless, unaware of any other studies showing among-population variation in lytic activity in insects, lack of such variation has been reported (Mucklow *et al*. 2004). Vogelweith *et al*. (2013b) found that in warmer geographical regions, the haemolymph has a higher ability to inhibit bacterial growth, which, however, differs from lytic activity in its mode of action (Galdiero *et al*. 2015). Indeed, while lytic activity measures the capability of lysozyme and other lytic enzymes to degrade dead bacterial cell wall, the enzymes involved in inhibiting live bacteria growth are different.

Our study suggests that the costs of high immunocompetence may in some specific cases be expressed as trade-offs between immunological and life-history traits. Survival to pupation increased with increasing PO in *P. populi* and lytic activity in *A. rumicis*. For example, higher immunocompetence (measured as increased survival rate) may have come at the cost of slower development (Janssens and Stoks 2014) and reduced pupal mass (Klemola *et al*.

2007; McNamara *et al*. 2013a,b). In contrast, the two immunological indices did not negatively correlate with one another. Nevertheless, a positive correlation between PO activity and lytic activity has been found in some studies (Freitak *et al*. 2009; Fedorka *et al*. 2013) as well as negative ones (Cotter *et al*. 2004; Freitak *et al*. 2007) in some others.

The absence of immune response is a phenomenon which is not easy to explain. In our results, however, lytic activity had a bimodal distribution: 40% of the haemolymph samples showed no lytic activity whatsoever. A similar pattern of occasional absence of lytic activity has been shown twice (Nokelainen *et al*. 2013; Dubuffet *et al*. 2015). This could indicate the costly nature of upregulating lytic activity (Schmid-Hempel 2011). Furthermore, it is possible that negative correlations between two immunological traits would have been discovered, if a bacterial insult would have been induced (Freitak *et al*. 2007). In addition, lysosome (one enzyme component of total lytic activity) has shown to inhibit proPO conversion into PO, causing the negative relationship between proPO and lytic activity (Rao *et al*. 2010). This has caused some controversy in PO as a suitable index to characterise immune response (Pauwels *et al*. 2011).

Environmental factors also affected both PO and lytic activity but did so in species- and condition-specific ways. In *A. rumicis*, PO activity increased at higher temperatures, and on the better host plant *C. arvense* (in terms of larval survival). Females also displayed higher PO activity than males. In the other two species, the host plant did not affect the immune parameters. The absence of host plant effect (cf. Vogelweith *et al*. 2011; Lampert 2012; Muller *et al*. 2015) may be related to our focus on constitutive immunity: it remains possible that host plant related differences would have been observed. For lytic activity, consistent with our observations on *P. populi* (but not the other two species), host plant has been shown to affect antimicrobial activity (Vogelweith *et al*. 2013b) and lytic activity has been show to increase with temperature (Lazzaro *et al*. 2008; Fedorka *et al*. 2016).

Correlations between different immunological traits and those between immunological and size-related traits were of species-specific nature without any consistent across-species trends. The reasons for such idiosyncratic patterns in immune responses remain complicated to interpret. As a practical point, such complex patterns, however, suggest that one should not solely rely on one species and a single immunological marker when aiming to reveal general trends about among-population differences in immune function. According to a widely appreciated hypothesis, population-specific immunological indices (PO and lytic activity) are suggested to indicate adaptations to different rates of parasitism (Tinsley *et al*. 2006; Seiter and Kingsolver 2013; Vogelweith *et al*. 2013b). The absence of uniform trends in different species is indeed consistent with this hypothesis: the assemblages of parasitoids that attack the larvae are highly species-specific, and differ between regions (Waage and Greathead 1989; Quicke 1997).

Novelty of the research

First, the present work is novel through making a number of original contributions to the research at the interface of life history ecology and developmental physiology. As a central contribution, the present thesis showed that, in insects, growth rate may not be a life history trait which is readily responding to ecologically based selection pressures. This clearly challenges some ideas which have become popular during recent decades. Indeed, our evidence indicates that the consistently higher body size in the southern (low latitude) populations is not attained through higher (differential) growth rates but is rather a result of uniform counter-gradient increase in development time. Our results are better consistent with the possibility that growth rates are maximised within physiological limits, rather than being optimised in response to ecological selective pressures. Our monitoring of the ontogenetic determination of the among-population size difference further supported the importance of longer development time in size acquisition: it showed that the larger body size is attained by an extended growth period in several larval instars. Such a detailed monitoring of larval development is novel in itself, and it also contributes to the emerging understanding that physiological constraints may substantially interfere with evolutionary and plastic changes in insect body size. Indeed, there must be limits on within-instar increase in body size as any major (both plastic and genetic) changes in body size appear to require adjusting larva growth in several instars. An important contribution of the present thesis is in advocating the view that evolutionary change in body size and development time is more readily attainable than changes in respective thermal reaction norms. This conclusion is based on the contrast between substantial among-population differences in these traits and the limited within-species variability in respective thermal reaction norms. Immunological indices were for the first time measured under strictly standardized conditions (time of the day, developmental stage), using several species at once. These indices were found to be variable within and among populations but the idea of an important role of immunological mechanisms as correlates of larval growth patterns was not supported.

Second, this work has proven the applicability of a number of novel methodological approaches; those should have the potential to be used in forthcoming studies on the subject. Our original way to achieve strict synchronization of larval development provides identical environmental conditions for the groups being compared, and allows one to isolate genetic differences. Rearing the larvae under different combinations of environmental factors proves that the among-population differences in life-history traits are consistent across environments. Measuring growth rate at a specific developmental stage (the second day of the final instar, in our case) properly considers the complex nature of insect growth curve, and allows one to obtain estimates of differential growth rates. Such measures have various advantages over the integral ones, and we propose that future studies should adopt this approach whenever

feasible. Furthermore, we demonstrate the potential of detailed monitoring of immature development of the insects to yield information which is useful also in the context of evolutionary ecology. The variable results of our immunological measurements despite the strictly standardised methodology caution against relying on only a single immunological parameter and a single species when aiming at revealing general trends. This indicates that the results of previous single-species studies on latitudinal differences in immunological traits cannot easily be generalised.

SUMMARY

This thesis focuses on evolutionary factors which shape animals' body sizes and related traits. For this purpose, we evaluated latitudinal variation in life-history traits, in respective thermal reaction norms and investigated whether such differences are associated with variation in immune capacity. We used a broad geographical range from northern to southern Europe (65°N to 41°N) and six moth species representing distantly related macrolepidopteran families. A *common garden* rearing with strictly standardised conditions was applied in our studies to isolate genetic differences in the traits measured, and to investigate their consistency across environmental conditions, and study species.

We studied latitudinal variation in differential growth rates based on short-term measurements in a specific ontogenetic stage, the 'free growth' period in the beginning of the last instar (**I**, **II**, **IV**). We found that differential growth rate did not vary among the populations (**I**, **II**). Based on these results, we challenge the popular opinion that growth rate is a trait that readily responds to environmentally based selective pressures. The invariability in growth rates was there despite consistent latitudinal differences in body size: the moths were genetically larger in the south than in the north (**I**, **II**). According to the 'temperature-size rule', ectotherms developing under higher temperatures tend to attain smaller adult sizes. Hence, the genetically larger size in the south may compensate for the environmentally driven size reduction in their native environment.

Our *common garden* rearing contributed to understanding the ontogenetic mechanisms behind among-population size differences. We showed that the larger body size in the south was attained through longer development times and not via increased growth rates (**I**). Furthermore, we conducted a detailed monitoring of the ontogenetic development from the egg to the pupa in one species, *Ematurga atomaria* (**II**). This was the species with the highest (twofold) among-population difference in body mass. We found that the size difference is accumulated in the course of several larval instars by means of longer instar-specific growth periods. Eggs were larger in the southern population as well. We, however, were able to refute the 'being larger from the beginning' hypothesis of the benefits of large egg size (**II**).

In addition to documenting the latitudinal variation in life-history traits, we aimed to determine how respective thermal reaction norms vary with latitude. Temperature had an effect on weight gain and development periods of the final instar larvae with strong differences among but much more limited differences within species (**III**). Evolvability of thermal responses in body size and development time may thus be limited at a shorter time scale. The evidence suggests that physiological factors tend to outweigh ecological adaptations as the drivers of the shape of reaction norms (**III**). An important role of physiological mechanisms was not, however, supported by the results of our immuno-

logical study (**IV**). In our study on among-population differences in immunological traits (**IV**), some indication of stronger immune responses in the north was found, but no consistent across species trends were discovered. Some case-specific (species or environmental condition) phenotypic correlations were still discovered between immune indices and life-history traits. The two immune indices measured were not correlated.

KOKKUVÕTE

Putukavastse kasvukiiruse evolutsiooniline ökoloogia: geograafilistest erinevustest biokeemiliste lõivsuheteni

Kehasuuruse ja sellega seotud elukäigutunnuste väärtusi kujundavate loodusliku valiku survete uurimine on osutunud üllatavalt keerukaks. Kui suurema kehasuuruse ja kiirema kasvu eelised on üsnagi iseenesestmõistetavad, ei ole selge, millised tegurid tekitavad vastupidiseid (tasakaalustavaid) valikusurveid ja osalevad seeläbi optimaalväärtuste kujunemises. Kui suuremast kehast ja kõrgemast kasvukiirusest oleks ainult kasu, peaks eeldama nende muutujate väärtuste pidevat suurenemist ajas, mida me ometigi ei näe. Loodusliku valiku mõistmiseks peab oskama seostada feno- ja genotüüpe keskkonnatingimustega. Üks võimalus selleks on tuvastada geograafilisi erinevusi uuritavate tunnuste väärtustes, kuna valikusurved erinevad populatsioonide vahel sageli ennustataval moel.

Putukate kehasuuruse varieeruvus eri laiuskraadide vahel on hästi dokumenteeritud. Kehasuuruse geograafiliste erinevuste kohta on teavet paljudes käsiraamatutes (nt määrajates), kuid leidub ka teemakohaseid teadustöid. Samas ei ole geograafilised trendid järjekindlad: tuvastatud on nii positiivseid kui negatiivseid korrelatsioone kehasuuruse ja laiuskraadi vahel. Negatiivseid fenotüübilisi korrelatsioone tõlgendatakse tavaliselt sellise valikusurve kõrvalproduktina, mis soosib kõrgematel laiuskraadidel lühemat arenguaega. Positiivseid fenotüübilisi korrelatsioone seostatakse sageli temperatuuriga: kõigusoojased organismid kasvavad külmematel temperatuuridel üldjuhul suuremaks. Lisaks võib piirkondade vahel erineda ka põlvkondade arv aastas: piirkonnas, kus lisageneratsioon on võimalik, võib valik surve soosida organisme kiiremini arenema, et kindlustada ka teise põlvkonna edukas läbimine.

Seni on vähe teavet ontogeneetiliste mehhanismide kohta, mis viivad populatsioonidevaheliste erinevuste väljakujunemisele kehasuuruses. Suuremat kehasuurust on võimalik saavutada kolmel erineval põhimõttelisel moel. Suurem on võimalik olla juba „algusest peale“ (muna või vastkoorunud vastsena). Alternatiivina võivad röövikud kas kasvada kiiremini või areneda kauem, mõnikord lisades vastsejärke. Hiljutised tööd on leidnud, et just kauem kasvamine on mehhanismiks, misläbi saavutatakse emaste suurem kehasuurus sugulise dimorfismi olemasolul. Vastav analüüs populatsioonidevaheliste kehasuuruse erinevuste ontogeneetilise tausta kohta on seni puudunud. Ka ei ole selge immuunökoloogiliste parameetrite võimalik roll populatsioonidevaheliste elukäigu-erinevuste vahendajana.

Käesoleva doktoritöö eesmärgiks oli uurida kehasuurusega seotud elukäigutunnuste ja vastavate termaalsete reaktsiooninormide varieeruvust laiuskraadilisel gradiendil ning seostada saadud tulemusi immunoloogiliste näitajatega. Võrdlesime kuue eri sugukondi esindava liblikaliigi geograafiliselt kaugeid populat-

sioone gradiendil Põhja-Euroopast (65°N) Lõuna-Euroopani (41°N). Liblikate röövikuid kasvatasime standardiseeritud disainiga *common garden* katses, mis võimaldas välja tuua geneetilised erinevused uuritavates tunnustes ning hinnata populatsioonidevaheliste erinevuste kooskõla eri liikidel. Katsetes registreerisime liblikaröövikute kasvukiiruse, arengukestuse ja saavutatud nukukaalu valmiku kehasuuruse mõõduna. Analüüsisime selliste isendi kasvu kirjeldavate muutujate erinevusi populatsioonide vahel ja nende väärtuste sõltuvust katsetingimustest. Lisaks uurisime ühe liigi puhul populatsioonidevaheliste kehasuuruse erinevuste ontogeneetilist kujunemist detailsemalt, monitoorides isendite arengut munast nukuni. Kolme liigi puhul võtsime ka hemolümfiproove immuunnäitajate hindamiseks, võrdlesime selliseid näitajaid populatsioonide vahel ning otsisime seoseid immuunparameetrite ja kasvuga seotud elukäigutunnuste vahel.

Varasemad tööd on leidnud putukavastsete kasvukiiruse puhul nii suurenemist kui vähenemist põhja-lõuna suunalisel gradiendil. Tulemuste ebajärjekindlust on usutavasti mõjutanud erinevused viisides, kuidas kasvukiirust mõõdetakse ja arvutatakse. Käesolevas töös pürgisime hindama röövikute hetkelist kasvukiirust, mille arvutamiseks tegime lühikese kestusega mõõtmisi selles arenguetapis, kus röövikute kasv on kiireim. Ühegi liigi puhul ei leidnud me populatsioonidevahelisi erinevusi hetkelises kasvukiiruses. Töö tulemused oponeerivad laialt levinud seisukohale, mille kohaselt kasvukiirus vastab kiiresti keskkonnast tulenevatele valikusurvetele. Seevastu tuvastasime geneetilisi populatsioonidevahelisi erinevusi kehasuuruses (**I**, **II**): lõuna poolt pärit isendid olid põhjapoolsete liigikaaslastega võrreldes suuremad. 'Temperatuuri-kehasuuruse reegli' kohaselt jäävad kõigusoojased loomad kõrgemal temperatuuril kasvades väiksemaks. Seetõttu võib suurem geneetiline kehasuurus lõunas olla evolutsioneerunud kompenseerimaks kõrgemast kasvutemperatuurist tulenevaid 'negatiivseid' keskkonnamõjusid.

Kirjanduses on vähe andmeid populatsioonidevaheliste suuruserinevuste ontogeneetilise tausta kohta. Käesoleva töö aluseks olev *common garden* metoodikaga katse näitas, et lõunapoolsete populatsioonide suurem kehasuurus oli põhjustatud pikemast arenguajast ning mitte kasvukiiruse erinevusest (**I**). Asja lähemaks uurimiseks jälgiti ühel liigil isendite arengut munast nukkumiseni (**II**). Töö viidi läbi võsavaksikul (*Ematurga atomaria*) ehk liigil, mille populatsioonidevahelised nukukaalu erinevused olid suurimad (kahekordsed). Näitasime, et lõunast pärit isendite suurem kehasuurus saavutatakse läbi suurema juurdekasvu ja pikema arenguaja akumuleeruvalt mitmes eri vastsejärgus; vastsejärkude arv populatsiooniti ei erine. Lisaks on lõuna pool suuremad ka võsavaksiku munad. Ootuspäratult ei kandunud erinevus munade suuruses üle hilisematesse arengustaadiumitesse: teise vastsejärgu röövikute kaal populatsiooniti ei erinenud. Seega lükkasime ümber hüpoteesi, et suurema munasuuruse eeliseks on anda parem stardipositsioon suuremaks kasvavatele lõunapoolsetele isenditele.

Lisaks elukäigutunnuste endi laiuskraadilisele varieeruvusele, tundsime huvi, kuidas varieeruvad vastavad termaalsed reaktsiooninormid. Temperatuuril oli ilmne mõju nii röövikute massi juurdekasvule kui ka arengukestusele, vastavad reaktsiooninormid erinesid liikide vahel, kuid mitte liikide

sees eri populatsioonide ja pesakondade vahel (**III**). Tulemustest võib järeldada, et nii kehasuuruse kui arengukestuse termaalsed reaktsiooninormid ei kaldu kiiresti evolutsioneeruma. Tulemust võib tõlgendada ka nii, et füsioloogilised faktorid kaaluvad üles keskkonnafaktoritest tulenevad valikusurved reaktsiooninormide kujule. Samas ei toeta meie immuunökoloogiline töö (**IV**) järeldust immuunmehhanismide olulisusest kasvuparameetrite kujunemises liblikaröövikutel.

Väitekirja viimases osas (**IV**) oligi eesmärgiks selgitada, kas laiuskraadilised erinevused elukäigutunnustes on seotud erinevusega röövikute immuunvõimekuses. Varasemad immuunvastuse geograafilisi erinevusi uurivad tööd on tuginenud vaid ühele liigile ning katsetingimuste standardiseerimine on sageli soovida jätnud. Meie töö seadis eesmärgiks seda viga parandada. Järjepidevaid liigiüleseid trende uuritud immuunparameetrites (fenooloksüdaasi aktiivsus ja lüütiline aktiivsus) meie töös siiski ei leitud, kuigi ilmnes väike tendents immuunvastuse tugevnemisele põhjapoolsete populatsioonide suunas liikudes. Leiti mõningaid liigi- ja keskkonnaspetsiifilisi fenotüübilisi korrelatsioone immuunparameetrite ja elukäigutunnuste vahel, kuid kaks uuritud immuunparameetrit omavahel ei korreleerunud (**IV**). Seega ei ole põhjust väita, et elukäigutunnuste laiuskraadilised erinevused (**I**, **II**, **III**) oleksid seotud erinevustega immuunvõimekuses. Veelgi enam, immuunparameetrite puhul ei õnnestunud ka meie standardiseeritud katsedisaini tingimustes leida seoseid elukäigu- ja immuunparameetrite vahel, mis oleksid järjepidevad üle keskkondade ja liikide.

Käesoleva doktoritöö peamiseks uudseks aspektiks on oponeerimine viimastel aastakümnetel levinud seisukohale, milles noorlooma kasvukiirust nähakse loodusliku valiku survetele kiiresti reageeriva elukäigutunnusena. Meie tulemused toetavad pigem seisukohta, et kasvukiirus on määratud peamiselt füsioloogiliste piirangute poolt ja maksimeeritud nende poolt seatud piires. Kui looduslik valik soosib suuremat kehasuurust, saavutatakse seeläbi kasvuperioodi pikenemise ning mitte kasvukiiruse tõstmise teel. Ka pakub töö lisatoetust seisukohale, et füsioloogilised piirangud ei võimalda oluliselt suurendada massi juurdekasvu ühe kasvujärgu piires: võsavaksiku näide tõendab, et evolutsioonilised muutused valmiku kehasuuruses eeldavad kasvuparameetrite muutusi mitmes eri arengujärgus. Samas ei leidnud kinnitust oletus, et populatsioonidevahelisi kasvuparameetrite erinevusi vahendavad immuunökoloogilised näitajad. Ilmnes, et erinevused immuunparameetrites on tugevalt liigi- ja keskkonnaspetsiifilised ja seetõttu tuleb suhtuda skeptiliselt varasemate, ühel liigil põhinevate tööde tulemuste üldistatavusse.

Doktoritöö tulemused võimaldavad kriitiliselt hinnata viise, kuidas putukavastse kasvukiirust on traditsiooniliselt defineeritud ja mõõdetud. Traditsioonilised integraalsed kasvukiiruse mõõdud jagavad (mõnikord matemaatiliselt teisendatud) lõppkaalu (enamasti nukukaal) kogu arenguajaga, mispuhul jääb arvestamata putukavastsete kasvukõvera eripära. Kuna putukavastse kasv on jagatud diskreetseteks kasvujärkudeks, on kasvukõver keerulise kujuga ning reaalne kasvamine toimub vaid teatud arenguperioodide jooksul. Seetõttu soovi-

tame evolutsioonilis-ökoloogilistes töödes kasutada lähendust hetkelisele (diferentsiaalsele) kasvukiirusele. Liblikaröövikute puhul osutus sobivaks lähenemine, kus kasvukiiruse arvutamise aluseks on massi juurdekasv kasvujärgu teise ööpäeva jooksul. Tagamaks selliste mõõtmiste võrreldavust, peame oluliseks katseisendite arengu ranget sünkroniseerimist. Taoline metoodika on rakendatav ja usutavasti tarvilik ka immuunökoloogia alastes uuringutes, kus on samuti vajalik mõõtetingimusi (kellaaeg, arengustaadium) ühtlustada.

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PUBLICATIONS

CURRICULUM VITAE

Name: Hendrik Meister
Date of birth: 04.03.1989
Citizenship: Estonian
Contact: Department of Zoology,
Institute of Ecology and Earth Sciences,
Vanemuise 46, 51014, Tartu, Estonia
E-mail: hmeister@ut.ee, hendrik.meister@gmail.com

Education:

2013–...	University of Tartu, PhD Zoology and Hydrobiology (Zoology)
2011–2013	University of Tartu, MSc Zoology and Hydrobiology
2008–2011	University of Tartu, BSc Biology
2000–2008	Tabasalu Gymnasium (*Golden Medal*)
1996–2000	Audentes Private School

Research interests: evolutionary ecology, immune ecology, butterfly conservation

Publications:

Freitak, D., Tammaru, T., Sandre, S.-L., **Meister, H.** and Esperk, T. Between-generation differences of immunocompetence in *Arachnia levana*. *Manuscript in preparation.*

Tammaru, T., **Meister, H.**, Välimäki, P. and Teder, T. Thermal reaction norms of larval growth in insects: physiological rather than ecological determinants. *Submitted manuscript.*

Meister, H., Hämäläinen, R., Valdma, D., Martverk, M. and Tammaru, T. 2017. How to become larger: ontogenetic basis of among-population size differences in a moth. – Entomol. Exp. Appl., in press. Doi 10.1111/eea.12634.

Lindman, L., Remm, J., **Meister, H.** and Tammaru, T. 2017. Host plant and habitat preference of *Euphydryas maturna* (Lepidoptera: Nymphalidae, Melitaeinae): evidence from northern Europe. Ecol. Entomol., in press.

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Meister, H., Lindman, L. and Tammaru, T. 2015. Testing for local monophagy in the regionally oligophagous *Euphydryas aurinia* (Lepidoptera: Nymphalidae). J. Insect Conserv. 19: 691–702.

Conference presentations:

Meister, H., Freitak, D., T., Esperk, T., Välimäki, P., Sandre, S.-L and Tammaru, T. Poster presentation 'Evolutionary ecology of insect growth: from geographic patterns to biochemical trade-offs' The 16th Congress of European Society for Evolutionary Biology. Groningen, Netherlands, 20th to 25th August 2017.

Meister, H., Tammaru, T., Sandre, S.-L. and Freitak, D. Oral presentation 'Latitudinal patterns of immune response in moths' The Graduate Seminar of Insect Evolutionary Ecology. Vana-Veski, Estonia, 4th to 6th May 2017.

Meister, H., Tammaru, T., Sandre, S.-L. and Freitak, D. Poster presentation 'Latitudinal patterns of immune response in moths' The 16th International Society for Behavioural Ecology. Exeter, U.K., 28th July to 3rd August 2016.

Meister, H., Esperk, T., Välimäki, P. and Tammaru, T. Oral presentation 'Latitudinal comparisons of life-history traits' Graduate Seminar of Insect Evolutionary Ecology. Kuke, Estonia, 17th to 19th May 2015.

Meister, H., Lindman, L. and Tammaru, T. Poster presentations 'Host preference of *Euphydryas aurinia*' The 7th International Conference on the Biology of Butterflies. Turku, Finland, 11th to 14th August 2014.

Dissertation supervised:

Juhan Heinma, bachelor thesis, 2015. *Among-population variation in traits of ecological immunology by the example of insects*. University of Tartu, Faculty of Science and Technology, Institute of Ecology and Earth Sciences, Department of Zoology.

Outreach:

Biology Experimental School Courses for University of Tartu the Gifted and Talented Development Centre, tutor 2013–2017.

International Science Night Event at the Department of Zoology, coordinator 2014–2017.

Animal ecology practical for the 53rd National Biology Olympiad, examinator.

Student research projects, supervisor:

- Laura Tammiste (*Ecology and conservation of Euphydryas maturna*),
- Hanno-Laur Kunnus (*Host plant effects on insect immunity*).

ELUKÄIK

Nimi: Hendrik Meister
Sünniaeg: 04.03.1989
Kodakonsus: Eesti
Kontakt: Zooloogia osakond,
Ökoloogia ja Maateaduste Instituut,
Vanemuise 46, 51014, Tartu, Eesti
E-post: hmeister@ut.ee, hendrik.meister@gmail.com

Haridus:

2013–...	Tartu Ülikool, PhD Zooloogia ja hüdrobioloogia (Zooloogia)
2011–2013	Tartu Ülikool, MSc Zooloogia ja hüdrobioloogia
2008–2011	Tartu Ülikool, BSc Bioloogia
2000–2008	Tabasalu Gümnaasium (*kuldmedal*)
1996–2000	Audentese Erakool

Peamised uurimisvaldkonnad: evolutsiooniline ökoloogia, immuunökoloogia, päevaliblikate looduskaitse

Publikatsioonide loetelu:

Freitak, D., Tammaru, T., Sandre, S.-L., **Meister, H.** & Esperk, T. Between-generation differences of immunocompetence in *Arachnia levana*. *Viimistlemisjärgus käsikiri.*

Tammaru, T., **Meister, H.**, Välimäki, P. & Teder, T. Thermal reaction norms of larval growth in insects: physiological rather than ecological determinants. *Submiteeritud käsikiri.*

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Konverentsiettekanded:

Meister, H., Freitak, D., Esperk, T., Välimäki, P. Sandre, S.-L. & Tammaru, T. Posterettekanne 'Evolutionary ecology of insect growth: from geographic patterns to biochemical trade-offs' konverentsil *16th Congress of European Society for Evolutionary Biology*. Groningen, Holland, 20.–25. august 2017.

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Juhendatud väitekiri:

Juhan Heinma, bakalaureusekraad, 2015. *Populatsioonidevahelised erinevused immuunökoloogilistes tunnustes putukatel*. Tartu Ülikool, Loodus- ja Tehnoloogiateaduskond, Ökoloogia ja Maateaduste Instituut, Zooloogia osakond.

Populaarteaduslik tegevus:

Tartu Ülikooli teaduskooli bioloogia õpikoja juhendaja 2013–2017.
'Teadlaste Öö' zooloogia osakonnas, koordinaator 2014–2017.
53. bioloogiaolümpiaadi praktilise osa 'loomaökoloogia' koostaja ja läbiviija.

Õpilaste uurimistööde juhendamine:

- Laura Tammiste (*Suur-mosaiikliblika ökoloogia ja looduskaitse*),
- Hanno-Laur Kunnus (*Toidutaime-eelistus varieeruvus ja mõju putukate immuunsusele*).

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