Life history traits, but not body size, vary systematically along latitudinal gradients on three continents in the widespread yellow dung fly

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Life history traits, but not body size, vary systematically along latitudinal gradients on three continents in the widespread yellow dung fly

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Large-scale clinal variation in body size and other life-history traits is common enough to have stimulated the postulation of several eco-geographical rules. Whereas some clinal patterns are clearly adaptive, the causes of others remain unclear. We present a comprehensive intraspecific population comparison for the cosmopolitan yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae) to check for consistent world-wide patterns. Common garden assessment of various life history traits permitted continental comparison of (clinal) quantitative genetic differentiation (Qst) with putatively neutral genetic differentiation (Fst) derived from field-caught flies. Latitudinal clines in fly development time, growth rate, and overwintering propensity were consistent among North American, European and Japanese populations. Increased winter dormancy incidence and duration at higher latitude, combined with a faster growth rate and shorter development time, suggest that flies are adapted to season length more than to temperature. The resulting body size clines, in contrast, were not very consistent; importantly, they were not negative, as expected under seasonal constraints, but flat or even positive clines. Quantitative genetic differentiation Q_{ST} exceeded neutral molecular variation F_{ST} for most traits, suggesting that natural selection plays a consistent role in mediating global dung fly life histories. We conclude that faster growth and development in response to shorter growing seasons at higher latitudes may indirectly counteract expected direct effects of temperature on body-size, potentially resulting in flat or inconsistent body size clines in nature.

Keywords: body size, development time, diapause, Fst, geographic differentiation, genetic differentiation, growth rate, latitudinal cline, life history, Qst

Introduction

Body size of closely related species, or populations within species, often increases with latitude (Bergmann's rule), although opposite clines are also common (Blackburn et al. 1999, Chown and Gaston 1999, Blanckenhorn and Demont 2004, Shelomi 2012). Systematic latitudinal variation in climate is generally presumed to cause these body size clines, but the specific underlying climatic causes, and whether body size is under direct selection or evolves as a correlated response to selection on co-varying traits, remain unclear. Body size is intimately intertwined with other life-history traits such as juvenile development time, growth rate and reproductive output (Nylin and Gotthard 1998), and thus all these traits, plus dormancy (diapause) and migration, co-evolve in seasonal environments as an interconnected life-history syndrome (Roff 1980, Tauber et al. 1986, Schmidt et al. 2005). Yet few studies investigate geographic clines of various traits in conjunction.

Whether geographic clines are adaptive depends on the trait and the putative environmental causes (Blanckenhorn and Demont 2004). Season length generally declines with latitude, constraining the period available for growth. Shorter seasons at high latitudes (or altitudes) thus select for faster growth and/or development. Selection caused by variation in season length can consequently generate two common patterns: converse (negative) Bergmann clines, according to which insects and other animals become smaller at higher latitudes (Masaki 1967, Blanckenhorn and Fairbairn 1995, Mousseau 1997, Blanckenhorn and Demont 2004, Shelomi 2012), and countergradient variation (also called latitudinal compensation), according to which animals compensate, primarily by increasing their growth rate, to maintain their (presumably optimal) body size as the season length declines (Levinton and Monahan 1983, Conover and Present 1990). Insect diapause also predictably increases at higher latitudes characterized by cool temperatures and short days, producing strong and often rapid evolutionary responses to season length variation (Masaki 1972, Bradshaw and Holzapfel 2001, Schmidt et al. 2005).

Whereas declining body size and faster growth with latitude is expected as season length shortens, it remains largely unclear whether and how (positive) Bergmann clines – larger body size at higher latitudes – can result from latitudinal variation in climate, and whether corresponding selection acts directly on body size or indirectly via the aforementioned co-varying juvenile life history traits. Bergmann's rule appears to be mediated directly by temperature (larger when cooler: Atkinson and Sibly 1997, Chown and Gaston 1999, Blanckenhorn and Demont 2004), and may also result as a possibly non-adaptive consequence of physiological processes at the cellular level (van der Have and de Jong 1996, Van Voorhies 1996). Thus, a general theory or mechanism explaining how clinal variation in temperature generates body size clines is lacking.

Many studies in numerous species have documented the existence of latitudinal body size clines on various continents (Blackburn et al. 1999, Blanckenhorn and Demont 2004, Shelomi 2012), though demonstrations of such clines evolving repeatedly in nature are rare and limited to widespread species, with *Drosophila* currently being the best (and only) example (Partridge and Coyne 1997, Huey et al. 2000, Klepsatel et al. 2014). However, while replicated evolution is suggestive of adaptation, it is not sufficient evidence. In particular, random differentiation among populations by neutral genetic drift always occurs in the background, such that natural selection has to be consistently stronger than genetic drift to produce local adaptations (Lewontin and Krakauer 1973). Comparisons of the genetic differentiation in quantitative traits (Q_{ST}) to that observed at neutral loci (F_{ST}) is a recently well-established indirect approach for evaluating the relative importance of selection versus genetic drift in natural populations. Such studies have revealed that Q_{ST} often exceeds F_{ST}, suggesting a greater role of diversifying or directional natural selection in shaping genetic differentiation within species (Merilä and Crnokrak 2001, McKay and Latta 2002, Chenoweth and Blows 2008, Leinonen et al. 2008, Whitlock and Guillaume 2009). F_{ST}/Q_{ST} comparisons are thus particularly helpful when assessing the relative contributions of selection and drift along latitudinal clines (Merilä 1997, Storz 2002, Palo et al. 2003, Demont et al. 2008, Kawakami et al. 2011, Shama et al. 2011, Hangartner et al. 2012, Klepsatel et al. 2014, Fabian et al. 2015).

We investigated whether latitudinal clines in a suite of interconnected life-history traits – diapause propensity, development time, growth rate and body size – are repeatable across three continents and three laboratory rearing temperatures (12°C, 18°C, 24°C) in the yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae; Fig. 1), a prominent and widespread model species in evolutionary ecology (Blanckenhorn 2009). Our laboratory common garden study tests for evolved, genetic clines, which from an evolutionary point of view is superior to merely assessing phenotypic patterns derived from field-caught specimens, as commonly utilized in macro-ecology (Chown and Gaston 2010). To address the repeatability of the underlying evolutionary mechanisms, we combine this with a F_{ST}/Q_{ST} approach contrasting quantitative (putatively adaptive) and molecular (putatively neutral) population genetic variation. We expected the likelihood of individuals entering diapause (at any given pre-winter condition) to increase as temperatures become cooler and seasons shorter with latitude (Bradshaw and Holzapfel 2001, Schmidt et al. 2005). We further tested whether clinal variation in body size is consistent across continents, and whether the observed clines follow the converse Bergmann patterns typically mediated by season length, thus implicating indirect selection on development time or growth rate, rather than positive Bergmann patterns putatively generated by temperature effects per se (Blanckenhorn and Demont 2004, Berger et al. 2011). Crucially, we expected traits under significant selection (with $Q_{ST} > F_{ST}$), particularly diapause
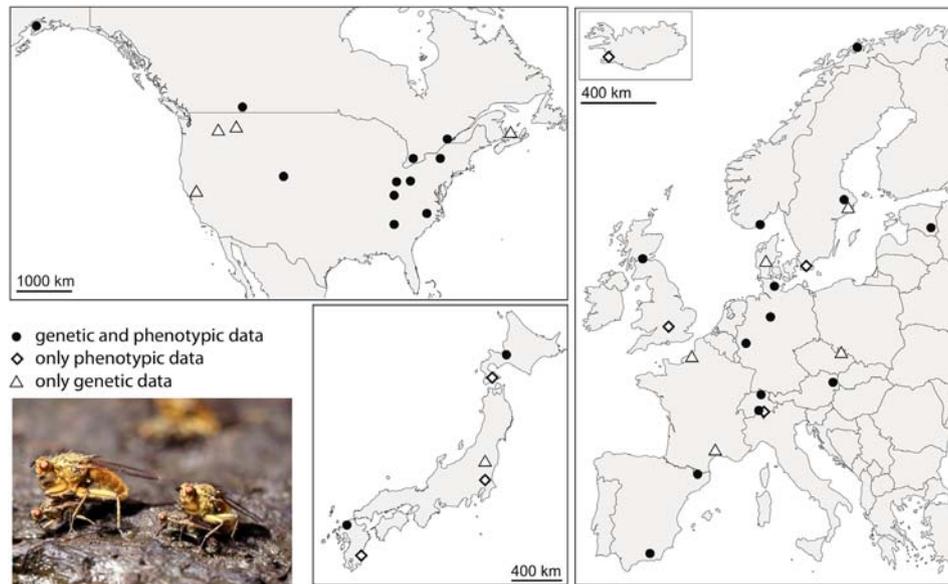
Figure 1. Yellow dung fly populations sampled in Europe, North America and Japan.

but possibly all others as well, to exhibit similar clines across geographic regions and rearing temperatures, whereas traits under minimal selection (low $Q_{ST} \approx F_{ST}$) should not show consistent clines unless they are tightly correlated with traits under selection.

Methods

Study species

Yellow dung flies are common throughout the northern hemisphere on and around cow pastures, a habitat that has become very widespread worldwide due to animal husbandry by humans. These flies prefer cool climates, occurring at high latitude sites as high as Spitzbergen, Greenland or Iceland in Europe, and Alaska in North America, where they are likely univoltine (one generation per year). In the warmer, southern areas of its distribution the species inhabits higher altitudes such as the Sierra Nevada in Spain or North America, producing at least four overlapping generations per year. In central European lowlands, each year features a spring (March–June) and an autumn season (September–November) generation, while during the hot midsummer (July and August) the flies largely disappear from the pastures due to their heat sensitivity, a pattern not evident at higher latitudes or altitudes (Blanckenhorn 2009, Blanckenhorn et al. 2010). Studies of Swiss populations indicate that altitudinal population differentiation is very weak while phenotypic plasticity is extensive (Blanckenhorn 2009). As these insects are good flyers, there is ample gene flow, presumably impeding local adaptation at least over short spatial scales across Switzerland (Kraushaar et al. 2002, Kaufmann et al. 2013). Molecular genetic differentiation of European populations is detectable but relatively weak (Demont et al. 2008).

Yellow dung fly adults are sit-and-wait predators of small flying insects and need to feed on prey to reproduce. Females spend most of their time foraging on nectar (for energy) and prey (for reproduction) in the vegetation surrounding pastures. Every few days they lay a clutch of eggs into fresh dung of large mammals, mainly cattle, on which larvae feed and wherein they develop. Many males typically wait at the dung to mate with incoming females. Larvae face unpredictable spatio-temporal variation in dung (i.e. food) quality and quantity, intra- as well as inter-specific competition, and dung drying, factors all greatly affecting the flies' growth, development and final adult body size, fostering great plasticity in all these traits. Yellow dung flies overwinter in the pupal stage, which has to be reached before the first frost (Blanckenhorn 2009, Blanckenhorn et al. 2010).

Population sampling and common garden rearing

We sampled approximately 1400 yellow dung flies from 45 sites (i.e. populations), 25 in Europe, 14 in North America, and 6 in Japan (Supplementary material Appendix 1 Table A1; Fig. 1). All flies were caught on haphazardly chosen cow pastures in the vicinity of collectors' residences with otherwise no special characteristics, either as copulating pairs by carefully lowering a vial over the insects, or by sweep-netting. European flies were sampled over three years (2003, 2007, 2009), North American flies were all sampled in 2009 (spring or autumn), and Japanese flies in spring 2013 (Supplementary material Appendix 1 Table A1). Live flies were then brought, and/or sent by express post, in these vials (containing sugar and humid toilet paper) in a cooler to one of the two experimental laboratories (see Blanckenhorn et al. 2010 for further details). Once there, flies were fed and allowed to copulate (again) with a random male from the same population within few days to generate a laboratory F1 generation (at 18°C,

12 h photoperiod, and 60% relative humidity) for our common garden rearing described below. Thereafter, the field-caught flies were preserved in pure ethanol and/or frozen at –80°C for later population genetic analysis of 10 polymorphic microsatellite loci to calculate F_{ST}, as described in detail by Schäfer et al. (2018). Supplementary material Appendix 1 Table A2 provides essential information on sample sizes, number of alleles, and mean observed (H_O) and expected (H_E) heterozygosity for each locus for North American, European and Japanese fly populations.

Common garden rearing was performed in temporal blocks (i.e. sampling years) in two different laboratories (Zürich and Lexington, KY) at the same conditions. At this point, flies from the various populations had been in the laboratory for variable amounts of time. By randomly crossing F1 females and males (but avoiding within-family matings) we produced F2 and, for some populations (Supplementary material Appendix 1 Table A1), later generation full-sib families to assess their life history traits in three distinct environments (Demont et al. 2008, Scharf et al. 2010). Full-sib larvae were split into constant 12°, 18° or 24°C regimes associated with 10, 12, and 14 h photoperiod (respectively; all at 60% relative humidity). We emphasize that the sole purpose of common garden rearing here was to document heritable clines largely free of random environmental noise, permitting direct comparisons across continents at the same, necessarily arbitrary environmental conditions chosen, while at the same time covering the full scope of phenotypic plasticity exhibited by this fly. Effects of photoperiod and temperature were disentangled in a previous study (Scharf et al. 2010) and were not the focus here.

Larvae were reared in plastic containers with overabundant (> 2 g per larva) homogenized and previously frozen cow dung. We checked daily for emerged adults to assess egg-to-adult survival and development times for all individuals. We also measured the hind tibia length of 1–6 emerged males and females per family (on average ca 3), a trait that well reflects overall body size of this species and is under direct and indirect selection (Blanckenhorn 2007, 2009). An approximate linearized estimate of growth rate was calculated by dividing hind tibia length by development time, for directly developing (i.e. non-diapausing) individuals only (Berger et al. 2011, Rohner et al. 2017).

Yellow dung flies raised at 12°C may enter diapause. Separation of directly and indirectly developing (i.e. diapausing) individuals in the 12°C treatment was based on their bimodal distribution of emergence times: development times < 70 d were classified as direct developers and those ≥ 70 d as diapausing (Demont and Blanckenhorn 2008). The number of days individuals spent diapausing at 12°C before emerging was here termed diapause duration. More details on handling and rearing methods can be found in Blanckenhorn et al. (2010).

Statistical analyses

All life history traits were analysed separately using full-sib family means (number of families given in Supplementary material Appendix 1 Table A1) or, alternatively, the number of populations as independent replicates to avoid pseudoreplication. We fitted generalized linear mixed full models with sex (a repeated measure because male and female offspring were siblings), rearing temperature (12°C, 18°C, 24°C), and continent as fixed effects, plus latitude or altitude or winter length as continuous covariates. As latitude, altitude, season length and even longitude are naturally highly correlated in this data set (Supplementary material Appendix 1 Table A1), multiple regressions cannot serve to rank the impact of these variables. We therefore gave priority to analysis of latitude, however reporting separate effects of the other covariates as well. The models additionally included laboratory generation, collection year (for Europe), and collection season (i.e. spring vs fall for North America) as blocking variables (all listed in Supplementary material Appendix 1 Table A1), which were often significant but not of interest here. Diapause traits were analysed similarly but without the factors sex and temperature.

In alternative analyses for the F_{ST}/Q_{ST} comparison, we entered population as a random effect instead of latitude (but never both because the two variables coincide): the former variance component estimates non-directional geographic population variation, whereas the latter, being a fraction of the former, estimates directional latitudinal variation. As diapause only occurred at 12°C, models for the proportion of diapausing individuals (model with binomial errors) and the duration of diapause did not include rearing temperature or sex.

Estimation of genetic variance and Q_{ST}

We computed population differentiation in quantitative traits (Q_{ST}) according to the formula $Q_{ST} = Var_{pop}/(Var_{pop} + 2Var_{within})$, where Var_{pop} is the observed component of variance between populations, and Var_{within} the observed component of additive genetic variance within populations (Spitze 1993, Merilä and Crnokrak 2001). Being based on full-sib families, we cannot evaluate the influence of dominance and maternal effects on our estimates of genetic variance (Lynch and Walsh 1998). Confidence intervals of Q_{ST} were calculated by a parametric bootstrap method adapted from O'Hara and Merilä (2005) and implemented in the R package 'QstFstComp' (<https://github.com/kjgilbert/QstFstComp>). Neutral population variation F_{ST} was calculated in a standard way as explained in Schäfer et al. (2018), from which we took the estimates for our F_{ST}/Q_{ST} comparison here.

Comparisons between F_{ST} and Q_{ST} values were performed following the bootstrapping method developed by Whitlock and Guillaume (2009), with corrections for unbalanced sample sizes and full-sib breeding design developed by Gilbert and Whitlock (2015) as implemented in the 'QstFstComp' package. This approach compares the observed $Q_{ST} - F_{ST}$ difference with its expected simulated distribution under a neutral hypothesis. Separately for Europe and North America (dropping Japan for reasons of low sample size), we generated 10 000 bootstrap replicates of the expected neutral

$Q_{ST} - F_{ST}$ distribution for each trait-treatment combination, from which we inferred the empirical p-value associated with our point estimates of $Q_{ST} - F_{ST}$. We also assessed the significance of the additive genetic variance estimates ($V_a = Var_{within}$) for all traits from the same bootstrapping procedure, using two times the dam (mother) variance component as V_a.

Results

Repeatable latitudinal clines in life history traits on three continents?

Latitudinal clines in diapause frequency and duration were marked, as expected due to previsusly documented strong viability selection exerted by winter duration (Blanckenhorn 2007, 2009). The average proportion of offspring per full-sib family entering diapause at 12°C (and 10 h light) steeply increased with latitude (Fig. 2a; $F_{1,790} = 236.06$, p < 0.001; analysis based on family means), which strongly reflects winter length (Supplementary material Appendix 1 Table A1). These clines were consistent in direction across continents but varied in magnitude (i.e. slope; estimates in Fig. 2a), as the cline was much shallower in Europe than in either Japan or North America (significant latitude by continent interaction: $F_{2,790} = 69.67$, p < 0.001). The mean proportion of flies entering diapause also varied between continents (continent main effect: $F_{2,790} = 62.07$, p < 0.001). Clines consistent in direction but variable in magnitude across continents were similarly evident for diapause duration (steepest in Japan and shallowest in Europe; latitude by continent interaction,

$F_{2,595} = 6.45$, p = 0.002; latitude effect: $F_{1,595} = 15.28$, p < 0.001; continent effect: $F_{2,595} = 5.92$, p = 0.003; Fig. 2b). Altitude as covariate (instead of latitude) was not significant in either case (p > 0.1), whereas winter length as covariate produced results similar to latitude (p < 0.001).

Directly developing, non-diapausing flies from higher latitudes had shorter egg-to-adult development times, as expected because seasonal time constraints amplify with latitude. The corresponding negative clines were generally consistent in direction but varied somewhat in their slopes among continents and temperatures (slopes and correlations in Table 1; Fig. 3; marginally significant overall effect based on populations as independent units but significant when based on full-sib families; continent and temperature interactions with latitude not significant in ANCOVA Table 2). Latitudinal slopes were more negative in North America and Japan than in Europe, where the slopes trended negative but were often not significantly different from zero (Table 1). However, at least in Europe the negative slopes were strongly driven by the high latitude populations; clines in development time are largely flat throughout most of the latitudinal range, but then decline at very high northern latitudes where flies are clearly univoltine (one generation per year) and cannot escape seasonal time constraints (Fig. 3; Roff 1980). (Note that at 12°C most (often all) offspring from the most northern populations entered diapause, explaining missing development times at that temperature in Fig. 3.) The separate relationships of development time with altitude and winter length were overall similarly negative but weaker and not significant (Table 2; compare Table 1, Supplementary material Appendix 1 Table A3).
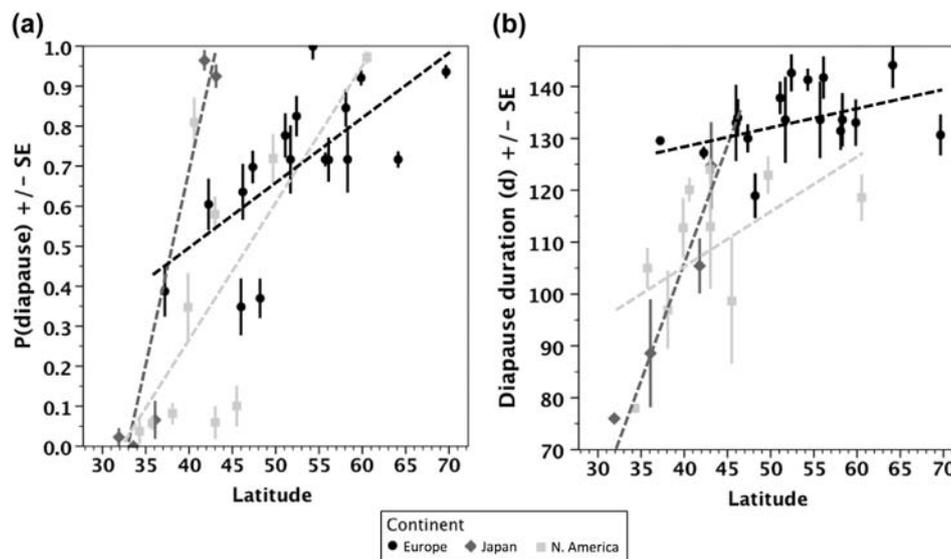

Figure 2. Mean ± SE proportion of yellow dung flies diapausing at 12°C (a) and their corresponding diapause duration (b) for latitudinal European, North American and Japanese populations. Both traits strongly increase with latitude. (a) Separate slopes (± SE) for Europe (0.017 ± 0.002; partial correlation $r = 0.36$), Japan (0.099 ± 0.005; $r = 0.90$), and North America (0.036 ± 0.003; $r = 0.59$; all p < 0.001); (b) Europe (0.26 ± 0.13; $r = 0.10$; p = 0.045), Japan (4.98 ± 2.46; $r = 0.28$; p = 0.050), and North America (0.48 ± 0.22; $r = 0.19$; p = 0.029).

Table 1. Sex- and temperature-specific regression slopes (*B*) and corresponding partial correlations (*r*) for development time, growth rate and hind tibia length with latitude based on family trait means (p < 0.05 in bold; p < 0.1 in bold italics), with overall means and 95% CI at the bottom. ([1] Still significant when test is based on populations as statistical units.)

Temperature	Sex	Development time		Growth rate ($\times 10^3$)		Hind tibia length	
		B	*r*	*B*	*r*	*B*	*r*
North America							
12	female	**−0.16**	**−0.17**	**0.30**	**0.30**	**0.005**	**0.29**[1]
18	female	−0.02	−0.07	**0.19**	**0.17**	**0.004**	**0.23**
24	female	**−0.08**	**−0.34**[1]	**0.77**	**0.44**[1]	**0.004**	**0.20**
12	male	**−0.17**	**−0.18**	**0.36**	**0.32**	**0.007**	**0.30**[1]
18	male	−0.01	−0.06	**0.20**	**0.17**	**0.004**	**0.20**[1]
24	male	**−0.08**	**−0.33**[1]	**0.94**	**0.43**[1]	**0.005**	**0.22**
Europe							
12	female	−0.03	−0.04	**0.10**	**0.09**	−0.001	−0.04
18	female	*−0.02*	*−0.08*	**0.13**	**0.14**	0.000	0.02
24	female	*−0.01*	*−0.08*	**0.30**	**0.24**[1]	**0.004**	**0.26**[1]
12	male	−0.06	−0.08	**0.17**	**0.19**[1]	0.001	0.04
18	male	**−0.04**	**−0.14**	**0.24**	**0.20**[1]	**0.002**	**0.11**
24	male	−0.01	−0.06	**0.34**	**0.21**[1]	**0.005**	**0.20**[1]
Japan							
12	female	−0.13	−0.19	0.11	0.10	**−0.006**	**−0.25**
18	female	−0.01	−0.03	−0.13	−0.07	−0.004	−0.17
24	female	0.03	0.13	−0.20	−0.11	0.000	−0.01
12	male	**−0.32**	**−0.52**[1]	**0.64**	**0.52**[1]	−0.001	0.02
18	male	−0.02	−0.06	0.25	0.13	−0.005	0.19
24	male	−0.02	−0.07	**0.40**	**0.16**	**0.010**	**0.23**
	mean	**−0.062**	**−0.124**	**+0.284**	**0.200**	**+0.003**	**0.119**
	95%CI	0.040	0.070	0.130	0.075	0.002	0.074

The relationship between latitude and adult body size (hind tibia length) was overall positive but least consistent in direction and slope and sometimes flat, featuring considerable variation between the sexes, temperatures and continents (Table 1; Fig. 3; see interaction terms with latitude in Table 2). Hind tibia length increased steeply with latitude in North American flies, only weakly so in Europe, but tended to decrease (non-significantly) in Japan (potentially related to the lower sample size). The separate effect of winter length was similar to latitude, while altitude was overall not significant and inconsistent (Table 2, Supplementary material Appendix 1 Table A3).

Growth rate (calculated as body size divided by development time) consistently increased with latitude across all continents (except Japanese females; Fig. 3), again with quantitative heterogeneity in the slopes across continents and rearing temperatures (Table 1, 2). Winter length again closely mirrored latitude while altitude trends were similar but weaker and more inconsistent (Table 2, Supplementary material Appendix 1 Table A3).

Q_{ST}/F_{ST} comparison

Neutral population differentiation of yellow dung fly populations, F_{ST}, based on 10 microsatellite markers is very low but significantly greater than zero overall (p < 0.001). The worldwide F_{ST} is 0.044 (95% CI: 0.034, 0.059), but differentiation was much lower within continents: 0.005 (95% CI: 0.002, 0.007) in Europe, 0.014 (95% CI: 0.001,

0.019) in North America, and 0.006 (95% CI: −0.003, 0.017) in Japan (elaborated in Schäfer et al. 2018; cf. Supplementary material Appendix 1 Table A2). Geographic quantitative genetic population differentiation, $Q_{ST,}$ of most traits was significantly greater than F_{ST} and tended to be higher for the diapause traits (Table 3; pairwise differences can be inferred from the confidence intervals given). However, the purely latitudinal component of this variation (i.e. the variance component derived from models with latitude as covariate, expressed as a fraction of the variance component of the corresponding model with population as a factor) typically explained only a small fraction, ca 20% (8–40%), of the total geographic variation in Q_{ST} (Table 3).

Discussion

We have shown for the widespread yellow dung fly that latitudinal clines in several interrelated life history traits are qualitatively reproducible across three continents in the northern hemisphere (Europe, North America, Asia), although the slopes of the clines varied considerably across continents. As these results are based on laboratory common garden rearing of multiple families at the same (arbitrary) conditions, they reflect evolved genetic differentiation, as opposed to merely phenotypic patterns derived from field-caught specimens as commonly utilized in macro-ecology (Jetz et al. 2009, Chown and Gaston 2010, Van Buskirk et al. 2011). Latitudinal relationships were most strongly positive
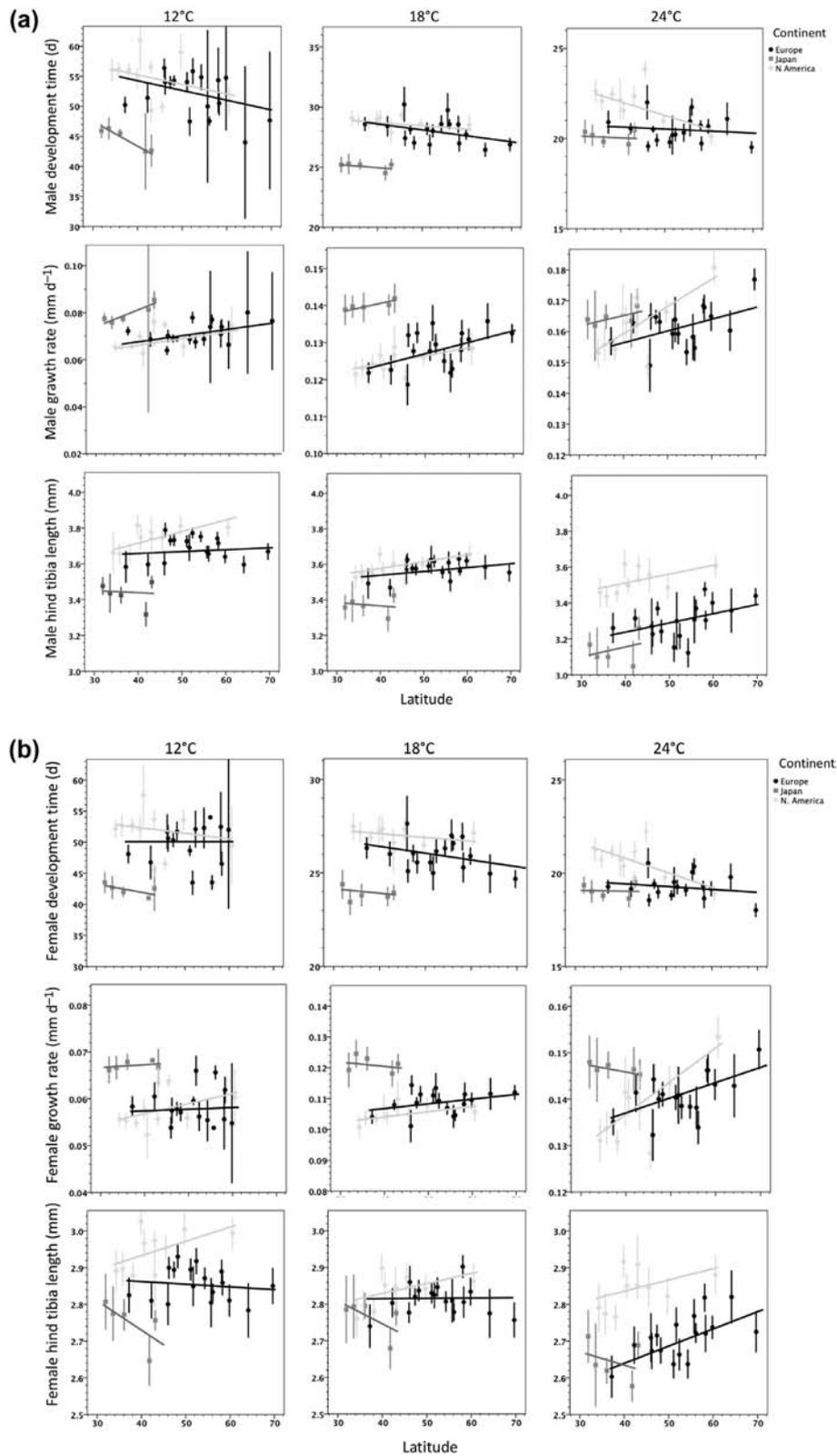
Figure 3. Mean ± 95% CI common-garden egg-to-adult development time (top), growth rate (center), and hind tibia length (bottom) of (a) male and (b) female yellow dung flies for latitudinal populations on three continents (different colors: Europe, Japan, North America) at three rearing temperatures (left to right: 12°C, 18°C, 24°C). Development time generally decreases and growth rate increases with latitude, while body size clines are inconsistent.

Table 2. Analysis of covariance (ANCOVA) tables for three life history traits. The separate effects of the (linear) covariates latitude, altitude and winter length are given, in general going in the same direction (sign of relationship in brackets). The last three blocking factors were tested against the number of full-sib families (n = 1829–2192), the remaining terms against the number of populations as the error (n = 81–83). p < 0.05 in bold; p < 0.1 in bold italics.

	df	Hind tibia length (mm)		Development time (d)		Growth rate (mm d^{-1}) × 10^{-4}	
		F	p	F	p	F	p
Intercept	1	4447.32	< 0.001	1787.94	< 0.001	622.86	< 0.001
Latitude	1	9.62	**0.003**[(+)]	2.97	***0.089**[(−)]*	22.68	**< 0.001**[(+)]
Altitude	1	2.29	0.134[(+/−)]	2.51	0.117[(−)]	2.62	0.109[(+)]
Winter length	1	7.03	**0.010**[(+)]	1.34	0.251[(−)]	17.39	**< 0.001**[(+)]
Continent	2	89.01	**< 0.001**	39.80	**< 0.001**	45.84	**< 0.001**
Temperature	2	96.51	**< 0.001**	1809.50	**< 0.001**	2883.75	**< 0.001**
Continent × temperature	4	8.05	**< 0.001**	10.67	**< 0.001**	2.85	**0.029**
Continent × latitude	2	2.60	***0.080***		ns		ns
Temperature × latitude	2		ns		ns		ns
Continent × temperature × latitude	4		ns		ns		ns
Sex	1	587.73	**< 0.001**	17.72	**< 0.001**	53.79	**< 0.001**
Sex × continent	2	66.33	**< 0.001**	3.28	**0.043**	3.80	**0.027**
Sex × temperature	2	136.96	**< 0.001**	16.81	**< 0.001**	96.73	**< 0.001**
Sex × continent × temperature	4	9.22	**< 0.001**		ns	4.56	**0.002**
Sex × latitude	1	10.31	**0.002**		ns	13.51	**0.002**
Sex × continent × latitude	2		ns		ns	9.93	**0.028**
Region (continent)	2	*3.29*	**0.038**	*15.65*	**0.017**	*16.44*	**< 0.001**
Collection block (continent)	3	*6.92*	**< 0.001**	*10.15*	**< 0.001**	*15.51*	**< 0.001**
Generation	7	1.20	0.297	7.96	**< 0.001**	5.63	**< 0.001**

for winter diapause traits (as also evident in the largest proportional effect of latitude for diapause incidence in Europe: Table 3), were consistent but rather shallow for larval growth rate (faster at higher latitudes) and development time (shorter at higher latitudes), and most inconsistent for body size (Fig. 3; Table 1). Estimates of Q_{ST} along the latitudinal clines were generally greater than estimates of F_{ST}, suggesting that the observed clines are more likely caused by natural selection than genetic drift.

Latitudinal clines in diapause, growth and body size

Clines in all measured traits are likely directly mediated by season length, which latitude well reflects (Supplementary material Appendix 1 Table A1), rather than temperature, as theory predicts greater propensity of entering diapause, faster growth, and shorter development in high latitude environments with short seasons (Roff 1980, Rowe and Ludwig 1991, Schmidt et al. 2005). Natural, i.e. particularly viability selection acting on diapause traits is known to be immediate and strong in many species, resulting in marked heritable clines in diapause incidence and/or duration (both of which should increase with winter length if adaptive: cf. Supplementary material Appendix 1 Table A1; Tauber et al. 1986, Bradshaw and Holzapfel 2001, Schmidt et al. 2005). Natural selection on juvenile growth and development is typically also substantial (Nylin and Gotthard 1998; see estimates for dung flies in Blanckenhorn 2007), despite considerable phenotypic plasticity assuaging such selection (Blanckenhorn 2009). Their wide latitudinal range notwithstanding, the experienced climatic conditions at the beginning and the end of the season may actually not differ so much for flies

at the various sites because the season begins later and ends earlier at higher latitudes (as winter length increases: Supplementary material Appendix 1 Table A1). Particularly in light of the species' great dispersal capacity mediating gene flow (Kaufmann et al. 2013, Schäfer et al. 2018), generally weaker selection may thus produce only slight differentiation

Table 3. Quantitative genetic geographic population differentiation estimates (Qst ± 95% CI) for six life history traits of North American and European yellow dung fly populations across both sexes and all temperatures (Japan omitted due to low sample size). The percentage of the total geographic variance among populations that is explained by latitude is also given. Bold values are significantly greater than the corresponding Fst value estimating neutral variation as outlined by Whitlock and Guillaume (2009; not applicable to the binary trait p(diapause)). Two estimates differ significantly roughly when the 95% CI of one value does not overlap the mean of the other.

	Qst(pop)	Low 95% CI	High 95% CI	% Variance (latitude)
North America				
p(diapause)	*0.142*	*0.050*	*0.250*	*19.2*
Diapause duration	**0.130**	0.011	0.342	13.4
Body size	0.057	0.004	0.136	24.3
Development time	**0.140**	0.038	0.260	26.5
Growth rate	**0.076**	0.012	0.165	38.2
Fst	*0.014*	*0.010*	*0.019*	
Europe				
p(diapause)	*0.111*	*0.040*	*0.200*	*39.9*
Diapause duration	**0.188**	0.068	0.367	10.7
Body size	**0.325**	0.122	0.567	8.0
Development time	**0.103**	0.041	0.178	12.5
Growth rate	**0.174**	0.024	0.380	12.3
Fst	*0.005*	*0.002*	*0.007*	

in growth, development and final body size, presumably because these traits are optimized according to similar costs and benefits everywhere, ultimately resulting in the rather shallow clines observed.

Body size (hind tibia length), in contrast, varied considerably with latitude, with specifics differing even qualitatively among continents (Fig. 3; Table 1). Although flies generally got larger with increasing latitude in North America, the Japanese clines were sometimes slightly negative, and the European clines largely flat or somewhat hump-shaped. Positive Bergmann clines (increasing body size with latitude) are inconsistent with selection caused by seasonal time constraints, which is predicted to lead to converse (i.e. negative) size clines (smaller at higher latitudes: Masaki 1967, Roff 1980, Mousseau 1997, Shelomi 2012), suggesting that seasonality is not an adequate general explanation for the body size clines documented here. Flat latitudinal body size clines may suggest perfect countergradient variation (Levinton and Monahan 1983, Conover and Present 1990), according to which the genetic body size of latitudinal populations presumably evolves to (precisely) compensate for any local environmental factors limiting phenotypic size. However, this postulates a global optimal body size, for which there is little evidence in yellow dung flies (Blanckenhorn 2007, 2009). Rather, adult male body size is under strong positive sexual selection and female size under strong positive fecundity selection in most populations, although the intensity of selection, including that of juvenile viability selection, varies considerably across space and time (Jann et al. 2000, Kraushaar and Blanckenhorn 2002, Blanckenhorn 2007, 2009). We see little reason why sexual and/or fecundity selection should get systematically stronger with latitude, which could explain the primarily positive Bergmann clines obtained, and we have no such evidence.

We can only speculate about the continental differences in the body size clines, which are consistently positive in North America but largely flat in Europe and Japan. Continental differences in molecular genetic variation suggest that North American dung flies have Eurasian ancestry (and not vice versa), and that the New World has likely been colonized quite recently (Schäfer et al. 2018). Colonization in North America presumably occurred from the (south)east to the northwest, such that body size tends to increase in the presumed direction of colonization in agreement with greater dispersal capacity of larger individuals, as postulated and found in a variety of taxa including bees and flies (Guédot et al. 2009, Zurbuchen et al. 2010, Rohner et al. 2015). While the Japanese sample size is too limited to permit firm conclusions, migration and gene flow may have equalized body sizes in the likely ancestral and therefore much older European fly populations, eventually resulting in weak to no latitudinal differentiation.

We stress that winter length (i.e. the inverse of season length: Supplementary material Appendix 1 Table A1) generally produced qualitatively and quantitatively similar effects as latitude (Table 2), whereas effects of the altitude at which the populations were sampled were expectedly in the same direction as those for latitude but much weaker and often not significant for all traits (Table 1, 2, Supplementary material Appendix 1 Table A3; Fig. 2, 3). In particular, altitude cannot help explain the lack of clear clines in Europe, as in connection with the heat sensitivity of yellow dung flies altitude and latitude are strongly negatively correlated (Supplementary material Appendix 1 Table A1). In general, in yellow dung flies all covariates considered here (latitude, altitude, winter length, even longitude) tend to be highly correlated, such that their effects cannot be effectively separated statistically.

Integrating all life history traits studied, we suggest that natural selection exerted by season length is foremost optimizing juvenile growth and development rates, and not body size per se. As these two juvenile traits determine final adult size, body size is initially merely indirectly selected, and later further optimized by sexual and fecundity selection at the adult stage (Blanckenhorn 2007, 2009). This implies that body size should be substantially influenced by correlational selection on the other juvenile traits and more difficult to optimize in general due to multiple conflicting selection pressures at various life stages. In this sense, the diverse (Bergmann, converse Bergmann, or flat) body size clines evident in nature for various species, and also here for a single species, may be regarded as a secondary consequence or epiphenomenon that is naturally more fickle and ultimately more difficult to predict or explain than the much clearer diapause, development time, and growth rate clines obtained here (Partridge and Coyne 1997, Huey et al. 2000, Blanckenhorn and Demont 2004). Nevertheless, yellow dung flies primarily display positive Bergmann size clines and not the negative clines predicted by season length effects (Mousseau 1997, Blanckenhorn and Demont 2004), implying that other factors must be at work. What remains possible is that Bergmann clines are non-adaptive consequences of the temperature dependence of physiological processes at the cellular level (Van Voorhies 1996, Atkinson and Sibly 1997). This was our prime motivation for assessing latitudinal population variation at three different temperatures, as we expected any physiological process to result in systematic clinal patterns in response to rearing temperature (James et al. 1997, Gilchrist and Huey 2004). While we found some heterogeneity in slopes across the three representative temperatures used, this variation was not systematic (Table 1). We therefore conclude that the slope plasticity found here for body size does not indicate a systematic physiological pattern, adaptive or not.

F_{ST} and Q_{ST} along the latitudinal clines

Worldwide neutral molecular differentiation, F_{ST}, in yellow dung flies proved low (ca 4.5%), and even lower (ca. 1%) within continents, although these values are significantly greater than zero due to our substantial sample sizes (elaborated in Schäfer et al. 2018; Supplementary material Appendix 1 Table A2). On average, we expected and largely found quantitative genetic population differentiation (Q_{ST})

to exceed F_{ST} across geographic regions and rearing temperatures (Table 3). This result supports a general role of natural selection in mediating consistent geographic and latitudinal differentiation of yellow dung fly life histories, even more so because it was repeatable across the continents (Merilä and Crnokrak 2001, Leinonen et al. 2008). As latitudinal differentiation necessarily explains only a fraction of the total geographic differentiation, latitudinal Q_{ST} is inevitably lower than Q_{ST} based on non-directional geographic population differentiation. However, beyond the relatively large latitudinal component of Q_{ST} for diapause incidence (ca 40%) and the rather small component for body size (8%) in Europe (which shows greatest overall variation of all traits: Table 1–3, Fig. 3), latitude explains ca 10–25% of the geographic variance for most traits assessed. Comparing Q_{ST} values among traits also does not reveal any marked patterns, other than values being on average lower in North America than in Europe (Table 3). We had expected that Q_{ST} would reveal which traits are subject to stronger (direct) selection, for which the relative latitudinal variance component should then be observably higher (e.g. for diapause vs body size). However, though the Q_{ST}/F_{ST} ratio consistently suggests that traits are under selection for differentiation along the cline, we see no clear patterns indicating which traits are consistently (i.e. across continents) under greatest selection (Table 3).

Reproducibility of geographic clines

Reproducibility of evolved patterns is necessary but not sufficient evidence for trait differentiation along clines being due to evolution by natural selection, as alternative constraint-based or demographic hypotheses can also produce consistent patterns (Flatt 2016, and references therein). For instance, as discussed above, secondary colonization from Europe may partly explain the North American Bergmann cline, possibly being mediated by better flight propensity of larger flies. Independent of the underlying cause, the evidence presented here that latitudinal clines in growth rate, development time and diapause in the widespread yellow dung fly are largely consistent on three continents is a major step towards elucidating their possible adaptive nature. Evidence for parallel evolution of independent intraspecific clines in nature, all else being alike, is very rare, primarily because it can be uncovered only in widespread species (Partridge and Coyne 1997). Parallel continental clines for body size (but no other traits) so far had been documented for *Drosophila subobscura*, which colonized from Europe to evolve similar body size clines in North and South America (Huey et al. 2000, Gilchrist et al. 2004), and in *D. melanogaster* across Europe, Africa, Australia, North and South America by various accumulated studies (James et al. 1997, Zwaan et al. 2000, Klepsatel et al. 2014). Whilst latitudinal clines in diapause propensity, growth rate and development time are primarily mediated by season length and clearly match theoretical predictions for adaptive traits shaped by natural selection (Roff 1980, Rowe and Ludwig 1991, Bradshaw and Holzapfel 2001, Schmidt et al. 2005), the adaptive nature of the slight (and sometimes totally flat)

Bergmann clines for body size obtained here remains ambiguous. We currently favour the hypothesis that they may be a secondary consequence of selection pressures shaping growth and development, and therefore are less consistent in general, not least also because Bergmann (presumably exerted by temperature) and converse Bergmann effects (exerted by season length) may combine to yield any intermediate outcome (Blanckenhorn and Demont 2004).

Latitude merely is a traditional proxy for systematic changes in climate on which some prominent historical biogeographic rules are based (Blackburn et al. 1999, Blanckenhorn and Demont 2004, Shelomi 2012). One might therefore think that such a proxy has become obsolete because site-specific climate data are now widely available world-wide. This is not so. Most crucially, as should have become evident from the above discussion, most of the key climatic variables (temperature, season length, insolation, UV radiation, humidity, etc.) change in conjunction along latitude such that they are necessarily highly correlated, generally too correlated so as to statistically separate them using multiple regressions. This was also the case here. As latitude is often obtainable even for poorly documented data sets in the literature, meta analyses therefore will continue to use this proxy for years to come. To elucidate the adaptive value and selective causes of intraspecific Bergmann clines, more studies of widespread species are needed that integrate various interrelated life history traits to document reproducible patterns on several continents.

References

Atkinson, D. and Sibly, R. M. 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. – Trends Ecol. Evol. 12: 235–239.

Berger, D. et al. 2011. High temperatures reveal cryptic genetic variation in a polymorphic female sperm storage organ. – Evolution 65: 2830–2842.

Blackburn, T. M. et al. 1999. Geographic gradients in body size: a clarification of Bergmann's rule. – Divers. Distrib. 5: 165–174.

Blanckenhorn, W. U. 2007. Case studies of the differential equilibrium hypothesis of sexual size dimorphism in dung flies. – In: Fairbairn, D. J. et al. (eds), Sex, size and gender roles. Evolutionary studies of sexual size dimorphism. Oxford Univ. Press, pp. 106–114.

Blanckenhorn, W. U. 2009. Causes and consequences of phenotypic plasticity in body size: the case of the yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae). – In: Whitman, D. W. and Ananthakrishnan, T. N. (eds), Phenotypic plasticity of insects: mechanism and consequences. Science Publishers, pp. 369–422.

Blanckenhorn, W. U. and Fairbairn, D. J. 1995. Life history adaptation along a latitudinal cline in water striders. – J. Evol. Biol. 8: 21–41.

Blanckenhorn, W. U. and Demont, M. 2004. Bergmann and converse Bergmann latitudinal clines in Arthropods: two ends of a continuum? – Integr. Comp. Biol. 44: 413–424.

Blanckenhorn, W. U. et al. 2010. Natural history and laboratory culture of the yellow dung fly, *Scathophaga stercoraria* (L.; Diptera: Scathophagidae). – J. Insect Sci. 10: 11.

Bradshaw, W. E. and Holzapfel, C. M. 2001. Genetic shift in photoperiodic response correlated with global warming. – Proc. Natl Acad. Sci. USA 98: 14509–14511.

Chenoweth, S. F. and Blows, M. W. 2008. Qst meets the G matrix: the dimensionality of adaptive divergence in multiple correlated quantitative traits. – Evolution 62: 1437–1449.

Chown, S. L. and Gaston, K. J. 1999. Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. – Biol. Rev. 74: 87–120.

Chown, S. L. and Gaston, K. J. 2010. Body size variation in insects: a macroecological perspective. – Biol. Rev. 85: 139–169.

Conover, D. O. and Present, T. M. C. 1990. Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. – Oecologia 83: 316–324.

Demont, M. and Blanckenhorn, W. U. 2008. Genetic differentiation in diapause response along a latitudinal cline in European yellow dung fly populations. – Ecol. Entomol. 33: 197–201.

Demont, M. et al. 2008. Molecular and quantitative genetic differentiation across Europe in yellow dung flies. – J. Evol. Biol. 21: 1492–1503.

Fabian, D. K. et al. 2015. Spatially varying selection shapes life history clines among populations of *Drosophila melanogaster* from sub-Saharan Africa. – J. Evol. Biol. 28: 826–840.

Flatt, T. 2016. Genomics of clinal variation in Drosophila: disentangling the interactions of selection and demography. – Mol. Ecol. 25: 1023–1026.

Gilchrist, G. W. and Huey, R. B. 2004. Plastic and genetic variation in wing loading as a function of temperature within and among parallel clines in *Drosophila subobscura*. – Integr. Comp. Biol. 44: 461–470.

Gilchrist, G. W. et al. 2004. A time series of evolution in action: latitudinal cline in wing size in South American *Drosophila subobscura*. – Evolution 58: 768–780.

Gilbert, K. J. and Whitlock, M. C. 2015. Q(ST)-F-ST comparisons with unbalanced half-sib designs. – Mol. Ecol. Res. 15: 262–267.

Guédot, C. et al. 2009. Relationship between body size and homing ability in the genus *Osmia* (Hymenoptera; Megachilidae). – Ecol. Entomol. 34: 158–161.

Hangartner, S. et al. 2012. The quantitative genetic basis of adaptive divergence in the moor frog (*Rana arvalis*) and its implications for gene flow. – J. Evol. Biol. 25: 1587–1599.

Huey, R. B. et al. 2000. Rapid evolution of a geographic cline in size in an introduced fly. – Science 287: 308–309.

James, A. C. et al. 1997. Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. – Genetics 146: 881–890.

Jann, P. et al. 2000. Temporal and microspatial variation in the intensities of natural and sexual selection in the yellow dungfly *Scathophaga stercoria*. – J. Evol. Biol. 13: 927–938.

Jetz, W. et al. 2009. Phenotypic population divergence in terrestrial vertebrates at macro scales. – Ecol. Lett. 12: 1137–1146.

Kaufmann, C. et al. 2013. Size-dependent insect flight energetics at different sugar supplies. – Biol. J. Linn. Soc. 108: 565–578.

Kawakami, T. et al. 2011. Natural selection drives clinal life history patterns in the perennial sunflower species, *Helianthus maximiliani*. – Mol. Ecol. 20: 2318–2328.

Klepsatel, P. et al. 2014. Similarities and differences in altitudinal versus latitudinal variation for morphological traits in *Drosophila melanogaster*. – Evolution 68: 1385–1398.

Kraushaar, U. and Blanckenhorn, W. U. 2002. Population variation in sexual selection and its effect on body size allometry in two species of flies with contrasting sexual size dimorphism. – Evolution 56: 307–321.

Kraushaar, U. et al. 2002. Geographical and altitudinal population genetic structure of two dung fly species with contrasting mobility and temperature preference. – Heredity 89: 99–106.

Leinonen, T. et al. 2008. Comparative studies of quantitative trait and neutral marker divergence: a meta-analysis. – J. Evol. Biol. 21: 1–17.

Levinton, J. S. and Monahan, R. K. 1983. The latitudinal compensation hypothesis: growth data and a model of latitudinal growth differentiation based upon energy budgets. II. Intraspecific comparisons between subspecies of *Ophryotrocha puerilis*. – Biol. Bull. 165: 699–707.

Lewontin, R. C. and Krakauer, J. 1973. Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphism. – Genetics 74: 175–195.

Lynch, M. and Walsh, B. 1998. Genetics and analysis of quantitative traits. – Sinauer Associates.

Masaki, S. 1967. Geographic variation and climatic adaptation in a field cricket. – Evolution 21: 725–741.

Masaki, S. 1972. Climatic adaptation and photoperiodic response in the band-legged ground cricket. – Evolution 26: 587–600.

McKay, J. K. and Latta, R. G. 2002. Adaptive population divergence: markers, QTL and traits. – Trends Ecol. Evol. 17: 285–291.

Merilä, J. 1997. Quantitative trait and allozyme divergence in the greenfinch. – Biol. J. Linn. Soc. 61: 243–266.

Merilä, J. and Crnokrak, P. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. – J. Evol. Biol. 14: 892–903.

Mousseau, T. A. 1997. Ectotherms follow the converse Bergmann's rule. – Evolution 51: 630–632.

Nylin, S. and Gotthard, K. 1998. Plasticity in life history traits. – Annu. Rev. Entomol. 43: 63–83.

O'Hara, R. B. and Merilä, J. 2005. Bias and precision in Q_{ST} estimates: problems and some solutions. – Genetics 171: 1331–1339.

Palo, J. U. et al. 2003. Latitudinal divergence of common frog (*Rana temporaria*) life history traits by natural selection: evidence from a comparison of molecular and quantitative genetic data. – Mol. Ecol. 12: 1963–1978.

Partridge, L. and Coyne, J. A. 1997. Bergmann's rule in ectotherms: is it adaptive? – Evolution 51: 632–635.

Rohner, P. T. et al. 2015. Distribution, diversity gradients and Rapoport's elevational rule in the black scavenger flies of the Swiss Alps (Diptera: Sepsidae). – Insect Conserv. Divers. 8: 367–376.

Rohner, P. T. et al. 2017. Critical weight mediates sex-specific body size plasticity and sexual dimorphism in the yellow dung fly *Scathophaga stercoraria*. – Evol. Develop. 19: 147–156.

Roff, D. 1980. Optimizing development time in a seasonal environment: the 'ups and downs' of clinal variation. – Oecologia 45: 202–208.

Rowe, L. and Ludwig, D. 1991. Size and timing of metamorphosis in complex life cycles: time constraints and variation. – Ecology 72: 413–427.

Schäfer, M. A. et al. 2018. Geographic clines in wing morphology relate to colonization history in New World but not Old World populations of yellow dung flies. – Evolution in press.

Scharf, I. et al. 2010. Effects of maternal and offspring environmental conditions on growth, development and diapause in latitudinal yellow dung fly populations. – Clim. Res. 43: 115–125.

Schmidt, P. S. et al. 2005. Geographic variation in diapause incidence, life-history traits, and climatic adaptation in *Drosophila melanogaster*. – Evolution 59: 1721–1732.

Shama, L. N. S. et al. 2011. Latitudinal and voltinism compensation shape thermal reaction norms for growth rate. – Mol. Ecol. 20: 2929–2941.

Shelomi, M. 2012. Where are we now? Bergmann's rule sensu lato in insects. – Am. Nat. 180: 511–519.

Spitze, K. 1993. Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. – Genetics 135: 367–374.

Storz, J. F. 2002. Contrasting patterns of divergence in quantitative traits and neutral DNA markers: analysis of clinal variation. – Mol. Ecol. 11: 2537–2551.

Tauber, M. J. et al. 1986. Seasonal adaptations of insects. – Oxford Univ. Press.

Van Buskirk, J. et al. 2011. Declining body sizes in North American birds associated with climate change. – Oikos 119: 1047–1055.

van der Have, T. M. and de Jong, G. 1996. Adult size in ectotherms: temperature effects on growth and differentiation. – J. Theor. Biol. 18: 329–340.

Van Voorhies, W. A. 1996. Bergmann size clines: a simple explanation for their occurrence in ectotherms. – Evolution 50: 1259–1264.

Whitlock, M. C. and Guillaume, F. 2009. Testing for spatially divergent selection: comparing Q_{ST} to F_{ST}. – Genetics 183: 1055–1063.

Zurbuchen, A. et al. 2010. Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. – Biol. Conserv. 143: 669–676.

Zwaan, B. J. et al. 2000. Cellular basis of wing size variation in *Drosophila melanogaster*: a comparison of latitudinal clines on two continents. – Heredity 84: 338–347.

Supplementary material (Appendix ECOG-03752 at <www.ecography.org/appendix/ecog-03752>). Appendix 1.