© 2020. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2020) 223, jeb219659. doi:10.1242/jeb.219659

RESEARCH ARTICLE

Consequences of rapid development owing to cohort splitting: just how costly is it to hurry?

Zoltán Rádai[1,*], Johanna Kiss[1], Agnieszka Babczyńska[2], Gábor Kardos[3], Ferenc Báthori[4], Ferenc Samu[5] and Zoltán Barta[1]

ABSTRACT

In cohort splitting, diverging sub-cohorts may show substantial differences in their growth and developmental rates. Although in the past, causes and adaptive value of cohort splitting were studied in detail, individual-level consequences of cohort splitting are still rather overlooked. Life history theory predicts that considerably increased growth and developmental rates should be traded off against other costly life history traits. However, it is not clear whether one should expect such associations in adaptive developmental plasticity scenarios, because natural selection might have promoted genotypes that mitigate those potential costs of rapid development. To address these contrasting propositions, we assessed life history traits in the wolf spider *Pardosa agrestis*, both collected from natural habitat and reared in laboratory. We found that some traits are negatively associated with developmental rates in spiders collected from the wild, but these associations were relaxed to a considerable extent in laboratory-reared specimens. In general, we observed no consistent trend for the presence of developmental costs, although some results might suggest higher relative fecundity costs in rapidly developing females. Our study provides a detailed approach to the understanding of individual-level consequences of cohort splitting, and to the associations between key life history traits in adaptive developmental plasticity scenarios.

KEY WORDS: Costs, Developmental plasticity, Life history evolution, Physiology, Trade-offs

INTRODUCTION

There is an immense diversity in the rate of post-embryonic development (the process during which individuals go through different stages of ontogeny from birth to maturation and reproduction) across the animal kingdom, and variation in developmental characteristics often occurs even within species (West-Eberhard, 2003). In the last two decades, it was established that developmental processes can show a high level of plasticity in response to environmental stimuli, and that this adaptive plasticity can contribute to the evolution of alternative developmental pathways (Moczek et al., 2011; Nettle and Bateson, 2015; West-Eberhard, 2003, 2005). One intriguing instance for adaptive developmental plasticity is when individuals belonging to the same age group within a population follow different trajectories of post-embryonic development, a phenomenon termed cohort splitting (Crowley and Hopper, 2015). Cohort splitting was observed in a number of species (for an extensive list, see Crowley and Hopper, 2015), and was described as a bifurcation of life histories, by which alternative developmental pathways of different durations emerge in the population. In past studies, the adaptive value of cohort splitting was mostly assessed either specifically for the species in which it occurred, or with respect to environmental characteristics more generally, such as owing to stochastic changes in environment quality (Crowley and Hopper, 2015). Although individual decision-making was proposed to be likely an important element of cohort splitting scenarios, empirical studies on the individual-level consequences of following either of the alternative developmental pathways arising from cohort splitting are still largely overlooked (Khelifa et al., 2019).

Alternative developmental pathways may entail different costs, arising from differences in how individuals balance their limited resources between life history traits in order to maximise their reproductive success. These costs manifest through the organisms' inability to simultaneously improve all their life history traits, leading to trade-offs between traits (e.g. investment to growth rate versus energy storages) that compete for the same pool of resources (Fischer et al., 2005; Stoks et al., 2006). Resource expenditure costs as such are the basis for allocation-based trade-offs central in life history theory (Agrawal et al., 2010; Stearns, 2000), and physiological mechanisms behind trade-offs in resource investment into different aspects of life history (e.g. survival versus reproduction) play crucial roles in shaping resource allocation patterns themselves (Cox et al., 2010). Notably, such costs ultimately translate into fitness costs, driving life history evolution by promoting resource allocation patterns that yield the highest lifetime fitness (English and Bonsall, 2019; Smith and French, 2017).

In cohort splitting, individuals of alternative cohorts might show different rates of development (life stage transitions, i.e. processes that entail differentiation) and/or growth (increase in size or number of somatic cells in a time unit) (David and Geoffroy, 2011; Martin et al., 1991; Watts and Thompson, 2012). Growth and development are resource-demanding processes, and their rates often show negative associations (trade-offs) with other costly traits that utilise the same resource pool, such as size at maturation, condition or efficiency of somatic maintenance (Bayne, 2000; De Block et al., 2008; Ficetola and De Bernardi, 2006; Peterson et al., 1999; Yearsley et al., 2004). An intriguing case of cohort splitting was observed in the wolf spider *Pardosa agrestis* (Westring 1861), in which the diverging sub-cohorts show considerable differences in developmental rate (Kiss and Samu, 2005). *Pardosa agrestis* is a semelparous epigeic (i.e. soil surface-residing) spider, common in Central and Eastern Europe, predominantly residing in arable

[1]MTA-DE Behavioural Ecology Research Group, Department of Evolutionary Zoology, University of Debrecen, H-4032 Debrecen, Hungary. [2]Department of Animal Physiology and Ecotoxicology, University of Silesia in Katowice, 40-007 Katowice, Poland. [3]Department of Medical Microbiology, Medical and Health Science Center, University of Debrecen, H-4032 Debrecen, Hungary. [4]Department of Ecology, University of Debrecen, H-4032 Debrecen, Hungary. [5]Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, H-1029 Budapest, Hungary.

*Author for correspondence (radai.zoltan@science.unideb.hu)

Z.R., 0000-0001-7011-5055; J.K., 0000-0001-5046-8910

Received 2 December 2019; Accepted 11 February 2020

Journal of Experimental Biology (2020) 223, jeb219659. doi:10.1242/jeb.219659

habitats, alkaline grasslands and marshes (Samu and Szinetár, 2002). It shows a yearly phenology in which individuals (after overwintering in juvenile or subadult stage) mature and reproduce during spring and early summer (Fig. 1, I). Cohort splitting takes place among the progeny originating from the early summer adults (Fig. 1, II), via asynchronous development of spiderlings, even within the same broods. Consequently, this case of cohort splitting results in the emergence of slowly (Fig. 1, IIb) and rapidly (Fig. 1, IIa) developing phenotypes in the population. Slowly developing spiderlings develop throughout the year, overwinter in juvenile or subadult stage, and mature and reproduce during spring/early summer of the next year. Rapidly developing spiderlings, however, have a much shorter life cycle, reaching maturity, producing offspring and perishing by late summer of the same year they hatched (Kiss, 2003; Kiss and Samu, 2002; Rádai et al., 2017b). The offspring of rapidly developing spiders develop alongside the slowly developing spiders, i.e. they overwinter, mature and reproduce during early summer of next year. Although not much is known about the proximate background of this case of cohort splitting as of now, past studies both on laboratory-reared spiders and spiders collected from natural habitats throughout the yearly phenology strongly indicate that day-length dynamics and ambient conditions (e.g. temperature) play important roles in evoking cohort splitting among the newly hatched spiderlings (Kiss, 2003; Kiss and Samu, 2002), which was proposed to pre-adapt *P. agrestis* into changeable arable habitats (Kiss and Samu, 2005).

Rapidly developing spiders finish their life cycles in a much shorter span (approximately one-third) of time than slowly developing ones. Based on past studies, one might hypothesise that such an increased rate of development is traded off with other life history traits, because of their competing resource demands (Zera and Harshman, 2001). For example, in damselflies, increased growth rates were found to negatively affect symmetric somatic development and dry mass build-up (De Block et al., 2008; Dmitriew and Rowe, 2005). Indeed, one might also argue that certain costs would be expected to be associated with rapid development, because if increased developmental and/or growth rate were to have no costs at all (but nevertheless would provide fitness advantages), they should have been fixed in the population. Furthermore, an enhanced pace of life was predicted to be associated with a specific 'syndrome' of life history characteristics, being associated with high metabolic and growth rate, precocious reproduction and low investment into somatic maintenance (Réale et al., 2010). Although between-species observations appear to generally support such trends, within-species interpretations of trait associations in light of organisms' pace of life remain ambiguous (Royauté et al., 2018), presumably owing to species-specific characteristics of natural history, sexual selection and/or inter- and intra-sexual competition for resources and mating opportunities (Dammhahn et al., 2018; Mathot and Frankenhuis, 2018). Notably, however, some studies suggest that such negative phenotypic correlations might not be apparent in 'good quality' individuals that can meet the energetic demands of the competing traits – the so-called 'big house, big car' effect (McNamara and Simmons, 2017; van Noordwijk and de Jong, 1986). For example, increased resource acquisition might mitigate the resource-allocation conflicts between the traits, hence masking the negative phenotypic association between them (Reznick et al., 2000). Indeed, increased resource acquisition might buffer developmental costs to other traits (Engqvist, 2007), and in adaptive developmental scenarios one might expect that the different developmental pathways have similarly efficient ways to balance their resources between costly traits. Also, theory predicts that individuals characterised by high metabolic rate and rapid development and growth may have a competitive edge over their 'slower' conspecifics when resources are less limiting (Careau et al., 2008; Salzman et al., 2018). As so, when food availability is not restricted, individuals of enhanced metabolism and growth rate may gain substantial fitness advantages, whereas in times of low food availability, they would be expected to show higher mortality if they were not able to meet the resource demands of a high metabolic rate (Biro and Stamps, 2010; Burton et al., 2011; Montiglio et al., 2018). So altogether it is still rather unclear what the individual-level consequences of rapid development might be, especially in an adaptive developmental plasticity scenario. For example, do rapidly developing individuals face such costs that must be paid in trade-offs against other, costly traits? Should we expect to see the predicted trait-association syndromes in specimens of *P. agrestis* in accordance with their (slow or rapid) life history?

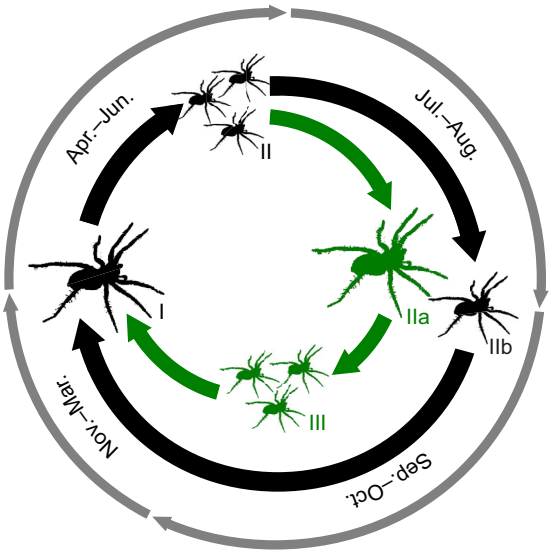

Fig. 1. Diagram depicting the yearly two-adult-peak phenology of *Pardosa agrestis*. The first adult peak (I) appears at spring/early summer, which produces the early summer (i.e. first) offspring generation (II). Some of the spiderlings from the early summer generation (II) will show rapid post-embryonic development and will mature during late summer (IIa), comprising a second peak of adults in the given year. These adults will reproduce and die before winter, but their offspring (III) will develop, overwinter and then mature in the next year. Meanwhile, the other group of spiderlings (IIb) will overwinter and mature in the next year. Figure modified from Rádai et al. (2017a,b).

To test the somewhat contradicting predictions of different aspects of life history theory, we conducted a study using the wolf spider *P. agrestis* as model organism by assessing a number of physiologically costly traits associated with condition and efficiency of somatic maintenance, and investigated whether the slow and rapid developmental types differ in these respects in the ways predicted by life history theory and the pace-of-life syndrome (POLS) hypothesis. We collected spiders from a natural habitat at both the early and late summer adult peaks, i.e. from slowly and rapidly developing sub-cohorts, respectively. To be able to test how developmental characteristics and life history traits are associated with one another within controlled conditions, we also reared spiderlings in the laboratory under two light cycle regimes that strongly affected developmental time and the occurrence of the rapidly developing phenotype itself. In both the wild-caught and

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laboratory-reared spiders, we assessed a number of important life history traits, namely adult size, fat reserves, cuticle melanisation, fecundity and immune parameters (see details in Materials and Methods, 'Tested traits'). Until now, studies using a composite approach to the individual-level consequences of cohort splitting have been rather scarce, and utilising both wild-caught and laboratory-reared individuals is arguably necessary for a more detailed understanding of this topic. Here, we aim to advance our understanding of the consequences of an enhanced developmental rate owing to cohort splitting by comparing several physiological and life history traits between spiders of slowly and rapidly developing sub-cohorts. Doing so, we wanted to test the hypotheses of high physiological costs of rapid development manifested as negative phenotypic associations between traits versus a 'big house, big car' scenario in which physiological costs (and hence negative phenotypic correlations) might be masked by good individual condition. Based on life history theory and the POLS hypothesis, we predicted that an enhanced developmental rate would bear substantial costs, manifested in decreased investment into somatic build-up and maintenance in rapidly developing spiders in comparison with slowly developing ones.

MATERIALS AND METHODS

Collection of spiders

Spiders were collected on multiple occasions, but always from the same population, in an uncultivated plot between Hajdúszoboszló and Nádudvar, Hungary (47°26′57.49″N, 21°18′01.96″E). Firstly, we collected 128 subadult individuals (penultimate larvae; 66 females and 62 males) between 21 March and 4 April 2017, and reared them until maturation in the laboratory. On 2 May 2017, we collected 111 cocoon-carrying females (i.e. females that mated, and carried the fertilised eggs in an egg sac). Later on we refer to specimens collected in March, April and May as slowly developing (spring) spiders. From the cocoon-carrying females collected in May, we randomly selected 12, the offspring of which we used in the first session of laboratory rearing (see 'Laboratory rearing sessions').

On 24 and 26 July 2017, we collected 43 subadult males and 47 subadult females, and reared them until maturity in the laboratory. We also collected a total of 92 cocoon-carrying females. In subsequent sections of this paper, we refer to spiders collected in July as rapidly developing (late summer) spiders. From the cocoon-carrying females, 19 spiders were randomly selected to provide spiderlings for the second laboratory rearing session (see 'Laboratory rearing sessions'). Over the course of our study we worked with a total of 902 spiders.

It should be noted that in the present study we termed spring adults as slowly developing spiders, although the whole spring/early summer adult generation is presumably a mixture of slowly developing spiders and the offspring of rapidly developing spiders, both from the previous year (see Introduction). In other words, it is possible that early summer spiderlings are composed of two types of spiders from the last year. To see whether this is so, we utilised model-based clustering using parametrised finite Gaussian mixture models (see Appendix), which revealed no bimodal distributions in prosoma size and melanisation (the two traits most influenced by developmental type; see Results) among the tested early summer spiders. This appears to indicate that either (i) there are no large, consistent differences in these traits between slowly developing spiders and the offspring of rapidly developing spiders, and/or (ii) owing to the early collection dates, mainly (or entirely) slowly developing spiders were collected.

Tested traits

The traits on which the effect of developmental rate was assessed in our study were adult body size, level of fat reserves, cuticular melanisation, levels of antimicrobial peptides and immunocompetence against gram-positive bacteria. Adult body size and fat content are generally considered as proxies of individual quality, especially in arthropods, where body size is less flexible owing to the rigid exoskeleton (Aisenberg and Peretti, 2011; Jakob et al., 1996; Kelly et al., 2014), and most often they are both positively correlated with survival and fecundity (Contreras-Garduño et al., 2006; Ellers and Alphen, 1997; Knapp and Uhnavá, 2014; Krams et al., 2011). Cuticular darkness, i.e. the extent of melanin incorporation in the cuticle, is an important characteristic in arthropods as well, found to be associated with developmental rate (Ma et al., 2008), fecundity (Ma et al., 2008; Roff and Fairbairn, 2013), and a number of immune-related traits (Bailey, 2011; Cotter et al., 2008; Rolff et al., 2005). Also, immunocompetence, as a vital part of somatic maintenance in multicellular organisms with the main role of upholding the somatic integrity of organisms, is firmly associated with fitness (Lee, 2006; Schmid-Hempel, 2003), and maintaining an efficient immune system bears considerable costs (Lochmiller and Deerenberg, 2000; Rauw, 2012; Sheldon and Verhulst, 1996). By measuring antimicrobial peptide levels (specifically cecropins and defensins, which are known to have important roles in invertebrate immunity; Hancock et al., 2006; Wu et al., 2018) and antibacterial activity against gram-positive bacteria, we wanted to assess the maintained level of immunocompetence, as we hypothesised that the costs of increased developmental rate in spiders of the rapidly developing cohort might interfere with maintenance costs of the humoral immune system (McKean and Lazzaro, 2011).

Laboratory rearing sessions

There were two laboratory rearing sessions, and in both of them we designed rearing conditions to match natural conditions as closely as possible, based on the methods described in previous publications (Kiss and Samu, 2002, 2005). In both rearing sessions, spiders were placed individually in plastic cups (ca. 25.5 cm^2 floor area, 4.5 cm wall height). Plastic cups (each containing only 1 specimen) were arranged in six large plastic boxes (60×40×20 cm in length, width and height). These large boxes were covered by non-transparent lids, and equipped with LED strips (Global JS-3528-30, 3000 K warm white, DC12V, 1000 mA) to provide light during the set daytime. In both rearing sessions we used two light-cycle regimes as treatments. In one of them, the length of the day cycle increased from 11 to 16 h over 11 weeks (mimicking early summer day length dynamics), while in the other group the day length decreased from 16 to 11 (mimicking late summer day lengths). By using these treatment groups we could manipulate the rate of the spiders' development, as it was shown previously that long day length promotes maturation among the spiders, while short day lengths appear to inhibit maturation (Kiss, 2003). Henceforward, we refer to the group receiving the increasing day length treatment as the 'rapid treatment group', and the spiders receiving the decreasing day length treatment as the 'slow treatment group'.

Each rearing session was divided into two phases. In the first phase, we started with 11 and 16 h day lengths for the rapid and slow treatment groups, respectively. One week after the start of the rearing experiment, we changed day lengths of the treatment groups once every week, increasing or decreasing day length by 30 min, according to the treatment. The first phase lasted for 11 weeks, and the day lengths converged to 16 and 11 h in the rapid and slow treatment groups, respectively. The second phase started 1 week

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after the last day length change of the first phase. During the second phase, we started to increase the day lengths in the slow treatment group by 30 min every week, increasing day length to 16 h, over 11 weeks. We did not alter the day length in the rapid group (i.e. day lengths were held constant at 16 h during the second phase). Applying the two rearing phases was necessary to evoke a developmental time difference (i.e. the appearance of slowly and rapidly developing spiders) in the laboratory.

All along both rearing sessions, spiders were provided water (wet cotton wool pieces) and food (springtails to first and second instar larvae, flightless fruit flies to larger spiders) *ad libitum*. Every 2 days we checked the spiderlings for exuvia (moults), during which we remoisturized the cotton wool, removed exuvia and dead prey, and delivered living prey for the spiders.

In the first session of laboratory rearing, we randomly selected 12 mated (i.e. cocoon carrying) spring females (from those collected in May 2017; see 'Collection of spiders'). Subsequently to the hatching of their offspring, we separated 24 randomly selected spiderlings from each female, i.e. we started the rearing with 288 spiderlings. Out of the 288 spiderlings, we randomly assigned 144 to the rapid treatment group, and the other 144 to the slow treatment group. From each mother, 12 offspring were assigned to each treatment group. Prior to all subsequent tests, we randomly assigned 72 spiders from each treatment group to the fat extraction treatment, and another 72 spiders from each treatment group to immune measurements. From the first day when a spider moulted to maturation, we collected all adult spiders that matured within a week, and used them for the designated tests (see 'Fat extraction' and 'Homogenate preparation' sections). The first rearing session lasted from 10 May to 25 November 2017. Henceforward we refer to this laboratory rearing session as the 'physiological test session', because spiders from this session were used for assessing physiological tests (see subsequent subsections). In the rearing laboratory, the temperature was 25.7±0.5°C (mean±s.d.) during the first rearing session.

The second rearing session was conducted to acquire precise data on the survival rate of spiderlings belonging to the different treatment groups, as we did not keep specific record of whether and when spiderlings perished throughout the physiological test session. In the following, we refer to the second laboratory rearing session as the 'survival test session', because spiders belonging to this session were reared to assess pre-maturation mortality patterns. From each of the 19 collected mated females, we selected an arbitrary number of spiderlings (15.6±7.4, mean±s.d., range: 4–33), resulting in 297 spiderlings. These spiderlings were randomly assigned to increasing and decreasing day length treatment groups, in a way so that from each mother, half of the selected spiderlings were assigned to the increasing, and the second half to the decreasing day length rearing group. The second rearing session lasted from 30 July 2018 to 8 January 2019. The rearing room temperature was 24.5±2.0°C (mean±s.d.) during the survival test session.

Fat extraction

Before fat extraction, the body mass of the living spiders was measured with accuracy to the tenth of a milligram. Subsequently, spiders were euthanised in −20°C. Fat extraction was performed according to the methodology of Aisenberg and Peretti (2011). Briefly, spiders were placed in 1.5 ml microtubes individually, and dried in a drying box at 60°C for 24 h. After that, we weighed the dried spiders (first dry mass), then pipetted 1 ml of chloroform on them, in which they were suspended for another 24 h. Following the chloroform soak, we disposed of the used chloroform and dried the spiders again for 24 h at 60°C. Finally, we measured again the dry mass of the spiders (second dry mass). For later analyses, we calculated the fat content of the body as the difference between the two dry masses, divided by the living mass. When fat extraction was complete, we put spiders in 0.5 ml 96% ethanol and stored them in −20°C for later measurements.

Homogenate preparation

For immune measurements, spiders were euthanised in −20°C, and their opisthosomas were homogenised in 50 µl of sterile, ice-cold 0.1 mol l^{-1} Sörensen buffer (pH 7.4). Spider prosomas were stored in 0.5 ml 96% ethanol, at −20°C, for later measurements. We stored homogenates on ice until centrifugation, and we centrifuged samples in 4°C at 10,000 ***g*** for 10 min. Subsequently, 2×20 µl supernatant was pipetted into separate microtubes: one for ELISA, and another for bacterial growth inhibition and cell wall lysis assays. Samples were stored at −70°C until tests.

Prosoma measurements

Prior to measurements, we dried prosomas on filter paper and gently removed the legs in order to make positioning easier and repeatable across specimens. Then, we placed the prosomas separately on a millimetre grid, next to a white standard photo paper and photographed them using a light box to ensure standard and repeatable light conditions. We used a Canon EOS 550D, with a Tamron 90 mm f/2.8 macro lens. On the pictures, we measured prosoma width as the widest segment along the dextro-sinister axis, using ImageJ (version 1.46a; Schneider et al., 2012). The extent of prosoma cuticular melanisation of the dorsal prosoma was evaluated by measuring the median grey value of the dorsal surface of the prosoma. In later analyses, we used a calculated estimate of cuticular darkness, with a range from 0 to 1, as:

$$1-\frac{(G-G_{\min})}{(G_{\max}-G_{\min})}, \quad (1)$$

where G is the median of grey pixel values of a given spider (ranging between 0 for black and 255 for white), and $G_{\min}$ and $G_{\max}$ are the smallest (minimum) and largest (maximum) of these medians across all individuals measured, respectively. In the analyses and results, we refer to this measure as the prosoma darkness, and it reflects the relative prosoma darkness of individuals compared with the most melanised specimen (having a prosoma darkness value equal to 1): values close to 1 indicate dark cuticle, while values close to 0 indicate brighter cuticle.

To make sure that all of the photos from all spider groups (spring, late summer, laboratory-reared increasing and decreasing day length group) are indeed comparable in their brightness, we randomly chose 10 photos from each group, measured the median grey value of a rectangle on the white standard paper (each rectangle was approximately the same size as the prosoma of a spider), and used the Conover–Iman test (https://cran.r-project.org/web/packages/conover.test/index.html) with Bonferroni's P-value correction to determine whether they vary substantially. We did not find significant differences between the white standard measures across the four photographing occasions (P>0.127 for all group-by-group comparisons).

Immune measures: cecropin B and β-defensin content

For cecropin B and β-defensin content, an indirect ELISA technique was applied, according to standard protocol (Crowther, 2009). Because of the small volume of some samples, we were not able to measure both proteins from all of the samples; therefore, for some

spiders the measurements of β-defensin were not feasible. Briefly, the wells of 96-well transparent flat-bottom plates (Corning; a separate plate for each antigen) were filled with 100 µl of supernatant samples of known protein concentration and incubated overnight. The potentially uncoated areas of the plates were then blocked with 1% bovine serum albumin (Sigma-Aldrich; 100 µl 1 h, 37°C). Anti-cecropin B and anti-β-defensin primary antibodies (anti-cecropin-B produced in rabbit, ab27571, Abcam; anti-β-defensin 3 produced in rabbit, D2444, Sigma-Aldrich; 100 µl, 1:1000, 2 h at 37°C) and then secondary antibody (goat anti-rabbit IgG polyclonal antibody, alkaline phosphatase conjugate, ADI-SAB-301-J, Enzo; 100 µl, 1:1000; 1 h at 37°C) were subsequently used. Between each application, the wells were washed four times with 0.05% Tween-20 detergent in Soerensen buffer, pH 7.4. Finally, after washing, the secondary antibody was replaced by 100 µl para-nitrophenylphosphate (Sigma-Aldrich) solution in 10 mmol l^{-1} diethanolamine buffer (pH 9.5, 0.5 h, room temperature). Primary and secondary antibodies as well as original homogenates were dissolved in protease inhibitor buffer (0.1 mol l^{-1} Sörensen buffer, pH 7.4; 100 ml buffer containing 32.5 mg sodium-azide, 140 µl β-mercaptoethanol, 4 mg phenylmethylsulfonyl fluoride). The contents of cecropin B and β-defensin were assessed spectrophotometrically at 405 nm using a Tecan Infinite M200 microplate reader. The contents of the detected proteins were expressed as absorbance values, which are proportional to the antigen contents in the samples. The comparability of the results was guaranteed by the application of the same total protein content per well for each sample and each antigen. For measuring total protein concentration of original samples, we used the modified Bradford method described by Ernst and Zor (2010).

Immune measures

Bacterial growth inhibition assay

A bacterial growth inhibition assay was used based on the methodology used by Castella et al. (2010). Briefly, we prepared a medium containing 1 g of bacto-tryptone, 1 g NaCl, 0.5 g yeast extract and 1 g agar in 100 ml distilled water. Prior to pouring the agar into Petri dishes, 50 µl from a 2.6×10^8 cells ml^{-1} suspension of *Micrococcus luteus* (obtained from the stock culture of the Department of Microbial Biotechnology, University of Debrecen) was added and mixed carefully. We used *M. luteus* because of its availability, and because it is frequently present in soil (Biskupiak et al., 1988; Sims et al., 1986); as *P. agrestis* is an epigeic species, it may often encounter *M. luteus* strains. We pipetted 2 µl from each spider's homogenate sample on the surface of the thin agar layer (using 6 ml of agar in a Petri dish of 100 mm diameter), in duplicates from each spider. Duplicates were placed in the same Petri dish in the case of all samples. Following the application of samples on the Petri dishes, they were incubated at 30°C for 24 h. Samples containing antimicrobial peptides inhibit bacterial growth; therefore, after an incubation period, when bacteria grows homogeneously on the surface of the agar, empty zones (so called inhibition zones) will persist where antimicrobial peptide containing samples were applied. The area of inhibition zones is proportional to the antimicrobial peptide content of the samples (Castella et al., 2010). Following the incubation, all Petri dishes were photographed (Figs S1-S4), and the areas of inhibition zones were measured using ImageJ.

We quantified the bacterial growth inhibition capacity for the analyses as follows. After measuring inhibition zone areas, we also measured the area of the given Petri dish. This was necessary because photographing was done from slightly different distances with respect to the Petri dishes, i.e. Petri dish areas covered different numbers of pixels, hence inhibition zones were not directly comparable. To correct for this, we divided inhibition zones (number of pixels within the zone) by the area of the given Petri dish (number of pixels within the whole Petri dish; Petri dish area was measured separately for each Petri dish photo, and the Petri dishes themselves were of the same size). Because all spider opisthosomas were homogenised in the same volume of buffer solution (i.e. larger spiders yielded more concentrated samples), this quantity was divided by the living mass of the spider which the given inhibition zone belonged to. This calculation resulted in a variable that is likely closely proportional to the bacterial growth inhibition power per milligram body mass. Finally, we centred this variable at zero by subtracting the mean of the variable from all values, and we scaled values by dividing them by the standard deviation of the variable (i.e. we performed mean centring and SD scaling). In later analyses, we used this variable as a quantification of bacterial growth inhibition power.

Cell wall lysis assay

Bacterial cell wall lysis assay was done based on the methodology described in Castella et al. (2010). We prepared a cell wall containing agar medium (1 g agar in 100 ml distilled water) with 5 mg ml^{-1} *Micrococcus lysodeikticus* (lyophilised *M. lysodeikticus*, ATCC no. 4698, Sigma-Aldrich), and 0.6 mg ml^{-1} ampicillin sodium. In this method, cell wall residues are homogeneously distributed in the agar, creating a thin, opaque layer. Samples containing lysozyme produce transparent clearing zones on the agar, and the area of clearing zones is proportional to the lysozyme content of the sample (Castella et al., 2010). We poured 5 ml of this medium into each Petri dish (100 mm in diameter) and applied 2 µl from each homogenate sample on the surface of the cell wall agar, in duplicates from each spider. Duplicates were placed in the same Petri dish in the case of each sample. Petri dishes were then incubated at 30°C for 24 h. Following the incubation, all Petri dishes were photographed, and the areas of clearing zones were measured using ImageJ. In later analyses we used a variable derived from the clearing zone the same way as in the case of bacterial growth inhibition (see above).

Statistical analyses

Statistical analyses were performed using R statistical software (version 3.5.2, https://www.r-project.org/), mainly with linear regression modelling. Some parameter estimates for variables in interaction that were not directly available from model result summaries were obtained by calculating estimated marginal means (EMMs) with the R package emmeans (https://cran.r-project.org/web/packages/emmeans/index.html). Non-significant interactions ($P\geq0.100$) were removed from the final models, and the reported estimates in the Results come from the reduced final models.

Prior to statistical tests, we visually checked grouped data and associations to be tested in order to identify potential outliers or anomalies. There was one anomalous data point in the bacterial growth inhibition measurements (value more than four standard deviations from the median) among the spiders from natural habitat (causing downward bias in effect size of male group, and upward bias in the standard error estimation for this group effect, rendering it non-significant), which was excluded from the analyses (Ruan et al., 2005).

We analysed data from natural and laboratory-reared spiders separately, mainly using linear regression models (LMs). In the analyses of spiders collected from the field, the used response variables were size (as prosoma width), fat content (proportion of

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fat in living mass), prosoma darkness (level of melanisation), levels of cecropin B and β-defensin, bacterial growth inhibition and cell wall lytic activity. In the models, we used season (spring or late summer) and status (male, virgin female or mated female) as explanatory (predictor) variables, except in the models on size and prosoma melanisation, in which we simply used sex instead of status (because female reproductive status is neither expected nor found to affect these traits; for all virgin versus mated female EMM, $P>0.110$). The interaction between the predictors was also included in the models. In an additional model, we tested how season affects female fecundity (number of offspring) using season and size as predictors. Season strongly affected size (see Results), but as a result, the interaction between season and size was not significant (i.e. regression slopes of size on fecundity did not differ between seasons); this case of correlation between predictors does not bias the results, nor does it cause interpretational problems. To confirm whether a fecundity difference is simply due to body size differences between slowly and rapidly developing females, we estimated their fecundity per size unit (i.e. we divided the number of offspring by body size), and used this as response variable in a simple linear regression model (LM), with season as a predictor.

In the analyses of spiders reared for physiological tests, we used linear mixed-effects models (LMMs; Kuznetsova et al., 2017) to control for kinship between spiders by defining the ID of mother spiders as a random effect. Firstly, we tested the effect of rearing treatment group on developmental time (specified as days from hatching to maturation) by fitting a model with developmental time as the response variable, and rearing group (slow or rapid treatment), sex (female or male) and their interaction as explanatory variables. Subsequently, we fitted an LMM in which growth rate (specified as adult size divided by number of moults, representing average increase in size per moulting) was the dependent variable, and treatment group, sex and their interaction were explanatory variables. In addition, we used a generalized LMM (GLMM; Bates et al., 2015) with Poisson error distribution to test whether spiders in the rapid and slow treatment groups differ in the number of moults (i.e. number of moults was the response variable, and rearing treatment was the explanatory variable), also controlling for sex, and the interaction of sex and developmental treatment.

Secondly, we fitted separate mixed-effects models using prosoma size, fat content, prosoma darkness, levels of cecropin B and β-defensin, bacterial growth inhibition and cell wall lytic activity as response variables. In the model on prosoma size, explanatory variables were rearing treatment, developmental time and sex, and the two-way interactions between the predictors. Because developmental time and rearing treatment were strongly correlated, we re-centred developmental time at zero within treatment groups by subtracting the mean developmental time of groups from all values within treatment groups, to eliminate the correlation between the explanatory variables. Developmental time was used in the linear regression on prosoma size instead of growth rate, because growth rate itself is a variable derived from prosoma size. In the other models, explanatory variables were rearing treatment, growth rate (re-centred within treatment groups) and sex. In these models we also included the interaction terms between rearing treatment and sex, rearing treatment and growth rate, and growth rate and sex.

Using the data from the survival test session, we tested how rearing treatment affected maturation incidence and mortality, by fitting mixed-effects Cox regression models (https://cran.r-project.org/web/packages/coxme/index.html) (using mother spiders' ID variable as random effect). We chose Cox regression instead of linear regression because (unlike among the spiders in the physiological test session) there were observations from non-mature specimens as well, and proportional hazards models can handle data in which some events and corresponding times (maturation and developmental time, respectively) are not observed. In order to be able to differentiate between juvenile and adult mortalities, also we fitted two additional binomial GLMMs, testing whether developmental treatment group affected the probability of dying before maturation: in the first binomial GLMM, we used the total mortality data from the survival test session, while in the second we used a subset, including only those spiders that died over the course of the rearing (i.e. as juveniles). Sex was not included as a co-factor either in the Cox models or in the GLMMs because identification of sex was not possible for juveniles.

RESULTS

Spiders collected from natural habitat

We found that slowly developing spiders are larger and have darker prosoma (although this difference was smaller, yet still significant, among males) and, although only among females (both virgin and mated), have more fat reserves (Table 1, Fig. 2A–C). Also, slowly developing females had much higher fecundity in comparison to rapidly developing females, having approximately 1.7 times more offspring on average [39.0±9.2 and 22.9±8.5 (mean±s.d.) offspring, for average sized slowly and rapidly developing females, respectively; Table 1, Fig. 2D]. Fecundity per body size unit was also significantly higher in slowly developing females than in rapidly developing females (LM: β=6.380, s.e.=0.964, t=6.62, $P<0.001$), indicating that developmental phenotype difference in fecundity was not exclusively due to differences in body size.

Table 1. Results from linear regression models on condition and fecundity, fitted using data from the spiders collected from natural habitat

Explanatory variables	Coefficients	s.e.	t	P
Size (prosoma width), n=385				
Intercept (slow development, female)	2.116	0.009	235.86	<0.001*
Developmental type (rapid)	−0.243	0.013	−18.35	<0.001*
Sex (male)	−0.100	0.014	−6.90	<0.001*
Fat content, n=97				
Intercept (slow development, virgin female)	0.085	0.005	18.77	<0.001*
Developmental type (rapid)	−0.015	0.006	−2.44	0.016*
Status (mated female)	−0.026	0.006	−4.23	<0.001*
Status (male)	−0.047	0.007	−7.07	<0.001*
Developmental type×Status (rapid, mated female)	−0.002	0.008	−0.20	0.841
Developmental type×Status (rapid, male)	−0.017	0.009	−1.84	0.068(*)
Prosoma darkness, n=385				
Intercept (slow development, female)	0.779	0.007	113.83	<0.001*
Developmental type (rapid)	−0.382	0.011	−33.87	<0.001*
Sex (male)	0.391	0.017	23.63	<0.001*
Developmental type×Sex (rapid, male)	−0.257	0.021	−12.06	<0.001*
Fecundity, n=80				
Intercept (slow development)	−52.859	15.307	−3.45	<0.001*
Developmental type (rapid)	−6.466	2.357	−2.74	0.008*
Size	43.772	7.262	6.03	<0.001*

Asterisks mark statistically significant effects ($P<0.05$); asterisks in parentheses mark marginally significant effects ($0.05<P<0.10$).

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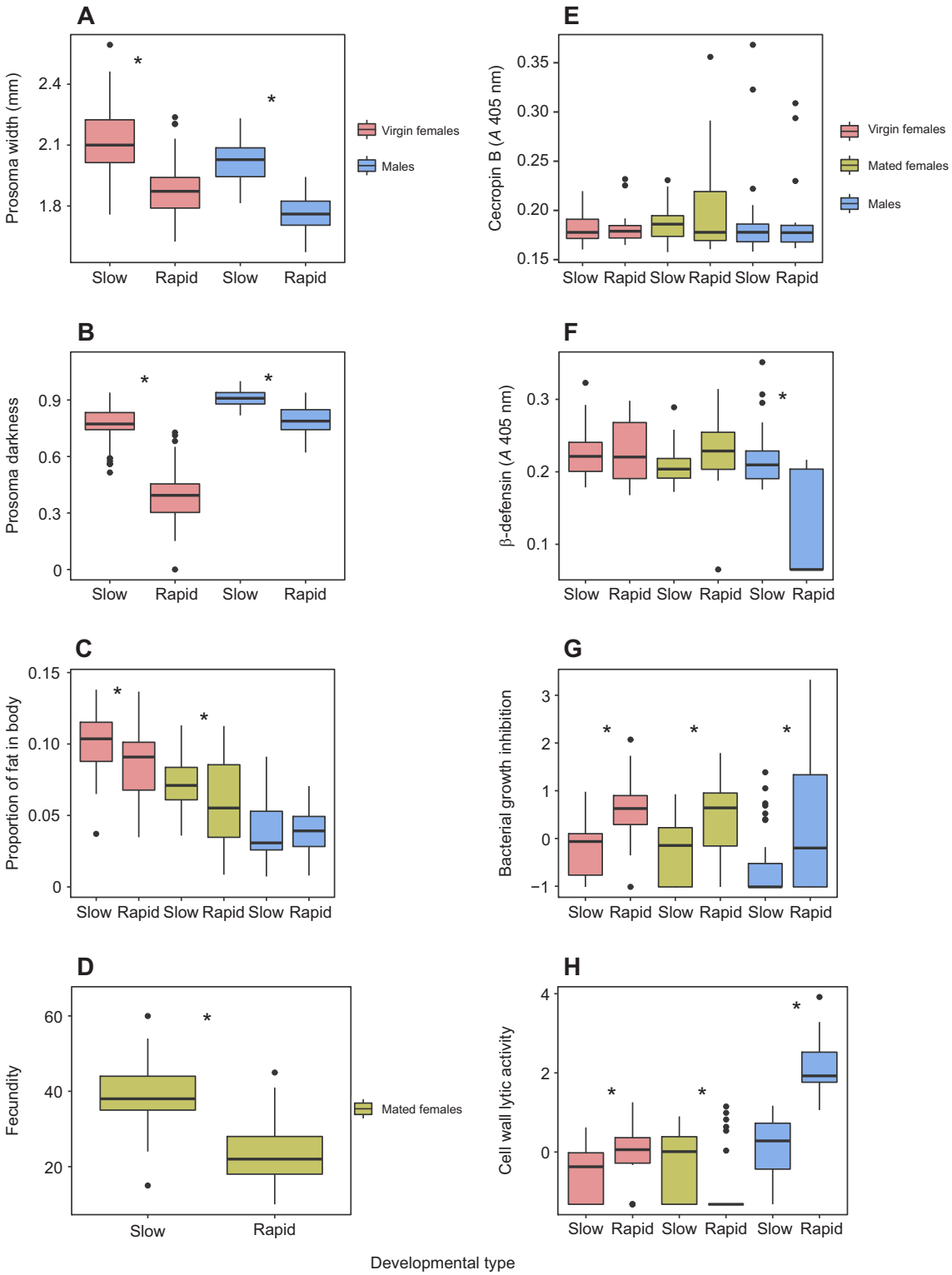

Fig. 2. Box plots visualising distributions of observed values for the assessed traits in slowly and rapidly developing spiders, collected from the natural habitat. Assessed traits, arranged from top-left to bottom-right, are (A) prosoma width, (B) extent of prosoma melanisation (prosoma darkness), (C) percentage of fat in the body, (D) fecundity, (E) level of cecropin B, (F) level of β-defensin, (G) bacterial growth inhibition and (H) cell wall lytic activity. Asterisks mark significant differences between developmental types within sexes (P<0.05). Vertical whiskers show minimum and maximum ranges, boxes represent the interquartile range between the first and third quartiles, horizontal solid lines show the median of the given value distribution, and individual dots represent outlier values.

There were no significant differences in cecropin B levels between sub-cohorts (Table 2, Fig. 2E). In males, rapidly developing spiders showed lower β-defensin levels compared with slow ones (EMM: β=−0.096, s.e.=0.018, t=−5.32, P<0.001), but we found no such difference in females (Fig. 2F). Unexpectedly, slowly developing spiders had weaker bacterial growth inhibition

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Table 2. Results from linear regression models on immune parameters, fitted using data from the spiders collected from the natural habitat

Explanatory variables	Coefficients	s.e.	*t*	*P*
Cecropin B, *n*=215				
Intercept (slow development, virgin female)	0.179	0.004	44.43	<0.001*
Developmental type (rapid)	0.007	0.004	1.53	0.129
Status (mated female)	0.008	0.005	1.74	0.083(*)
Status (male)	0.007	0.005	1.25	0.212
β-defensin, *n*=181				
Intercept (slow development, virgin female)	0.227	0.006	38.33	<0.001*
Developmental type (rapid)	−0.001	0.010	−0.06	0.949
Status (mated female)	−0.020	0.008	−2.69	0.008*
Status (male)	−0.009	0.009	−0.94	0.348
Developmental type×Status (rapid, mated female)	0.022	0.014	1.62	0.108
Developmental type×Status (rapid, male)	−0.095	0.021	−4.57	<0.001*
Bacterial growth inhibition, *n*=210				
Intercept (slow development, virgin female)	−0.229	0.112	−2.05	0.041*
Developmental type (rapid)	0.801	0.116	6.90	<0.001*
Status (mated female)	−0.106	0.132	−0.80	0.423
Status (male)	−0.350	0.147	−2.38	0.019*
Cell wall lytic activity, *n*=211				
Intercept (slow development, virgin female)	−0.517	0.126	−4.10	<0.001*
Developmental type (rapid)	0.483	0.204	2.37	0.019*
Status (mated female)	0.239	0.158	1.51	0.132
Status (male)	0.563	0.180	3.13	0.002*
Developmental type×Status (rapid, mated female)	−1.094	0.268	−4.08	<0.001*
Developmental type×Status (rapid, male)	1.616	0.294	5.50	<0.001*

Asterisks mark statistically significant effects (P<0.05); asterisks in parentheses mark marginally significant effects (0.05<P<0.10).

than rapidly developing spiders (Table 2, Fig. 2G). The effect of developmental type and sex was a bit more complex in cell wall lytic activity, as males and virgin females of the slowly developing sub-cohort were characterised by smaller values than in the rapid sub-cohort, while cell wall lytic activity of rapidly developing mated females was dramatically lower than in slowly developing mated females (and, in fact, in any other group; Fig. 2H).

Laboratory-reared spiders

Physiological test session

In total, 218 spiders matured in the first laboratory rearing session (119 and 99 spiders in the rapid and slow treatment groups, respectively). Rearing photoperiod treatments had strong impact on both developmental time and growth rate: spiders reared in rapid treatment group matured in 84.0±25.2 days (mean±s.d.) on average, while spiders in the slow treatment group reached adulthood in 125.7±20.3 days (mean±s.d.), an almost 50% increase (Table 3, Fig. 3D). By the end of the first phase of the rearing, 4% of spiders in the slow group matured (n=4), while 62% of the spiders in the rapid group reached maturity (n=74). Treatment group did not affect significantly the number of moulting, and sexes did not differ in the mean number of moults (Table 3). Males were smaller, were characterised by much darker prosomas and had lower fat content than females (Fig. 3A–C, Table 3). Additionally, males matured sooner than females in the slow treatment group (EMM: β=12.021, s.e.=4.250, t=2.83, P=0.005), but not in the rapid treatment group (EMM: β=0.756, s.e.=3.850, t=0.20, P=0.845).

Spiders in the rapid treatment group did not differ in size from specimens in the slow treatment group (Fig. 3A). Females in the rapid treatment group were brighter (i.e. had less melanised cuticle) than females in the slow treatment group (EMM: β=0.061, s.e.=0.018, t=3.31, P=0.001), while there was no such difference in males (EMM: β=0.009, s.e.=0.019, t=0.46, P=0.648; Fig. 3B). Growth rate itself was positively associated with prosoma melanisation in both treatment groups (Table 3). In the rapid treatment group, spiders showed marginally significantly higher fat content than those in the slow treatment group (Fig. 3C). Also, growth rate was positively associated with fat content in both groups (Table 3).

No significant differences were found between treatment groups either in β-defensin levels (Fig. 3F) or in bacterial growth inhibition power and cell wall lytic activity (Table 4, Fig. 3G,H). However, the cecropin B levels in spiders reared in the rapid treatment group were significantly lower than in the slow treatment group (Table 4, Fig. 3E).

Survival test session

In total, 212 spiders matured in the second laboratory rearing session, 121 and 91 in the rapid and slow treatment groups, respectively. Throughout the entire rearing session, mortality among the rapid treatment group was higher than in the slow treatment group (Cox regression: β=0.805, s.e.=0.150, z=5.36, P<0.001), but this was due to the fact that spiders matured much sooner in this group (Cox regression: β=1.149, s.e.=0.143, z=8.03, P<0.001), and hence had shorter life cycles and perished as adults sooner than those developing slowly. Indeed, there was no difference between rearing treatment groups in likelihood of dying during the first rearing phase (Cox regression: β=−0.204, s.e.=0.345, z=−0.59, P=0.560). Even more surprisingly, based on the binomial GLMMs, probability of perishing before maturation was lower in the rapid treatment group than in the slow treatment group, both among the total reared population (binomial GLMM: β=−0.809, s.e.=0.277, z=−2.92, P=0.004) and among those that died during the rearing session (binomial GLMM: β=−1.982, s.e.=0.331, z=−5.99, P<0.001).

DISCUSSION

In our study, we quantified a number of important traits in slowly and rapidly developing sub-cohorts of *P. agrestis* to assess what physiological consequences might be associated with rapid post-embryonic development. Life history theory and the POLS hypothesis predicts that a substantially increased developmental (and growth) rate would incur high physiological costs, e.g. in the form of negative phenotypic covariance between costly traits utilising the same resource pool. It is also predicted by theory that these costs would translate into fitness costs (i.e. decreased survival or effective reproductive success), which appear to be rather rational assumptions, because if no costs were associated with such an enhanced developmental rate, it would be likely to invade, and become fixed in, the populations.

Our results are multifarious, as we have found some evidence implying physiological costs associated with the rapid developmental cohort, while in some traits we found no consistent effect of developmental type, or even found a positive link with rapid development. One of the most prominent differences between slowly and rapidly developing spiders was apparent in their cuticular darkness, as rapidly developing specimens had brighter prosomas, both among natural and laboratory-reared spiders (although in the latter the difference was milder, and statistically significant only in

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Table 3. Results from linear regression models on development and condition, fitted using data from the physiological test session

Explanatory variables	Coefficients	s.e.	*t*	*P*
Developmental time, *n*=218				
Intercept (slow treatment, female)	130.798	4.208	31.08	<0.001*
Rearing treatment (rapid treatment)	−46.134	3.976	−11.60	<0.001*
Sex (male)	−12.021	4.242	−2.83	0.005*
Rearing group×Sex (rapid treatment, male)	11.265	5.733	1.97	0.050*
Growth rate, *n*=218				
Intercept (slow treatment, female)	0.321	0.006	57.23	<0.001*
Rearing treatment (rapid treatment)	0.013	0.006	2.32	0.022*
Sex (male)	−0.008	0.006	−1.48	0.140
Size (prosoma width), *n*=218				
Intercept (slow treatment, female)	2.182	0.0196	111.10	<0.001*
Rearing treatment (rapid treatment)	0.002	0.015	0.12	0.908
Developmental time (re-scaled within treatment groups)	0.001	0.0005	2.51	0.013*
Sex (male)	−0.181	0.015	−11.79	<0.001*
Sex×Developmental time (male)	−0.002	0.0007	−2.93	0.004*
Fat content, *n*=109				
Intercept (slow treatment, female)	0.084	0.004	21.65	<0.001*
Rearing treatment (rapid treatment)	0.007	0.004	1.82	0.072(*)
Growth rate (re-scaled within treatment groups)	0.100	0.041	2.43	0.017*
Sex (male)	−0.020	0.004	−5.49	<0.001*
Prosoma darkness, *n*=218				
Intercept (slow treatment, female)	0.708	0.021	33.05	<0.001*
Rearing treatment (rapid treatment)	−0.061	0.018	−3.31	0.001*
Growth rate (re-scaled within treatment groups)	0.434	0.165	2.63	0.009*
Sex (male)	0.104	0.019	5.35	<0.001*
Rearing treatment×sex (rapid treatment, male)	0.052	0.026	1.96	0.052(*)

Asterisks mark statistically significant effects (P<0.05); asterisks in parentheses mark marginally significant effects (0.05<P<0.10).

females). Melanin incorporation into the cuticle has multifaceted roles. For example, in arthropods, the level of cuticular melanisation is associated with the efficiency of thermoregulation and water retention (Rajpurohit et al., 2008; Watt, 1969). Also, thermal regime-dependent changes in cuticle melanisation are often associated with changes in the efficiency of melanin production-related elements of the immunity, hinting at a positive association between cuticular melanin content and pathogen resistance (Fedorka et al., 2013; Kutch et al., 2014). Indeed, in mealworm beetles, heavily melanised individuals were found to be able to mount a stronger melanotic encapsulation response upon repeated immune activation, and the simulated parasite attack had a much less detrimental impact on other life history traits, such as fecundity or longevity, implying a positive association between condition and cuticle melanisation (Krams et al., 2016). Brighter prosomas among rapidly developing spiders might indicate that the level of melanin production and incorporation in the cuticle is depressed in them. Melanin production was shown to have considerable costs in arthropods (Ethier et al., 2015; Hooper et al., 1999; Roff and Fairbairn, 2013; Siva-Jothy, 2000; True, 2003; but see Roulin, 2016), and negative phenotypic correlations between cuticular melanisation and developmental rate, along with other traits, were found by several studies in arthropods (Busso et al., 2017; Cotter et al., 2008; Ma et al., 2008). Lower melanin content of the prosomal cuticle could be a result of an energetic or physiological conflict between increased developmental rate and melanin production. Presumably, lower availability of precursor molecules, or lower expression and/or activity of contributing enzymes, such as phenoloxidase, might be at play behind this pattern (Armitage and Siva-Jothy, 2005; Bailey, 2011; Thompson et al., 2002). It also may be possible that in rapidly developing spiders, the timespan during which melanin is produced and incorporated into the new cuticle (prior to the moulting) is simply shorter, hence there is less time to build melanin into the cuticle. Interestingly, however, among laboratory-reared spiders, growth rate (increase in body size per moults) was positively associated with prosoma melanisation, and in fact, the difference in melanisation between rapid and slow treatment group spiders was quite mild. These results might imply that an increased rate of building up the soma is associated with an increased rate of melanin-incorporation into the prosomal cuticle, but when resource availability is limited the production of melanin is more restricted, hence the prosoma darkness is substantially lower in rapidly developing spiders from the natural habitat. Notably, the thermal environment may also have contributed to the milder developmental group differences in prosoma melanisation among the laboratory-reared spiders. The hypothesis of thermal melanism predicts that melanin incorporation into the cuticle will increase in colder environments; therefore, a stable, warm environment might have reduced cuticle melanisation in laboratory-reared spiders (Fedorka et al., 2013). However, when comparing the prosoma darkness measurements between laboratory-reared and wild-caught spiders, we did not find generally lower melanisation among laboratory-reared spiders (Table A1).

Notably, in a previous study, we found evidence for a negative association between developmental rate and a melanin-based immune function in *P. agrestis*, as the time from hatching to maturation was positively correlated with the extent of melanotic encapsulation of a nylon monofilament (Rádai et al., 2018). Although we do not have information about how cuticular darkness and encapsulation efficiency are associated in *P. agrestis*, evidence from other arthropods suggests that they could be positively correlated (Bailey, 2011; Fedorka et al., 2013; Krams et al., 2016; Prokkola et al., 2013), providing further (although indirect) support for the common physiological mechanisms of cuticle melanisation and encapsulation, which may be in energetic conflict with developmental rate.

In other arthropods, not only cuticle melanisation, but also adult size was found to be negatively correlated with developmental rate (Ma et al., 2008; Windig, 1999). Similarly, we found that rapidly

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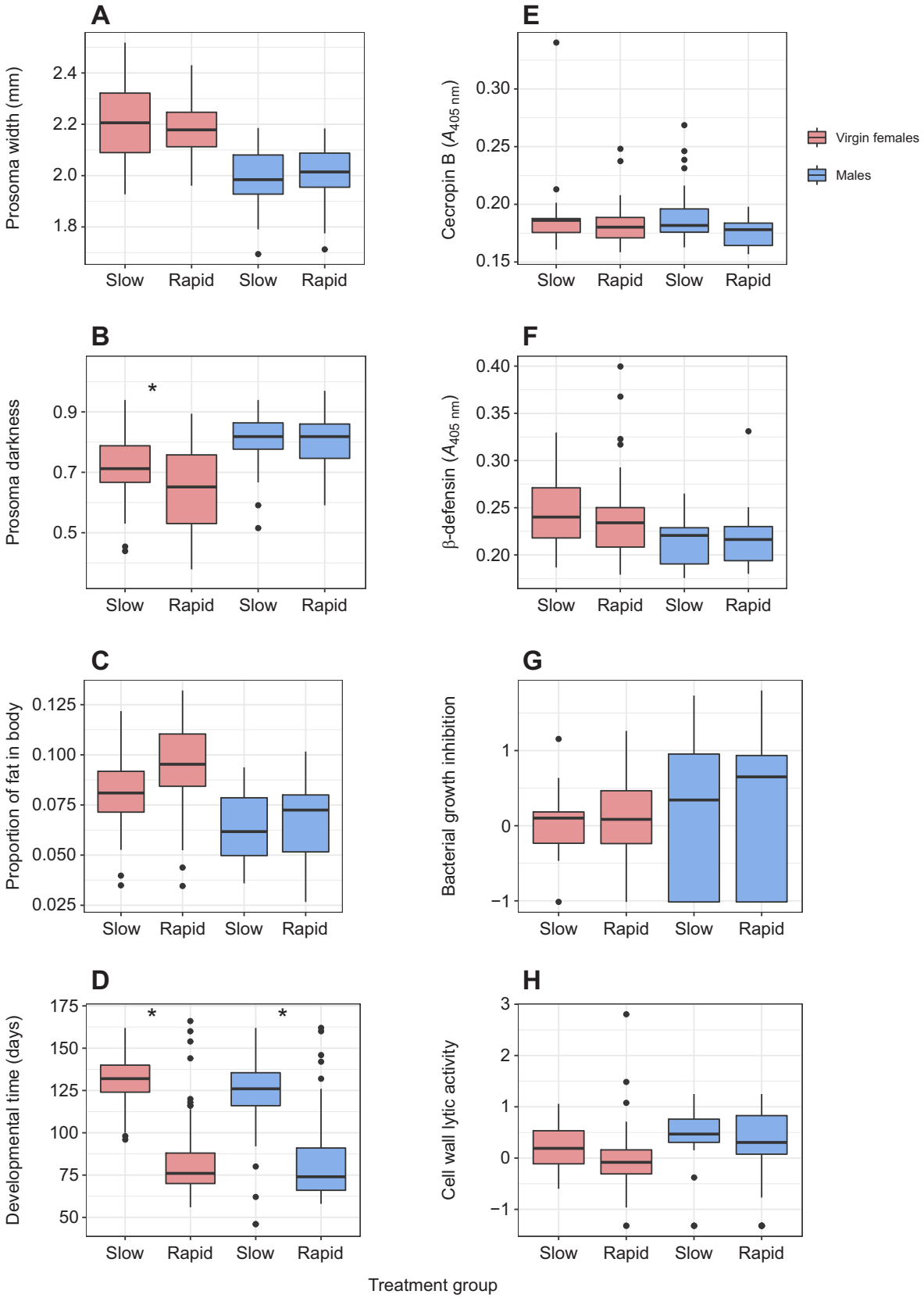

Fig. 3. Box plots visualising distributions of observed values for the assessed traits of spiders in the slow and rapid treatment groups, reared in laboratory (physiological test session). Assessed traits, arranged from top-left to bottom-right, are (A) prosoma width, (B) extent of prosoma melanisation (prosoma darkness), (C) percentage of fat in the body, (D) developmental time as days from hatching to maturation, (E) level of cecropin B, (F) level of β-defensin, (G) bacterial growth inhibition and (H) cell wall lytic activity. Asterisks mark significant differences between developmental treatment groups within sexes (P<0.05). Vertical whiskers show minimum and maximum ranges, boxes represent the interquartile range between the first and third quartiles, horizontal solid lines show the median of the given value distribution, and individual dots represent outlier values.

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Table 4. Results from linear regression models on immune parameters, fitted using data from the physiological test session

Explanatory variables	Coefficients	s.e.	*t*	*P*
Cecropin B, *n*=108				
Intercept (slow treatment, female)	0.194	0.005	42.08	<0.001*
Rearing treatment (rapid treatment)	−0.013	0.005	−2.62	0.010*
Growth rate (re-scaled within treatment groups)	−0.019	0.064	−0.30	0.767
Sex (male)	−0.005	0.005	−0.98	0.329
β-defensin, *n*=95				
Intercept (slow treatment, female)	0.244	0.008	29.97	<0.001*
Rearing treatment (rapid treatment)	−0.001	0.008	−0.15	0.885
Growth rate (re-scaled within treatment groups)	−0.078	0.115	−0.68	0.501
Sex (male)	−0.027	0.008	−3.27	0.002*
Bacterial growth inhibition, *n*=108				
Intercept (slow treatment, female)	0.110	0.173	0.64	0.528
Rearing treatment (rapid treatment)	−0.029	0.149	−0.20	0.844
Growth rate (re-scaled within treatment groups)	−2.662	2.099	−1.27	0.207
Sex (male)	0.067	0.148	0.45	0.651
Cell wall lytic activity, *n*=109				
Intercept (slow treatment, female)	0.168	0.143	1.18	0.242
Rearing treatment (rapid treatment)	−0.183	0.149	−1.23	0.221
Growth rate (re-scaled within treatment groups)	0.021	1.950	0.01	0.992
Sex (male)	0.185	0.147	1.26	0.211

Asterisks mark statistically significant effects (P<0.05).

developing spiders collected during late summer had smaller prosomas than slowly developing spiders collected in spring. This result seems to imply that the costs of a higher developmental rate precluded building up the same body size as in slowly developing specimens. For rapidly developing specimens to achieve the same size at maturation as slowly developing ones, growth rate (i.e. somatic tissue built in a given time interval) has to be substantially increased (Dmitriew, 2011). Of course, increased growth rate may be apparent not just when rapidly and slowly developing spiders have the same adult size; in fact, rapidly developing spiders with relatively higher growth rate may still achieve smaller body size given that their time period of growth is considerably shorter. Increased developmental and growth rates are both often negatively associated with adult body size (Blanckenhorn, 1998; Gotthard et al., 1994), and energetic (i.e. resource allocation) limitations play a key role in these relationships (Fischer et al., 2005). In the case of spiders reared in the laboratory, we did not find differences in body size between spiders in the rapid and slow treatment groups, but growth rate was considerably higher in rapidly developing spiders than slow ones. This suggests that in the laboratory, spiders could reach high growth rates, by which they were able to attain adult sizes similar to those of slowly developing specimens. Because food availability was not limited during rearing, it seems to be plausible that rapidly developing laboratory-reared spiders might have been able to achieve adult body sizes similar to those of slowly developing spiders owing to increased acquisition of resources. Indeed, in a previous study, we found that rapidly developing spiders are more voracious foragers (Rádai et al., 2017a), which, combined with unconstrained food availability, might have helped rapidly developing spiders in the laboratory to grow to a similar body size as slowly developing specimens. When resources are less limited, enhancement of both developmental and growth rates may be possible without trading them off with adult size (Dmitriew and Rowe, 2005). This is a quite important result, because it reflects that rapidly developing spiders have the latent capacity to reach the same adult size as slowly developing ones, even at much higher rates of growth and development. As such, among females collected from their natural habitat, 23 out of the 103 rapidly developing spiders were as large or larger than the average female size among the slowly developing spiders. Being in accordance with the 'big house, big car' scenario (Reznick et al., 2000; van Noordwijk and de Jong, 1986), this shows that physiological and energetic costs linked with the rapid life history cohort can be mitigated to a considerable extent by increased resource acquisition. Also note that laboratory-reared rapidly developing spiders had more fat reserves than spiders in the slow treatment group, and growth rate was positively associated with fat reserves, suggesting that rapidly developing specimens are not only more voracious, but are also more prone to build up fat reserves when resources are not limiting. However, when resources are limited, fat reserve build-up might be halted, which would explain the result that in spiders collected from natural habitat, we observed lower fat reserve levels in rapidly developing spiders.

In the case of immune measures, although there were some differences between slowly and rapidly developing spiders collected from natural habitat, it is difficult to draw a general conclusion. Among spiders collected from nature, only in male β-defensin levels did we find lower values in rapidly developing specimens. However, rapidly developing spiders showed more efficient bacterial growth inhibition than slowly developing specimens. A similar pattern was observed in cell wall lytic power, although mated females of the rapidly developing cohort were characterised by extremely low values, lower than in any other group. Mated females had already laid eggs prior to the tests, so the prominent decrease in their cell wall lytic capacity might suggest the presence of an energetic conflict between this element of immunity and reproductive investment. Indeed, among rapidly developing mated females that did not produce measurable cell wall lytic zones, fecundity was higher, indicating a trade-off between investment into reproduction and investment into bacterial cell wall lytic capacity (Table A2). The observation that the dramatic decrease in cell wall lytic capacity was apparent only among rapidly developing females might hint at a three-way interaction between developmental rate, reproduction and antibacterial capacity in *P. agrestis* females, i.e. reproductive investment has higher relative immunity costs among rapid females. Additionally, fecundity of mated *P. agrestis* females was negatively associated with bacterial growth inhibition and cell wall lytic activity, although the latter was only marginally significant (Table A3). Such a negative association was observed in the cricket *Allonemobius socius*, and in the pholcid spider *Physocyclus dugesi* as well, as mated females showed considerable decrease in their hemolymph lytic activity (Calbacho-Rosa et al., 2012; Fedorka et al., 2004). Although there are fewer studies on the

Table A1. Linear regression results, in which prosoma darkness is the response variable, while sex, developmental type (or treatment) and spider type (collected from nature, or reared in the laboratory) are predictors; all two- and three-way interactions between the predictors are also included

Contrasts (laboratory spiders – natural spiders)	Coefficient	s.e.	*t*	*P*
Rapid females	0.252	0.016	16.21	<0.001*
Slow females	−0.055	0.016	−3.47	0.003*
Rapid males	0.015	0.020	0.73	0.885
Slow males	−0.105	0.019	−5.62	<0.001*

Estimated marginal means for the contrasts between natural-collected and laboratory-reared spiders are shown. *P*-values are adjusted with Tukey's method. Asterisks mark statistically significant effects (P<0.05).

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Table A2. Results of the logistic regressions on the probability of not producing measurable bacterial growth inhibition and cell wall lysis, using fecundity and developmental type as explanatory variables; interaction of the explanatory variables was not significant in the model of bacterial growth inhibition and thus was excluded

Explanatory variables	Coefficients	s.e.	*z*	*P*
Bacterial growth inhibition				
Intercept (slow development)	2.479	1.237	2.00	0.045*
Fecundity	−0.044	0.029	−1.51	0.131
Developmental type (rapid)	−1.900	0.731	−2.60	0.009*
Cell wall lytic activity				
Intercept (slow development)	−2.121	1.985	−1.07	0.285
Fecundity	0.044	0.049	0.89	0.373
Developmental type (rapid)	2.774	2.418	1.15	0.251
Fecundity×Developmental type (rapid)	−0.183	0.090	−2.04	0.041*

Asterisks mark statistically significant effects (*P<0.05).

trade-off between reproduction and immunity in spiders, this trade-off is rather well studied and known among insects (Schwenke et al., 2016).

Among the laboratory-reared spiders, we found no differences between slow and rapid group specimens either in β-defensin, bacterial growth inhibition or cell wall lytic activity, nor were these measures found to be associated with growth rate itself. Although we found lower cecropin B levels in rapid treatment spiders, this difference was quite small (see Fig. 3E), and no such difference was observed among spiders collected from the natural habitat. In contrast, higher antibacterial capacity in rapidly developing spiders from the natural habitat might suggest that those spiders able to mature before winter could also invest more into this element of immunity, possibly owing to their higher resource acquisition. Indeed, in some studies on arthropods, it appears that increased food availability might enhance the efficiency of some immune mechanisms (e.g. Simmons, 2012; but see also Kelly and Tawes, 2013). The absence of a significant difference between laboratory-reared slow and rapid groups in their bacterial growth inhibition power and cell wall lytic activity seems to suggest that when resources are not limited, the developmental types may achieve quite similar levels in the assessed immune elements. This result is quite similar to what was observed in the case of body size, and might hint at the important role of individual quality and resource acquisition in shaping these life history traits in such a cohort splitting scenario.

The result that adult body size of females was positively correlated with the number of their offspring is unsurprising. More interesting is the finding that besides variation owing to body size, developmental pathway also explained a significant amount of variation in fecundity. On average, slowly developing females had 1.5 times higher fecundity per body size unit than rapidly developing ones (LM parameter estimates were 12.11 and 18.49 offspring per millimetre prosoma width for rapid and slow females, respectively). These indicate that the difference in fecundity is not entirely due to the body size difference between slowly and rapidly developing females. One possibility is that physiological costs of rapid development impair the capacity of adult females to produce eggs. Notably, differences between slowly and rapidly developing females in egg sizes also might contribute to fewer offspring in rapid females, owing to the trade-off between egg number and size (Lim et al., 2014), meaning that rapidly developing females may invest relatively more into the size of eggs. Although it is not clear whether we should expect the same in *P. agrestis*, in the cricket *Gryllus firmus*, a significant negative association was observed between developmental time and egg size (Roff and Sokolovska, 2004). Unfortunately, currently we do not have any information about the egg (or hatchling) sizes from either slowly or rapidly developing females, so this notion remains to be assessed in future studies. It is also worth noting that a decreased investment into fecundity may contribute to similar (or, apparently, even higher) levels of self-preservation in rapidly developing spiders as slowly developing ones, as a substantial proportion of resources that otherwise would be invested into reproductive tissues can be allocated into somatic maintenance. By doing so, rapidly developing spiders might be able to mitigate the costs of enhanced post-embryonic development to some extent, which would otherwise need to be paid in the currency of somatic maintenance.

Table A3. Results from the linear regression with prosoma size, bacterial growth inhibition, cell wall lytic activity and developmental type as explanatory (predictor) variables, and fecundity as response; interactions between the predictors were not significant, and therefore were excluded

Explanatory variables	Coefficients	s.e.	*t*	*P*
Intercept (slow inhibition)	36.501	1.711	21.34	<0.001*
Developmental type (rapid)	−13.081	2.024	−6.46	<0.001*
Bacterial growth inhibition	−3.313	1.310	−2.53	0.015*
Cell wall lytic activity	−2.101	1.221	−1.72	0.092(*)
Size (re-scaled)	4.968	0.813	6.11	<0.001*

Asterisks mark a statistically significant effect (*P<0.05); asterisks in parentheses mark marginally significant effects (0.05<P<0.10).

The result that spiders in the rapid developmental treatment group exhibited higher overall mortality during the survival test may appear to hint at the survival costs of rapid development itself, but additional modelling results make it clear that this result emerges simply from their shorter life spans. Indeed, spiders in the rapid treatment group did not exhibit higher juvenile mortality than slowly developing spiders; quite the contrary. In other words, although longevity (life span) is shorter in rapidly developing spiders, we observed no survival cost related to the rapid development. Although the survival cost of rapid development might have been mitigated to some extent by the *ad libitum* food availability, past observations also did not show differences in mortality between slowly and rapidly developing spiders, neither in the laboratory nor in rearing conditions close to those in the natural habitat (Kiss, 2003). These observations may seem somewhat perplexing at first, as high growth rate was proposed (and, in a number of studies, found) to have considerable physiological costs that often manifest as increased mortality among rapidly developing individuals (reviewed by Dmitriew, 2011). Given that cohort splitting supposedly is an adaptive form of developmental plasticity in *P. agrestis*, it seems likely that juvenile survival costs of rapid development might have been decreased over the course of evolution in this species, promoting resource acquisition and allocation patterns that mitigate such costs. In future studies it will be quite important to assess metabolic rate measures of both slowly and rapidly developing spiders, how it relates to condition and survival, and how physiological costs (predicted to be associated with higher developmental and growth rates) are manifested under more resource-limiting treatment regimes.

Overall, our findings seem to indicate that the rapid post-embryonic development in the rapidly developing sub-cohort of *P. agrestis* spiders might not entail strict and general costs on the assessed life history characteristics, as opposed to some predictions of life history theory. Instead, individuals appear to change their resource allocation, and presumably even resource acquisition patterns as well, in accordance with their developmental pathway

after cohort splitting. As a likely explanation, we propose that, because cohort splitting is a result of adaptive developmental plasticity, *P. agrestis* adapted physiological and behavioural mechanisms helping to mitigate developmental costs to a considerable extent. Arguably, there is a large number of potential factors that may play a role in shaping life history traits and their associations with one another, and indeed the occurrence of cohort splitting itself, not assessed in the present study. In future studies, detailed assessments of the proximate and ultimate mechanisms evoking such adaptive developmental plasticity, and how they translate to individual differences in life history traits (and ultimately to fitness), will most certainly benefit from integrating genetic, epigenetic and ecological perspectives. For example, future studies should assess how food (or micro- and macro-nutrient) availability affects life history traits and trade-offs between them within sub-cohorts of different developmental rates, to determine to what extent high quantity and/or quality of food is able to mitigate resource allocation-based trade-offs predicted by theory. Intraguild and intraspecies competition may be important factors as well that may affect food availability, and hence potentially shape fitness consequences of taking on either of the developmental phenotypes. Also, a more detailed picture on how well slowly and rapidly developing spiders fare in different ecological conditions (e.g. with regards to predators or parasites) may help us better understand the individual-level consequences of an enhanced pace of life. Furthermore, a better understanding on the genetic components of cohort splitting would be quite important. Ongoing empirical and theoretical investigations suggest that engaging in rapid development by spiderlings in response to environmental stimuli varies consistently between mothers, hinting at genetically determined variation in how responsive spiderlings are to environmental cues, i.e. in how likely they will develop rapidly in the presence of rapid development-promoting stimuli. Intriguingly, such a mechanism could give way for a case of plastic bet-hedging on the part of early summer females from which cohort splitting spiderlings originate. Finally, the competing hypotheses on what drives the fecundity difference between slowly and rapidly developing spiders should be resolved in future studies in order to gain a better understanding of how cohort splitting affects fecundity (and, indeed, reproductive success) in *P. agrestis*, and to complement our general understanding on the physiological and fitness consequences of cohort splitting scenarios. We argue that this spider species will provide an excellent model organism for studies furthering our knowledge of life history evolution and the consequences of adaptive plasticity in development.

APPENDIX

Model-based clustering using parametrised finite Gaussian mixture models

Spring and early summer adults presumably are a mixture of two life history strategies from the previous year, namely (1) slowly developing spiders and (2) offspring of rapidly developing spiders. If differences in developmental times were to consistently affect prosoma size and/or melanisation, we might see bimodal distributions in these traits. We tested whether such bimodal distributions can be detected by model-based clustering (Scrucca et al., 2016) in prosoma size and melanisation. We ran models separately for males and females. For both male and female data subsets, models were run with 1 to 9 clusters (i.e. 9 models for each subset), and best models were selected based on Bayesian information criterion (BIC) scores; similarly to other information criteria (e.g. Akaike or deviance), models with small scores are preferred. The best models were unimodal for both females (n=66, BIC=186.31, β_{size}=2.165, $\beta_{darkness}$=0.777) and males (n=62, BIC=321.82, β_{size}=2.023, $\beta_{darkness}$=0.913).

Prosoma melanisation

We did not find general support for the thermal melanism hypothesis (Table A1). Indeed, among rapidly developing females, laboratory-reared specimens had darker (i.e. more strongly melanised) cuticle in comparison to females collected from the natural habitat. This appears to support the resource-limited hypothesis of prosoma melanisation, and to contradict the thermal melanism hypothesis, because in the latter we would expect to see weaker melanisation among laboratory-reared individuals owing to the high and stable environmental temperature (Fedorka et al., 2013).

Fecundity and immune parameters

We assessed the association between fecundity and the two antibacterial immune measures in mated females, using data from mated females collected from natural habitat during early and late summer (i.e. slowly and rapidly developing females, respectively; n=80, see Table A2).

Acknowledgements

We owe thanks to a large number of people for their help in different segments of our study: Dávid Nagy, Péter Kiss, Edvárd Mizsei, Dr Ádám Zoltán Lendvai, Dr Jácint Tökölyi, Zsófia Tóth, Alex Váradi, Rita Váradi-Rácz, Melinda Babits, Mester Valéria, Dr Judit Bereczki, Pál Mikecz, Dr Ilona Mészáros, Tamás Plaszkó, Anita Király, Márta Tóth Gábor Lászlóné, Dr Tamás Emri, Dr Attila Bácsi, Dr Ádám Kiss, Dr Monika Tarnawska, Dr Jacek Francikowski, Michał Krzyżowski and Bartosz Baran. We are grateful for the constructive comments on the manuscript from two anonymous reviewers.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: Z.R., J.K., A.B., G.K., F.B., F.S., Z.B.; Methodology: Z.R., J.K., A.B., G.K., F.B., Z.B.; Formal analysis: Z.R., J.K., A.B.; Investigation: Z.R., J.K., A.B., G.K., F.B., F.S., Z.B.; Resources: Z.B.; Data curation: Z.R.; Writing - original draft: Z.R., J.K., A.B., G.K., F.B., F.S., Z.B.; Writing - review & editing: Z.R., Z.B.; Visualization: Z.R.; Supervision: F.S., Z.B.; Funding acquisition: Z.B.

Funding

The study was supported by the Higher Education Institutional Excellence Program of the Ministry of Human Capacities in Hungary, within the framework of the FIK-Lendület Behavioural Ecology Research Group thematic program of the University of Debrecen.

Data availability

Datasets used in our analyses have been uploaded to figshare: https://doi.org/10.6084/m9.figshare.9942533.v1.

Supplementary information

Supplementary information available online at http://jeb.biologists.org/lookup/doi/10.1242/jeb.219659.supplemental

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