

Secondary contact and local adaptation contribute to genome-wide patterns of clinal variation in *Drosophila melanogaster*

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Abstract

Populations arrayed along broad latitudinal gradients often show patterns of clinal variation in phenotype and genotype. Such population differentiation can be generated and maintained by both historical demographic events and local adaptation. These evolutionary forces are not mutually exclusive and can in some cases produce nearly identical patterns of genetic differentiation among populations. Here, we investigate the evolutionary forces that generated and maintain clinal variation genome-wide among populations of *Drosophila melanogaster* sampled in North America and Australia. We contrast patterns of clinal variation in these continents with patterns of differentiation among ancestral European and African populations. Using established and novel methods we derive here, we show that recently derived North America and Australia populations were likely founded by both European and African lineages and that this hybridization event likely contributed to genome-wide patterns of parallel clinal variation between continents. The pervasive effects of admixture mean that differentiation at only several hundred loci can be attributed to the operation of spatially varying selection using an F_{ST} outlier approach. Our results provide novel insight into the well-studied system of clinal differentiation in *D. melanogaster* and provide a context for future studies seeking to identify loci contributing to local adaptation in a wide variety of organisms, including other invasive species as well as temperate endemics.

Keywords: adaptation, *Drosophila melanogaster*, latitudinal clines, parallelism, secondary contact

Received 19 December 2014; revision received 29 October 2015; accepted 2 November 2015

Introduction

All species live in environments that vary through time and space. In many circumstances, such environmental heterogeneity can act as a strong selective force driving adaptive differentiation among populations. Thus, a major goal of evolutionary and ecological genetics has been to quantify the magnitude of adaptive differentiation among populations and to identify loci underlying

adaptive differentiation in response to ecologically relevant environmental variation.

Phenotypic and genetic differentiation between populations has been examined in a variety of species. In some cases, patterns of differentiation are directly interpretable in the context of circumscribed environmental differences that occur over short spatial scales (Richardson *et al.* 2014). For instance, differences in salinity experienced by freshwater and marine populations of sticklebacks have led to the identification of key morphological, physiological and genetic differences between replicate pairs of populations (Kitano *et al.* 2010; Jones *et al.* 2012). Similarly, pigmentation morph

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frequency closely tracks variation in substrate colour for a variety of species (reviewed in Gray & McKinnon 2007), thereby providing an excellent opportunity to directly relate environmental variation to phenotypic and genetic differentiation.

Patterns of genetic and phenotypic variation have also been examined in species arrayed along broad geographical transects such as latitudinal clines (Endler 1993). In this paradigm, the goal has often been to identify the phenotypic and genetic basis for adaptation to temperate environments. In certain cases, it has been possible to directly relate latitudinal variation in specific environmental variables to aspects of phenotypic and genetic differentiation (e.g. photoperiod and critical photoperiod or flowering time: Bradshaw & Lounibos 1977; Stinchcombe *et al.* 2004). In general, the collinearity of multiple ecological and environmental variables along latitudinal clines often complicates the direct relationship of environmental variation with specific phenotypic and genetic differences. Nonetheless, because many genetically based phenotypic clines within species often mirror deeper phylogenetic differentiation between endemic temperate and tropical species, it is clear that populations distributed along latitudinal clines have adapted to aspects of temperate environments (Gibert *et al.* 2001).

Latitudinal clines have been extensively studied in various drosophilid species, most notably *Drosophila melanogaster*. Parallel clines in morphological (Rajpurohit *et al.* 2008; Telonis Scott *et al.* 2011), stress tolerance (Hoffmann *et al.* 2002; Schmidt *et al.* 2005) and life history traits (Schmidt *et al.* 2005; Lee *et al.* 2011b) have been identified in *D. melanogaster* populations distributed along multiple continents. These phenotypic clines demonstrate that flies from poleward locales are generally more hardy albeit less fecund, reflecting a classic trade-off between somatic maintenance and reproductive output (Schmidt *et al.* 2005) that would be alternately favoured between populations exposed to harsh winters versus more benign tropical environments. Extensive clinal variation in various genetic markers has also been identified (Sezgin *et al.* 2004; Hoffmann & Weeks 2007). In some cases, clinal genetic variants have been directly linked to clinally varying phenotypes (Schmidt *et al.* 2008; Paaby *et al.* 2010, 2014; Lee *et al.* 2013), whereas in other cases parallel clinal variation at genetic markers has been documented across multiple continents (Turner *et al.* 2008; González *et al.* 2010; Reinhardt *et al.* 2014). Taken as a whole, there is abundant evidence that local adaptation to spatially varying selection pressures associated with temperate environments has shaped clinal patterns of phenotypic and genetic variation in *D. melanogaster*.

Demographic forces can also heavily shape patterns of clinal variation (Endler 1993), and the recent demographic history of *D. melanogaster* may be relevant to our understanding of clinal patterns of genetic variation in this species. *D. melanogaster* is an Afro-tropical species (David & Capy 1988) that has colonized the world in the wake of human migration. Population genetic inference suggests that Eurasian populations of *D. melanogaster* diverged from ancestral sub-Saharan populations approximately 15 000 years ago (Li & Stephan 2006) and eventually migrated eastward across Asia, arriving to Southeast Asia approximately 2500 years ago (Laurent *et al.* 2011). *D. melanogaster* invaded the Americas and Australia within the last several hundred years and likely colonized these continents in their entirety quickly (Bock & Parsons 1981; Keller 2007). Historical records suggest that *D. melanogaster* colonized North America and Australia each in a single event (Bock & Parsons 1981; Keller 2007).

In contrast, however, population genetic (Caracristi & Schlötterer 2003; Duchon *et al.* 2013; Kao *et al.* 2015; Pool 2015) and morphological evidences (Capy *et al.* 1986; Ferveur *et al.* 1996; Coyne *et al.* 1999; Takahashi *et al.* 2001; Rouault *et al.* 2004; Yukilevich & True 2008) suggest that, for the Americas at least, there were multiple colonization events with some migrants coming from Africa and some from Europe. While there is less evidence that Australian populations of *D. melanogaster* form a secondary contact zone, such a scenario is not implausible given the high rates of human migration and intercontinental travel during the 19th century. Alternatively, it is possible that Australia was colonized in a single event, but that these invading flies represent a recent mixture of distinct ancestral lineages. Thus, if North America and Australia represent secondary contact zones, having experienced immigration from highly differentiated and locally adapted ancestral populations such as those from Europe and Africa (Pool *et al.* 2012), many genetic variants would appear clinal, even in the absence of spatially varying selection pressures (Endler 1993; Caracristi & Schlötterer 2003).

Investigating whether North America and Australia represent secondary contact zones is therefore crucial for our understanding of the extent of spatially varying selection operating on this species. We note that the secondary contact zone model and adaptive differentiation as evolutionary forces that generate and maintain clinal variation in North America and Australia are not mutually exclusive. Notably, one plausible model is that dual or mixed-lineage colonization of these continents generated patterns of clinal variation and spatially varying selection has subsequently slowed the rate of genetic homogenization among populations. Accordingly, we sought to investigate whether genome-wide patterns of

clinal genetic variation in North America and Australia show signals of secondary contact and local adaptation.

We find that both North American and Australian populations show several genomic signatures consistent with secondary contact and suggest that this demographic process is likely to have generated patterns of clinal variation at a large fraction of the genome in both continents. Despite this genome-wide signal of recent admixture, we find evidence that spatially varying selection has shaped patterns of allele frequencies at some loci along latitudinal clines using an F_{ST} outlier approach. Some of these F_{ST} outliers are in or near genes previously identified to underlie life history and stress tolerance traits. However, in other cases, previously identified clinal functional polymorphisms were not identified as F_{ST} outliers demonstrating the inherent challenge of identifying the genetic targets of spatially varying selection. We discuss these findings in relation to the well-documented evidence of spatially varying selection acting on this species as well as the interpretation of patterns of genomic variation along broad latitudinal clines in general.

Materials and methods

Genome-wide allele frequency estimates

We utilized novel and publically available genome-wide estimates of allele frequencies of *D. melanogaster* populations sampled worldwide (Fig. 1; Table S1, Supporting information). Allele frequency estimates of six North American populations are described in Bergland *et al.* (2014). Allele frequency estimates of three European populations are described in Bastide *et al.* (2013) and Tobler *et al.* (2014). Allele frequency estimates from 20 African populations with full genome sequence are described in Pool *et al.* (2012). Allele frequency estimates of two Australian populations, Tasmania (~41°S) and Queensland (hereafter Queensland S1; ~16°S), are described in Kolaczowski *et al.* (2011). Allele frequency estimates from two additional Australian populations are reported here for the first time. Allele frequency estimates from these additional Australian populations were made by pooling ten individuals from each of 22 isofemale lines originating from Innisfail (hereafter Queensland S1; 17°S) or Yering Station (hereafter Victoria; 37°S), Australia (isofemale lines kindly provided by A. Hoffmann). Sequencing library preparation and mapping followed methods outlined in Bergland *et al.* (2014). In several analyses, *D. simulans* was used as an outgroup. In these analyses, we used genome-wide allele frequency estimates from a worldwide collection of *D. simulans* (Begun *et al.* 2007), using genome-wide *lift-over* files reported in Bergland *et al.* (2014).

We performed SNP quality filtering similar to the methods presented in Bergland *et al.* (2014). Briefly, we excluded SNPs within 5 bp of polymorphic indels, SNPs within repetitive regions, SNPs with average minor allele frequency less than 15% in both North America and Australia, SNPs with low (<5) or excessively high read depth (>2 times median read depth) and SNPs not present in the *Drosophila* Genetic Reference Panel (Mackay *et al.* 2012). African samples were not quality-filtered for read depth because allele frequency estimates from these samples were derived from sequenced haplotypes and not pooled samples. Regions of inferred admixture (Pool *et al.* 2012) in African samples (i.e. introgression of European haplotypes back to African populations) were removed from analysis.

Estimation of the population tree

We calculated Nei's genetic distance (Nei 1972) between each pair of populations as,

$$-\log \left(\frac{\sum p_1 \cdot p_2}{\sqrt{\sum p_1^2} \cdot \sqrt{\sum p_2^2}} \right),$$

where p_1 and p_2 are allele frequency estimates in populations 1 and 2, and generated a population tree using the neighbour-joining algorithm implemented in the R (R Core Team 2014) package APE (Paradis *et al.* 2004). To generate bootstrap values for each node, we randomly sampled 10 000 SNPs 100 times. We generated estimates of the population tree using the whole genome and for each chromosome focusing on SNPs that occur within the large, cosmopolitan inversions (Corbett-Detig & Hartl 2012), outside these inversions or both.

Estimation of the proportion of African and European ancestry

We used a simple linear regression method to estimate the proportion of African and European ancestry for each North American and Australian population. This method is inspired by an approach outlined in Alkorta-Aranburu *et al.* (2012) where each North American and Australian (derived) population is modelled as a linear combination of one African and one European (ancestral) population using an intercept-free regression model. Ancestry coefficients were calculated by drawing 100 sets of 5000 random SNPs per derived population per pair of ancestral populations. We generated ancestry estimates using the whole genome and for each chromosome separately focusing on SNPs that occur within the large, cosmopolitan inversions, outside these inversions or both.

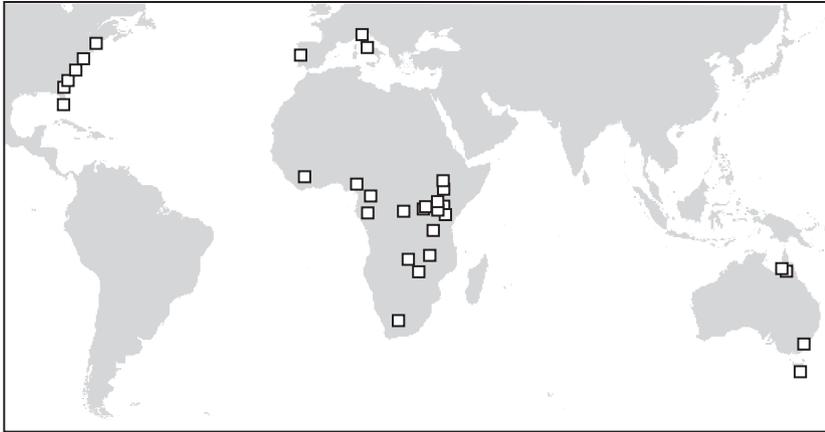


Fig. 1 Map of collection locales (squares) of *D. melanogaster* populations used in this study.

To verify that our regression-based method of ancestry proportion estimation is accurate and robust to various demographic scenarios, we simulated several demographic models using coalescent simulations as implemented in *ms* (Hudson 2002). To assess the accuracy of estimated ancestry proportion, we first simulated a three-population model with one ancestral population, one ancient population that diverged from the ancestral population $0.1N_e$ generations ago and one derived population that resulted from admixture between the ancestral and ancient populations $0.001N_e$ generations ago. In these simulations, we varied the proportion of lineages in the derived population that originated from the ancient and ancestral populations. A graphical model of this demographic history is presented in Fig. S2 (Supporting information), and *ms* code is available from DataDryad under accession doi:10.5061/dryad.7440s.

Next, we sought to verify that single- and dual-colonization scenarios produce distinctive ancestry proportion clines. In these models, we simulated an ancient population that diverged from an ancestral population $0.1N_e$ generations ago. In the single-colonization scenario, a newly derived population was founded from the ancestral population and a series of four additional populations were derived from this initial newly derived population through a series of serial founder events. In the dual-colonization scenario, two newly derived populations were founded by either the ancestral or the ancient population. These newly derived populations each gave rise to another newly derived population through a serial founder event, and a fifth newly derived population resulted from the merger of these later populations. For both single- and dual-colonization models, migration was either modelled as a stepping-stone process between neighbouring newly derived populations or as a panmictic process between all newly derived populations. For either migration model, Nm was set to 1. A graphical representation of

these models can be found in Fig. S3 (Supporting information), and *ms* code is available from DataDryad under accession doi:10.5061/dryad.7440s. In the single- and dual-colonization models, we varied the age of initial colonization of the newly derived populations and the extent of the population bottleneck during the initial and serial founder event(s). We note that parameters used for *ms* simulations are not intended to reflect the actual demographic history of *D. melanogaster*, but rather reflect a variety of plausible values for *D. melanogaster* and other organisms.

Formal tests of admixture

To further assess whether newly derived North America and Australian populations result from admixture of European and African lineages of flies, we performed formal tests of admixture using the f_3 and D statistics (Reich *et al.* 2009; Patterson *et al.* 2012). Briefly, these statistics assess whether a proposed tree topology is consistent with the data.

The f_3 statistic, denoted (C; A, B), assesses whether the data are consistent with the topology ((A, C), B) or ((B, C), A). A significantly negative f_3 statistic demonstrates that the data are consistent with both topologies, thus indicating that population C is derived from an admixture event from populations A and B or populations closely related to A and B. However, nonsignificant or positive f_3 statistic does not preclude the possibility of admixture between tested populations, particularly if there has been extensive drift in the focal population (Patterson *et al.* 2012).

The D statistic, which is derived and conceptually similar to the ABBA-BABA statistic (Patterson *et al.* 2012), assesses whether the data are consistent with the proposed topology ((W, X), (Y, Z)). In general, a significantly positive D statistic indicates that population W results from admixture between populations X and Y, or populations closely related to X and Y. A

significantly negative D statistic can indicate that population X results from admixture between populations W and Y , or populations closely related to W and Y . While, in general, the sign of significant D statistics can be difficult to interpret, the most general interpretation is that the data do not conform to the proposed $((W, X), (Y, Z))$ topology.

There are three possible D statistics for any four populations: $((W, X), (Y, Z))$, $((W, Y), (X, Z))$ and $((W, Z), (X, Y))$. In our analysis, we used $D. simulans$ as an outgroup (population Z in this notation). Accordingly, the first two D statistics are most easily interpretable in our analysis. In this context, population W represents the newly derived North American or Australian population and populations X and Y represent the putative source African and European populations, respectively. To simplify the interpretation of these D statistics, and to provide a conservative analysis of admixture, we report the D statistic corresponding to the minimum absolute D of $((W, X), (Y, Z))$ and $((W, Y), (X, Z))$.

For each North American and Australian population, we calculated f_3 and D using each European population as one putative ancestral population and each African population as the other putative ancestral population. f_3 and D statistics were calculated using the `THREEPOP` and `FOURPOP` programs included in `TREEMIX` version 1.13 (Pickrell & Pritchard 2012) with 500 bootstrap replicates using a block size of 500 SNPs. Multiple testing correction was performed using the Bonferroni correction applied across all P -values from f_3 or D statistics. f_3 and D statistics were calculated for the whole genome and separately for the X and autosomes.

F_{ST} outlier identification

We used the T_{F-LK} statistic (Bonhomme *et al.* 2010) to identify F_{ST} outliers among North American populations and Australian populations, separately. This statistic is related to the classic Lewontin–Krakauer test for F_{ST} outliers, under the assumption that the distribution of F_{ST} is proportional to a χ^2 distribution with degrees of freedom equal to one less the number of populations examined (Lewontin & Krakauer 1973). Various assumptions underlying the Lewontin–Krakauer test have been criticized (Robertson 1975), and the T_{F-LK} statistic attempts to correct for these by conditioning the distribution of F_{ST} values on the inferred underlying population tree. Under a variety of demographic scenarios (Bonhomme *et al.* 2010; Mita *et al.* 2013), including secondary contact (Lotterhos & Whitlock 2014), the T_{F-LK} statistic has generally been found to have low false-positive and high true-positive rates (i.e. power) in F_{ST} outlier detection. It is unknown, however, how T_{F-LK} or any outlier-based test statistic performs in more

complicated scenarios including secondary contact between populations that have adapted to similar spatially varying selection pressures as found between the focal populations. Multiple testing correction for the T_{F-LK} statistic was performed using the false discovery rate methods implemented in the `Q-VALUE` package (Storey & Tibshirani 2003).

Differentiation and rates of parallelism

To assess rates of codifferentiation, we calculated the odds that SNPs fell above a range of F_{ST} quantile thresholds in North America, Australia or the Old World (i.e. Europe vs. Africa). We compared this value to the odds of codifferentiation from 500 sets of randomly selected SNPs that were matched to the focal SNPs by recombination rate (Comeron *et al.* 2012), chromosome, inversion status (at the large, cosmopolitan inversions *In(2L)t*, *In(2R)NS*, *In(3L)Payne*, *In(3R)K*, *In(3R)Payne*, *In(3R)Mo*, *In(X)A* and *In(X)Be*), average read depth and heterozygosity. To control for the possible autocorrelation in signal along the chromosome, we divided the genome into nonoverlapping 50-Kb blocks and randomly sampled, with replacement, one SNP per block 500 times.

Results

Data

We examined genome-wide estimates of allele frequencies from ~32 populations of *D. melanogaster* sampled throughout North America, Australia, Europe and Africa (Fig. 1). Our analyses largely focused on patterns of variation in North American and Australian populations and, consequently, we primarily focus on two sets of SNP markers. First, we utilized allele frequency estimates at ~500 000 high-quality SNPs that segregate at intermediate frequency (MAF > 15%) in North America. The second set was composed of ~300 000 SNPs that segregate at intermediate frequency in Australia. For analyses that examine patterns of polymorphism in both North America and Australia, we examined SNPs that were at intermediate frequency in both continents, yielding a data set of ~130 000 SNPs. Because of the low sequencing coverage in the Australian populations, it is unclear whether the reduced polymorphism in that continent reflects the demographic history of those populations or experimental artefact. Although our analysis primarily focused on patterns of polymorphism in North America and Australia, we also examined allele frequency estimates at both sets of polymorphic SNPs in populations sampled in Europe and Africa.

Genomic signals of secondary contact

We performed a series of independent analyses to examine whether North America and Australia represent secondary contact zones of European and African populations of *D. melanogaster*. First, we constructed a neighbour-joining tree based on genome-wide allele frequency estimates from populations sampled worldwide using 100 sets of 10 000 randomly sampled SNPs. Neighbour-joining trees were generated for SNPs residing within inversions, outside inversions or both genome-wide (Fig. 2) and for each chromosome separately (Text S1, Supporting information). As expected, African populations exhibited the greatest diversity (Pool *et al.* 2012) and clustered at the base of the tree, while European populations clustered at the tip. Similar to previously published reports (Caracristi & Schlotterer 2003; Nunes *et al.* 2008), North American and Australian populations generally clustered between African and European populations in a branching pattern collinear with latitude (Fig. 2), consistent (Kopelman *et al.* 2013) with the model that both North American and Australian populations result from secondary contact of European and African ones. In contrast to Reinhardt *et al.* (2014), we find that Australian and North American populations are not reciprocally monophyletic (Fig. 2).

In general, the topology of these population trees was independent of whether SNPs within or outside the

cosmopolitan inversions were included in the analysis. One notable exception was the placement of the Portuguese population which falls within the North American samples when examining SNPs within inversions. In addition, the topology of the African populations varied depending on the set of SNPs under consideration. However, the bootstrap support for the nodes among African populations is generally low, likely stemming from small sample sizes in these populations. In chromosome-specific analyses (Text S1, Supporting information), nodes that do not agree with the genome-wide analysis generally had low bootstrap support. We note that our treatment of the effects of the inversions on patterns of clinality is preliminary because we are performing a coarse binning of SNPs within or outside inversions rather than a finer partitioning based on distance from inversion breakpoints.

Next, we calculated the proportion of African ancestry in North American and Australian populations by modelling these populations as a linear combination of African and European ancestry (Table S1, Supporting information). Neutral coalescent simulations using *ms* found that this method accurately estimates ancestry proportions (Fig. S1, Supporting information). These simulations also revealed that a cline in ancestry proportion is unlikely under a single-colonization scenario, even with extensive drift (Fig. S2A–A", C–C", Supporting information), yet is stable and persistent under a dual-colonization scenario for long periods of time

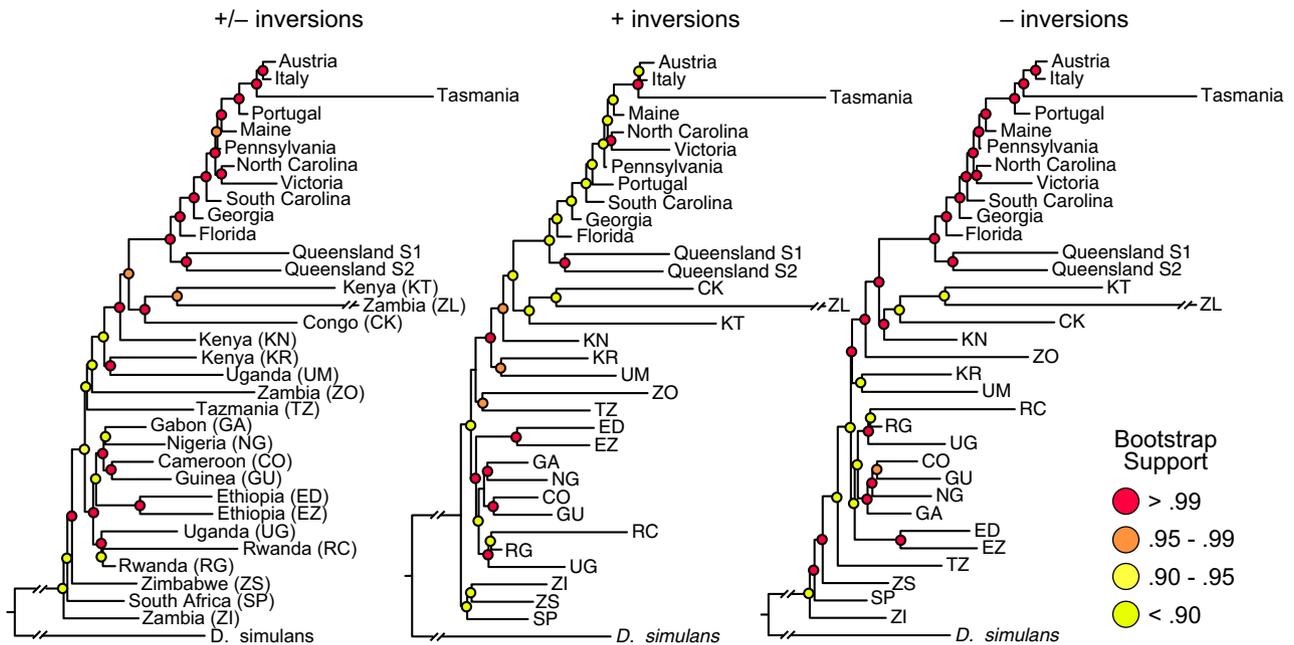


Fig. 2 Estimated population tree of sampled locales. Loci were sampled across all chromosomes, focusing on SNPs residing within (+), outside (-) large, cosmopolitan inversions or both (\pm). See Fig. S1 (Supporting information) for population trees based on each chromosome.

following initial colonization (Fig. S2B–B', D–D', Supporting information) regardless of migration patterns among newly derived populations.

In general, the proportion of African ancestry in North American and Australian populations is negatively correlated with latitude (Fig. 3). Conversely, the proportion of European ancestry is positively correlated with latitude (Fig. 3). Ancestry estimates for one mid-latitude North American population (Pennsylvania) do not significantly differ between samples of flies collected in the spring and fall (Fig. 3; Table S1, Supporting information), suggesting that large-scale migration of flies up the East Coast does not occur on a seasonal basis, consistent with previous findings (Bergland *et al.* 2014).

One notable exception to the general ancestry patterns occurs when using a single population from Zambia (population ZL from Pool *et al.* 2012) as a putative ancestral, source population. This population is notable in that it has an exceptionally long branch in our neighbour-joining analysis (Fig. 2), possibly indicating that it may be highly diverged from other African populations or that sparse sampling ($n = 1$) of this population resulted in imprecise allele frequency estimates.

Nonetheless, the general pattern of decreasing African ancestry with increasing latitude remains when using nearly any combination of African and European populations and for SNPs inside inversions, outside inversions or both sampled genome-wide or on any particular autosome. Average European and African

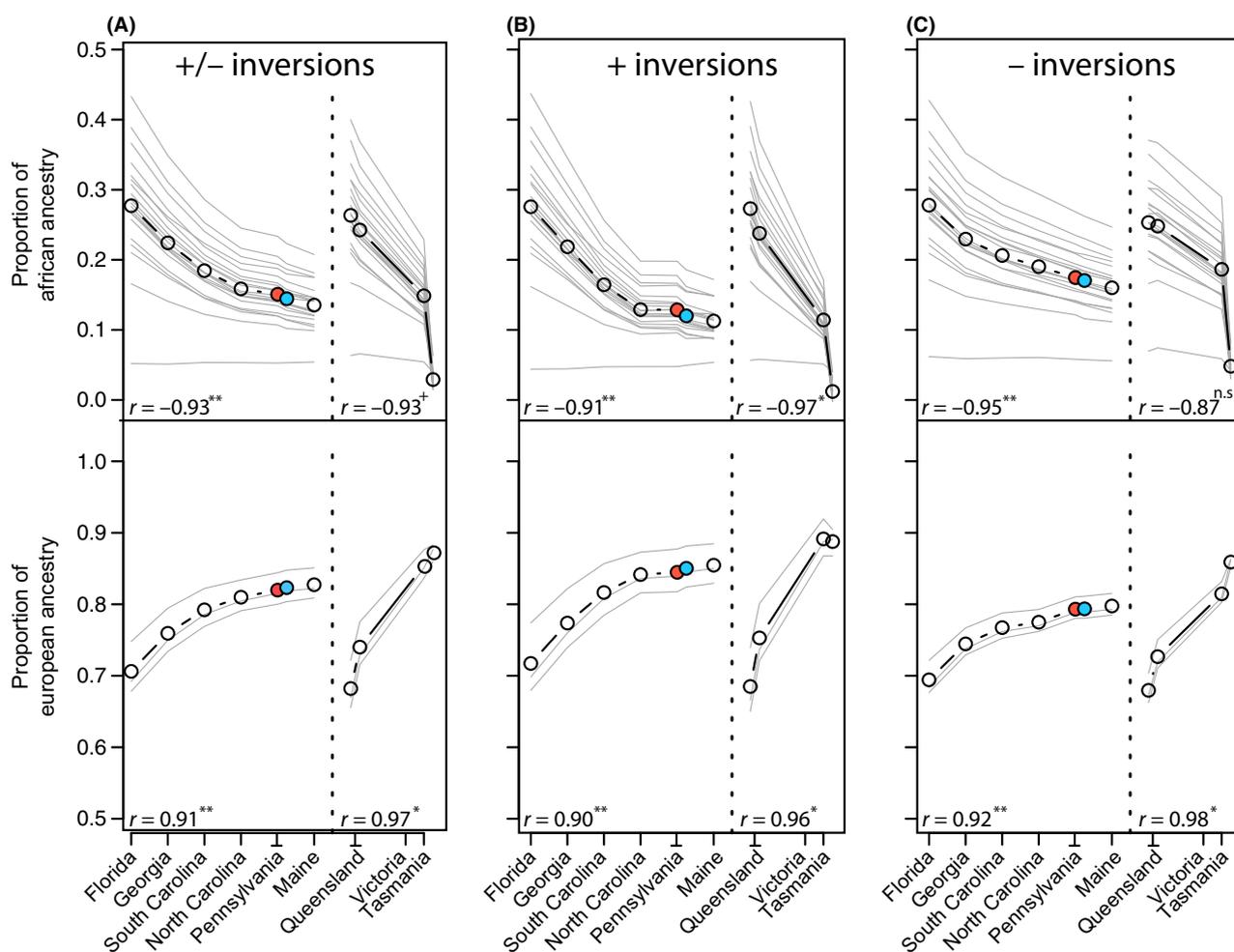


Fig. 3 Proportion of African and European ancestry in North American and Australian populations. Thin grey lines represent the ancestry estimate for any particular African or European population averaged over all European and African populations. Black line represents the average ancestry proportions from all African and European populations. The blue circle represents the spring Pennsylvania sample, and the red circle represents the fall Pennsylvanian sample. Correlations (r) between the average ancestry estimates (thick black line) and latitude are reported (P -value < 0.01 :**; P -value < 0.05 :*; P -value < 0.1 :⁺; P -value > 0.1 :^{n.s}). See Table S1 (Supporting information) for ancestry estimates for each pairwise combination of African and European population, and for SNPs on each chromosome, and with locations inside and/or outside inversions.

ancestry for each North American population is significantly correlated with latitude when assessed genome-wide (Fig. 3) and for each chromosome separately (Fig. S3, Supporting information), independent of inversion status. Average ancestry clines in Australia are similar in magnitude to North American clines and are significantly or marginally correlated with latitude for most chromosome/inversion comparisons (Fig. 3; Fig. S3, Supporting information). Intriguingly, ancestry clines along the X-chromosome are less pronounced for both North America and Australia, although they are still significantly correlated with latitude for North American populations. The attenuated ancestry cline along the X-chromosome is consistent with analyses presented in Kao *et al.* (2015) and Pool (2015) and is also consistent with reduced level of significantly clinal polymorphisms along the X in North America (Bergland *et al.* 2014; Reinhardt *et al.* 2014). The mechanisms generating an attenuated ancestry cline along the X-chromosome are unclear but may result from reduced variation along the X (Andolfatto 2001) or increased rate of adaptive substitution on this chromosome (Ávila *et al.* 2014) perhaps driven by an increased rate of Dobzhansky–Muller incompatibilities (Lachance & True 2010; Pool 2015). Finally, we note that our estimate of ancestry proportion of the North Carolina population is in agreement with estimates made using other methods and data sets (Duchen *et al.* 2013; Kao *et al.* 2015; Pool 2015).

We calculated f_3 and D statistics (Reich *et al.* 2009; Patterson *et al.* 2012) – commonly referred to as formal tests of admixture – for each North American and Australian population using each sampled European and African population as a putative source population. We observe significantly negative f_3 statistics (Fig. S4A, Table S2, Supporting information) for each North American population when using the Italian or Austrian (Fig. 4) populations as European source populations and various African populations as the alternate source population. Significant admixture from Portuguese population into North America was only observed in southern populations using the f_3 statistic (Fig. 4A; Table S2, Supporting information). f_3 statistics were not significantly negative for the Australian populations individual, and the large positive values could be interpreted as a consequence of drift following colonization of Australia (Patterson *et al.* 2012) or experimental differences between Australian samples and North American ones. For instance, allele frequency estimates of Australian samples were generated by sequencing multiple individuals from isofemale lines that had been maintained as laboratory stocks, whereas North American samples were generated by sequencing wild-caught male flies or sons of isofemale lines that had been maintained in the

laboratory for less than five generations. One consequence of this difference in experimental protocol is that the Australian isofemale lines may have undergone extensive drift in the laboratory, possibly contributing to positive f_3 statistics. In addition, Australian samples have lower read depth than North American samples. To assess the effect of read depth, we performed in silico pooling of the two northern Australian samples (Queensland S1 and S2) or the two southern Australian samples (Victoria and Tasmania). This produces negative f_3 statistics that are significantly lower than zero at nominal $P < 0.05$, but not significant after multiple test correction (Fig. 4A; Fig. S4, Supporting information). Downsampling of North American samples to average read depth in Australia did not substantially alter the observed f_3 statistics (results not shown).

D statistics for each North American and Australian population were significantly different from zero using various combinations of European and African populations as putative source populations (Fig. 4B; Fig. S4, Table S2, Supporting information). Similar to the f_3 analysis, the Portuguese population shows little evidence of admixture into populations sampled in northern North America. It is worth noting that evidence of admixture using the f_3 and D statistics does not conclusively demonstrate that the sampled donor populations (i.e. the European and African populations) are the actual donor populations. Rather, evidence of admixture using these statistics implies that either the sampled donor populations or unsampled yet closely related populations are likely the donor populations. f_3 and D analyses consistently resolved West African populations as the most likely source of the African component of North American and Australian flies. In addition, f_3 and D analyses failed to reject the null hypothesis of admixture when examining only the X-chromosome (Table S2, Supporting information).

Taken together, these results are consistent with the scenario in which both North America and Australia represent secondary contact zones between European and African lineages of *D. melanogaster*. Although others (David & Capy 1988) have proposed a secondary contact model for Australian *D. melanogaster* before, here we present the first statistical evidence supporting that model. Our results confirm an earlier model (David & Capy 1988), as well as phenotypic (Yukilevich & True 2008) and genetic (Caracristi & Schlötterer 2003; Kao *et al.* 2015) evidence, that low-latitude locales in North America are more genetically similar to African *D. melanogaster* than higher-latitude populations in North America are. One possible demographic model to explain this pattern is that European flies colonized northern North America, whereas southern North America was colonized directly by African flies, by

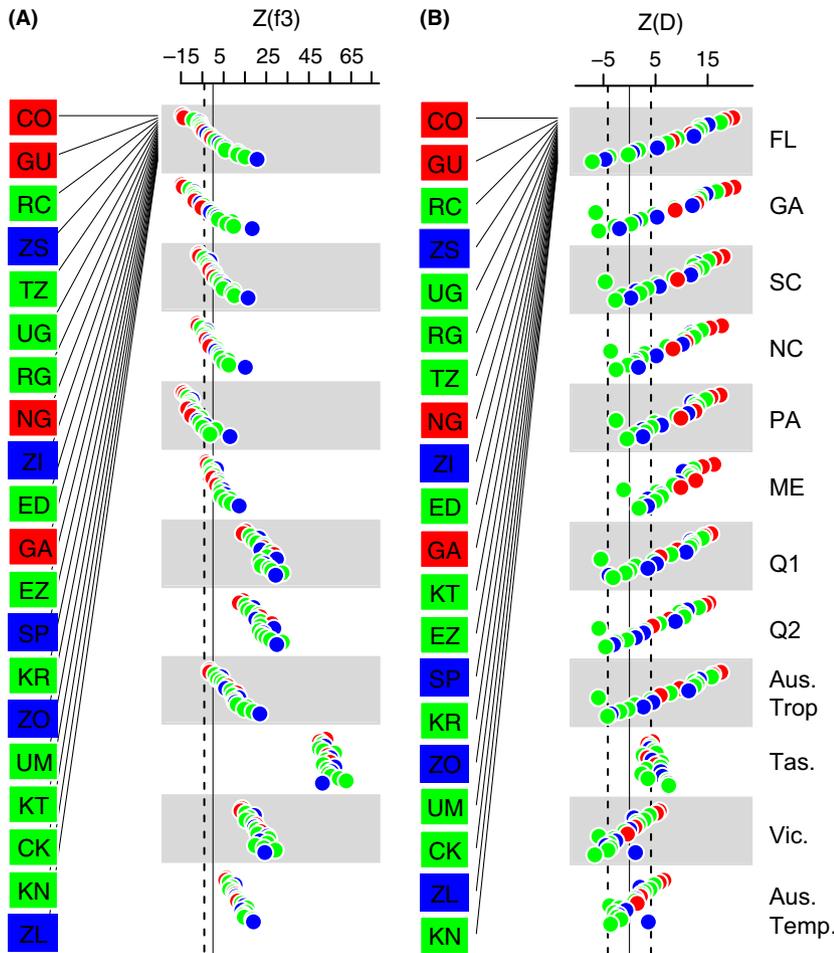


Fig. 4 Observed standardized Z score of (A) f_3 and (B) D statistics for each North American and Australian population when considering combinations of African and European populations and a putative donor population. For simplicity, only the Austrian population is shown as a putative European donor population. See Fig. S4 and Table S2 (Supporting information) for every pairwise combination of European and African populations. Dashed line represents the Bonferroni significance threshold at $\alpha < 0.05$. Values below the threshold in (A) are significantly different from 0. Values above or below the threshold in (B) are significantly different from 0. African populations are colour-coded based on location: West Africa (red); Central Africa (green); Southern Africa (blue).

Caribbean populations, which are themselves admixed between European and African lineages (Yukilevich & True 2008; Yukilevich *et al.* 2010; Kao *et al.* 2015), or both.

Under this secondary contact scenario, we would expect that a large fraction of the genome varies clinally. Indeed, among North American populations of *D. melanogaster*, approximately one-third of all common SNPs, on the order of 10^5 , are significantly clinal at false discovery rate $< 10\%$ (following the analysis in Bergland *et al.* 2014). However, these results do not preclude the existence of spatially varying selection that could also be acting among these populations which could explain the patterns of differentiation reported for some loci (e.g. Sezgin *et al.* 2004; Hoffmann & Weeks 2007; Paaby *et al.* 2010, 2014) and could slow the rate of homogenization of allele frequencies at neutral polymorphisms throughout the genome among clinally distributed populations. Unfortunately, similar analysis of the extent of clinality in Australia is not possible at the moment because we lack genome-wide allele frequency estimates from intermediate-latitude populations in that continent.

Genomic signals of local adaptation along latitudinal gradients

Our previous analysis supports the model that the demographic history of *D. melanogaster* has contributed to genome-wide patterns of differentiation among temperate and tropical populations of *D. melanogaster* living in North America and Australia. Regardless of this putative demographic history, multiple lines of evidence suggest that populations of flies living along broad latitudinal gradients have adapted to local environmental conditions that may be associated with aspects of temperate environments (see Discussion). Accordingly, we performed several tests to assess whether there is a strong, observable genomic signal of local adaptation.

First, we sought to identify F_{ST} outliers using the T_{F-LK} method that attempts to identify SNPs subject to spatially varying selection while maintaining a high power and low false-positive rate (Bonhomme *et al.* 2010; Mita *et al.* 2013; Lotterhos & Whitlock 2014). This method models the distribution of F_{ST} values after conditioning on the observed population tree among the

sampled populations. We identified several hundred significantly differentiated SNPs in North America (Fig. 5; Table S3, Supporting information), some of which are in or near genes that have been previously implicated in adaptation to spatially varying selection pressures (e.g. *Abd-B*, Fabian *et al.* 2012) or likely affect life history traits and correlates (e.g. *AlstR*, *TyR*, *DopR*, *sNPF*) via modulation of endocrine signalling (Bergland 2011). Intriguingly however, the amino acid polymorphism in *cpo* (3R: 13793588) previously implicated in clinal variation in diapause propensity (Schmidt *et al.* 2008) is significantly differentiated prior to, but not following, multiple testing correction in our F_{ST} outlier analysis ($T_{F-LK} = 16.2$, P -value = 0.006, q -value = 0.52). Similarly, the extensively studied threonine/lysine polymorphism (Kreitman 1983; Powell 1997) that encodes the fast and slow allozyme variants at alcohol dehydrogenase (*Adh*, 2L:14617051) is not significantly differentiated following multiple testing correction in our F_{ST} outlier analysis ($T_{F-LK} = 17.3$, P -value = 0.004, q -value = 0.45).

While a limited number of polymorphisms were identified as significantly differentiated among North American populations, no significantly differentiated SNPs were observed among the Australian populations after correcting for multiple testing (Fig. 5). Note that the genome-wide average F_{ST} among North American populations is lower than among Australian populations (0.025 vs. 0.08, respectively), suggesting that the lack of significantly elevated F_{ST} values in Australia is not due to a lack of population differentiation but rather a high genome-wide differentiation likely caused by recent secondary contact.

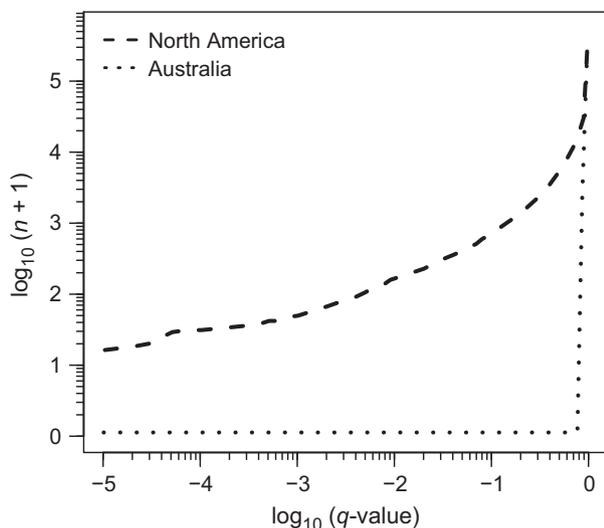


Fig. 5 Number of significantly differentiated SNPs in North America and Australia at various FDR (q -value) thresholds.

The exact number of SNPs with significantly elevated F_{ST} in any particular continent will be subject to a variety of considerations including the number of sampled populations, the precision of allele frequency estimates and the power of particular analytic methods to detect outlier F_{ST} . Some of these factors vary between our North American and Australian samples, and thus, our power to detect significant elevation of F_{ST} will vary between continents. Therefore, we investigated the general patterns of differentiation and parallelism between the sets of populations sampled in North America and Australia. In addition, we also examined the patterns of differentiation and parallelism between these continents and populations sampled from the Old World (i.e. Europe and Africa).

For these analyses, we first examined whether SNPs that were highly differentiated among one set of populations were also differentiated in another set (hereafter, 'codifferentiated'). To perform this analysis, we calculated the odds ratio (see Materials and methods) that SNPs fell above a particular quantile threshold of the F_{ST} distribution in any two sets of populations (Fig. 6A). We performed this analysis for SNPs that fell either within or outside the large cosmopolitan inversions. We find that SNPs that are highly differentiated in North America are also highly differentiated in Australia. In addition, we find that SNPs that are highly differentiated in either North America or Australia are also highly differentiated between Europe and Africa. Although patterns of codifferentiation are higher among SNPs within the large, cosmopolitan inversion than for SNPs outside the inversions, the qualitative patterns remain the same for either SNP class, suggesting that clinal variation in inversions per se does not drive the observed high levels of codifferentiation.

SNPs that are codifferentiated among temperate and tropical populations in North America, Australia or the Old World can be differentiated in a parallel way or at random among each geographical region. We show here that there is a high degree of parallelism at the SNP level, genome-wide, among polymorphisms that are highly differentiated in any two sets of populations (Fig. 6B). Patterns of parallelism at highly codifferentiated SNPs are similar among SNPs within or outside the large cosmopolitan inversions, again suggesting that clinal variation in inversions is not driving genome-wide patterns of parallelism.

High rates of codifferentiation and parallelism among temperate and tropical populations sampled throughout the world can be interpreted in two ways. On the one hand, these patterns could be taken as evidence of parallel adaptation to aspects of temperate environments. On the other hand, these patterns are consistent with the model presented above that North American and

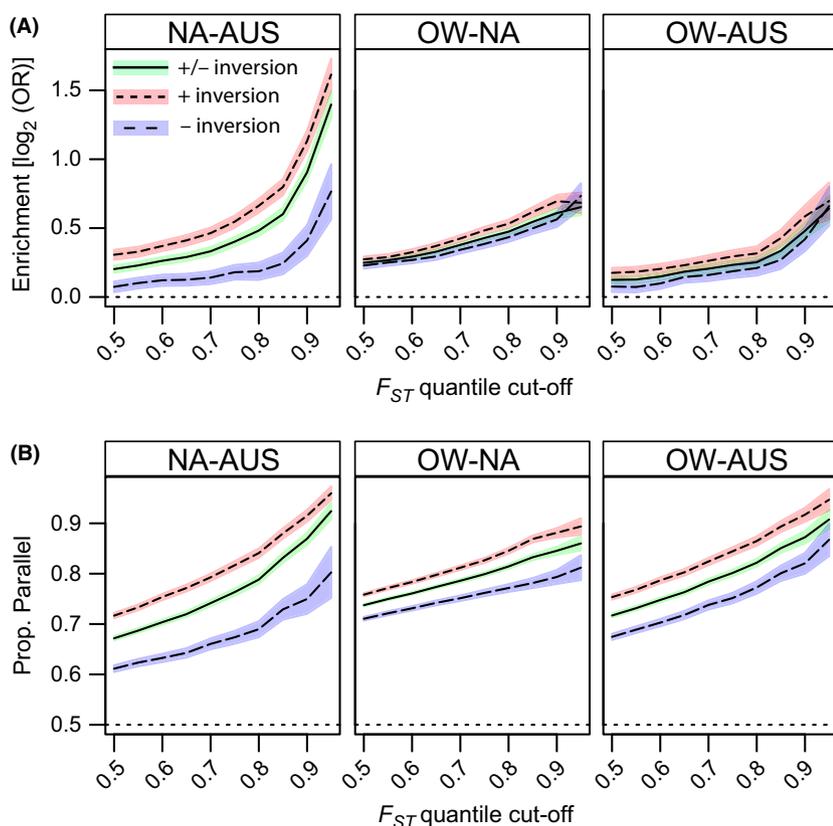


Fig. 6 Patterns of codifferentiation and parallelism between North American, Australian and the Old world populations. (A) \log_2 odds ratio that SNPs fall above the F_{ST} quantile cut-off in both sets of populations (NA: North America; AUS: Australia; OW: Old World). (B) Proportion of SNPs that vary in a parallel way given that they fall above the F_{ST} quantile cut-off in both sets of populations. Confidence bands represent 95% confidence intervals.

Australian populations are the result of recent secondary contact between European and African lineages of flies (see Results: Genomic signals of secondary contact). Most likely, parallelism at any particular polymorphism is due to a combination of similar or analogous demographic history and shared selection pressures between temperate and tropical locales in North America, Australia and Europe/Africa.

Discussion

Herein, we report results from a series of analyses that (i) examine whether populations of *D. melanogaster* sampled throughout North America and Australia show signatures of recent secondary contact between European and African lineages and (ii) examine whether there is a genomic signal of spatially varying selection acting along latitudinal gradients. We find that both North America and Australia show several signatures of secondary contact (Figs 2–4). Notably, high-latitude populations are closely related to European populations, whereas low-latitude populations are more closely related to African ones. This result implies that a large portion of clinal variation within these continents could, in principal, be generated by the dual colonization of both North America and Australia. Consistent

with this view, SNPs that are highly differentiated between temperate and tropical locales in either North America or Australia are also highly likely to be differentiated in a parallel way between Europe and Africa (Fig. 6). In addition, we report that genome-wide scans for significantly differentiated polymorphisms identified a limited number of outlier loci (Fig. 5). Taken together, our results support the model that recent secondary contact in North America and Australia has likely generated clinal variation at a large fraction of polymorphisms genome-wide and that spatially varying selection acting at a moderate number of loci acts to slow the rate of genomic homogenization between geographically separated populations.

Secondary contact and the generation of clinal variation in allele frequencies

Recent secondary contact between formerly (semi-)isolated populations is a potent force that can generate clinal variation genome-wide (Endler 1993). In *D. melanogaster*, high levels of genetic differentiation have been observed between temperate and tropical populations sampled in North America and Australia (Turner *et al.* 2008; Kolaczowski *et al.* 2011; Fabian *et al.* 2012; Reinhardt *et al.* 2014). In North America at least, most of

these highly differentiated SNPs vary clinally (i.e. in an approximately monotonic fashion along latitudinal gradients at false discovery rate <10%; Bergland *et al.* 2014). Moreover, surveys of allele frequencies along latitudinal clines in both North America and Australia at allozymes (Sezgin *et al.* 2004), SNPs (Sezgin *et al.* 2004; Bergland *et al.* 2014; Lavington *et al.* 2014), microsatellites (Gockel *et al.* 2001) and transposable elements (González *et al.* 2010) have repeatedly demonstrated that approximately one-third of all surveyed polymorphisms are significantly clinal in either continent. At face value, the high proportion of clinal polymorphisms throughout *D. melanogaster*'s genome suggests that demographic processes such as secondary contact have contributed to the generation of clinal variation in this species among recently colonized locales (Bock & Parsons 1981; Keller 2007).

We note that signatures of secondary contact are stronger for North American populations than for Australian populations. While historical records from North America (Keller 2007) and Australia (Bock & Parsons 1981) suggest a single point of colonization of *D. melanogaster*, results from morphological, behavioural and genetic studies reported here and elsewhere (Caracristi & Schlötterer 2003; Rouault *et al.* 2004; Duchon *et al.* 2013; Kao *et al.* 2015) suggest that a dual-colonization scenario is more likely. At least for the Americas, active trade between Europe and western Africa supports the model that North America represents a secondary contact zone. Given the extent of trade between North America, Europe and Africa, a simple model of a dual invasion and a single secondary contact event in North America is unlikely. A more plausible model is that North American populations represent a mixture of multiple European and African lineages.

Australia did not experience the same types of trade with the Old World, and throughout the 19th century, intercontinental travel to Australia was primarily restricted to British ships. However, British ships travelling to Australia ported in South Africa, in India and, after the opening of the Suez Canal, in East Africa (Bach 1976). This raises the possibility that secondary contact between European and African fruit fly lineages could have occurred immediately prior to the successful colonization of Australia by *D. melanogaster* in the mid-nineteenth century (Bock & Parsons 1981). Under this mixed-lineage, single-colonization scenario, rapid ecological sorting of colonizing lineages to temperate and tropical niches (Agosta & Klemens 2008) may have created a gradient where European flies were initially predominant at high latitudes and African flies predominant at low latitudes within Australia.

It is possible that the weaker signal of secondary contact in Australian populations is caused by a different

form of secondary contact than occurred in North America, unsampled source populations or both. Alternatively, given the lack of historical migration between Africa and Australia and the weaker signals of admixture in Australia, it is also possible that Australia does not represent a secondary contact zone for *D. melanogaster* and that genetic differentiation in this continent is driven by local adaptation and range expansion following colonization (Kennington *et al.* 2003). Conclusively determining the colonization history of Australia will thus require extensive worldwide sampling. Regardless, given that the observed ancestry clines in Australia (Fig. 3) are concordant with those in North America and that our simulations suggest that such ancestry clines are unlikely to occur from single colonization, it seems plausible that Australia does represent a secondary contact zone.

Although secondary contact is capable of generating patterns of clinal variation genome-wide, clines generated through this demographic process are transient. As admixed populations approach migration–selection equilibrium, clines at neutral loci should attenuate. Moreover, once at equilibrium, neutral differentiation should be minimal (Slatkin 1987) for species such as *D. melanogaster* where Nm has been estimated to be on the order of ~ 1 (Yamazaki *et al.* 1986; Singh & Rhomburg 1987) and long-distance dispersal is believed to be frequent (Coyne & Milstead 1987).

Thus, the critical question in determining whether the vast amount of clinal variation in North American and Australian flies has been generated by demography or selection is whether this species is at migration–selection equilibrium in these continents. There are several reasons why we suspect this species is not at equilibrium yet. First, *D. melanogaster* appeared in North America and Australia in the mid- to late nineteenth century (Bock & Parsons 1981; Keller 2007), or on the order of 1000 generations ago, assuming approximately 10 generations per year (Pool 2015). If Nm is on the order of 1, and population size within either continent is on the order of 10^6 or more (Karasov *et al.* 2010), it would take at least on the order of 10 000–100 000 generations for these populations to reach half way to equilibrium (Whitlock 1992; Whitlock & McCauley 1999). Thus, from a simple demographic perspective, it would seem unlikely that *D. melanogaster* has reached migration–selection–drift equilibrium.

Others have suggested that non-African populations of *D. melanogaster* are not at equilibrium. In general, non-African populations of *D. melanogaster* show a reduction in diversity coupled with an excess of rare variants (Mackay *et al.* 2012). This genome-wide pattern is consistent with a population bottleneck during colonization followed by population expansion. Others have

noted that non-African populations of *D. melanogaster* also have higher levels of linkage disequilibrium (LD) than expected under the standard neutral model (Andolfatto & Przeworski 2000; Haddrill *et al.* 2005; Langley *et al.* 2012), whereas LD in African populations is more consistent with demographic equilibrium (Andolfatto & Wall 2003 *cf.* Langley *et al.* 2012). Although genome-wide elevation of LD could be caused by various factors including pervasive positive or negative selection, admixture might possibly generate this signal.

Previous studies examining departure from equilibrium models in *D. melanogaster* have concluded that caution should be taken when conducting genome-wide scans for positive selection given the nonequilibrium nature of this species (Andolfatto & Przeworski 2000). Notably, demographic forces such as population bottlenecks can, in principal, mimic many of the signatures left by some types of adaptive evolution. A complementary approach to quantify the magnitude of adaptive evolution and to identify loci subject to selection is to identify polymorphisms that are differentiated between populations that are subject to divergent selection pressures. However, results presented here demonstrate that for *D. melanogaster* at least, signatures of adaptive evolution from genome-wide patterns of differentiation along latitudinal clines in newly derived populations in North America and Australia should likely be taken with a similar degree of caution as traditional scans for recent, positive selection.

Spatially varying selection and the maintenance of clinal variation in allele frequencies

Whereas secondary contact is capable of generating clinal variation, spatially varying selection is required for its long-term maintenance. There is little doubt that populations of *D. melanogaster* living along broad latitudinal clines in temperate environments have adapted to spatially varying selection pressures. Support for the idea of local adaptation along latitudinal clines comes from three main lines of evidence.

First, certain phenotypes show repeatable clines along latitudinal and altitudinal gradients that mirror deeper phylogenetic variation among temperate and tropical species. For instance, aspects of body size vary clinally in North America (Coyne & Beecham 1987) and Australia (Kennington *et al.* 2003) as well as along altitudinal/latitudinal clines in India (Bhan *et al.* 2014) and altitudinal clines within Africa (Pitchers *et al.* 2013; Klepsatel *et al.* 2014; Fabian *et al.* 2015). Given such patterns of parallelism within and among continents, including within the ancestral African range, the most plausible explanation is that parallel selection pressures

have generated these patterns of latitudinal and altitudinal variation. These intraspecific clines mimic interspecific patterns among temperate and tropical endemic drosophilids following Bergmann's rule (Blanckenhorn & Demont 2004; Shelomi 2012), again implicating that natural selection has shaped some of these patterns of genetically based, phenotypic variation.

Second, certain genetic and phenotypic clines in *D. melanogaster* have shifted over decadal scales. Shifts in these clines are consistent with adaptation to aspects of global climate change wherein alleles common in low-latitude populations have become more prevalent in high-latitude ones over the last 20 years (Hoffmann & Weeks 2007).

Finally, here we identify several hundred polymorphisms in North America that are significantly differentiated after accounting for demography (see Results, Fig. 5 and Table S3, Supporting information). Although the function of many of these polymorphisms is presently unknown, several are within the genes known to affect life history traits and correlates that vary among temperate and tropical populations (see Results). In addition, others have identified enrichment of clinal variants among putatively functional classes of polymorphisms (e.g. Machado *et al.* 2015).

The identification of significantly differentiated SNPs within North America can be taken as evidence of local adaptation to spatially varying selection pressures. Moreover, the observation that two SNPs (one in *cpo* and one in *Adh*) that each likely contribute to local adaptation fall in an upper, but not extreme, tail of the F_{ST} distribution suggests that there are many more ecologically relevant and functional polymorphisms that have contributed to local adaptation in *D. melanogaster* than can be identified reliably with any outlier F_{ST} approach. Most likely, the signal of high differentiation caused by spatially varying selection at these SNPs is masked by recent admixture that has contributed to a high level of differentiation genome-wide. In the light of these results, we suggest that scans for local adaptation based on patterns of genetic differentiation in *D. melanogaster* are an important first step in identifying adaptively differentiated clinal polymorphisms, but that additional evidence, such as functional validation (Schmidt *et al.* 2008; Paaby *et al.* 2010, 2014; Lee *et al.* 2011a; Sgró *et al.* 2013; Ullastres *et al.* 2015), should be gathered before concluding that differentiation is caused by adaptive processes.

Conclusions

It has long been recognized that genetic differentiation among populations can be caused by both adaptive and demographic (neutral) processes (Wright 1943). Due to

D. melanogaster's large effective population size (Karasov *et al.* 2010), high migration rate (Coyne & Milstead 1987) and rapid decay of linkage disequilibrium (Mackay *et al.* 2012), others have concluded that differentiation among populations sampled along latitudinal gradients is primarily caused by spatially varying selection (reviewed in Adrion *et al.* 2015). Work presented here supports the notion that spatially variable selection does contribute to some differentiation among populations.

However, several genome-wide signatures presented here (Figs 2–4) and elsewhere (Caracristi & Schlötterer 2003; Duchon *et al.* 2013; Kao *et al.* 2015; Bergland *et al.* 2014) indicate that populations of flies in North America and Australia likely result from admixture of European and African lineages. High-latitude (temperate) populations in North America and Australia appear to be more closely related to European populations, whereas low-latitude (tropical) populations are more closely related to African ones (Figs 2 and 3), suggesting that admixture occurred along a latitudinal gradient and that this demographic event generated clinal genetic variation at approximately 1/3 of all common SNPs (Bergland *et al.* 2014). These colonizing lineages of flies were likely already differentially adapted to the temperate and tropical conditions that they encountered in North America and Australia. Consequently, the recent demographic history of this species in North America and Australia is collinear with both local adaptation within these newly colonized continents and among the ancestral ranges. One practical consequence of the collinearity of demography and adaptation is that the identification of clinality or parallelism at any particular locus cannot be taken as definitive evidence of spatially varying selection.

The collinearity of demography and adaptation may be a general feature of a variety of species. For instance, the successful colonization of novel locales by invasive species such as *D. melanogaster* is often facilitated by multiple waves of invasion. In addition to increasing propagule pressure (Simberloff 2009), multiple independent invasions are also thought to facilitate invasion success by buffering the loss of genetic diversity that accompanies bottlenecks associated with colonization. Moreover, some evidence suggests that rapid adaptation following invasion (reviewed in Dormontt *et al.* 2011) often results directly from hybridization between independently invading subpopulations (reviewed in Lee 2002). If invasive species are widely distributed in their native range (Bates *et al.* 2013), this raises the possibility that successful invasions may often result from admixture of populations that are already differentially adapted to selection pressures that vary along broad spatial gradients in the introduced ranges.

The collinearity of demography and adaptation may also occur in temperate endemic species. This phenomenon may be particularly true in marine taxa as a result of admixture between high-latitude refugial populations and low-latitude populations following the last glacial maxima ~20 000 years ago (Bernatchez & Wilson 1998; Maggs *et al.* 2008). For these species, secondary contact between high- and low-latitude populations would result in clinal variation at neutral loci as well as at loci that contribute to adaptation along latitudinal gradients (e.g. Adams *et al.* 2006). In this scenario, neutral and adaptive genetic clines may be indistinguishable.

The ability to identify targets of spatially varying selection is now possible in many model and nonmodel species (Li *et al.* 2008; Savolainen *et al.* 2013). While these genome-wide approaches can be extremely powerful, great care must be taken to ensure that signals of local adaptation are uniquely identifiable and independent from pervasive (but often subtle) signals of demography. F_{ST} outlier approaches can in some cases enable the detection of loci that underlie local adaptation following complex and confounding demographic scenarios. However, as we show here, loci known to underlie functionally relevant phenotypic variation are not necessarily detected as statistically significant outliers, likely due to the pervasive signals of recent demographic events. To ameliorate these concerns, we suggest that proposed genetic targets of spatially varying selection be functionally verified or that patterns of spatial variation at loci known a priori to underlie fitness-related phenotypic variation be investigated. Alternatively, the genomic targets of local adaptation can be identified by examining population differentiation over small spatial scales (Richardson *et al.* 2014) or over short time periods (Bergland *et al.* 2014) that are likely to be orthogonal to the demographic history of the focal species.

Acknowledgements

We thank Joyce Kao, Heather Machado and Annalise Paaby for insightful comments on earlier versions of this manuscript. We thank Ary Hoffmann for graciously providing isofemale lines from Australia. Finally, we thank Richard Hudson for help with *ms* and Nick Patterson and Peter Ralph for assistance in interpreting *D* statistics. AOB was supported by an NIH National Service Research Award (F32 GM097837). RT's stay in Stanford was kindly supported by the Austrian Science Fund (FWF). JG is a Ramon y Cajal Fellow (RYC-2010-07306) supported by grants from the European Commission (Marie Curie CIG PCIG-GA-2011-293860) and from the Spanish Government (Fundamental Research Projects Grant BFU-2011-24397). This work was supported by NSF DEB 0921307 (awarded to PS) and NIH R01GM089926 (awarded to PS and DP).

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A.O.B. designed and performed the analysis and wrote the manuscript. R.T. performed analysis of admixture. J.G. constructed sequencing libraries for Australian samples. R.T., J.G., P.S. and D.P. edited the manuscript.

Data accessibility

Raw sequence data for newly resequenced Australian populations are available under NCBI Sequence Read Archive (SRA) Study Accession no. SRP064620. Raw sequence data from previously resequenced Australian populations are available under NCBI SRA Accession no. SRA012285.16. Raw sequence data for North American populations is available under NCBI SRA Accession no. PRJNA256231. Assembled sequence data for African populations were obtained from <http://www.dpgp.org>. Raw sequence data for Austrian and Italian populations are available from the European SRA under Accession no. ERP001827. Raw sequence data for the Portuguese population are available from the European SRA under Accession no. PRJEB4689. Allele frequency data for all populations in a flat 'csv' format, genetic distances between population and *ms* are available from DataDryad under accession doi:10.5061/dryad.7440s.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1. Verification that the linear model method of ancestry proportion estimation is accurate.

Fig. S2. Verification that the linear model method of ancestry proportion estimation is robust under various demographic scenarios.

Fig. S3. Ancestry estimates for each chromosome partitioned by inversion status.

Fig. S4. Observed standardized Z score of (A) f_3 and (B) D statistics for each North American and Australian population when considering every possible combination of African and European populations and a putative donor population.

Table S1. Ancestry estimates for each pairwise combination of populations.

Table S2. f_3 and D statistics for each pairwise combination of African and European populations.

Table S3. Annotated table of F_{ST} outlier SNPs at $q < 0.15$ in North America.

Text S1. Neighbour-joining trees for each chromosome in Newick format.