



Largely flat latitudinal life history clines in the dung fly *Sepsis fulgens* across Europe (Diptera: Sepsidae)

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Abstract

Clinal variation in body size and related life history traits is common and has stimulated the postulation of several eco-geographical rules. Whereas some clinal patterns are clearly adaptive, the causes of others remain obscure. We investigated intra-specific body size, development time and female fecundity (egg size and number) clines across 13 European populations of the dung fly *Sepsis fulgens* spanning 20° latitude from southern Italy to Estonia in a genetic common garden approach. Despite very short generation times (ca. 2 weeks at 24 °C), we found a converse Bergmann cline (smaller size at higher latitudes). As development time did not change with latitude (flat cline), integral growth rate thus likely declines towards the pole. At the same time, early fecundity, but not egg size, increased with latitude. Rather than being mediated by seasonal time constraints, the body size reduction in the northernmost flies from Estonia could suggest that these are marginal, edge populations, as when omitting them the body size cline became flat as well. Most of the other sepsid species investigated to date also show flat body size clines, a pattern that strikingly differs from *Drosophila*. We conclude that *S. fulgens* life history traits appear to be shaped by similar environmental pressures and selective mechanisms across Europe, be they adaptive or not. This reiterates the suggestion that body size clines can result as a secondary consequence of selection pressures shaping an entire life history syndrome, rendering them inconsistent and unpredictable in general.

Keywords Body size · Development time · Egg size · Fecundity · Geographic differentiation · Genetic differentiation · Latitudinal cline · Life history

Introduction

The study of large-scale geographic variation in life history traits, first and foremost body size, has a long tradition in evolutionary ecology (Partridge and Coyne

1997; Blackburn et al. 1999; Chown and Gaston 1999; Blanckenhorn and Demont 2004; Shelomi 2012). Body size of closely related species often increases with latitude, known as Bergmann's rule (Bergmann 1847), and corresponding intra-specific trends, sometimes called James' rule (James 1970), also commonly occur. Nevertheless, opposite, so-called converse Bergmann clines also exist (Mousseau 1997; Blanckenhorn and Demont 2004; Shelomi 2012). All these patterns are generally thought to be caused by systematic latitudinal climate variation, although the specific underlying environmental variables and causal mechanisms are frequently unclear. This is because body size is intimately entwined with other life history traits such as juvenile development time and growth rate, which ultimately co-determine final adult body size (Nylin and Gotthard 1998; Blanckenhorn 1999). Moreover, as body size affects most if not all life history and physiological traits of an organism in a concerted syndrome, several reproductive traits (egg size, egg number, etc.) also tend to exhibit geographic clines (e.g. Azevedo

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et al. 1996; Armbruster et al. 2001; Lardies et al. 2010). Though there are many exceptions, higher temperatures usually result in smaller body sizes in ectothermic organisms, an entirely plastic physiological response of unclear adaptive value (named temperature-size rule: Stevenson 1985; Atkinson 1994; van der Have and de Jong 1996; Van Voorhies 1996; Horne et al. 2015, 2018). While temperatures systematically decrease with latitude, season length decreases too, thus constraining an individual's growth period and hence final body size, producing adaptive converse Bergmann size clines especially in large species with long generation times, such as many beetles, grasshoppers and butterflies (smaller towards the poles: Blanckenhorn and Demont 2004; Nygren et al. 2008; Shelomi 2012). Analogous effects are exerted by altitude, albeit at much more condensed spatial scales, such that expected patterns are often not found (Shelomi 2012; Klepsatel et al. 2014; Horne et al. 2018). It follows that several environmental factors (temperature, season length, etc.), more or less independently, act in conjunction on the entire life history syndrome of any particular species, resulting in diverse and potentially intermediate clinal patterns that are ultimately difficult to predict (Blanckenhorn and Demont 2004). More studies of geographic and clinal life history variation are therefore needed.

Although the literature by now boasts many studies on latitudinal clines for a great number of taxa, in insects work on the “model species” *Drosophila* tends to dominate (Shelomi 2012; Schilthuizen and Kellermann 2014; Flatt 2016; Horne et al. 2018). Such strong bias towards *Drosophila* cannot be representative from a biodiversity perspective, especially because Dipterans are very derived phylogenetically (see Misof et al. 2014). This occurs also because small insects, such as *Drosophila*, can be studied as genetic lineages under common garden conditions in the laboratory. Such work therefore tests for evolved, genetic clines, which from an evolutionary point of view is superior to merely phenotypic patterns derived from field-caught specimens, as commonly utilized in macro-ecology (Chown and Gaston 2010). In *Drosophila*, Bergmann clines are the rule within species (e.g. James et al. 1997; Huey et al. 2000; Shelomi 2012; Klepsatel et al. 2014; Fabian et al. 2015; Table 1), and apparently also across species (Rohner et al. 2018a). This can be expected in small species with short generation times, which consequently are multivoltine (multiple generations per year) over most of their distributional range (Blanckenhorn and Demont 2004). By contrast, larger insects such as beetles, grasshoppers or water bugs with typically long development times relative to the available season length often have univoltine life cycles (one generation per year; e.g. Masaki 1967; Blanckenhorn and Fairbairn, 1995; Mousseau 1997; Sota et al. 2000), and thus more likely exhibit converse Bergmann clines because they regularly experience

seasonal time limitations forcing them to abbreviate their development at the expense of a reduction in final body size (Blanckenhorn and Demont 2004).

Sepsid “black scavenger” or “dung” flies (Diptera: Sepsidae) are common and widespread worldwide, with many species in the tropics (Ang et al. 2013; see <http://sepsidnet-rmbr.nus.edu.sg/> for photographs). On average they are a bit larger than *Drosophila* (3–8 mm body length), and multivoltine throughout most of their range. Sepsids generally depend on decaying organic material for reproduction and larval development, often breeding in livestock faeces (e.g. cow dung; Pont and Meier 2002), a common habitat in agricultural grasslands. In populating ephemeral habitats, sepsids are ecologically similar to *Drosophila* (Blanckenhorn 1999; Rohner et al. 2018a, b). Adult sepsids feed on dung (for protein) and nectar (for energy), and therefore, as *Drosophila*, are anautogenous income breeders, although males (but not females) likely produce their first batch of gametes already during the juvenile phase (Teuschl and Blanckenhorn 2007). Not least because they are easy to rear and have short generation times, sepsid flies are fast becoming model organisms in behaviour, ecology, and evolution (e.g. Teuschl and Blanckenhorn 2007; Puniamorthy et al. 2009, 2012, 2014; Berger et al. 2013; Rohner et al. 2015; Esperk et al. 2016; Busso and Blanckenhorn 2018). So far, the few studies of latitudinal variation in this group yield inconsistent evidence (summarized in Table 1). Puniamorthy et al.'s (2012) study suggests a Bergmann cline in European but not North American *Sepsis punctum* (see also Dmitriev and Blanckenhorn 2012), and Berger et al. (2013) show a cline in development rate in European *S. punctum* that is consistent with seasonal time constraints operating at higher latitudes (faster in northern Europe). No (i.e. flat) clines are evident in European *S. cynipsea* (Rohner et al. 2016) and *S. thoracica* (Busso and Blanckenhorn 2018).

Sepsis fulgens is one of the most ubiquitous, entirely black sepsid species in Europe about which almost nothing is known so far (Pont and Meier 2002; Fig. 1). We here examine clinal body size, development time and female fecundity (egg number and size) variation of 13 *Sepsis fulgens* populations across roughly 20° latitude from southern Italy to Estonia. Similar to *Drosophila* studies, we took a genetic common garden approach by working with iso-female lines (i.e. the laboratory offspring of single field-caught females) to investigate evolved clines. We additionally assessed the extent of phenotypic plasticity in the above traits by rearing flies at a range of 2–4 laboratory temperatures, thus evaluating the temperature-size rule (Atkinson 1994; Horne et al. 2015, 2018). As this sepsid species is relatively small (body length ca. 4 mm), it features multiple generations per year (e.g. > 8 overlapping generations in lowland Switzerland (pers. obs.); and 2–3 generations per year even at higher latitude in Moscow, Russia: 55.45°N, 37.36°E; Pont and

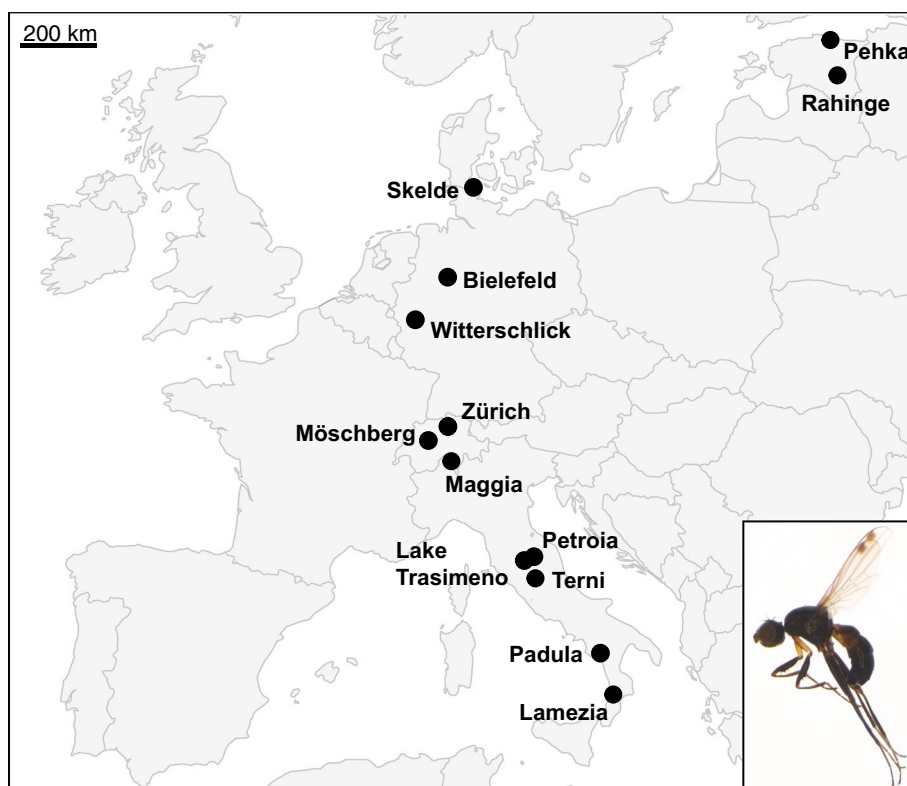
Table 1 Comparison of clinal studies of Drosophilidae and Sepsidae (Diptera)

Sepsidae	Sex ^a	Geographic range	Temp.	Lat. range	Slope	SE	95% CI low	95% CI hi	No. pops	References
<i>Sepsis cynipsea</i>	M and F	Europe	24	43–60	0.00008	0.00050	–0.00091	0.00106	7	Rohner et al. (2016)
<i>Sepsis fulgens</i>	M and F	Europe	18	38–60	–0.00849	0.00502	–0.01833	0.00134	11	This study
<i>Sepsis neocynipsea</i>	M and F	North America	24	33–44	0.00324	0.00469	–0.00596	0.01243	6	Rohner et al. (2016)
<i>Sepsis punctum</i>	M and F	Europe	18	43–60	0.01471	0.00493	0.00506	0.02437	7	Berger et al. unpublished
	M and F	North America	18	34–46	0.00144	0.00629	–0.01088	0.01376	6	Berger et al. unpublished
<i>Sepsis thoracica</i>	M and F	Europe	18	38–60	0.00239	0.01787	–0.03264	0.03741	15	Busso and Blanckenhorn (2018)
Drosophilidae										
<i>Drosophila kikkawai</i>	F	Indian subcontinent	24	7–31	0.01868	0.00156	0.01562	0.02174	9	Karan et al. (1998)
<i>Drosophila melanogaster</i>	F	Eurasia	25	38–58	0.00210	–	–	–	16	Imasheva et al. (1994)
<i>Drosophila serrata</i>	F	Eastern Australia	25	14–34	0.11400	0.00760	0.09910	0.12890	19	Hallas et al. (2002)
	M	Eastern Australia	25		0.06900	0.01550	0.03862	0.09938		
<i>Drosophila simulans</i>	F	Eastern Australia	25	25–42	0.00337	0.00100	0.00141	0.00533	10	Arthur et al. (2008)
	M	Eastern Australia	25		0.00370	0.00100	0.00174	0.00566		
<i>Drosophila subobscura</i>	F	Europe	20	37–56	0.00200	0.00060	0.00082	0.00318	11	Calboli et al. (2003)
	M	Europe	20		0.00250	0.00060	0.00132	0.00368		
	F	North America	20	35–50	0.00190	0.00070	0.00053	0.00327	11	
	M	North America	20		0.00040	0.00060	–0.00078	0.00158		
	F	South America	20	29–45	0.00210	0.00070	0.00073	0.00347	10	
	M	South America	20		0.00150	0.00070	0.00013	0.00287		
<i>Zaprionus indianus</i>	F	Indian subcontinent	24	9–31	0.01190	0.00230	0.00739	0.01641	10	Karan et al. (2000)
	M	Indian subcontinent	24		0.01120	0.00420	0.00297	0.01943		

All slopes refer to wing length (mm/deg. latitude; rescaled from other structural traits for sepsids). Slopes that differ significantly from 0 are in bold

^aM and F: average slope for both sexes

Fig. 1 Map indicating sampling sites of our 13 European *Sepsis fulgens* populations



Meier 2002; cf. Table 2), such that seasonal time limitations would generally not exert strong selection except in the last generation(s) before winter. We thus expected this species to show a Bergmann cline (larger towards the pole), and no countergradient pattern of accelerated development as found for the larger *S. punctum* by Berger et al. (2013; Blanckenhorn and Demont 2004). Consequently, following the expected body size pattern, we also expected either more or larger eggs in populations from higher latitudes.

Methods

Fly cultures and maintenance

Sepsis fulgens Meigen, 1826 (Diptera: Sepsidae) is a eurytopic species occurring throughout Europe, North Africa and the Middle East. The species is commonly found on cow, pig and horse dung, as well as on manure piles (Pont and Meier 2002). Contrary to many sepsids, the species is always entirely black (see http://sepsidnet-rmbr.nus.edu.sg/Sepsis_fulgens.html; Fig. 1).

We studied flies from 13 latitudinal field populations from southern Italy to Estonia (Fig. 1; Table 2 lists some standard climate characteristics of the collections sites). Live wild-caught females from these populations were collected and brought to our laboratory at different times mainly during

the seasons 2013 and 2014. Cultures were subsequently kept as iso-female lines (i.e. the descendants of one wild-caught female) for multiple (> 15) generations in the laboratory (ca. 3–4 weeks per generation) before being used in our experiment in 2015. We had several (5–13) replicate families (i.e. iso-female lines) per population (Table 2), each housed in separate 1-l plastic containers that were regularly supplied with fresh cow dung, sugar, and water ad libitum, and kept in a climate chamber at 24 ± 1 °C, 60% relative humidity, and 13:11 h light:dark cycle. Assessing iso-female lines instead of wild-caught females minimises environmental variation influencing the phenotype, thus exposing mainly genetic variation, which remains best preserved in the laboratory by this culturing method.

Laboratory rearing experiments

Two rearing experiments were performed. The first rearing served to analyse body size and development time of both sexes, using 11 populations (cf. Fig. 1): Lamezia, Padula, Terni, Trasimeno, Maggia, Möschberg, Zürich, Bielefeld, Skelde, Rahinge, and Pehka (Table 2). Iso-female lines were provided with fresh dung in their rearing containers, into which multiple females could lay eggs for a duration of up to 4 h. These egg masses were then split randomly among four environments differing in temperature only (5–100 eggs per mass culture dish). The larvae were subsequently allowed to

Table 2 Characteristics and climate conditions at the sampling locations of our 13 populations

Population	Collection (YYYY.M)	No. lines	Latitude	Longitude	Altitude (m)	Winter length ^a (d)	Temperature ^b (°C)			Annual precipitation (mm)
							Mean annual (bio1)	Mean annual range (bio7)	Mean of warmest quarter (bio10)	
Lamezia (I)	2013.4	13	38.92	16.25	244	15	17.6	22.0	24.8	864
Padula (I)	2013.4	13	40.22	15.65	720	128	13.2	22.6	20.6	764
Terni (I)	2012.9	7	42.57	12.62	132	119	14.6	28.1	22.7	853
Lake Trasimeno (I)	2012.9	5	43.13	12.1	258	155	13.3	27.3	21.4	792
Petroia (I)	2014.8	6	43.25	12.55	513	157	12.2	26.0	20.5	936
Maggia (CH)	2013.5	4	46.25	8.7	372	172	10.8	26.6	19.0	1240
Möschberg (CH)	2013.4	11	46.91	7.63	518	200	7.5	25.2	15.4	1067
Zürich (CH)	2013.6	8	47.34	8.55	489	189	9.5	25.8	17.6	1077
Witterschlick (D)	2014.7	6	50.69	7.02	96	186	9.6	23.6	16.9	773
Bielefeld (D)	2013.6	12	52.03	8.53	105	196	8.8	23.1	16.2	833
Skelde (DK)	2013.8	5	54.85	9.73	11	215	8.3	21.5	15.7	750
Rahinge (Est)	2014.6	7	58.37	26.7	72	238	4.9	31.3	15.9	600
Pehka (Est)	2013.5	12	59.48	26.37	57	240	4.7	31.1	15.7	618

^aMean number of days below 10 °C (assumed developmental threshold)^bBioclimatic variables 1, 7, 10 from: <http://worldclim.org>; Hijmans et al. (2005)

develop in overabundant and uniform defrosted cow dung at 60% relative humidity and long photoperiods (≥ 13 h) at constant 12, 18, 24 or 30 °C in 50 ml glass vials. Vials were checked daily for offspring until no more individuals emerged. The thorax length (length of the scutum plus the scutellum) of two randomly chosen offspring males and two offspring females was imaged and measured as an index of body size. The structural outline of the thorax best reflects overall body mass as it contains the important flight muscles.

The second rearing focused on female reproductive traits of eight latitudinal populations: Lamezia, Petroia, Padula, Maggia, Möschberg, Zürich, Witterschlick, and Rahinge (Fig. 1). Male and female flies from any given iso-female line, reared at constant 24 °C, 60% relative humidity and 13 h photoperiod, were paired randomly and subsequently kept in one of two environments differing in temperature only (60% relative humidity and photoperiod ≥ 13 h); for each replicate iso-female line, two pairs were kept in separate 50 ml glass vials with fresh dung and sugar at 18 °C, and two pairs at 30 °C. Every day the dung was checked for eggs. As soon as eggs were laid (=day of first reproduction), they were counted and five of them imaged, and the pair was provided with fresh dung for another day. In case the female laid additional eggs on the second day, we considered them part of the same clutch (as sepsids do not always lay discrete clutches within 24 h). Thereafter, females were frozen and measured. From this experiment, we obtained data on adult age at first reproduction (days), the total number of laid eggs in the first 2 days (i.e. first clutch size, or early fecundity), egg size (length and width, from which egg volume was calculated using the formula for a spheroid: $(1/6)\pi \times \text{length} \times \text{width}^2$). For convenience, fore tibia length was used as an index of female body size; correlations among all structural length traits (including thorax) of *S. fulgens* exceed $r > 0.97$ in both males and females ($n > 40$ individuals).

All images (thorax, eggs, foreleg) were taken with a Leica MZ 12 stereomicroscope and a Leica DC490 digital camera. Measurements were performed using the program tpsDIG2 (© F. J. Rohlf 2010) and the program PAST (© O. Hammer 2003). All analyses were conducted with the software IBM SPSS Statistics 23 (SPSS Inc.).

Results

Development time and body size (thorax length) were analysed with sex and rearing temperature as fixed factors and latitude as a continuous covariate with univariate ANOVAs (general linear models with underlying normal error distribution), using line (i.e. family) means (of typically two individuals per sex) as independent data points (and global error), because this is a genetic study and we were not

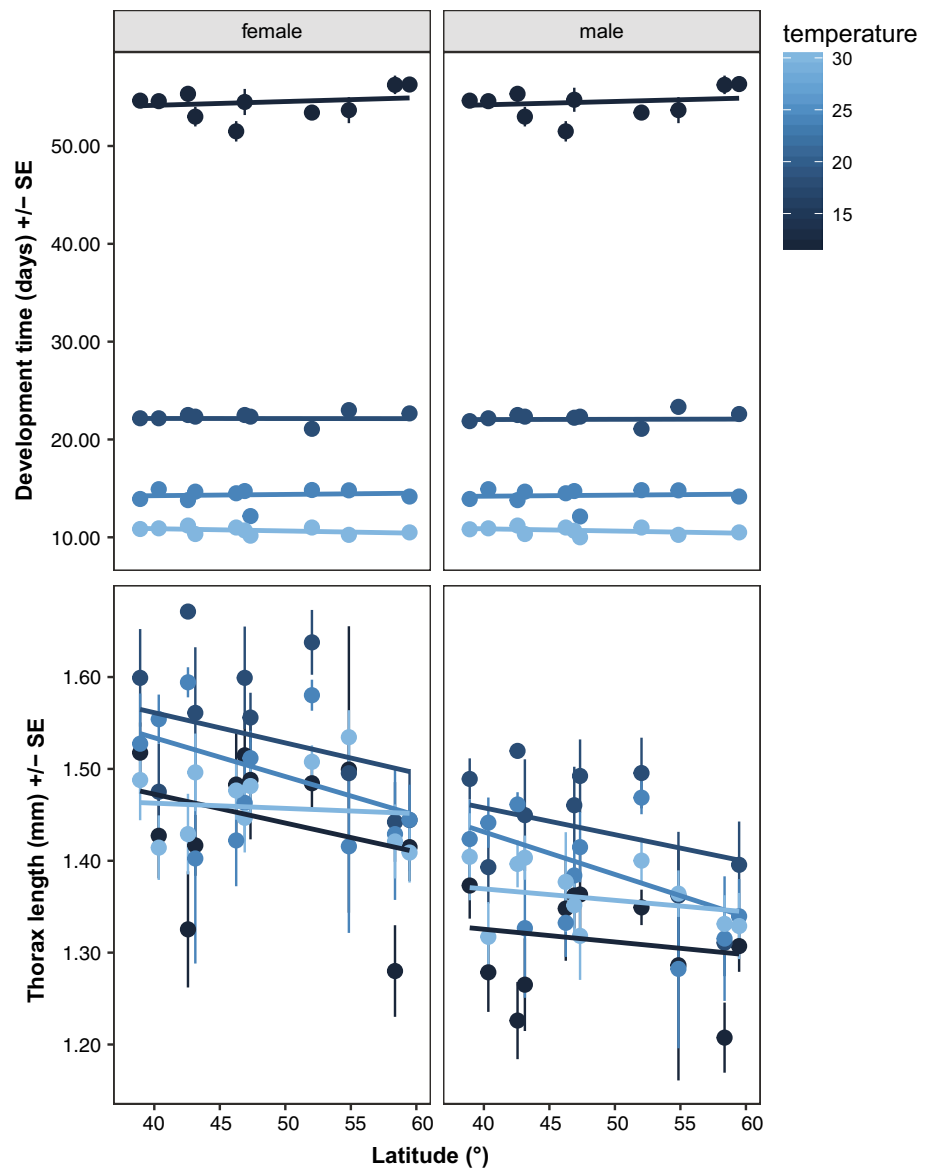
specifically interested in within-line phenotypic variation. Development time did not change with latitude, i.e. the cline was flat ($F_{1,551} = 0.47$, $p > 0.3$; Fig. 2), with no significant interactions (e.g. temp \times latitude: $F_{3,544} = 1.78$, $p = 0.149$; all other interactions $p > 0.2$). In contrast, body size of the flies clearly decreased with latitude ($F_{1,648} = 14.38$, $p < 0.001$; Fig. 2), again revealing no significant interactions (temp \times latitude: $F_{3,651} = 1.16$, $p > 0.3$; all other interactions $p > 0.3$). Development time strongly increased as temperature decreased ($F_{3,551} = 477.83$, $p < 0.001$; Fig. 2), while body size was largest at 18 °C, followed by 24 °C, and smallest at both extreme temperatures of 30 and 12 °C ($F_{3,648} = 2.29$, $p = 0.067$, Fig. 2). As usual in insects, females are larger than males in this species ($F_{1,648} = 117.27$, $p < 0.001$). Sexual size dimorphism (calculated as the logarithm of the ratio between female and male thorax length) decreased from cold to warm temperatures ($F_{1,310} = 9.98$, $p = 0.002$); however, this pattern was only due to males being much smaller at 12 °C, as sexual size dimorphism remained constant from 18 to 30 °C. When calculating an analogous index for the development time difference between the sexes, there was no difference between the sexes ($F_{1,201} = 0.08$, $p = 0.146$), and the development time difference did not vary strongly with temperature, although there was a trend of males taking overall longer to develop at cold temperatures ($F_{1,201} = 3.70$, $p = 0.056$; Fig. 2).

The female reproductive trait experiment was similarly analysed with temperature as fixed factor, and latitude, latitude² (to test for nonlinearity), and female size (tibia length) as continuous covariates, again using line means (of typically two females) as independent data points. Flies from all populations first reproduced after approximately the same amount of time, but oviposition occurred faster when warmer [18 °C: 6.62 ± 0.073 (SE) days; 30 °C: 4.18 ± 0.076 days], although this difference was not significant ($F_{1,76} = 2.48$, $p = 0.119$). Early fecundity (square-root-transformed number of eggs laid in the first 2 days) was unaffected by rearing temperature ($F_{1,76} = 0.11$, $p = 0.737$) but increased with body size ($F_{1,76} = 7.54$, $p = 0.008$) and latitude ($F_{1,76} = 13.30$, $p < 0.001$), and additionally showed a negative latitude² (i.e. hump-shaped, concave) effect ($F_{1,76} = 12.55$, $p = 0.001$), suggesting that the increase in fecundity with latitude could be asymptotic (Fig. 3). In contrast, cube-root-transformed egg volume was not affected by any of these factors (Figs. 3, 4). Consequently, no trade-off between the number and size of laid eggs was found.

Discussion

Despite *S. fulgens*' short generation time (2 weeks at 24 °C, 3 weeks at 18 °C; Fig. 2), we did not find the expected Bergmann size cline (or James' rule) in Europe, but rather

Fig. 2 Population mean \pm SE egg-to-adult development time (top) and body size (thorax length; bottom) for females (left) and males (right) of 11 latitudinal *Sepsis fulgens* populations at four constant laboratory temperatures (12, 18, 24, 30 °C)



a converse Bergmann cline (smaller size toward the north pole; cf. Blanckenhorn and Demont 2004). As development time did not change with latitude (Fig. 2), this implies that growth rate does not accelerate towards the pole, although our measurements are restricted to integral measurements of growth, which have been shown to sometimes differ from more precise growth rate measures (Tammara and Esperk 2007; Rohner et al. 2017). Nevertheless, this result is opposite to what was found for the closely related but larger *S. punctum* (Berger et al. 2013), and also for the much larger yellow dung fly (Blanckenhorn et al. 2018). Rather than being mediated by seasonal time constraints, which would imply an adaptive response (Roff 1980; Kivelä et al. 2011), the size decline in the northernmost flies from Estonia alternatively suggests that these could be marginal, edge populations in terms of environmental factors such as temperature,

food, precipitation, or irradiation, etc.; when omitting the two Estonian populations from the analysis (cf. Fig. 1), the European body size cline became flat as well. We therefore conclude that the clinal patterns found here are not necessarily adaptive, as body size, development time, fecundity and egg size are similar across all of Europe, hence presumably shaped by similar environmental pressures everywhere, or alternatively by stabilizing ecological selection relating to these flies' ephemeral food resource (dung). However, we have no direct evidence for the latter, and in the related dung fly *Sepsis cynipsea* overall balancing selection could not be found in an extensive study of one single Swiss population (Blanckenhorn 2007). Unfortunately, we could not obtain *S. fulgens* from more northern sites to verify whether these populations are indeed marginal and/or subject to seasonal time constraints there.

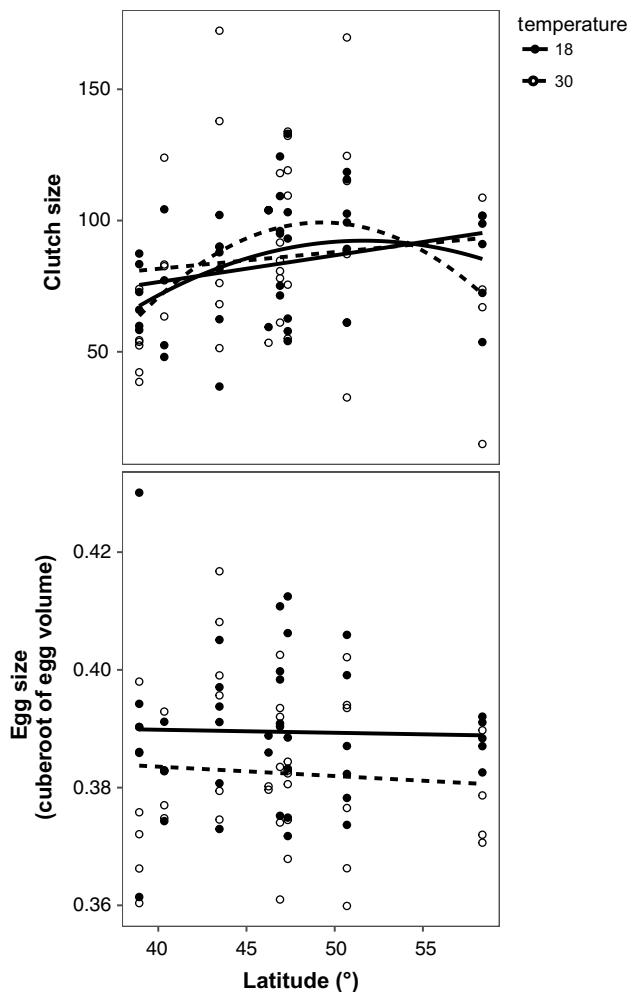


Fig. 3 Line mean number of eggs laid (i.e. first clutch size; top) and size of eggs (bottom) for eight latitudinal populations of lab-reared *Sepsis fulgens* at 18 or 30 °C

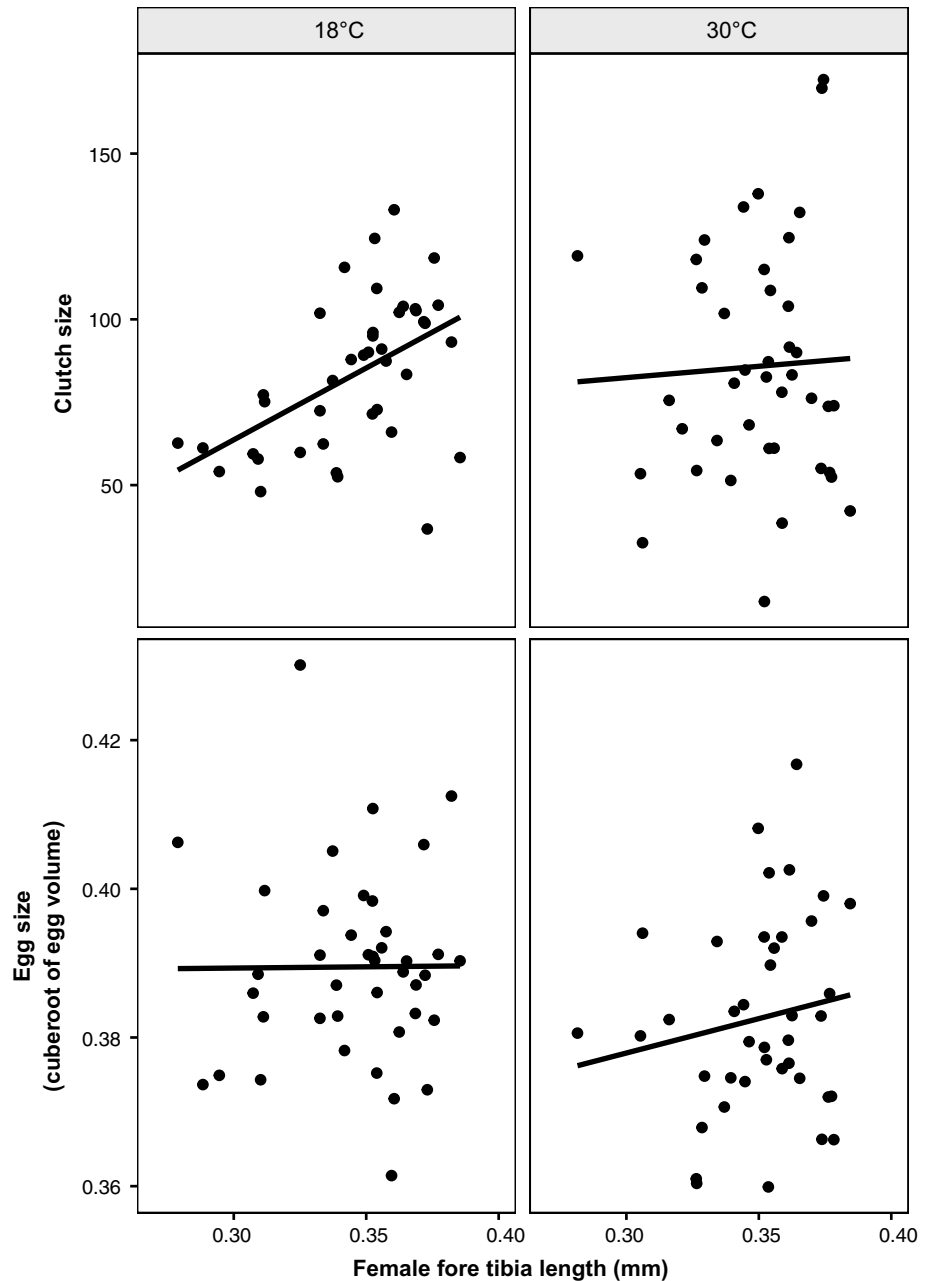
Clear body size clines also lack in the related *S. thoracica* and *S. cynipsea* in Europe (Rohner et al. 2016; Busso and Blanckenhorn 2018; Table 1). This situation for sepsids contrasts with that of various *Drosophila* species, the model insects also in evolutionary ecology, which generally show positive Bergmann (or James') clines within species that even have been demonstrated to re-evolve in relatively short time after invasion of a new continent (James et al. 1997; Huey et al. 2000; Zwaan et al. 2000; Klepsatel et al. 2014; Fabian et al. 2015; Flatt 2016; Table 1). Our common garden study implies that all the patterns found for *S. fulgens* (as well as the above-mentioned two other sepsid species) are genetic, i.e. evolved, as is also the case in the listed *Drosophila* studies. As sepsid flies are comparable to *Drosophila* in terms of nutrition ecology, life history, size, dispersal capacity and culturing methods (cf. Blanckenhorn 1999; Rohner et al. 2018a, b), this supports the overall conclusion that the evolution of clinal life history patterns

and their underlying mechanisms are complex and not easily predictable in general. In the end, therefore, we are left without being able to identify the mechanisms that mediate the cline differences between the two fly species summarized in Table 1 (cf. Blanckenhorn 1999).

Even though *S. fulgens* flies became smaller, they laid more but not larger eggs at higher latitudes, at least initially between 40° and 50° latitude, beyond which early fecundity declined again in the potentially marginal Estonian populations (Fig. 3). This occurred despite the fact that within populations larger flies produced more eggs (Fig. 4), as is typical in insects (Honek 1993). Berger et al. (2008) showed that the realized fecundity of butterflies can be limited by cool weather, an effect that might select for increased size-specific early fecundity in insects at high latitudes. It could also be that northern flies invest more in female abdomens at the expense of thorax length, but we have no data on abdomen size, which is generally difficult to measure in insects because it is so flexible. Lacking sex by latitude interactions further suggest no sex-specific shifts in investment into the thorax relative to other body parts in females from high latitudes. Egg size also appears to be independent of body size in this species. Derived from Bergmann's rule and the temperature-size-rule (Stevenson 1985; Atkinson 1994; Partridge et al. 1994; van der Have and de Jong 1996; Van Voorhies 1996; Horne et al. 2015), our expectation was that the cooler northern climate would promote the evolution of larger eggs, a plastic response found e.g. in yellow dung flies (Blanckenhorn 2000; Blanckenhorn and Heyland 2004) and *D. melanogaster* (Azevedo et al. 1996), but this was not the case here. Our results also do not support the hypothesis that more extreme climates select for larger eggs providing offspring a competitive advantage during early development (Fox and Czesak 2000). That clutch size increases with latitude while egg size remains unaffected further implies the absence of a size-number trade-off, which is often expected (Smith and Fretwell 1974), perhaps due to seasonal time constraints on this income breeder's life history at higher latitudes (Berger et al. 2008). Again, it seems that clines in reproductive parameters (fecundity and egg size) can turn out equally variable and unpredictable depending on species and environments (cf. Fox and Czesak 2000; e.g. Bauerfeind et al. 2018).

Despite the patterns documented here for *S. fulgens* being heritable, phenotypic plasticity in response to temperature was extensive for development time and body size (Fig. 2), albeit not for reproductive traits (Fig. 3). While development time lengthens strongly and expectedly as temperatures drop, body size did not strictly follow the temperature-size rule in this species (smaller when warmer: Atkinson 1994), as it was maximal at intermediate temperatures of 18 and 24 °C but lower at 12 and 30 °C (Fig. 2). For the related *S. cynipsea*, the lower temperature threshold at which development

Fig. 4 Line mean number of laid eggs (top) and size of eggs (bottom) of *Sepsis fulgens* females from eight latitudinal populations lab-reared at 18 °C (left) or 30 °C (right) as a function of body size (fore tibia length)



ceases has been estimated to be roughly 9 °C (Blanckenhorn 1999); 12 °C appears to be a marginal temperature for *S. fulgens* as well, at which flies require 55 days to complete development to adulthood (Fig. 2). When reared at temperatures above this threshold, sexual size dimorphism did not vary with temperature, a common pattern in insects (Hirst et al. 2015). The increase in size dimorphism at 12 °C further suggests that the sexes do differ in their temperature-size response at such marginal temperatures: males took longer time to emerge and emerged smaller, so they seem to do worse at cool temperatures. In contrast, rearing temperature of adult females did not affect fecundity here, as is often not the case and thus not expected, whereas egg size was,

albeit non-significantly, reduced at 30 °C relative to 18 °C (Fig. 3), as expected on physiological grounds related to processes presumably mediating the temperature-size rule (Van Voorhies 1996; Atkinson and Sibly 1997; Fox and Czesak 2000; Blanckenhorn 2000; Fischer et al. 2003; Blanckenhorn and Heyland 2004; Garrad et al. 2016).

Flat latitudinal clines for body size, development time and/or growth rate in *S. fulgens* as demonstrated here do not necessarily imply that phenotypic clines based on field-caught specimens are also flat. As also shown here, all these traits are considerably plastic, such that lower average temperatures at high latitudes, often combined with low population densities and hence more abundant food per capita,

could suffice to produce Bergmann clines (Blanckenhorn and Demont 2004). Unfortunately, we have no good data on field flies, as we concentrated on collecting live specimens. To convincingly demonstrate phenotypic (i.e. macro-ecological) clines based on field samples in this small and very plastic fly would require numerous specimens sampled systematically at all locations over the season. This is no trivial task. We thus cannot present a phenotypic field cline in addition to the genetic cline. Furthermore, while climate information for our sampling locations is available (Table 2), such data usually do not serve to single out the responsible environmental factors for a given cline, as the crucial parameters, season length and temperature, are typically highly correlated with latitude (and altitude) so that multiple regression approaches necessarily fail. Latitude thus remains the most easily assessable proxy (see Busso and Blanckenhorn 2018; Blanckenhorn et al. 2018).

In conclusion, latitudinal clines in body size, development time and reproductive traits in European *S. fulgens* populations are mostly flat, implying no systematic life history shifts across a wide range of climates. This may relate to these flies being relatively small and ubiquitous, because their substrate (livestock dung) is also ubiquitous in agricultural grasslands that are very common in Europe. Seasonal time constraints therefore probably do not exert strong selection shaping the life history of even the northernmost populations, where these flies still feature multiple generations per season (as, e.g. in Moscow; op. cit.). As sepsid flies are widespread in the tropics (Pont and Meier 2002; Ang et al. 2013), northern European climes likely are secondary, marginal habitats for them, though not in terms of food (i.e. dung) availability. Despite being bad fliers, sepsids are widespread such that gene flow should nevertheless be extensive, hampering local adaptation of populations (Busso and Blanckenhorn 2018). It remains puzzling that sepsids, being ecologically similar to drosophilids also in terms of dispersal capacity and thus gene flow between populations, show very different clinal life history patterns. So, this result reiterates the interpretation that clinal life history patterns are complex and not easily predictable in general, as temperature effects (presumably fostering positive Bergmann clines) and season length effects (fostering converse Bergmann clines) may cancel out, ultimately possibly producing the largely flat clines observed here (Blanckenhorn and Demont 2004).

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Author contribution statement JR, WUB and PTR conceived and designed the study, and contributed all to the statistical analysis and the writing of the manuscript. JR and PTR performed the experiments.

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