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Geographic clines in wing morphology relate to colonization history in New World but not Old World populations of yellow dung flies

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Geographic clines offer insights about putative targets and agents of natural selection as well as tempo and mode of adaptation. However, demographic processes can lead to clines that are indistinguishable from adaptive divergence. Using the widespread yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae), we examine quantitative genetic differentiation (Q_{ST}) of wing shape across North America, Europe, and Japan, and compare this differentiation with that of ten microsatellites (F_{ST}). Morphometric analyses of 28 populations reared at three temperatures revealed significant thermal plasticity, sexual dimorphism, and geographic differentiation in wing shape. In North America morphological differentiation followed the decline in microsatellite variability along the presumed route of recent colonization from the southeast to the northwest. Across Europe, where *S. stercoraria* presumably existed for much longer time and where no molecular pattern of isolation by distance was evident, clinal variation was less pronounced despite significant morphological differentiation ($Q_{ST} > F_{ST}$). Shape vector comparisons further indicate that thermal plasticity (hot-to-cold) does not mirror patterns of latitudinal divergence (south-to-north), as might have been expected under a scenario with temperature as the major agent of selection. Our findings illustrate the importance of detailed phylogeographic information when interpreting geographic clines of dispersal traits in an adaptive evolutionary framework.

KEY WORDS: **Allometry, biogeography, developmental canalization, dispersal, gene flow, insect flight.**

Understanding how populations adapt to heterogeneous environments has always been a challenge for evolutionary biologists. Adaptation is of special importance in species with large distribution ranges and/or recent range expansions during which populations typically face multiple novel ecological conditions (Fisher 1930; Wright 1931; Carson and Templeton 1984). Along environmental gradients in temperature, humidity, or seasonality, adaptation frequently generates predictable clines in fitness-associated traits and/or reaction norms across a species' range (Endler 1977, 1986; Barton 1999). Geographic clines of species with high dispersal ability are of particular interest for identifying putative agents and targets of natural selection, since their persistence requires strong selection gradients to counterbalance the homogenizing effects of gene flow. In the extreme case, gene flow can prevent any genetic differentiation, but may favor the evolution of enhanced phenotypic plasticity instead. Although

phenotypic plasticity and genetic divergence have been historically often regarded as conceptually distinct, the more recent literature has emphasized the importance of a better understanding of how both processes interact in shaping the evolutionary trajectories of quantitative genetic traits (West-Eberhard 2003; Pigliucci 2005; Räsänen and Kruuk 2007; Crispo 2008; Chevin and Lande 2011). Depending on the nature of the interaction, phenotypic plasticity may speed up, slow down, or have little effect on rates of evolutionary change (Price et al. 2003; West-Eberhard 2003; Ghalambor et al. 2007). For example, while adaptive plasticity facilitates genetic divergence via accommodation or assimilation (Waddington 1953; Braendle and Flatt 2006; Suzuki and Nijhout 2006), and allows for large phenotypic differences among populations despite gene flow, nonadaptive plasticity can promote counter-gradient genetic variation and sometimes acts opposite to genetic divergence (Hendry et. al. 2001; Hendry 2016; Schmid and Guillaume 2017).

When inferring the evolutionary history of adaptation along ecological gradients, it is important to consider that by generating a pattern of isolation by distance, neutral demographic processes such as migration and drift can result in geographic clines that are indistinguishable from adaptive divergence (Wright 1978; Slatkin 1987; Hewitt 2004). One widely used approach to differentiate between adaptive and nonadaptive scenarios of trait divergence is to contrast geographic patterns of quantitative genetic differentiation (Q_{ST}) with patterns of genetic differentiation at neutrally evolving markers (F_{ST}) (Spitze 1993; Merilä and Crnokrak 2001; McKay and Latta 2002; Storz 2002, Ovaskainen et al. 2011; Gilbert and Whitlock 2015). Such comparisons should be particularly informative for traits influencing dispersal and gene flow, and hence the spatial distribution of neutral allelic variation.

In pterygote insects, dispersal is tightly linked to flight performance. This is most obvious in species with discrete wing polymorphisms, where winged morphs typically invade and colonize new habitats, but later become replaced by wingless morphs due to physiological costs associated with the development and maintenance of the flight apparatus (Harrison 1980; Roff and Fairbairn 1991; Zera and Denno 1997). Similar but subtler evolutionary patterns can occur in species with continuous variation in wing size and shape. For example, flight morphology and physiology of butterflies not only correlate with dispersal and genetic structure (Hill et al. 1999; Hanski et al. 2004; Hughes et al. 2007), but also with a range of ecological factors including host plant specificity, predation risk, habitat structure, and climate (Chai and Srygley 1990; Norberg and Leimar 2002; Sekar 2012; Garca et al. 2017).

In dipterans the vast majority of research on the phenotypic and genetic diversification of wing morphology has centered on species of *Drosophila*. Investigations of natural populations suggest climatic adaptation as an important driving force behind the formation of wing (i.e., body) size clines in several species (e.g., *D. simulans*: Capy et al. 1993; *D. kikkawai*: Karan et al. 1998; *D. buzzatii*: Dahlgaard et al. 2001; *D. serrata*: Hallas et al. 2002; *D. melanogaster*: Klepsatel et al. 2014). These clines can evolve quickly, within decades, as shown for *D. subobscura* after its colonization of the New World in the early 1980s (Huey et al. 2000; Gilchrist et al. 2001; Fragata et al. 2010). How selection has shaped naturally occurring allelic variation for wing shape is less clear. Over macro-evolutionary time scales, wing shape has evolved extremely slowly relative to neutral expectations (Houle et al. 2017). Over short-term evolutionary time scales, however, part of the shape variation may diverge neutrally due to genetic drift or selection on genetically correlated traits. For example, a significant proportion of the clinal variation in wing vein positioning in *D. melanogaster* probably originates from selection on wing (or body) size (Gilchrist and Partridge 2001), and the contrasting wing shape clines between New and Old World populations of *D. subobscura* likely evolved as a correlated response to chromosomal inversion clines associated with a bottleneck during the invasion of the Americas by flies of Eurasian origin (Fragata et al. 2010; Simões et al. 2015). Similar wing shape clines related to inversions have been documented for *D. melanogaster* (Kapun et al. 2016). Although wing shape among populations may to some degree diverge neutrally, there is evidence for local adaptation in certain wing shape dimensions (e.g., Hoffmann and Shirrifs 2002; Schiffer et al. 2004; Moraes and Sene 2007). For example, in Australian populations of *D. melanogaster* wing aspect ratio exhibits latitudinal clinal variation that mimics plastic responses to developmental temperature (Azevedo et al. 1998). As flies raised at cold temperatures show improved cold-flight performance, this clinal variation likely harbors some adaptive value (Frazier et al. 2008). All evidence available thus suggests a rich interplay between adaptive and demographic processes in shaping the evolution of dipteran wing morphology, but studies that have contrasted geographic patterns of wing shape differentiation against neutral genetic markers remain scarce, even in *Drosophila*.

Using a population genetic framework derived from microsatellite analysis we here examine the evolutionary forces affecting quantitative genetic variation in geometric wing morphology in the widespread yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae) at worldwide scale. Previous studies of central and northern European dung fly populations documented substantial phenotypic plasticity in response to developmental temperature and significant latitudinal clines in a number of life-history traits, including development time, growth rate, and pupal diapause induction, which appear to have evolved in response to climate and seasonal time constraints (Demont et al. 2008; Scharf et al. 2010; Berger et al. 2011; Blanckenhorn et al.

2018). The absence of significant population structure and isolation by distance at neutral nuclear markers (Kraushaar et al. 2002; Demont et al. 2008) supports an adaptive scenario of life-history diversification across Europe despite the species' high inherent dispersal capability (Kaufmann et al. 2013). Here, we investigate whether similar geographic clines exist for wing morphology, and if so, whether populations on different continents (North America, Europe, and Japan) evolved convergent clines in response to similar latitudinal temperature gradients. By rearing flies of both sexes in a laboratory common garden at three developmental temperatures, we further investigate the degree of temperature-mediated phenotypic plasticity and sexual wing shape dimorphism. In addition to testing for effects of temperature, sex, size, and latitude on wing shape, we quantify the similarity (i.e., their alignment in morphospace) of these effects and investigate their interrelations and consistency across the species' range.

Materials and Methods

STUDY SPECIES

The yellow dung fly, a relatively large dipteran species with pronounced male-biased sexual size dimorphism (Borgia 1981; Jann et al. 2000; Ding and Blanckenhorn 2002), is distributed throughout the northern hemisphere and has served as model organism in numerous evolutionary, ecological, and behavioral studies since the early 1960s (Parker 1970; Ward 2007; Blanckenhorn 2009). Like many other scathophagids, this fly has adapted to cooler climates and invaded arctic regions in the Old and New Worlds (Vockeroth 1987; Šifner 2008). The species depends on the availability of fresh dung of cattle and other large herbivores, on which males wait for and mate with females. In central European lowlands the flies disappear from the pastures during the hottest months of the year to forage for pollen and nectar (for energy) or prey (for protein) on small insects such as *Drosophila* in cooler microhabitats, a pattern that is not observed at high elevation or latitude (Sigurjónsdóttir and Snorrason 1995; Blanckenhorn 2009; Blanckenhorn et al. 2001).

Given this fly's influence on various theoretical and empirical research disciplines (e.g., Simmons et al. 2001; Zuk et al. 2014), surprisingly little is known about its biogeographic and demographic history. Allozyme and microsatellite studies documented extremely low genetic differentiation across Central and Northern Europe, most certainly related to large effective population sizes and the flies' high inherent dispersal capability (Kaufmann et al. 2013) minimizing genetic drift effects (Kraushaar et al. 2001; Demont et al. 2008). Also, cytochrome *c* oxidase subunit I sequences of 14 specimens collected from North America, Europe, and Japan showed no geographic signal implying very low degrees of molecular differentiation across large parts of the species range (Bernasconi et al. 2008). Bernasconi et al. (2008) further discuss that *S. stercoraria* is probably of Old World origin and that the nearly cosmopolitan current distribution across the Northern hemisphere is probably human-mediated due to the species' strong association with cattle farming and shipment. Indeed, most recent introductions by humans and their livestock have been reported for Puerto Rico and Newfoundland, making recent colonization of the Americas plausible (Cuny 1983; Morris 1983).

GEOGRAPHIC SAMPLING, GENOTYPING, AND COMMON GARDEN REARING

The flies used for molecular and morphological analyses originated from 39 populations collected across North America ($N = 15$), Europe ($N = 18$), and Japan ($N = 6$) covering geographic distances of ca. 5700, 3800, and 1500 km, respectively (Fig. 1; see Table S1 for additional information on collection dates and sampling sites). Except for two Japanese populations, all flies used for molecular analysis were collected at the same date and pasture as the flies that were brought alive to the laboratory for our common garden experiments (Table S1).

For the molecular analysis we genotyped 1082 field-collected specimens originating from 36 populations across North America ($N = 15$), Europe ($N = 18$), and Japan ($N = 3$) at 10 polymorphic microsatellite loci. This sampling encompasses a much larger set of European populations collected de novo over a different geographic range (cf. Kraushaar et al. 2002; Demont et al. 2008), and for the first time addresses the population genetic structure of *S. stercoraria* at the global scale. DNA extraction and microsatellite amplification procedures are outlined in detail in the supplement and followed the protocols described in Garner et al. (2000), Watts et al. (2005), and Demont et al. (2012). Table S2 additionally provides information on the sample sizes, the number of alleles, and the mean observed (H_O) and expected (H_E) heterozygosity for each locus across North America, Europe, and Japan.

We quantified phenotypic and genetic variation in wing morphology based on 696 full-sib families originating from 28 populations (North America: $N = 11$; Europe: $N = 12$; Japan: $N = 5$) raised at three developmental temperatures (12°C, 18°C, 24°C). Sample sizes ranged from 8 to 61 families per population (see Table S1) yielding a total of 7135 flies used for morphometric analysis. For logistic reasons, common garden rearing was conducted in four experimental blocks. Common garden rearing of European flies took place in 2007 and 2009 at the University of Zurich. North American flies were assessed in 2009 at the University of Kentucky, and Japanese flies in 2013 in Zurich (see Table S1). Due to this spatio-temporal blocking, patterns of cross-continental morphological differentiation could, for example, be caused by using dung of different quality, and thus need to be interpreted cautiously. This issue, however, should not apply to

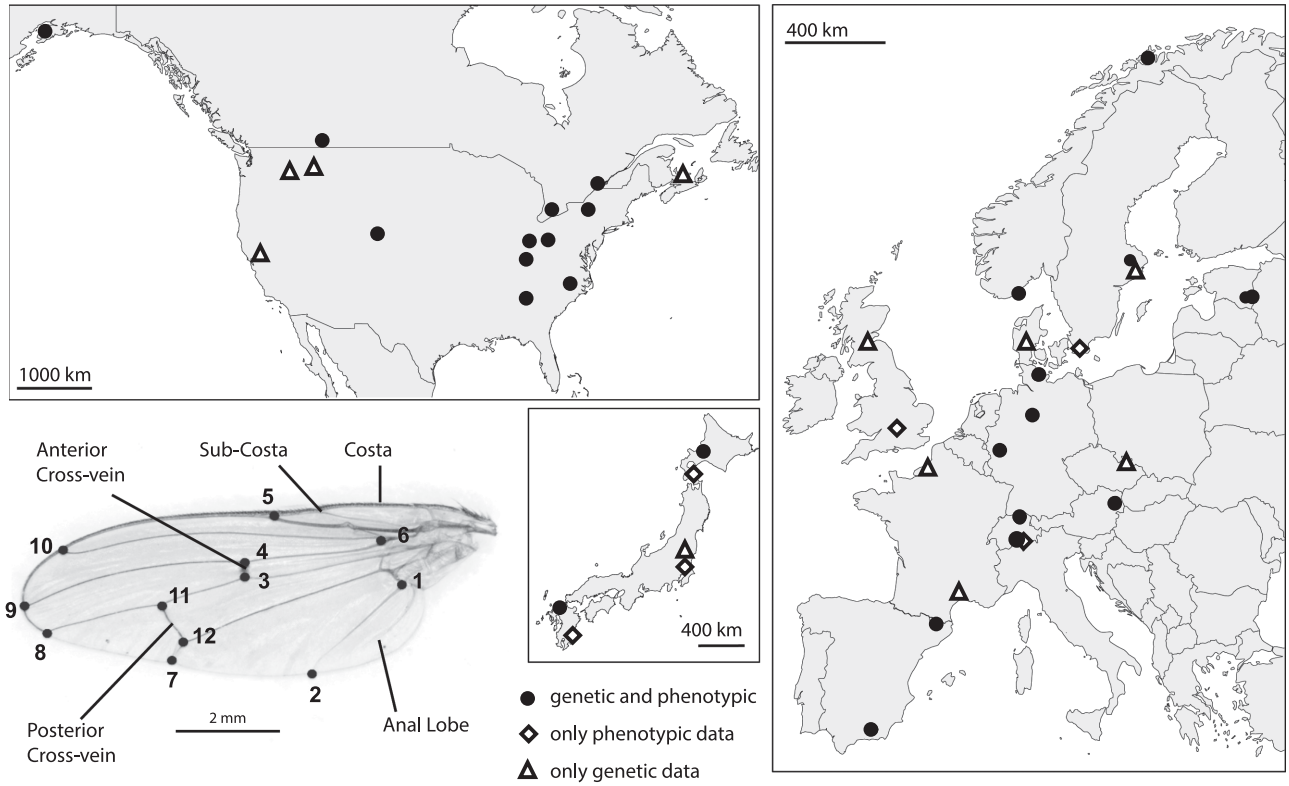

Figure 1. Sampling locations of North American, European, and Japanese *S. stercoraria* used for molecular and morphometric analyses. Geometric wing morphology was analyzed based on 12 landmarks.

the patterns of geographic variation within continents, as potential block effects could in this case be controlled for statistically. We are therefore confident that our results on temperature-dependent phenotypic plasticity, sexual dimorphism, and parallel wing shape clines are robust.

Feeding, breeding, and rearing conditions followed the standard protocols described in Blanckenhorn et al. (2010). Further details on the common garden rearing of the European flies, which were previously used to analyze patterns of quantitative genetic differentiation and thermal plasticity in female sperm storage morphology and correlated life-history traits (development time and growth rate), are published in Berger et al. (2011), so we only provide a brief summary here. Prior to common garden rearing, offspring of approximately 20 field-collected mating pairs per population were bred for one generation at 18°C to negate possible carry-over maternal effects relating to varying environmental conditions in the wild. A randomly chosen F1 female of each family raised in the laboratory was then mated with a random single F1 male from another family to produce F2 clutches for our common garden experiment. The F2 clutches were then split into three batches of 10–14 eggs, which were transferred to transparent plastic containers filled with superabundant and previously frozen cow dung to assess larval growth and development at unlimited conditions. Containers were then randomly assigned to different climate chambers set to constant 12°C, 18°C, or 24°C and 14 h light period. After 17 days of incubation (the minimum development time at 24°C), vials were checked daily for emerging flies, which were stored at –20°C in Eppendorf tubes until preparation of their wings.

MOLECULAR DATA ANALYSIS

We quantified the degree of genetic differentiation resulting from the separation among continents relative to that originating from the differentiation between populations within continents by performing AMOVA using ARLEQUIN 2.0 (Schneider et al. 1997). Variance components were tested for statistical significance by permuting genotypes among populations and continents (F_{ST}), by permuting genotypes among populations but within continents (F_{SC}), and by permuting populations among continents (F_{CT}) 10,000 times. In addition we used Bayesian clustering as implemented in the computer software BAPS 5.3 to infer population genetic structure (Corander and Marttinen 2006; Corander et al. 2006). This method uses a stochastic optimization algorithm to find the best posterior genetic partitioning. The number of genetic clusters can then be identified by changes in log marginal likelihood at a given probability for the best partitioning. We ran the analyses with prior distributions of K (1–37) genetic clusters and used population identity as a rather uninformative spatial prior. We ran 10,000 updates after a burn-in phase of 5000 iterations. Because of the stochastic nature of the algorithm, five independent runs were carried out to check the robustness of our results.

To detail similarities and differences in genetic structure across North America and Europe, we performed two additional types of analyses. First, we performed a series of Mantel tests (Manly 1991) comparing matrices of pairwise F_{ST}-values (across all loci and for each locus individually) with matrices of pairwise geographic distances. Pairwise F_{ST} values were estimated according to Weir and Cockerham (1984). Statistical significance was determined by permuting genotypes among populations 10,000 times. The corresponding 95% confidence limits were obtained by resampling loci with replacement. This conservative procedure does not assume Hardy–Weinberg equilibrium and allows for linkage among loci. All calculations were carried out with version 3.12 of the MICROSATELLITE-ANALYZER software (Dieringer and Schlötterer 2003). Spherical geographic distances were obtained from version 1.2 of the GEOGRAPHIC DISTANCE MATRIX GENERATOR (Ersts 2001). The subsequent Mantel tests were carried out with the program ZT (Bonnet and Van de Peer 2002) using 10,000 randomizations for evaluating statistical significance. Second, we tested for latitudinal and longitudinal patterns in expected heterozygosity H_E and allelic richness using ordinary least-squares regressions. Allelic richness was estimated based on a minimum sample size of five randomly selected genotypes (10 alleles) across loci and populations using FSTAT version 2.9.3 (Goudet 1995). Because genotype information of three markers (SsCa1, SsCa16, LIST9-004) was missing for some populations (see Table S2), all analyses were redone based on the seven-marker subset to evaluate statistical robustness. As results were nearly identical for the two data sets, and unless stated otherwise, we only report the results of the complete dataset, which included missing data.

MORPHOMETRIC ANALYSIS OF WING SHAPE

Wing shape was analyzed using 12 landmarks extracted from images photographed by a *Leica DM105* light microscope (Fig. 1). Landmarks were digitized using version 2.14 of the software tpsDig2 (Rohlf 2009). Landmark (LM) data were aligned using Generalized Procrustes Analysis (GPA) using version 2.17 of the morphometric package PAST (Hammer et al. 2001). As composite measure we retained raw wing centroid sizes (in mm) so that size-related (allometric) shape aspects could be explored. Prior to subsequent analyses we tested for potential block effects of experimental year (Europe only) and the observer who digitalized the landmarks, even though digitalization was conducted in a randomized fashion. While effects of year and observer were statistically significant, they only explained a minor fraction of the total variance. Nevertheless, we repeated all analyses using both the raw data and the residuals (controlled for observer and block). Since all results were nearly identical, we only report the results of the analyses based on the raw data.

To analyze global patterns of phenotypic and genetic variation in wing morphology we used a Procrustes ANOVA following Klingenberg et al. (2002). That is, we first computed individual ANOVAs for each of the 24 Procrustes (x and y) coordinates in SPSSv23. In each ANOVA, we used temperature, sex, family nested within population, and population nested within continent (setting population and family as random effects) as predictor variables. Subsequently, the Sums of Squares (type III) of each model term were summed across all the 24 ANOVAs. Procrustes Mean Squares were calculated by dividing the latter by the adequate degrees of freedom (df times 24). To assess the significance of model terms, we computed parametric *F*-tests as proposed by Goodall (1991).

We tested for geographic wing shape clines within continents by using the multivariate regression approach implemented in MorphoJ (Klingenberg 2011). Prior to analysis we averaged all Procrustes coordinates per population and regressed these means against latitude and longitude using centroid size as covariate (controlling for potential allometric body size effects). Statistical significance was tested by means of randomizations (10,000 ×). Due to the limited number of Japanese populations we focused on the comparison between North America and Europe, but nevertheless included Japanese populations in graphical illustrations of shape vector comparisons. Given significant correlations between latitude and longitude in our morphological data set (North America: $r = -0.81$, $N = 11$; Europe: $r = +0.75$, $N = 12$; Fig. 1), we explored latitudinal and longitudinal effects separately, thus accepting some degree of redundancy. Similarly, altitude correlated strongly with latitude ($r = -0.80$, $N = 12$) and longitude ($r = -0.71$, $N = 12$) across European sampling locations. These correlations were less pronounced and statistically nonsignificant in North America (latitude: $r = -0.06$; longitude: $r = -0.22$; both $N = 11$). However, since altitudinal differentiation patterns of important life-history traits such as pupal diapause induction or development time are absent or extremely small in *S. stercoraria* (Blanckenhorn 1997, 1998), we focused on broad-scale geographic variation only.

VECTOR CORRELATIONS

To compare the effects of rearing temperature, sex, size, and latitude on wing shape, we calculated correlations among their shape vectors. We used a multivariate generalized linear mixed model with Markov Chain Monte Carlo sampling (R-package "MCMCglmm" (Hadfield 2010)) to first estimate the effects of each variable on shape. Principal components (PCs) were used to represent overall shape instead of the original Procrustes coordinates, as PCs are by definition orthogonal at the level of the individual and thus do not introduce multicollinearity, which simplifies multivariate analysis. The first 20 PCs were used to quantify all shape variation in our dataset, since Procrustes superimposition of the 24

x and y coordinates results in a deficiency of four ranks: two ranks are removed due to size and rotation information, and two for the position. We fitted separate MCMCglmms for each continent using family and population as random effects. The off-diagonal elements of the variance-covariance matrix for the random effects were set to zero (using the *idh()* function of MCMCglmm) given the orthogonal structure of the PCs at the individual level. Uninformative priors based on population identity were used for the residual errors and random effects (R, G1, G2: $v = 0.10^{-6}$). Models were run for 220,000 iterations using a thinning interval of 100, and the first 20,000 iterations were discarded (burn-in). This resulted in 1000 posterior estimates of the effects of each factor (sex, temperature, and latitude) on the 20 PCs, and these posteriors thus allowed us to calculate 95% credible intervals and Bayesian *P*-values for all pair-wise vector correlations.

Q_{ST} – F_{ST} COMPARISONS

We next estimated the degree of quantitative genetic differentiation between populations (Q_{ST}) within continents to evaluate the role of drift in affecting wing morphology. Note that due to our full-sib family design genetic variance components extracted here might contain nonadditive effects as well, and hence potentially underestimate (rather than overestimate) Q_{ST} (Whitlock 1999; Goudet 2008). We performed a multivariate Q_{ST}-F_{ST} comparison analysis with the MCMC Bayesian method of Ovaskainen et al. (2011) implemented in the R-packages RAFM and DRIFTSEL (Karhunen et al. 2013). We used their "S-test" to look for a signal of selection on population phenotypic differentiation. This S-statistic varies between 0 and 1, where 0.5 means neutral differentiation (not significantly different from that expected based on F_{ST} differentiation); values <0.2 indicate stabilizing selection, while values >0.8 indicate divergent selection. We performed separate analyses for North American and European populations. In each case we tested for differentiation of the first three, five, and seven PCs, with sex and temperature as fixed effects. We used the results of 15 MCMC chains, running 160,000 iterations and sampling every 20 iterations after discarding the first 80,000 as burn-in. Because the multivariate analyses of more than three PCs mostly failed to provide consistent results, we additionally ran complementary univariate Q_{ST}-F_{ST} analyses on the first 7 PCs, which cumulatively explained 79.4% of the total wing shape variation. S-test values were averaged over three MCMC chains for each trait, which were run with 80,000 iterations and a burn-in of 8000 and a thinning of 20.

Results

MICROSATELLITE VARIABILITY AND POPULATION STRUCTURE

Genetic analyses revealed low but statistically significant geographic differentiation between and within continents best illustrated by the AMOVA results. Only 3.3 (95% CI [2.4, 4.9]) percent of the total molecular variance resulted from the differentiation among continents and 1.1 (95% CI [0.9, 1.3]) percent could be attributed to the differentiation among populations within continents. The remaining 95.6 percent of the variance was localized within populations. Bayesian analysis of population structure supported three distinct genetic clusters corresponding to the three continents with high probability $P > 0.999$. Further comparison of pairwise F_{ST}-values indicated a greater similarity between North American and European populations (mean $F_{ST} = 0.05$; 95% CI [0.043, 0.058]) relative to the other comparisons (America vs Japan: mean $F_{ST} = 0.11$; 95% CI [0.095, 0.124]; Europe vs Japan: mean $F_{ST} = 0.08$; 95% CI [0.065, 0.086]). Similar results were obtained from cross-continental comparison of the number of shared alleles. North American populations shared a higher proportion of alleles with flies of European (mean = 0.63; 95% CI from [0.627, 0.638]) than of Japanese origin (mean = 0.52; 95% CI [0.510, 0.536]). The mean proportion of shared alleles between European and Japanese populations was 0.59 (95% CI [0.572, 0.600]). All North American alleles were found to be present in European populations (not shown). Private alleles unique to Europe were typically low in frequency (< 10%), whereas common alleles (frequency >10%) were always present in all geographic areas. This resulted in reduced allelic richness (mean = 4.47, 95% CI [4.329, 4.606]) and heterozygosity H_E (mean = 0.72, 95% CI [0.701, 0.735]) of North American compared to European populations (mean allelic richness = 4.95, 95% CI [4.853, 5.045]), mean $H_E = 0.75$, 95% CI [0.740, 0.761]); Fig. 2, see also Table S2). Due to the small number of Japanese populations genotyped, allelic variability patterns remain less conclusive for Asia: mean allelic richness = 4.82, 95% CI [4.455, 5.176], mean $H_E = 0.75$, 95% CI [0.747, 0.789].

In North America, pairwise F_{ST}-values were tightly correlated with geographic distances among populations (Mantel test: $r = 0.90$, $P < 0.001$; Fig. 2). Significant isolation by distance was detected for 8 of the 10 loci analyzed (all: $P \leq 0.01$), suggesting that this is a genome-wide phenomenon and not confined to a few blocks of DNA owing to genetic hitchhiking along an ecological gradient. In contrast, no systematic spatial genetic structure was found in Europe (Mantel test: $r = 0.17$, $P = 0.172$; Fig. 2).

The pattern of isolation by distance in North America was accompanied by a significant decline in allelic richness from the southeast towards the northwest (effect of longitude: $r = 0.82$, 95% CI [0.518, 0.936]; latitude: $r = –0.69$, 95% CI [–0.888, –0.275]; Fig. 2) as well as a decrease in mean H_E (longitude: $r = 0.81$, 95% CI [0.428, 0.920]; latitude: $r = –0.73$, 95% CI [–0.896, –0.311]; both $N = 15$). The pattern in Europe was different, as neither longitude nor latitude explained a significant fraction of variation in allelic richness (longitude: $r = 0.21$, 95% CI [–0.287, 0.615]; latitude: $r = 0.04$, 95% CI [–0.435, 0.498]) or H_E

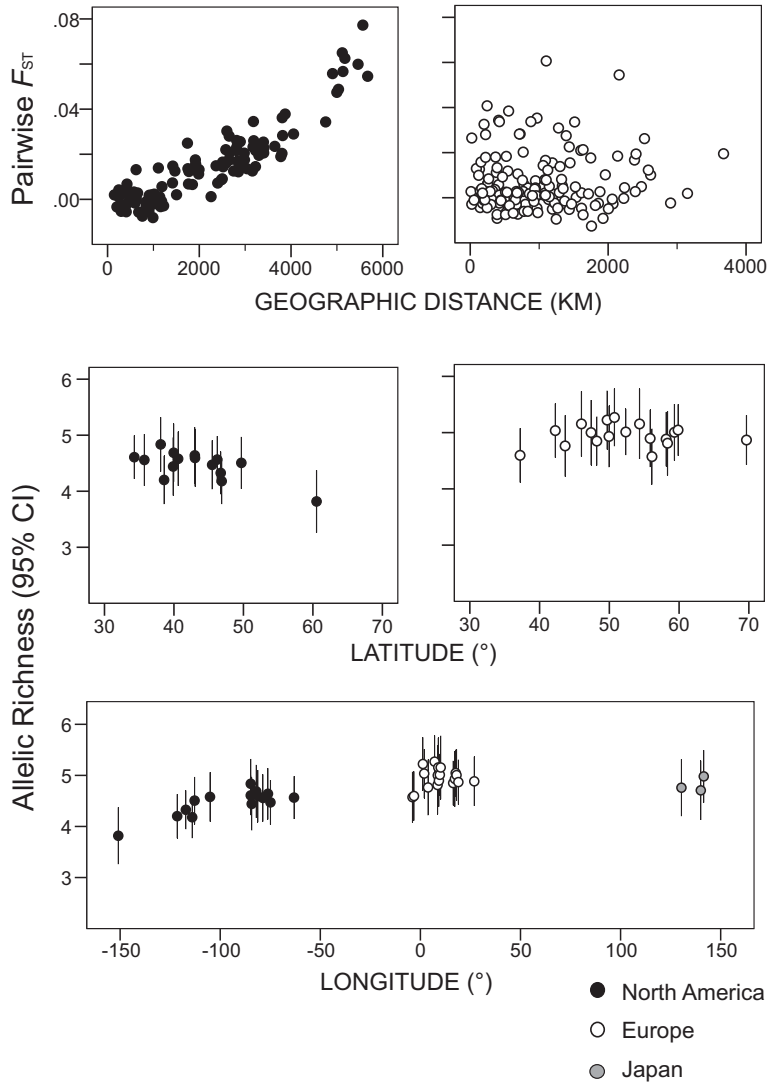

Figure 2. Correlations between pairwise F_{ST} values and geographic distances for European (open circles) and North American (black circles) *S. stercoraria* populations. Latitudinal and longitudinal variation in mean allelic richness is plotted based on 10 randomly sampled alleles per locus.

(longitude: $r = 0.25$, 95% CI [–0.92, 0.611]; latitude: $r = 0.32$, 95% CI [–0.264, 0.630]; both $N = 18$). In this context, however, it is important to consider that phylogeographic histories of cold-adapted species, which often survived in multiple glacial pockets further north, are in general difficult to reconstruct (Hewitt 2004; Bhagwat and Willis 2008). This certainly applies to species with good dispersal capacity like *S. stercoraria* (Kaufmann et al. 2013) and will require genomic data with much higher resolution than the moderate number of microsatellites genotyped here.

EFFECTS OF TEMPERATURE, SEX, AND GEOGRAPHY ON WING MORPHOLOGY

Procrustes ANOVA revealed significant variation of wing shape and size depending on the continent and population of origin, sex, and developmental temperature (Table 1). Main effects and two-way interactions terms were statistically significant throughout, though partial effect sizes of the interactions between continent, sex, and temperature regarding wing shape were small in magnitude relative to the main effects. At the population level, two-way interactions showed larger partial effect sizes, indicating that thermal plasticity and sexual shape dimorphism are more variable among populations within continents than between continents. Family effects (across temperature and sex) were highly significant, demonstrating substantial standing genetic variation encoding for wing shape within natural populations of *S. stercoraria*.

Developmental temperature primarily affected the outline of the wing independent of induced changes in wing centroid size (Fig. 3). Flies raised at 12°C developed wider wings at the anal lobe (LM 2) and more downwardly curved wing tips compared

Table 1. Results of nested mixed model ANOVA for wing centroid size and multivariate Procrustes ANOVA for wing shape.

	Wing size					Wing shape				
	MS	df	F	P	η_p^2	MS	df	F	P	η_p^2
Continent	23.92	2	11.06	<0.001	0.45	6.75^{-03}	48	22.78	<0.001	0.61
Temperature	110.69	2	149.83	<0.001	0.85	3.35^{-03}	48	53.93	<0.001	0.63
Sex	993.77	1	5546.45	<0.001	0.99	9.15^{-03}	24	257.99	<0.001	0.87
C × T	7.48	4	10.01	<0.001	0.43	2.85^{-04}	96	4.55	<0.001	0.22
C × S	1.69	2	9.46	0.001	0.38	5.42^{-05}	48	1.53	0.013	0.08
T × S	6.18	2	60.50	<0.001	0.62	1.53^{-04}	48	5.12	<0.001	0.11
C × T × S	1.09	4	10.68	<0.001	0.36	3.36^{-05}	96	1.12	0.202	0.05
Population (C)	2.64	25	2.60	<0.001	0.41	3.52^{-04}	600	2.56	<0.001	0.32
T × P (C)	0.94	50	8.87	<0.001	0.90	7.32^{-05}	1200	2.50	<0.001	0.70
S × P (C)	0.20	25	1.91	0.025	0.48	3.85^{-05}	600	1.31	<0.001	0.39
T × S × P (C)	0.11	50	1.22	0.142	0.01	2.96^{-05}	1200	1.04	0.193	0.01
Family (P(C))	0.41	668	4.70	<0.001	0.33	1.15^{-04}	16032	4.01	<0.001	0.30

η_p^2 denotes the partial effect size of each factor in the model.
Continent, temperature, and sex were treated as fixed and population and family as random effects.

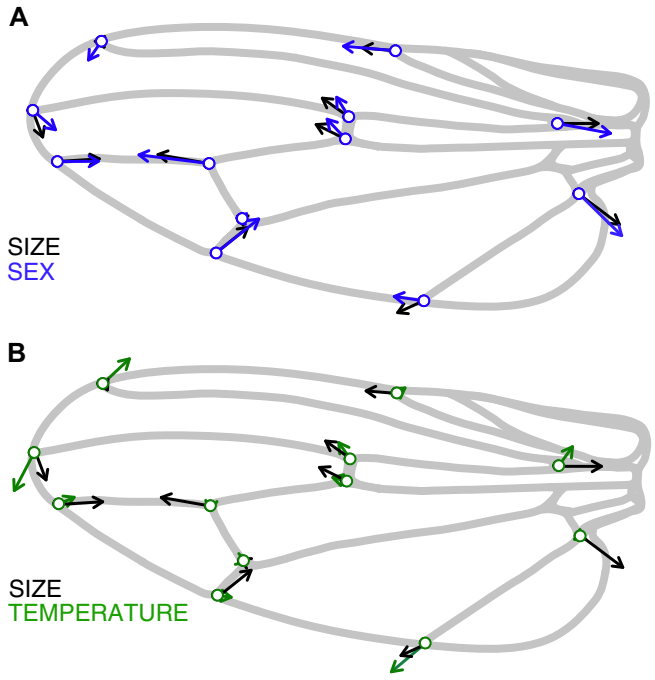

Figure 3. Landmark displacement vectors for the effects of (A) sex and (B) temperature in *S. stercoraria*. While wing shape dimorphism primarily results from larger wing centroid (i.e., body) sizes of males than females (blue vs black arrows), shape changes induced by cool developmental temperature (12°C) are mostly size independent (green vs black arrows).

to flies that developed at 18°C and 24°C. Sexual wing shape dimorphism scaled allometrically with wing size, equally involving marginal and inner wing node positions, particularly the position of the posterior cross-vein and the Cu-vein (LM1 and LM2). Females also had wider wings at the fourth medial vein (LM7) and more roundish wing tips (Fig. 3).

CLINAL VARIATION IN WING SHAPE

Multivariate regression analysis revealed a significant wing shape cline across North America (Fig. 4), which was more pronounced in latitudinal ($R^2 = 0.39$, $P < 0.001$) than longitudinal direction ($R^2 = 0.22$, $P = 0.044$). While clinal variation in latitudinal direction was mainly caused by the relative positioning of the anterior cross-vein and by size variation of the sub- and costal wing cells, longitudinal differentiation involved displacements of outer wing node positions as well. We also explored the extent to which clinal variation of wing shape evolved independently of wing size by performing a multivariate regression on residuals in which effects of centroid size were partialled out. The cline was still significant, although latitude explained a smaller fraction of the variation ($R^2 = 0.28$, $P = 0.045$). After controlling for centroid size, clinal variation in longitudinal direction turned marginally nonsignificant ($R^2 = 0.21$, $P = 0.064$).

Across Europe latitude explained only a small and statistically nonsignificant fraction of the shape variation, independent of whether we accounted for allometric effects ($R^2 = 0.06$, $P = 0.618$) or not ($R^2 = 0.05$, $P = 0.729$). Although longitude explained a larger proportion of the geographic variation in wing shape, multivariate regressions were nonsignificant before ($R^2 = 0.13$, $P = 0.209$) and after partialling out the effects of centroid size ($R^2 = 0.11$, $P = 0.277$). When testing for clinal variation in wing centroid size, we found that wing size tends to increase from the southeast toward the northwest in North America (latitude: $y = 7.49 + 0.01x$, $R^2 = 0.28$, $P = 0.092$; longitude:

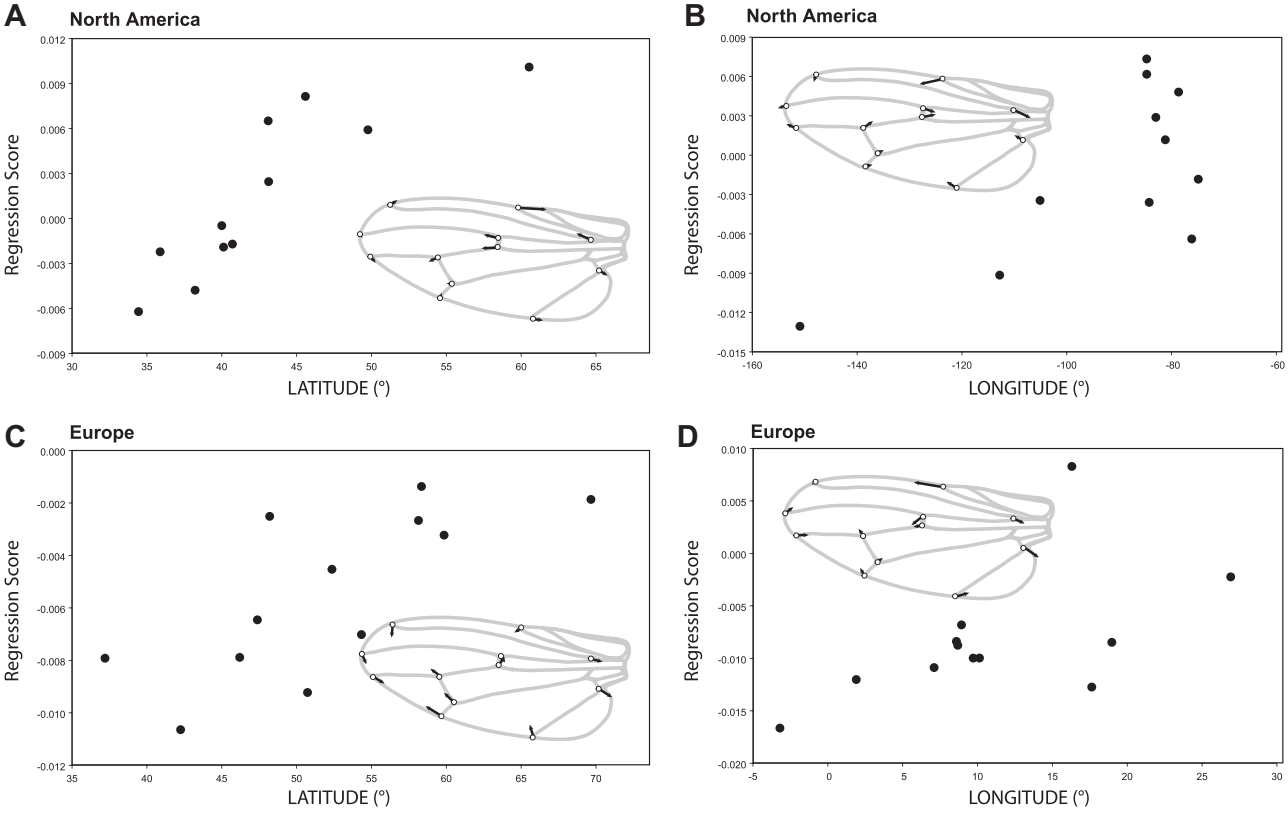

Figure 4. Latitudinal (left) and longitudinal (right) variation of wing shape (across all temperatures and sexes) for North American and European populations of *S. stercoraria*. Mean wing shape differences were multiplied by five for illustrative purposes.

y = 7.72–0.002, $R^2 = 0.14$, $P = 0.259$; both $N = 11$), whereas in Europe clines are almost flat (latitude: y = 7.94–0.003x, $R^2 = 0.07$, $P = 0.401$; longitude: y = 7.77 + 0.000, $R^2 = 0.00$, $P = 0.955$; both $N = 12$; see also Fig. S2).

VECTOR CORRELATIONS

We quantified the similarity of the effects of rearing temperature, sex, centroid size, and latitude on wing shape across continents by calculating correlations among the effect vectors (Fig. 5A). The effects of centroid size, sex, and temperature on wing shape correlated strongly between continents, demonstrating that temperature plasticity, sexual dimorphism, and allometry are highly conserved across the species' range. In contrast, the latitudinal shape variation was not consistent across continents, suggesting no common pattern of climate adaptation.

Within continents the shape vectors of temperature and centroid size did not correlate among populations in Europe and North America, while there was a rather weak, yet significant, effect in Japanese flies (Fig. 5B). Hence, temperature plasticity was generally independent of allometry, demonstrating that temperature effects are not merely due to larger body sizes at cool temperatures (temperature-size-rule: Atkinson 1994; Blanckenhorn 2009). In contrast, sexual dimorphism in wing shape was strongly associated with body size on all continents (Fig. 5B; see also Fig. 3). Sexual shape dimorphism is therefore mostly driven by the pronounced sexual size dimorphism in this species. Furthermore, latitude and temperature effects were largely unrelated. Any clinal variation in shape is therefore likely not associated with local adaptation to cool environments even in the presence of significant clinal variation for wing shape, as is the case in North America.

Q_{ST}—F_{ST} COMPARISONS

To test for an effect of local adaptation on phenotypic divergence across populations in North America and Europe, we compared the quantitative genetic divergence (Q_{ST}) of wing principal components (PCs) with the genetic divergence expected under drift and demography (F_{ST}). Although statistical power is limited by the number of populations sampled (rather than the number microsatellites genotyped; see, e.g. Whitlock and Guillaume 2009), and possibly also due to potential nonadditive genetic effects, multivariate Q_{ST}-F_{ST} analysis suggests that at least part of the clinal population differentiation pattern has a genetic basis and is likely caused by divergent selection acting across the species' range. The results of the S-tests performed for the three first PCs (averaged over 15 MCMC chains) were indicative of divergent

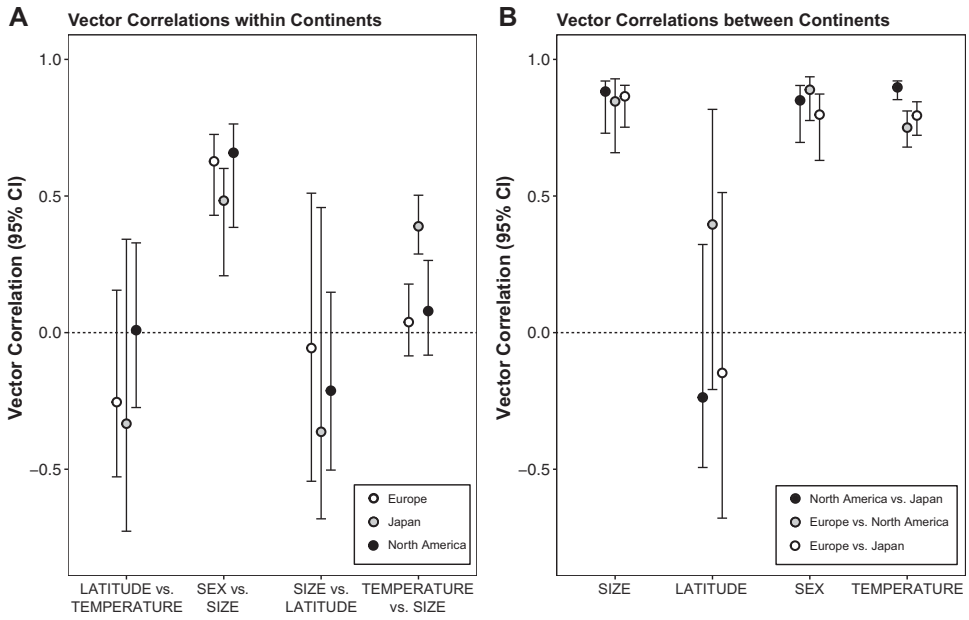

Figure 5. Vector correlations of the effects of temperature, sex, latitude, and wing centroid size on wing shape within and between continents in *S. stercoraria*. Mean coefficient vectors for pairwise comparisons were derived from a generalized model with error bars representing the 95% credible intervals for each comparison. (A) Vector correlations within continents indicate that sexual wing shape dimorphism is to a significant extent explained by variation in wing size in this species with pronounced male biased size dimorphism, whereas other vector correlations were weaker and mostly nonsignificant. (B) In contrast, latitudinal effects indicate no parallel wing shape clines on the different continents, and temperature, sex, and allometric scaling effects were highly conserved across the species' range.

selection in North America, with $S_{NA} = 0.93$ (± 0.16 SD), and Europe, with $S_{EU} = 0.999$ ($\pm 1.6^{-4}$ SD). However, analyses using five and seven PCs (PC1 to PC5, or PC1 to PC7) did not produce consistent results across the 15 MCMC chains and were therefore discarded. Furthermore, the univariate Q_{ST}-F_{ST} analyses showed that more trait shape dimensions harbor a signal of local adaptation in Europe than North America. While in Europe the first four PCs, which cumulatively explained 60 percent of the total shape variation, indicated a signature of local adaptation, in North America only PC2 and PC3, accounting for 28.7 percent of the shape variation, were significant with S statistics >0.8 (see Table S3 and Fig. S1 for corresponding landmark displacement vectors of the various PCs).

Discussion

Our worldwide study of morphological and molecular variation in the yellow dung fly *Scathophaga stercoraria* highlights two salient results. First, dung fly populations are clearly differentiated in wing morphology and microsatellites. Geographic patterns of morphological differentiation include significant clinal variation in wing shape across the New but not the Old World. Second, the shape of the wing margin exhibits significant phenotypic plasticity in response to developmental temperature, but plastic responses (hot-to-cold) do not mirror patterns of latitudinal differentiation (south-to-north) as might have been expected under a synergistic scenario of adaptive plasticity and adaptive genetic divergence. Furthermore, in contrast to sexual wing shape dimorphism, which can be mostly attributed to variation in wing (i.e., body) size, temperature-induced shape changes are largely size-independent and mainly affect the wing margin. In the following, we first consider adaptive and nonadaptive scenarios that might have caused the contrasting patterns of clinal variation in the New and Old Worlds, and then discuss our findings on phenotypic plasticity and sexual wing shape dimorphism in a similar context.

Morphometric and molecular analyses indicate that dung fly populations from different continents form distinct morphological and genetic entities. While the degree of cross-continental morphological differentiation is difficult to evaluate due to the common garden rearing having taken place in different laboratories, within continents we detected significant clinal variation of wing shape in the New World, but not (or to a lesser degree) the Old World. Correspondence with patterns of microsatellite variation across North America suggests that the wing shape cline relates to the species' biogeographic history. The absence of private microsatellite alleles in combination with reduced allelic diversity implies that North American dung flies have Eurasian ancestry

and that the New World has been colonized quite recently. This is similar to several other insects, like the house fly *Musca domestica* (Morris 1983), that were introduced from the western Palearctic region to the United States and Canada over the past 500 years (Sailer 1983; Liebold et al. 1995; Nimelä and Mattson 1996). Most recent introductions of *S. stercoraria* by humans and their livestock have been reported for Puerto Rico (Cuny 1983) and Newfoundland (Morris 1983), making recent colonization of the Americas highly plausible (cf. Bernasconi et al. 2008). This scenario is consistent with the greater molecular similarity of North American with European rather than Japanese flies, and with the decline in microsatellite variability from the Atlantic coast toward the Pacific Northwest following putative routes of colonization. Regardless of the details, the strong pattern of isolation by distance across North America clearly demonstrates that long-distance gene flow is restricted to some extent and, assuming similar dispersal abilities on the different continents, that recent colonization history has influenced the spatial genetic structure of *S. stercoraria* in the New World.

Theoretical and empirical studies of colonization processes suggest that rates of dispersal should increase in marginal relative to core populations (Hill et al. 2011; Shine et al. 2011; Kubisch et al. 2013; Fronhofer and Altermatt 2014; Lombaert et al. 2014). It is therefore plausible that the wing shape cline across North America has a similar explanation in that certain phenotypes disproportionally contributed to dispersal and colonization. For example, yellow dung fly body size tends to increase in the presumed direction of colonization (see also Blanckenhorn et al. 2018), in agreement with empirical findings indicating greater dispersal distances of larger organisms in a variety of taxa including bees and flies (Guédot et al. 2009; Zurbuchen et al. 2010; Rohner et al. 2015). Wing shape also exhibits significant latitudinal and (weaker) longitudinal differentiation in North America independent of centroid size, mainly involving the relative position of the anterior cross-vein and size variation of the sub- and costal wing cells that might affect the stiffness and aerodynamic properties of the wing (see Fig. 1). However, in light of the general uncertainty about the influence of wing venation patterns on flight performance traits (e.g., Ennos 1889; Combes and Daniel 2003; Hedrick et al. 2015), it is also possible that the North American cline has somehow resulted from random genetic drift associated with the colonization process. Similar conclusions were reached for the contrasting wing shape clines in *D. subobscura*, which have been ascribed to the bottleneck associated with the invasion of the New World by flies of Eurasian ancestry (Fragata et al. 2010; Simões et al. 2015).

In Europe, where *S. stercoraria* presumably has existed for much longer time than in North America, and where no molecular pattern of isolation by distance was evident, clinal variation in wing shape was less pronounced and statistically nonsignificant. When studying wing shape variation of nine *D. melanogaster* populations collected across ancestral Sub-Saharan Africa, Pitchers et al. (2013) detected significant altitudinal, latitudinal, and longitudinal clines, which accounted for only one to seven percent of the total wing shape variation, respectively. By examining 12 European *S. stercoraria* populations collected over a comparable geographic range, we found that latitude and longitude explained a similar, low degree of the morphological variation despite significant population differentiation. If natural selection is involved, as indicated by multi- and univariate Q_{ST}-F_{ST} analyses, these findings imply that broad-scale climatic selection gradients shaping dipteran wing morphology are rather weak compared to the strong selection gradients that are often observed to influence the evolutionary trajectories of important life-history traits, such as diapause incidence in *Drosophila* (Schmidt et al. 2005) or *Scathophaga* (Demont et al. 2004; Scharf et al. 2010; Blanckenhorn et al. 2018). Although southern populations were collected at higher elevation in Europe, potentially weakening latitudinal selection gradients, this explanation seems unlikely to apply to *S. stercoraria* given the fly's dispersal ability and absence of significant altitudinal differentiation patterns in life-history traits across the Swiss Alps (Blanckenhorn 1997, 1998). Also, shape vector comparisons revealed no evidence for parallel latitudinal clines on the different continents, which is not surprising given the multifaceted nature of selection regimes that might act on insect flight and the different biogeographic and demographic histories of the study populations. That wing shape can diverge quickly in response to selection or drift is illustrated by quantitative genetic studies of *Drosophila* demonstrating a highly polygenic basis of largely additive effects encoding for intraspecific variation in wing vein positioning (Zimmermann et al. 2000; Weber et al. 2001; Mezey et al. 2005), and lack of significant genetic constraints in any direction of phenotypic space (Weber 1990, 1992; Mezey and Houle 2005; see also Houle et al. 2017) even though shape variation is integrated over the entire fly wing (Klingenberg and Zaklan 2000).

Adaptive responses to variable but predictable environments involve the evolution of phenotypic plasticity, and considerable interest centers on how phenotypic plasticity and genetic divergence interact in allowing populations to persist in heterogeneous environments (Pigliucci 2005; Räsänen and Kruuk 2007; Crispo 2008). In *S. stercoraria* we found the margin of the wing (rather than inner wing vein positions) to respond plastically to developmental temperature: flies raised at cold temperature (12°C) developed wider wings at the wing base and more downwardly curved wing tips than flies raised at warmer temperatures (18°C and 24°C). Using targeted RNA interference, Ray et al. (2016) modified the shape of the *D. melanogaster* wing far beyond the species' natural spectrum and found that fruit flies with artificially high wing aspect ratio showed improved aerial agility relative

to the wild-type phenotype, and that this improvement imposes metabolic costs. Frazier et al. (2008) further demonstrate that flies developing at cold temperature have improved flight performance in the cold due to wing shape changes. Since metabolism and muscular efficiency, including wing-beat frequency, increase with temperature in flies (Reed et al. 1942; Hargrove 1980; Unwin and Corbet 1984), the phenotypic plasticity of wing shape in *S. stercoraria* might have evolved in response to similar flight performance trade-offs. However, this hypothesis requires functional testing, particularly because here shape effect vectors of temperature and latitude were not correlated, as could have been expected under a synergistic scenario of adaptive thermal plasticity and adaptive genetic divergence (Price et al. 2003; Gahlambor et al. 2007; Schmid and Guillaume 2017). In this context, however, it is worth noting that shape effect vectors integrate over the entire wing, potentially masking functionally relevant plastic and genetic shape changes accruing by phenotypic changes with little or no effect on flight performance.

Irrespective of their adaptive significance, our results clearly demonstrate that thermal plasticity of wing shape in *S. stercoraria* is conserved across the species' range and is not merely a function of temperature-induced changes of wing size, as is possibly the case for sub-Saharan *D. melanogaster*, where shape effect vectors of developmental temperature and latitude were found to strongly correlate with wing centroid size (Pitchers et al. 2013; see also Gilchrist et al. 2000). Contrary to thermal plasticity, sexual dimorphism in wing shape contained a significant allometric component. In *S. stercoraria* sexual competition on dung (their breeding substrate) is usually intense but variable, which is largely responsible for the male-biased size dimorphism in this species (Borgia 1981; Jann et al. 2000; Ding and Blanckenhorn 2002). Alternative mating tactics of small-sized males off the dung, however, also exist in nature (Pitnick et al. 2009; Gress et al. 2014). Therefore, spatially and temporally varying sexual selection regimes on male body size probably have contributed to the sexual wing shape dimorphism in *S. stercoraria* by way of allometric scaling, explaining the correlation between wing shape and size dimorphism found here.

In conclusion, by integrating molecular and morphometric data, our comprehensive study of the widespread yellow dung fly revealed significant variation in wing shape and size depending on geographic origin, sex, and developmental temperature. In agreement with theoretical expectations we found a strong relationship with biographic history in North America, which has presumably been colonized quite recently by dung flies of Eurasian ancestry. By contrast in Europe, where *S. stercoraria* has resided for much longer time and no molecular pattern of isolation by distance was evident, clinal variation in wing morphology is less pronounced and statistically nonsignificant. Furthermore, patterns of latitudinal wing shape differentiation (south-to-north) are largely unrelated to thermal responses (hot-to-cold), implying no synergistic scenario of adaptive genetic population divergence and adaptive phenotypic plasticity along broad-scale temperature gradients. Laboratory and field experiments are needed to fully evaluate the functional significance and fitness consequences of different wing shape phenotypes under varying environmental (particularly temperature) conditions.

AUTHOR CONTRIBUTIONS

M.A.S. and W.U.B. conceived and lead the research. They plus D.B, P.T.R, A.K., S.S.B, and C.W.F. performed the rearing in Zürich or the USA, and/or contributed to the collection of morphometric and microsatellite data. P.T.R. and M.A.S. analyzed the morphometric data, F.G. performed the Q_{ST}-F_{ST} analyses and M.A.S. analyzed the microsatellite data. M.A.S., P.T.R., F.G., and W.U.B. wrote the article, with input by all others. All authors have seen and agreed to the submission and publication of this manuscript.

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DATA ARCHIVING

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Sampling locations, collection dates and geographical information on the *S. stercoraria* populations used for molecular and morphometric analyses.

Table S2. Sample sizes (N: genotypes; populations), total number of alleles per locus (NA), mean observed (HO) and expected (HE) heterozygosity at 10 microsatellite loci across North American, European and Japanese populations of S. stercoraria.

Table S3. Univariate Q_{ST}-F_{ST} comparisons of the first 7 PCs describing wing shape in *S. stercoraria* using the MCMC Bayesian approach described in Ovaskainen et al. (2011; Genetics 189: 621–729) and implemented in the R packages RAFM and DRIFTSEL (Karhunen et al. 2013; Mol. Ecol. Res. 13: 746–754).

Figure S1. Landmarks used to describe wing shape in *S. stercoraria*.

Figure S2. Latitudinal and longitudinal variation of wing centroid size (averaged across temperatures and sexes) for North American and European populations of *S. stercoraria*.