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Replicated latitudinal clines in reproductive traits of European and North American yellow dung flies

Stephanie S. Bauerfeind¹, Martin A. Schäfer¹, David Berger^{1,2}, Wolf U. Blanckenhorn^{1,4} and Charles W. Fox^{3*}

¹ *Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstr. 190, 8057 Zurich, Switzerland*

² *Evolutionary Biology Centre, University of Uppsala, Norbyvägen 18D, 752 36, Uppsala, Sweden*

³ *Entomology Department, University of Kentucky, Lexington KY 40546-0091, USA; Orcid: 0000-0002-7545-7967*

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Abstract

Geographic variation in phenotypic traits is commonly correlated with spatial variation in the environment, e.g., seasonality and mean temperature, providing evidence that natural selection generates such patterns. In particular, both body size and egg size of ectothermic animals are commonly larger in northern climates, and temperature induces plastic responses in both traits. Size-independent egg quality can also vary with latitude, though this is rarely investigated. For the widespread yellow dung fly (*Scathophaga stercoraria*; Diptera: Scathophagidae), we investigated whether there are latitudinal clines in reproductive traits (clutch size, egg size and egg composition), whether these clines are due to variation in body and/or egg size, and whether such clines replicate across continents (North America, Europe). Egg size consistently increased with latitude, an effect largely explained by body size of the mother, while clutch size did not; overall reproductive effort thus increased with latitude. Both the absolute and relative (correcting for egg size) amount of egg protein increased with latitude, egg glycogen decreased with latitude, while latitudinal trends for egg lipids and total egg energy content were complex and non-linear. Altitude sometimes showed relationships analogous to those of latitude (egg proteins and glycogen) but occasionally opposite (egg size), possibly because latitude and altitude are negatively related among populations of this cold-adapted species. There was no evidence of a trade-off between egg size and number across latitudinal populations; if anything, the relationship was positive. Importantly, all traits, including body and egg size, varied with rearing temperature (12°C, 18°C, 24°C), generally following the temperature-size-rule. Clines based on common garden rearing, thus reflecting genetic differentiation, were qualitatively but not always quantitatively consistent between two continents, and were similar across rearing temperatures, suggesting they evolved due to natural selection, although the concrete selective mechanisms involved require further study.

Key words: egg size, fecundity, egg composition, lipid, protein, glycogen, glucose, reproductive investment; latitude; geographic differentiation; life history; altitude

Introduction

Systematic geographic variation of animal life history traits, such as body size, egg size or diapause propensity, has been observed across species, and among populations within species, for a variety of ectothermic organisms. These macro-ecological patterns generally covary with, and are often caused by, environmental gradients that covary with latitude or altitude, such as mean annual temperature and/or season length, both of which decrease towards the poles (James et al. 1997; Blackburn et al. 1999; Chown & Gaston 1999, 2010; Blanckenhorn & Demont 2004; Gaston et al. 2008). Latitudinal clines in body size and life history traits, and analogous altitudinal clines (e.g., Berven 1982; Tanaka & Brookes 1983; Chown & Klok 2003; Berner et al. 2004), are generally regarded as examples of the systematic action of natural selection. However, the specific ecological variables generating this selection, and the mechanisms by which those variables mediate selection, are often unclear (Chown & Gaston 1999; Roff 2002; Hoffmann & Weeks 2007; Stillwell 2010). In particular, the life history traits that covary with latitude and altitude may not be the direct targets of selection. Understanding the causes of these clines thus requires consideration of the underlying physiological traits that may be the actual targets of selection (e.g., heat tolerance: Hoffmann et al. 2002; Castañeda et al. 2004; Deutsch et al. 2008; Santos et al. 2011).

The most prominent examples of latitudinal clines relate to Bergmann's rule, the common observation that body size increases with latitude within and among species (Bergmann 1847; Blackburn et al. 1999). These commonly observed latitudinal clines may be adaptive responses to selection, with larger body size favoured and thus evolving in colder climates (Atkinson & Sibly 1997). That clines in some species have independently evolved to be concordant on different continents (e.g., Huey et al. 2000, Gilchrist et al. 2004) is consistent with this, though the link between temperature and selection on body size is not well established. Although the adaptive significance of being larger at higher latitudes continues to be debated, physiological plasticity in response to temperature is widely demonstrated to influence body size (Atkinson & Sibly 1997; Blackburn et al. 1999; Ashton 2004; Chown & Gaston 2010). Warmer temperatures speed up virtually all physiological processes and thus influence most aspects of the ecology and evolution of ectotherms (Van Voorhies 1996; van der Have & de Jong 1996; Atkinson & Sibly 1997; Hoffmann et al. 2003; Sinclair et al. 2003). Most ectotherms mature at smaller body sizes when reared at warmer temperatures (the so-called temperature-size-rule: Atkinson 1994; Partridge et al. 1994, 1995; Forster et al. 2011), and therefore have larger body sizes in cooler climates, thus providing a

mechanistic link to Bergmann's rule (Blanckenhorn & Demont 2004; Horne et al. 2015). This commonly observed plasticity in body size may be an adaptive response to selection if larger body size is favoured by selection in colder climates (Atkinson & Sibly 1997). These physiological arguments generally also apply to cells, such that plasticity in body size (and eggs and sperm; e.g., Blanckenhorn 2000; Fox & Czesak 2000; Blanckenhorn & Hellriegel 2002) may be mediated temperature effects on cell size rather than cell number (Partridge et al. 1994; James et al. 1995, 1997; Stevenson et al. 1995; Blanckenhorn & Llaurens 2005). However, the effects of temperature on plasticity in and selection on body size are often counteracted by the reduction in season length with increasing latitude. Season length limits the period of growth and hence ultimately constrains the maximum body size that can be achieved (elaborated in Blanckenhorn & Demont 2004), such that organisms can grow to be larger at lower latitudes (for any equivalent number of generations per year; Roff 1980; Kivelä et al. 2013).

As body size affects most if not all life history (i.e., development time, growth rate, reproductive output, etc.) and physiological traits (e.g., metabolic rate) of an organism in a concerted syndrome, it is unsurprising that several of these traits also exhibit geographic clines, potentially in conjunction with but also sometimes independent of body size. Examples include stress resistance, diapause or reproductive investment (Blanckenhorn & Fairbairn 1995; Karan et al. 1998; Hoffmann et al. 2002; Schmidt & Paaby 2008; Ducatez et al. 2013). The egg output (clutch size or lifetime fecundity) of several vertebrate species (birds, reptiles, fish) increases with latitude, which is believed to result from stronger seasonality (and concomitant changes in food availability) and/or changing predation regimes (see Cooper et al. 2005; Jetz et al. 2008, and references therein). Because fecundity (i.e., ovariole number) generally increases with the female's body size in insects (Honek 1993), and because of an allocation trade-off between egg size and number (Smith & Fretwell 1974; Roff 2002), any species exhibiting body size clines is expected to also exhibit clines in either egg number or egg size, if only for their association with body size. Evidence generally supports this, but there are exceptions. For example, fecundity increases with latitude in most studied species (Lardies & Castilla 2001; Azevedo et al. 1996; Starmer et al. 1997; but see Rhainds et al. 2010) but decreases with latitude in some others (Lardies & Wehrtmann 2001). Similarly, clines exhibiting larger eggs at higher latitudes have been reported for multiple species (Azevedo et al. 1996; Armbruster et al. 2001; Lardies et al. 2010), but some species show opposite clines (small eggs at high latitude) or no clines in egg size (Blanckenhorn & Fairbairn 1995; De Block et al. 2008; Sniegula et al. 2016). Moreover, insects often lay

smaller egg sizes when reared or ovipositing at higher temperatures (Ernsting & Isaaks 1997, 2000; Blanckenhorn 2000; Fox & Czesak 2000; Fischer et al. 2003a,b). Just as is the case for body size clines, the underlying mechanisms and adaptive value of egg size variation still remain to be fully explained (Ernsting & Isaaks 2000; Fox & Czesak 2000; Fischer et al. 2003a).

Latitudinal clines may also occur in size-independent traits such as egg quality, though investigations of variation in egg composition across latitudes are scarce (see e.g., Ruuskanen et al. 2011 for birds). Altitudinal variation in egg composition has been, for instance, reported for the snail *Arianta arbustorum* (Baur & Baur 1997, 1998), and a recent meta-analysis revealed latitudinal variation in egg development in stoneflies (Plecoptera), mayflies (Ephemeroptera) and dragonflies/damselflies (Odonata) (Rotvit & Jacobsen 2013), implying (but not directly demonstrating) some latitudinal variation in egg composition for these groups. That egg composition varies with temperature has been documented (e.g., Geister et al. 2008 for butterflies), though whether this generates variation in egg composition over a latitudinal gradient is unknown. Egg size variation does not always reflect qualitative changes in egg composition (McIntyre & Gooding 2000), confounding the interpretation of geographic variation in egg size and correlated life history traits. Most importantly, variation in egg composition may be ecologically and evolutionarily more important than variation in egg size *per se* (Begon & Parker 1986; Bernardo 1996; Fox & Czesak 2000; Giron & Casas 2003; Fox 2006; Bauerfeind et al. 2007; Karl et al. 2007).

The yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae) is widespread throughout the northern hemisphere, making this species highly suited for broad-scale geographic and latitudinal investigations. Previous studies revealed very low molecular population differentiation and high levels of gene flow in Europe and North America (Kraushaar et al. 2002; Demont et al. 2008; Schäfer et al. 2018). In contrast, quantitative life history traits (body size, development time, growth rate, and diapause characteristics) appear to be consistently differentiated worldwide, exhibiting heritable latitudinal clines in multiple traits that are replicated across continents when reared under common garden conditions (Blanckenhorn & Demont 2004; Scharf et al. 2010; Blanckenhorn et al. 2018). The degree to which clines are replicable across continents varies among traits (Blanckenhorn et al. 2018), suggesting that at least some traits (diapause and growth rate) are under substantial selection and evolved in response to environmental variables that covary with latitude.

By sampling various yellow dung fly populations across Europe and North America (Table 1) and subjecting them to common garden laboratory rearing, we here address several

questions. (1) Given repeatable latitudinal clines in juvenile life history traits in yellow dung flies (Blanckenhorn et al. 2018), are there likewise clines in adult reproductive traits (egg number, size and energy content)? Following patterns for other species, and given slight Bergmann body size clines (larger bodies at higher latitudes) in *S. stercoraria* (Blanckenhorn & Demont 2004; Blanckenhorn et al. 2018), we expected flies to lay larger clutches and/or eggs with increasing latitudinal origin of the populations. We further expected analogous increases in egg energy content, so we examine the various components of the egg (egg protein, glycogen, glucose and lipid) to test for clinal variation in egg composition. (2) We also test whether clines in life history traits are replicable across continents (Europe, North America), as would be expected if observed clines are generated by selection driven by latitudinally-covarying climatic variables. (3) Given the well-established thermal effects on life history traits discussed above, we finally investigate whether observed clinal patterns are consistent across rearing temperatures (i.e., parallel).

Methods

Study organism

Yellow dung flies are common on and around cattle pastures throughout the northern hemisphere. These flies prefer cool climates, occurring at high latitudes up to Spitzbergen, Greenland and Iceland in Europe, or Alaska in North America. In the southern, Mediterranean areas of its distribution, the species inhabits higher altitudes such as the Sierra Nevada in Spain and North America. In central European lowlands, each year features a spring (March – June) and an autumn season (September – November), while during the hot midsummer (July and August) the flies largely disappear from the pastures due to their heat sensitivity. In contrast, flies are active throughout the summer at higher latitudes or altitudes (Blanckenhorn 2009; Blanckenhorn et al. 2010). European populations are geographically differentiated in various traits and in general very plastic (Demont et al. 2008; Scharf et al. 2010), whereas corresponding altitudinal differentiation in Switzerland is very weak in comparison (Blanckenhorn 1997, 2009). As these insects are good flyers (Kaufmann et al. 2013) gene flow is extensive, presumably impeding local adaptation at least at short spatial scales (Kraushaar et al. 2002).

Yellow dung fly adults are anautogenous income breeders and sit-and-wait predators of small flying insects. Females spend most of their time foraging for nectar (for energy) and prey (for the protein necessary 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. Their mating system is best characterized as resource defence polygynandry, with elements of scramble and contest competition among males as well as female choice (Parker 1978; Ward 2000). Many males typically wait at fresh dung pats 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. 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

Flies were collected along latitudinal clines in Europe and North America spanning ca. 3600 km or 32° latitude and 2900 km or 26° latitude, respectively. A total of 9 European (EU) and 11 North American (NA) populations were sampled. European flies were collected over two years (2007, 2009), whereas North American flies were all sampled in 2009 (spring or autumn; Table 1). Common garden rearing was subsequently performed in two spatio-temporal blocks in Zürich, Switzerland (EU flies) and in Lexington, Kentucky (NA flies).

Field-collected flies were brought alive to the laboratory, where they were allowed to copulate with a random male to establish our laboratory populations based on offspring from a minimum of 10 mated females per site. Prior to our experiment all populations were maintained at 18°C, 60% relative humidity, and 14 h light. These first-generation lab-reared offspring (F1) were crossed (within populations, but avoiding within-family matings) to generate F2 – F5 generation full-sib families. Our experiments were done with F5 generation flies for some populations (Tromsö, Uppsala, Estonia, Zürich, Sierra Nevada, Pyrenees), and with F2 flies for all others (Table 1). These standard common garden procedures should have eradicated any carry-over maternal effects from the field that might have been present due to the specific season, environmental conditions, or voltinism stage, etc., at the time of fly collection.

For our experiment, full-sib clutches (of 30 – 90 eggs) were split into constant 12°, 18° or 24°C regimes (with 60 % relative humidity and 14 h photoperiod) to investigate phenotypic plasticity in response to temperature (Blanckenhorn & Demont 2004; Demont et al. 2008). Larvae were reared in family groups in translucent plastic containers with overabundant (> 2 g per larva) and homogenized, previously frozen cow dung. We checked daily for emerged adults to score egg-to-adult development times, and measured hind tibia

length of all individuals as an index of body size. More details on rearing methods can be found in Blanckenhorn & Demont (2004) or Blanckenhorn et al. (2010).

Upon eclosion, flies were kept individually in 100 ml glass vials and provided with water, sucrose and *Drosophila spp.* as food in ample supply. Females were allowed a pre-reproductive period of up to 20 days to become sexually mature, develop and lay eggs (Blanckenhorn et al. 2010). During this time they were mated in their vial with a haphazardly chosen male from the same population but a different family, adding a smear of cow dung for oviposition. Because adult flies were confined together for an extended period to mate, some pairs likely mated more than once. If mating did not take place, the procedure was repeated the following day using a different male. Males were removed after mating, and females allowed to deposit their clutch of eggs. We only used the first clutch of each female as our most reliable, secure and comparable estimate of each female's fecundity (Jann & Ward 1999), as is standard procedure in this and other species (Blanckenhorn 2000; Blanckenhorn & Heyland 2004). The female's hind tibia length was measured after oviposition. Eggs were first counted using a stereo microscope (first clutch size). A number of haphazardly picked eggs were then removed for egg size and content analyses, cleaned and rinsed with water and insect Ringer solution. Of these, 10 eggs each were collected for separate analyses of (1) glycogen, glucose and lipid content, and (2) protein content (described below). For egg size estimation, length and width of 3-5 eggs per clutch were measured using a digital camera connected to a binocular microscope. The resulting images were analysed using ImageJ 1.38x (NIH, USA). Mean egg volume was calculated assuming an ellipsoid as $(1/6) \cdot \pi \cdot \text{length} \cdot \text{width}^2$ (Blanckenhorn & Heyland 2004). The remaining eggs of a clutch were counted and allowed to develop at the specified temperature environments to generate an estimate of egg-to-adult survivorship per clutch (EU populations only).

Egg content analyses

For the analyses of egg protein, glycogen, lipid and glucose content, we followed the classic methods of Van Handel (1985a,b) and Minari & Silversmit (1963; consider Foray et al. 2012, for an update). Ten eggs of a given clutch were homogenized in 200 μ l of 2% Na₂SO₄, which absorbs and precipitates the glycogen. Glycogen was then quantified photometrically by anthrone reaction with 0.1% glucose in 25%EtOH as the calibration standard. After a 1:1 chloroform-methanol extraction, lipid content was measured using a vanillin-phosphoric acid reaction with 0.1% soybean oil in chloroform as the standard. From the remaining supernatant glucose content was quantified again by anthrone reaction. The

total (i.e., structural plus free) protein content was measured as total nitrogen by Nesslerization of Kjeldahl digests of eggs with 0.1N ammonium sulfate as the standard, and was then back-calculated to the amount of protein by multiplying with 6.25 (assuming an average nitrogen content of all amino acids of 16%; Minari & Silversmit 1963). To facilitate direct comparison, the energetic values of all components were converted to calories with 1 calorie (= 4.187 Joules) corresponding to 250 μ g of carbohydrates or protein and 110 μ g of lipids.

Statistical analyses

Individual data stemming from 1 – 7 females per family (on average 2 – 3) were averaged, with these full-sib family means treated as the lowest level of independence (number of families given in Table 1). We averaged across families to calculate population means.

We fitted two sequential linear models. First we examined variation among populations, between continents and among rearing temperatures (12 °C, 18 °C, 24 °C). We used the model $\text{Trait} = \text{Continent} + \text{Population} + \text{Rearing temperature} + \text{Population} \times \text{Temperature}$, with temperature and continent as fixed effects and population as a random effect (nested within continent), considering family means as our lowest level of independence. We note that the term Continent in this model confounds two factors, the collection location of the flies (North America versus Europe) and the laboratory in which the flies were reared (North American flies in Kentucky, USA, European flies in Zürich, Switzerland). The extent to which results are the same between continents (non-significant Continent effect in our models) provides insight into the generality of the observed patterns, but significant Continent effects could reflect differences between fly populations and/or between the laboratories in which the flies were reared.

We next explored the influence of latitude, altitude, longitude and season length (Table 1) in explaining variation among populations, treating each population as a single data point, using the model $\text{Trait} = \text{Continent} + \text{Latitude} + \text{Altitude} + \text{Longitude} + \text{Rearing temperature} + \text{interactions}$; continent and rearing temperature were treated as fixed effects, and all other variables were covariates. Because some traits varied non-linearly with latitude, we also included latitude^2 in models for which this quadratic term was significant. Other variables, such as body size, egg size and clutch size, were then added to the best fit models to test specific hypotheses about the role of these variables in explaining observed clines (e.g.,

whether clinal variation in egg size or clutch size could be explained by clinal variation in body size).

Data transformation was not necessary for any outcome variable considered (residuals normally distributed). All statistical analyses were done with SAS 9.4 (SAS Proc Mixed and SAS Proc GLM). Throughout, all means are given ± 1 SE.

Results

Body size and egg-to-adult survival

Flies reared at cooler temperatures were consistently larger-bodied (Figure 1; Table 2). Body size also varied significantly among yellow dung fly populations, and differed between the continents, with flies from North America generally larger than flies from Europe (Figure 1; Table 2), though interpretation of this between-continent difference is limited because fly rearing was performed in different labs at different times (albeit at the same growth chamber conditions). Unsurprisingly, the response to temperature varied among fly populations, with some populations being more phenotypically plastic than others (significant temperature*population interaction; Figure 1; Table 2a).

On average, fly body size increased with latitude (Figure 1) across all three rearing temperatures (non-significant latitude*temperature interaction; Table 2b). However, the relationship between body size and latitude was complex, presenting a significant negative quadratic term in the model (i.e., a concave relationship; Table 2). A previous study (Blanckenhorn et al. 2018), using a substantially larger dataset than the one examined here, similarly observed a hump-shaped relationship and a significant difference in the linear slope of the latitudinal cline between North America and Europe, a difference visible in Figure 1 but not statistically significant in our analysis based on population means (latitude*continent interaction *ns*: Table 2b). Body size also varied with season length (when adding season length to the model in Table 2b; $F_{1,50} = 9.48$, $P = 0.003$), but including variation in season length in the model was not adequate to explain all of the variation explained by latitude (latitude remained significant: $F_{1,50} = 9.21$, $P = 0.004$). However, latitude and season length covary substantially so this analysis should be interpreted with caution.

Egg-to-adult survival (only available for the European populations) averaged 0.64 ± 0.03 (95% CI) across populations and rearing temperatures, and generally decreased with rearing temperature, but did not vary systematically with latitude (except for the lower survival of flies from the northernmost population, Tromsø: Figure S1).

Variation in clutch size, egg size and reproductive investment

Rearing temperature had strong effects on fly reproductive output: first clutch size (number of eggs), egg volume and total reproductive effort all declined with increasing temperature (Figure 2; Table 2). Clutch size, egg size and total reproductive effort also varied significantly among populations (within continents), though the specifics of how they varied depended on temperature (significant population*temperature interactions for all three traits; Figure 2; Table 2).

Within populations, larger flies laid more eggs (when adding body size to the model in Table 2a: $F_{1,927} = 40.5$, $P < 0.001$) and larger eggs ($F_{1,902} = 6.34$, $P = 0.01$) than smaller flies, and thus allocated more total biomass to reproduction ($F_{1,902} = 42.6$, $P < 0.001$). Accounting for body size variation was sufficient to explain the among-population variation in clutch size (which turned non-significant: $F_{18,927} = 1.43$, $P = 0.11$), while significant variation among populations (within continents) in egg size ($F_{18,902} = 6.19$, $P < 0.001$) and total reproductive investment ($F_{18,902} = 2.40$, $P < 0.001$) remained. Interestingly, there was no evidence that egg size trades off with clutch size, either within populations (when adding egg size to the clutch size model in Table 2a, the relationship was positive, opposite the predicted direction: $F_{1,971} = 3.12$, $P = 0.08$) or among populations ($F_{1,17} = 1.51$, $P = 0.24$; Table 2b). Both relationships remained non-significant when accounting for variation within and among populations in female size (as covariate).

We found no evidence that mean clutch size of a population varied with latitude, altitude, or longitude (Figure 2; Table 2b), and these relationships remained non-significant after accounting for among population variation in body size ($F_{1,51} = 0.44$, $P = 0.70$). In contrast to clutch size, there was a positive, albeit weak, latitudinal cline for egg size; females from higher latitudes laid larger eggs (Figure 2; Table 2b). However, this relationship was asymptotic (significantly negative, i.e., concave, quadratic slope; Table 2b), with the slope of the increase declining with latitude. This relationship became non-significant after including mean population body size (which also increased with latitude) into the model ($F_{1,50} = 2.45$, $P = 0.12$), suggesting that most of the increase in egg size with latitude is because flies are larger at higher latitudes. Interestingly, egg volume declined slightly with increasing altitude of the source population, opposite to the corresponding latitudinal effect (Table 2b), a relationship remaining significant when accounting for mean population body size ($F_{1,50} = 5.95$, $P = 0.02$). Reproductive effort, which is the product of clutch size and egg volume, did not vary with latitude, altitude or longitude (Table 2b). We found no evidence that populations from different latitudes responded differently to rearing temperature, as there

were no significant temperature*latitude interactions for the three reproductive traits (Table 2b). Season length did not explain any of the variation among populations in egg size, clutch size or total reproductive effort ($P > 0.42$ for each) and did not change any of the patterns reported above.

Egg composition

Rearing temperature influenced nearly all energy components of the egg. The most consistent pattern was that total egg energy content declined with increasing rearing temperature (Figure 2; Table 3a), in agreement with the influence of temperature on egg size (see above). The rearing temperature effect remained the same and significant ($F_{2,606} = 8.31$, $P < 0.001$) after accounting for the positive effect of egg size on egg energy content; that is, eggs contained proportionally more energy per unit egg size when females were reared at lower temperature (Figure 2). The specific components of the egg contributing to the higher energetic content of eggs for flies reared at low temperature varied considerably between North America and Europe (Figure 3). Egg lipids generally decreased with rearing temperature (Figure 3), whereas egg proteins were lowest at 12 °C, and egg glycogen content decreased with rearing temperature in North American flies but increased with temperature in European flies (Figure 3; Table 3b).

All four egg constituents – total caloric energy content, proteins, lipids and glycogen – varied significantly among populations within continents (Table 3a), and all three subcomponents differed between European and North American flies (Table 3a). Within populations, larger eggs on average contained more total energy ($F_{1,606} = 105.4$, $P < 0.001$), more protein ($F_{1,606} = 5.93$, $P = 0.02$), and more lipid ($F_{1,606} = 9.74$, $P = 0.002$), but not more glycogen ($F_{1,606} = 0.06$, $P = 0.81$) (egg volume added as covariate to the models in Table 3b).

Within populations, egg protein content was negatively correlated with both egg lipid content ($F_{1,621} = 1665.0$, $P < 0.001$) and glycogen content ($F_{1,620} = 16.4$, $P < 0.001$), whereas glycogen and lipid content were uncorrelated ($F_{1,621} = 1.79$, $P < 0.18$) (controlled for egg size and rearing temperature). The relationship between egg protein and egg lipid varied among populations ($F_{18,621} = 14.4$, $P < 0.001$) and with rearing temperature ($F_{2,621} = 5.51$, $P = 0.004$), whereas the relationship between egg protein and glycogen content did not vary with either of these variables ($P > 0.2$ for each). Among populations, egg protein content was negatively correlated with egg lipid content ($F_{1,52} = 443.1$, $P < 0.001$), as it was within populations; this relationship differed between continents ($F_{1,52} = 17.8$, $P < 0.001$) but did not vary with rearing temperature ($F_{1,52} = 1.87$, $P = 0.16$). Egg protein was also correlated with glycogen content in

North America but not Europe (continent*covariate interaction; $F_{1,52} = 7.10, P = 0.01$). Lipid and glycogen were uncorrelated among populations ($F_{1,52} = 0.20, P = 0.66$), just as they were within populations (all analyses controlled for egg size and rearing temperature).

The *absolute* amount of total egg energy varied with latitude, but the relationship was quite complex (Figure 2): there was a significant negative quadratic (hump-shaped) term in the model (Table 3b) that dominates the shape of the relationship, and remains significant ($F_{1,49} = 8.27, P = 0.006$) after accounting for geographic variation in egg size (which also showed a concave relationship with latitude). The linear slope of the relationship between egg energy and latitude changed from being slightly negative when ignoring the quadratic relationship (Figure 2; $F_{1,51} = 12.3, P = 0.001$; slope = -0.015 ± 0.007 calories per degree latitude) to slightly positive in the model including the quadratic term (Table 5; slope = 0.07 ± 0.02 calories per degree latitude); both slopes were unaffected by rearing temperature (no significant temperature*latitude interactions). Season length did not explain variation in egg energy content ($F_{1,49} = 1.33, P = 0.25$).

Of the various egg components, the clearest relationships with latitude were for protein and glycogen (Figure 3): egg protein content increased linearly with latitude, and glycogen content decreased with latitude, both effects being consistent across rearing temperatures and continents (non-significant latitude*rearing temperature interactions; Table 3b), and remaining significant (and in the same direction) after accounting for geographic variation in egg size ($F_{1,50} = 8.7, P = 0.005$ and $F_{1,49} = 5.53, P = 0.02$). Egg protein and glycogen content varied with altitude in the same direction as observed for latitude (higher egg protein and lower egg glycogen at higher altitudes), though only the variation in egg protein with latitude remained significant after correcting for variation in egg size. The relationship between lipid content and latitude was less clear: though the slope was significantly positive overall (more lipid at higher latitude), the relationship is obscured by the substantial non-linearity with intermediate latitude populations having most lipid in their eggs (Figure 3). Both absolute (Table 3b) and relative (egg size corrected: $F_{1,49} = 11.4, P = 0.001$) lipid content of eggs was generally lower at higher altitudes. There was no evidence that any of these egg components varied with season length of the populations ($P > 0.16$).

Discussion

Body size varies geographically in the yellow dung fly, generally increasing with latitude, albeit often only weakly (Fig. 1; Blanckenhorn & Demont 2004; discussed in more detail in Blanckenhorn et al. 2018). Here we examined whether adult reproductive traits of

yellow dung flies, which commonly covary with body size, vary along a latitudinal cline. We tested whether these patterns are repeatable across continents, and examined the degree to which the observed geographic variation is explained by clines in body size (for egg size and fecundity) or egg size (for egg constituents). We found that egg size generally increases with latitude on both continents (North America and Europe), but the increase was largely explainable by the cline in body size (larger flies laid larger eggs both within and among populations; Figure 2). In contrast, neither clutch size nor total reproductive effort varied in a consistent manner with latitude (though both did vary among populations; Figure 2). Both the absolute and relative (correcting for egg size) amount of protein in eggs generally increased with latitude, and the amount of glycogen with eggs generally decreased with latitude (Figure 3). Rearing temperature in our common garden experiment affected nearly all traits quantified here, but did not generally change the observed latitudinal clines or relationships between traits.

The most consistent (across rearing temperatures) and geographically repeatable cline we found here was the increase in egg size with latitude, though the slope of the increase declined with increasing latitude. However, the increase in egg size with latitude was largely explained by an increase in fly body size with latitude – larger flies laid larger eggs both within and among populations. Within populations, larger eggs on average contained more total energy, but the relationship between egg energy content and latitude was quite complex. Unexpectedly, although clutch size varied among populations, it did not covary with latitude and did not trade-off with egg size among populations. The positive latitudinal cline in egg size of yellow dung flies, albeit largely explained by variation in body size of the mother, matches clines observed for some other ectotherms in the literature when reared in a common garden (Azevedo et al. 1996; Armbruster et al. 2001; Parry et al. 2001; Lardies et al. 2010; reviewed in Fox & Czesak 2000), with few of these clines replicable across continents (*Drosophila* and yellow dung flies). Given that egg size generally has large effects on hatchling size, and that hatchling size can be under substantial selection (especially in stressful environments: Fox & Czesak 2000), it seems likely that these observed clines are generated in part by variation in selection caused by some environmental variables, such as temperature or seasonality, that covary with latitude. In particular, experimental evolution studies have demonstrated that egg size evolves quickly and repeatedly when rearing temperature is manipulated (Azevedo et al. 1996), although the specific mechanism generating the selection remains uncertain. That low temperatures favour the evolution of larger eggs is now widely accepted (Armbruster et al. 2001; Fischer et al. 2003b) and may

provide an explanation for the clines observed in many ectotherms. However, numerous examples exist in which ectotherms show either no cline in egg size with latitude/altitude (e.g., Hein et al. 2015; Kasimatis & Reginos 2016; Sniegula et al. 2016), or a cline in which eggs get smaller at higher latitude or altitude (Bauer & Bauer 1998; Johnston & Leggett 2002; Kokita 2003; Blanckenhorn & Fairbairn 1995), indicating that the causes of clines are not universal.

In contrast, it is nearly universally observed that females produce larger eggs when reared at lower temperature (Blanckenhorn 2000; Ernsting & Isaaks 1997, 2000; Fischer et al. 2003a; Bownds et al. 2010), as also observed here. More generally, yellow dung flies responded plastically to rearing temperature by changing a whole suite of inter-related reproductive traits – flies reared at lower temperature matured at larger body size, laid more and larger eggs, and those eggs contained proportionally more energy per unit egg size. These effects of rearing temperature are in accordance with the temperature-size-rule, which describes a general decrease in body and cell size with increasing temperature (Atkinson 1994; Atkinson & Sibly 1997; Horne et al. 2015). Mechanistically, and contrary to the temperature-size-effect on body size (Atkinson & Sibly 1997), these temperature effects on reproductive traits are most easily explained by overall increased metabolic energy demands at higher temperatures, leaving less energy to be invested into egg size, its energy content, and ultimately also egg numbers (clutch size). Nevertheless, observed clines (or lack thereof) obtained here for reproductive traits were largely consistent in slope at the three rearing temperatures used (12 °C, 18 °C, 24 °C). That is, there were no significant temperature by latitude (by continent) interactions, indicating that common physiological mechanisms must be at work at a broad range of temperatures (cf. Introduction).

Few studies have examined how egg composition varies within or among invertebrate populations, and whether it varies systematically with geography. Those studies have found that egg composition varies with egg size (Capinera et al. 1977; Karl et al. 2007), maternal age (McIntyre & Gooding 2000; Muller et al. 2017), rearing temperature (Geister et al. 2008, 2009), diet (Newcombe et al. 2015), and altitude (Baur & Baur 1998), and that different egg components can vary somewhat independently among populations (Baur & Baur 1997). Unfortunately, too few studies have examined variation along any particular environmental axis to allow generalizations about how egg constituents vary. Further, although it is often assumed that offspring hatching from larger eggs have higher fitness, few studies have examined how specific aspects of egg composition influence offspring performance (Newcombe et al. 2015). In yellow dung flies, we found that larger eggs generally contain

more lipid and more protein compared to smaller eggs, though these two components also traded off with each other (both within and among populations) after controlling for variation in egg size. Protein content of eggs generally increased and glycogen content generally decreased with latitude after accounting for variation in egg size, whereas latitudinal variation in lipid content was complex. That the clines for egg protein and egg glycogen were generally consistent in direction (though not magnitude) between continents suggests that some variable covarying with latitude, such as temperature or seasonality, influences selection on egg composition. However, what form this selection takes, and even whether this observed geographic variation in egg composition is indeed under selection, is unclear. Blanckenhorn et al. (2007, 2012) found in the laboratory and the field (respectively) that more energy reserves conferred survival advantages to adult flies, but egg size was not measured in those studies, nor which specific egg components (if any) were responsible for the survival advantage. Lipid content of eggs predicts diapause incidence in yellow dung flies (Scharf et al. 2010); we thus might predict lipid content of eggs to increase with latitude, but this was not clearly observed here. Further experiments examining how egg composition mediates offspring survival, development and diapause are necessary to clarify the adaptive significance of the variation in egg composition observed here.

With a substantial data set replicated with yellow dung flies from two continents reared in common laboratory conditions at three temperatures, we identified repeatable latitudinal clines in body size, egg size and egg composition, suggesting that selection varying with latitude contributes to generating geographic trends in these traits. In contrast, other traits, notably clutch size and egg energetic content, either did not vary with latitude, or exhibited variation with latitude that was not consistent between continents. We also observed significant non-linear latitudinal changes in some traits. Several studies have demonstrated more complex, non-linear latitudinal clines, including hump-shaped (Laugen et al. 2005; also evident here in Europe for body size: Fig. 1), non-monotonic (Long & Singh 1995) or saw-toothed patterns (Masaki 1967; Mousseau & Roff 1989; Kivelä et al. 2013). Hump-shaped patterns, as observed here for body size, egg size, egg energy content and egg lipoids, may indicate combined selective effects of temperature and season length (Blanckenhorn & Demont 2004). Disentangling the fitness consequences of temperature and season length, and identifying which components of fly life history are under direct or indirect selection (if at all), requires additional experimental study.

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Fig. 1. Family mean ($\pm$ SE) hind tibia length of the mothers as a function of latitude for 9 European and 11 North American yellow dung fly populations.

Fig. 2. Family mean ($\pm$ SE) reproductive traits as a function of latitude for 9 European and 11 North American yellow dung fly populations.

Fig. 3. Family mean ($\pm$ SE) absolute protein, lipid and glycogen content of 10 eggs as a function of latitude for 9 European and 11 North American yellow dung fly populations.

Fig. S1. Proportion egg-to-adult survival ($\pm$ SE) for 15 European populations as a function of latitude (full European data set of Blanckenhorn et al. 2017).

Table 1. Sampled populations of *Scathophaga stercoraria* used for common garden assessment of reproductive traits.

City	State	Year	Latitude	Longitude	Altitude (m)	Season length (d)	No. families	Collectors
<i>Europe</i>								
Sierra Nevada	Spain	2007	37.20	-3.21	1290	274	20	J. Moya-Laraño & L. Corcobado
Pyrenees	Spain	2007	42.25	1.91	830	268	21	Jordí Moya-Laraño
Locarno	Switzerland	2009	46.26	8.69	300	267	23	Martin Schäfer
Zürich	Switzerland	2007	47.38	8.75	536	228	18	David Berger
Hannover	Germany	2009	52.43	9.53	56	225	22	Martin Schäfer
Estonia	Estonia	2007	58.14	26.91	81	186	28	Toomas Tammaru
Grimstad	Norway	2009	58.34	8.59	10	198	14	Ane Laugen
Uppsala	Sweden	2007	59.73	17.70	16	187	28	Luc Bussière & Ane Laugen
Tromsø	Norway	2007	69.68	18.72	5	122	21	Arne Nilssen
<i>North America</i>								
White	Georgia	2009s	34.29	-84.75	273	334	13	Wolf Blanckenhorn
Raleigh	N. Carolina	2009s	35.74	-78.66	90	301	28	Matt Bertone & Keith Bayless
Lexington	Kentucky	2009s	38.09	-84.74	272	256	30	Charles Fox
Dayton	Ohio	2009f	39.88	-84.27	292	222	16	Stefan Lüpold
Columbus	Ohio	2009s	40.01	-83.16	260	222	11	Woody Foster
Fort Collins	Colorado	2009f	40.60	-105.10	1672	215	16	Dale Broder
Syracuse	New York	2009f	42.97	-76.15	133	211	38	Scott Pitnick
London	Ontario	2009s	43.03	-81.19	304	199	13	Jay Whistlecraft
Alfred	Ontario	2009s	45.50	-74.90	59	191	23	Simon Lachance
Lethbridge	Alberta	2009f	49.67	-112.75	985	183	26	Paul Coghlin & Kevin Floate
Soldotna	Alaska	2009f	60.53	-150.92	1	143	15	Matt Browser

Season length is expressed as days with average temperature above 6° C (developmental threshold) based on climate records.

s = spring; f = fall

Table 2. Effects of continent, temperature (fixed factors) and population (A)(as random factor; based on family means) or latitude (b: as covariate, based on population level means) on first clutch size, egg size and total reproductive effort (clutch * egg size) in 20 populations of European (EU) and North American (NA) yellow dung flies (*Scathophaga stercoraria*).

	Hind tibia length			Clutch size			Egg volume			Reproductive effort		
	df	F	P	df	F	P	df	F	P	df	F	P
A)	R ² = 0.54			R ² = 0.10			R ² = 0.33			R ² = 0.28		
Continent	1,18	27.7	<0.001	1,18	6.17	0.02	1,18	7.42	0.01	1,18	8.61	0.009
Population (continent)	18,928	16.8	<0.001	18,1018	1.74	0.03	18,972	9.05	<0.001	18,972	4.50	<0.001
Rearing temperature	2,928	51.6	<0.001	2,1018	37.9	<0.001	2,972	52.5	<0.001	2,972	75.6	<0.001
Pop*Temp(continent)	38,9288	3.51	<0.001	38,1018	1.95	<0.001	38,972	1.72	0.005	38,972	1.96	<0.001
B)	R ² = 0.75			R ² = 0.41			R ² = 0.68			R ² = 0.61		
		3.86	0.06									0.6
Continent	1,51			1,52	0.00	0.95	1,51	1.59	0.21	1,52	0.16	9
		17.3	<0.001									0.7
Latitude(linear)	1,51			1,52	0.34	0.56	1,51	9.64	0.003	1,52	0.09	6
Latitude(quadratic)	1,51	16.0	<0.001	-	NA ¹	-	1,51	9.37	0.004	-	NA ¹	-
		1.04	0.31									0.7
Latitude(lin)*continent	1,51			1,52	0.13	0.72	1,51	4.47	0.04	1,52	0.12	3
		2.52	0.09									0.0
Rearing temperaure	2,51			2,52	2.81	0.07	2,51	1.96	0.16	2,52	3.06	6
		1.24	0.30									0.5
Latitude*temperature	2,51			2,52	1.13	0.33	2,51	1.04	0.36	2,52	0.58	6
		1.30	0.26									0.0
Longitude	1,51			1,52	0.91	0.34	1,51	12.2	0.001	1,52	3.34	7
		0.31	0.58									0.1
Altitude	1,51			1,52	0.38	0.54	1,52	6.23	0.02	1,52	2.14	5

¹The quadratic term was deleted when non-significant

Table 3. Effects of continent, temperature (fixed factors) and population (A; based on family means) or latitude (B: as covariate, based on population level means) on egg energy (total, protein, lipid, glycogen) content in 20 populations of European (EU) and North American (NA) yellow dung flies (*Scathophaga stercoraria*).

	Egg energy content			Egg proteins			Egg lipids			Egg glycogen		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
A)	R ² = 0.54			R ² = 0.38			R ² = 0.38			R ² = 0.35		
Continent	1,18	2.33	0.14	1,18	10.4	0.005	1,18	5.69	0.03	1,18	34.2	<0.001
Population (continent)	18,617	28.1	<0.001	18,617	9.18	<0.001	18,617	11.33	<0.001	18,617	2.26	0.002
Rearing temperature	2,617	32.1	<0.001	2,617	6.86	0.001	2,617	2.99	0.051	2,617	12.7	<0.001
Pop*Temp(continent)	37,617	1.37	0.08	37,617	1.13	0.27	37,617	1.08	0.34	37,617	4.29	<0.001
B)	R ² = 0.61			R ² = 0.55			R ² = 0.48			R ² = 0.49		
Continent	1,50	6.73	0.01	1,51	1.25	0.27	1,50	0.60	0.44	1,50	2.97	0.09
Latitude(linear)	1,50	11.9	0.001	1,51	8.94	0.004	1,50	5.78	0.02	1,50	4.82	0.03
Latitude(quadratic)	1,50	15.9	<0.001	-	NA ¹	-	1,50	7.54	0.008	1,50	4.30	0.04
Latitude(lin)*continent	1,50	11.2	0.002	1,51	0.40	0.53	1,50	4.51	0.04	1,50	1.61	0.21
Rearing temperaure	2,50	1.19	0.31	2,51	1.40	0.56	2,50	0.48	0.62	2,50	4.02	0.02
Latitude*temperature	2,50	0.36	0.70	2,51	1.19	0.31	2,50	0.35	0.70	2,50	2.99	0.06
Longitude	1,50	11.3	0.002	1,51	0.82	0.37	1,50	4.55	0.04	1,50	1.32	0.26
Altitude	1,50	35.8	<0.001	1,51	11.0	0.002	1,50	12.4	<0.001	1,50	0.01	0.93

¹ The quadratic term was deleted when non-significant