bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

1 # Allelic polymorphism at *foxo* contributes to local adaptation in
2 # *Drosophila melanogaster*

3

4

5 Nicolas J. Betancourt[1], Subhash Rajpurohit[1,2], Esra Durmaz[3,4], Daniel K. Fabian[5,6],
6 Martin Kapun[3,4], Thomas Flatt[3,4,*], and Paul Schmidt[1,*]

7

8 [1] Department of Biology, University of Pennsylvania, Philadelphia, USA

9

10 [2] Ahmedabad University, Division of Biological and Life Sciences, Ahmedabad, India

11

12 [3] Department of Ecology and Evolution, University of Lausanne, Lausanne,
13 Switzerland

14

15 [4] Department of Biology, University of Fribourg, Fribourg, Switzerland

16

17 [5] Department of Genetics, University of Cambridge, Cambridge, United Kingdom

18

19 [6] European Bioinformatics Institute (EMBL-EBI), Hinxton, United Kingdom

20

21 * Co-correspondence:
22 Paul Schmidt, Fax: +1 215 898 8780, E-mail: schmidtp@sas.upenn.edu
23 Thomas Flatt, Fax: +41 21 692 4165, E-mail: thomas.flatt@unil.ch

24

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

25
26 **Abstract:** The insulin insulin-like growth factor signaling pathway has been
27 hypothesized as a major determinant of life history profiles that vary adaptively in
28 natural populations. In *Drosophila melanogaster*, multiple components of this
29 pathway vary predictably with latitude; this includes *foxo*, a conserved gene that
30 regulates insulin signaling and has pleiotropic effects on a variety of fitness-
31 associated traits. We hypothesized that allelic variation at *foxo* underlies genetic
32 variance for traits that vary with latitude and reflect local adaptation. To evaluate this,
33 we generated recombinant outbred populations in which the focal *foxo* allele was
34 homozygous and fixed for either the allele common at high latitude or low latitude
35 and the genomic background was randomized across 20 inbred lines. After eight
36 generations of recombination, experimental populations were phenotyped for a
37 series of traits related to gene function. Our results demonstrate that natural allelic
38 variation at *foxo* has major and predictable effects on body size and starvation
39 tolerance, but not on development time. These patterns mirror those observed in
40 natural populations collected across the latitudinal gradient in the eastern U.S.:
41 clines were observed for starvation tolerance and body size, but development time
42 exhibited no association with latitude. Furthermore, differences in size between *foxo*
43 genotypes were equivalent to those observed between populations sampled from the
44 latitudinal extremes, although contribution to the genetic variance for starvation
45 tolerance was less pronounced. These results suggest that allelic variation at *foxo* is
46 a major contributor to adaptive patterns of life history variation in natural populations
47 of this genetic model.
48 Keywords: *foxo*, cline, size, starvation tolerance, genetic architecture

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

49

50 ## 1. INTRODUCTION

51 Elucidating the molecular, mechanistic basis of adaptive differentiation for
52 complex traits in natural populations remains a fundamental goal in evolutionary
53 biology. Outlining the genotype-phenotype map and subsequent investigation into
54 mechanism requires, to some extent, identification of the genes and variants that
55 underlie genetic variance realized in the wild. However, fitness traits are often
56 complex, with a highly polygenic architecture (e.g., Arnegard et al. 2014; McCown et
57 al. 2014; Savolainen et al. 2013). The likelihood of effectively mapping complex traits
58 to causative polymorphism varies between two classical viewpoints regarding
59 architecture for quantitative traits: many loci of small effect vs. few loci of large effect
60 (Rockman 2012; Wellenreuther and Hansson 2016; Roff 2017; Boyle et al. 2017;
61 Barton et al. 2017). While these perspectives may be artificially polarized, many
62 empirical advances in understanding the mechanistic basis of adaptation in sexual,
63 outbred populations include both a clear identification of traits that drive local
64 adaptation as well as an apparently simple genetic architecture, with at least one
65 locus of major effect (e.g., Colosimo et al. 2005; Comeault et al. 2015; van't Hof et
66 al. 2016; Lamichhaney et al. 2016; Jones et al. 2018). In such examples, alleles
67 segregating at a locus of major effect can then be examined for functional
68 differences that affect performance and fitness (e.g., Laurie and Stam 1988;
69 Manceau et al. 2011; Cheviron et al. 2012; Chakraborty and Fry 2016). What is
70 unclear, however, is whether alleles at loci underlying local adaptation are typically of
71 large effect, or whether the effect size of such naturally occurring variants is
72 effectively negligible for any molecular or functional investigation (e.g., Lewontin
73 1974; Rockman 2012).

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

74

75 Body size is a trait commonly associated with fitness (Brown et al. 1993;
76 Blanckenhorn 2000; Bonnet et al. 2017). In a variety of taxa, size also varies
77 predictably across environmental gradients such as those associated with latitude
78 (James et al. 1995; Huey et al. 2000; Ashton 2002; Blanckenhorn and Demont 2004;
79 Stillwell et al. 2007); such clines suggest that body size is affected by spatially
80 varying selection and contributes to local adaptation (Partridge and Coyne 1997;
81 Stillwell 2010). While size-related traits are in general highly polygenic (e.g., Boyle et
82 al. 2017), major effect loci have also been observed (e.g., Sutter et al. 2007). In
83 particular, multiple components of the insulin insulin-like growth factor signaling
84 pathway (IIS) can regulate size (e.g., Colombani et al. 2005; Sutter et al. 2007); in
85 particular, the forkhead box-O transcription factor gene *foxo* is a major regulator of
86 IIS and impacts size as well as a variety of other traits associated with fitness
87 (Libina, Berman, & Kenyon, 2003; Kramer, Davidge, Lockyer, & Staveley, 2003;
88 Kramer, Slade, & Staveley, 2008; Hwangbo, Gersham, Tu, Palmer, & Tatar, 2004;
89 Fielenbach & Antebi, 2008; Mattila, Bremer, Ahonen, Kostiainen, & Puig, 2009).
90 Thus, the analysis of variation in body size offers an excellent system in which to
91 examine aspects of genetic architecture and the integration between allelic and
92 phenotypic patterns in natural populations.

93

94 In *Drosophila melanogaster*, body size increases with increasing latitude on
95 multiple continents (Coyne and Beecham 1987; James et al. 1995, 1997; Karan et
96 al. 1998). Such latitudinal patterns are mirrored by altitudinal clines where size
97 increases with increasing altitude (Fabian et al. 2015; Lack et al. 2016). These
98 parallel and replicated patterns suggest that patterns of size variation are adaptive

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

99 and directly associated with thermally mediated selection (reviewed in Stillwell 2010).
100 However, it remains unknown why small body size is associated with high fitness at
101 low latitudes and large size with high fitness at high latitudes. De Jong and
102 Bochdonavits (2003) hypothesized that adaptive patterns of size variation are driven
103 primarily by one or more components of the IIS pathway; the simple prediction is that
104 any causative variants would also exhibit pronounced and replicated allele frequency
105 clines. Analysis of PoolSeq data has shown that multiple IIS genes (e.g., *Pi3K*, *foxo*,
106 *InR*) are segregating for many alleles that vary predictably with latitude in *D.*
107 *melanogaster* (Kolaczkowski et al. 2011; Fabian et al. 2012; Bergland et al. 2014;
108 Kapun et al. 2016; Machado et al. 2018). The question is whether these clinal alleles
109 are distinct with respect to gene function and, at least in part, underlie the observed
110 patterns of local adaptation in size (Paaby et al. 2014).
111
112 Here, we examine the functional significance of naturally occurring allelic
113 variation at the forkhead box-O transcription factor gene *foxo* and evaluate whether
114 the effects of allelic variation are consistent with patterns of variation among natural
115 populations. We use a specific *foxo* allele, previously identified in PoolSeq data as
116 being strongly clinal in the eastern U.S. (Fabian et al. 2012), as a marker for
117 candidate alleles of functional significance. If size in *D. melanogaster* is highly
118 polygenic with no loci of major effect, then alleles segregating at *foxo* should be
119 effectively and functionally equivalent in laboratory- or field-based functional assays.
120 However, if *foxo* is a major locus for body size in this species, then effect size may
121 be of sufficient magnitude to be detectable. If allelic variation at *foxo* substantially
122 contributes to phenotypic variation and local adaptation, then the effects of *foxo*
123 alleles on phenotype should also be concordant with patterns observed in natural

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

124 populations. Such patterns should also be trait-specific and restricted to those
125 related to gene function.
126
127

128 ## 2. MATERIAL AND METHODS

129 ### 2.1 Identification of SNPs/alleles that vary predictably with latitude

130 Fabian et al. (2012) identified a series of SNPs in *foxo* that exhibited high F_{ST}
131 in pooled sequencing of natural populations derived from Florida (low latitude),
132 Pennsylvania (mid latitude), and Maine, U.S.A. (high latitude). From this analysis, we
133 identified a candidate *foxo* allele based on the nucleotide state at two SNPs
134 spanning approximately 2kb. Further details are provided in Durmaz et al. (2018).
135 To further examine clinal patterns associated with the focal low- and high-
136 latitude *foxo* haplotypes, we analyzed ten populations collected along the latitudinal
137 gradient of the U.S. east coast and sequenced as pools (Bergland et al. 2014; Kapun
138 et al. 2016; Machado et al. 2018). We restricted our analyses to high-confidence
139 SNPs that were polymorphic in the *Drosophila* Genetic Reference Panel (DGRP)
140 dataset (Mackay et al. 2012; Langley et al. 2012) and isolated a total of 1372 SNPs
141 located inside or within 2kbp up- and downstream of the annotated *foxo* gene. To
142 provide a null context for allele frequency differentiation, we isolated 20,000 SNPs
143 located in short introns (<60bp) and at least 100 kbp distance from the breakpoints
144 of common cosmopolitan inversions (Corbett-Detig et al. 2012) that are consistent
145 with patterns of evolutionary neutrality (Parsch et al. 2010; Clemente & Vogl 2012).
146 For each of these neutral and *foxo*-associated SNPs, we tested for significant
147 correlations between latitude and allele frequencies using generalized linear models
148 (GLM) with a binomial error structure of the form: $y_i = L + \varepsilon_i$, where y_i is the allele

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

149 frequency of the ith SNP, L is the continuous factor "Latitude" and ε_i is the binomial
150 error of the ith SNP. We further assessed whether allele frequency changes of the
151 two candidate SNPs that constitute our focal haplotype were more clinal than neutral
152 SNPs or other SNPs located within or in the proximity of *foxo*. To this end, we
153 compared the -$\log_{10}(p)$-values from the GLMs for each of the two focal SNPS to
154 distributions of -$\log_{10}(p)$-values from GLMs of either neutral SNPs or non-candidate
155 SNPs associated with *foxo.* We subsequently calculated empirical cumulative
156 distribution functions (ECDF; with total area =1) based on -$\log_{10}$(p)-values from all
157 neutral or non-candidate *foxo* SNPs in *R* (R Development Core Team 2009). To test
158 if the significance values associated with the two focal SNPs were greater than the
159 95 percentiles of each significance distribution, we integrated over the area under
160 each ECDF with values larger than the significance of each candidate SNP (see gray
161 areas limited by red dashed lines in Figure 1) and subtracted the integral value from
162 1, which represents the total area of the ECDF.

163 To investigate and visualize the relative patterns of allele frequency change
164 for the two focal SNPs with respect to all other SNPs located inside or in close
165 proximity to *foxo*, we conditioned the alleles with lower frequencies in Florida
166 compared to the population in Maine for each *foxo*-associated SNP. Allele
167 frequencies in Florida were set to zero and we then calculated the allele frequency
168 differences relative to Florida for all other populations.

169

170 **2.2 Constructing recombinant population cages**

171 Based on the combination of the results of Fabian et al. (2012) and the
172 DrosRTEC (*Drosophila* Real-Time Evolution Consortium) sequencing effort
173 (Machado et al. 2018), we identified individual lines in the DGRP (Mackay et. al

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

174 2012) that were homozygous for the candidate *foxo* allele that was at high frequency
175 in high-latitude populations (hereafter, the high-latitude allele), and lines that were
176 fixed for the *foxo* allele that was at high frequency in low-latitude samples (hereafter,
177 the low-latitude allele). Scripts for this filtering of the DGRP based on nucleotide
178 state and locus are provided in the raw data (Data Dryad submission XXXXX). Two
179 biological replicate population cages, denoted as sets A and B, were established
180 using 20 independent lines per cage per *foxo* allele; thus, each cage was
181 constructed with a completely distinct and independent set of inbred lines. Each
182 population cage was founded using 10 individuals of each sex, from age- and
183 density-controlled cohorts, from each of the 20 inbred DGRP founding lines.

184 Biological replicates were further split into two experimental replicates in the
185 first generation of founding and cultured independently thereafter. After
186 establishment, each population cage was cultured at a population size of ~2000
187 adults on standard cornmeal-molasses medium for 8 discrete generations of
188 outcrossing under conditions of constant temperature (25˚C) and photoperiod
189 (12L:12D) in Percival I36VL incubators. Thus, at the end of the experimental period,
190 we generated replicate population cages in which the focal *foxo* allele was
191 homozygous and fixed for either the high-latitude or low-latitude allele and the
192 genomic background was randomized across the 20 inbred lines used to found each
193 respective cage (Paaby et al. 2014; Behrman et al. 2018). To further test for
194 genome-wide patterns of genetic differentiation among the recombinant outbred
195 populations (ROP) fixed for either the high or low-latitude haplotypes, we calculated
196 SNP-wise F_{ST} based on the method of Weir and Cockerham (1984) for sets A and B
197 separately using custom software. After the eight generations of density-
198 standardized culture under standardized environmental conditions, we established

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

199 replicate density-controlled vial cultures (30 ± 10 eggs/vial) for subsequent
200 phenotyping of the high-latitude and low-latitude *foxo* alleles.
201
202 **2.3 Isofemale lines from natural populations**
203 Thirty isofemale lines were randomly selected from each of six previously
204 collected outbred populations along the east coast of the U.S. to serve as a
205 latitudinal comparison to the recombinant *foxo* populations (described in Rajpurohit
206 et al. 2017, 2018: Homestead, FL (HFL), Jacksonville, FL (JFL), Charlottesville, VA
207 (CVA), Media, PA (MPA), Lancaster, MA (LMA), and Bowdoin, ME (BME)). The
208 individual lines from each population were maintained on a standard 21d culture
209 regime under the same environmental conditions as the *foxo* recombinant cages.
210 Prior to phenotyping, each isofemale line was cultured for two generations at low
211 density (30±10 eggs/vial) at 25˚C, 12L:12D; in the third generation, freshly eclosed
212 flies were collected in daily cohorts and used in the phenotypic assays described
213 below.
214
215 **2.4 Phenotype assays**
216 In all assays, *foxo* recombinant cages were tested simultaneously at three
217 independent timepoints and the data partitioned into blocks. For assessment of
218 starvation resistance, virus infection of the cages precluded running three
219 independent blocks, and a single timepoint was included in the analysis. For the
220 natural populations, all lines from all populations were assayed simultaneously for all
221 phenotypes using discrete 1d cohorts for each phenotype.
222 *Starvation resistance.* For the *foxo* recombinant outbred populations, embryos
223 were collected from each cage in two replicate glass culture bottles and density was

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

224 standardized at 150±10 eggs per bottle. Isofemale lines from the natural populations
225 were transferred into replicate vials and density standardized at 30±10 embryos per
226 vial. All culture was done under standard conditions of 25˚C and a photoperiod of
227 12L:12D. Upon eclosion, mixed sex daily cohorts were collected over 3d and
228 subsequently aged to 5d. The flies were then separated by sex into replicate groups
229 of 10 and placed into glass vials equipped with a small cotton ball saturated with 1
230 mL of water. Samples were placed in an incubator at 25˚C, 12L:12D and mortality
231 was recorded at four timepoints per day (9AM, 1PM, 5PM, and 9PM) until all flies
232 had died. The *foxo* cages were assayed in three replicates per cage population; all
233 isofemale lines from each of the natural populations were also assayed.
234 *Development time.* For the *foxo* recombinant outbred populations, eggs were
235 collected from each cage over a 3h window using large Petri dishes containing
236 standard medium supplemented with live yeast. The collected eggs were then
237 counted and distributed in groups of 30 into three replicate vials per cage. For the
238 natural populations, embryos from all isofemale lines were similarly collected over 3h
239 in small collection receptacles. Embryos were counted and distributed to new
240 collection vials, with density also standardized at 30 embryos per vial. All
241 experimental material was subsequently cultured as previously described in Percival
242 I36VL incubators with constant and standardized humidity, temperature, and
243 photoperiod. All experimental vials were checked four times daily (9AM, 1PM, 5PM,
244 9PM); eclosion events and sex were recorded.
245 *Body size/morphology.* For both the *foxo* cage as well as the natural
246 populations, flies from the development assay were transferred to new vials, allowed
247 to mate and age for 5d post eclosion, then were preserved in 95% ethanol in
248 eppendorf tubes for subsequent size measurements. From these preserved

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

249 samples, 10 flies for each sex were randomly sampled and measured from each
250 *foxo* ROP cage and 5 flies for each sex were measured for each isofemale line from
251 the natural populations. Body size measurements (thorax length and wing area)
252 were recorded using a Leica MZ9.5 microscope mounted with an Olympus DP73
253 camera with CellSens standard measuring software. Thorax length was measured
254 as the longest length across the dorsal shield; wing area was defined as a polygon
255 using a standardized series of veinous landmarks. The ratio of total wing area to
256 thorax length, indicative of wing loading (Azevedo et al. 1998; Gilchrist et al. 2000),
257 was also calculated and subsequently analyzed.
258

259 **2.5 Statistical analysis**

260 For the natural populations, data were analyzed separately by sex. Isofemale
261 line was considered a random variable and all data were analyzed using a restricted
262 maximum likelihood ANOVA with population as a fixed effect. For all other traits
263 other than starvation tolerance, experimental block (N=3) was also included as a
264 covariate. For the *foxo* experimental population cages, a similar nested ANOVA was
265 run independently for both sexes in which *foxo* allele, biological replicate (set), and
266 experimental replicate (cage) were included as predictors.
267

268 **3. RESULTS**

269 **3.1 DrosRTEC Pooled Population Genome Sequencing**

270 By analyzing extensive genome-wide Pool-Seq data from ten populations
271 sampled along the U.S. east coast generated by the DrosRTEC consortium
272 (Bergland et al. 2014; Kapun et al. 2016; Machado et al. 2018), we show that
273 numerous SNPs associated with *foxo* exhibit steep latitudinal clines and extensive

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

274 differentiation as a function of geography (Figure 1). Notably and consistent with
275 previous observations by Fabian et al. (2012), the two focal *foxo* candidate SNPs
276 that are highlighted by black and red lines in Figures 1A and 1B, respectively, exhibit
277 strong allele frequency change between the populations at lowest and highest
278 latitudes. In fact, clinal patterns of the two candidate SNPs were more pronounced
279 than 95% of neutrally evolving SNPs located in short introns (>95.94% for
280 3R:9,892,517 and >98% for 3R:9,894,559, respectively; Figure 2 A, B) and 91% of
281 all non-candidate SNPs located within or close to *foxo* (>91.22% for 3R:9,892,517
282 and >96% for 3R:9,894,559, respectively; Figure 2 C, D).

283 In establishing the recombinant outbred populations (ROPs) using the DGRP
284 panel of inbred lines, the goal was to use candidate SNPs as markers of functional
285 effects for naturally occurring alleles or haplotypes at this locus (Paaby et al. 2014;
286 Berhman et al. 2018). The utility of this method is predicated on using a sufficient
287 number of independent inbred lines such that no other position in the genome, other
288 that the candidate site(s), is fixed or highly differentiated between experimental sets.
289 In Figure 3, F_{ST} between experimental cages (*foxo* allele AT vs. *foxo* allele GG) is
290 plotted as a function of chromosomal position for all SNPs segregating in the
291 biological replicate sets A (Figure 3A) and B (Figure 3B). While there are multiple
292 sites on each chromosome arm with F_{ST} > 0.4 between the sets of lines used to
293 construct the alternative *foxo* allelic cages, only the candidate sites are fixed
294 between the cages that comprise the allelic states.

295

296 **3.2 Clinal variation in natural populations**

297 *Body size.* In the six sampled natural populations, thorax length was highly
298 distinct among populations (Table 1), and these patterns of differentiation exhibited a

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

299 positive association with latitude (Figure 4C). Wing area exhibited qualitatively
300 identical patterns, as did the ratio of wing area to thorax length (Table 1, Figure 4D).
301 Sexes were analyzed separately due to dimorphism and the potential for differential
302 allometry, but exhibited highly similar patterns of size variation among populations
303 and as a function of latitude. As expected, these results are consistent with previous
304 associations between latitude and body size in *D. melanogaster* (Coyne and
305 Beecham 1987; James et al. 1995; de Jong and Bochdanovits 2003).
306
307 *Starvation resistance.* As with body size, starvation tolerance was highly
308 variable among isofemale lines within populations yet exhibited a robust association
309 with geography for both sexes (Table 1, Figure 3A). The increase in starvation
310 tolerance as a function of increasing latitude appears more pronounced for females
311 than males, although patterns are qualitatively identical and no heterogeneity of
312 slopes was detected (analysis not shown). The patterns of increasing tolerance with
313 increasing latitude is consistent with other aspects of stress tolerance in North
314 American populations (Schmidt and Paaby 2008), but opposite to what has been
315 observed on the Indian subcontinent (e.g., Karan et al. 1998). Starvation tolerance
316 does not appear to vary predictably with latitude in other assayed geographic regions
317 (Robinson et al. 2000; Hoffmann et al. 2001).
318
319 *Development time.* In contrast to the patterns observed for size and starvation
320 tolerance, development time did not vary predictably with latitude. For both males
321 and females, significant variation was observed among lines and among populations
322 but there was no association with latitudinal origin (Table 1, Figure 4B). We did,
323 however, observe distinct patterns of development time among the replicate

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

324 experimental blocks, despite controlling for density and culture conditions. This
325 suggests that the trait is affected by additional environmental variables,
326 measurement or other experimental error, or a combination of the two. It should be
327 noted, however, that in examination of the experimental blocks individually, no
328 significant association between development time and latitudinal origin of the
329 population was observed. This is in contrast to patterns of seasonal variation, which
330 demonstrate predictable change in development time as a function of time of
331 collection (Behrman et al. 2015). All raw data for the phenotyping of these natural
332 collections are available (Dryad Accession: XXXXXXX).
333

334 **3.3 Phenotypic differentiation between *foxo* alleles**

335 *Body size*. Thorax length and wing area were both highly distinct between the
336 high and low-latitude *foxo* genotypes (Table 2). Significant heterogeneity was
337 present between the replicate sets A and B, as expected based on distinct
338 composition of founding inbred lines, as well as among experimental replicates that
339 were cultured independently since cage initiation. Despite these two sources of cage
340 effects, the differences between *foxo* genotypes were consistent with expectations
341 based on geography: the genotypes homozygous for the high-latitude *foxo* allele
342 were significantly larger than genotypes homozygous for the low-latitude allele
343 (Figure 4). The ratio of wing area to thorax length did not show any further
344 differentiation between genotypes, but as with the two traits independently,
345 demonstrated significant and predictable differences between the high and low-
346 latitude *foxo* genotypes (Figure 4H). Furthermore, the observed differences between
347 *foxo* genotypes are strikingly similar in effect size to the trait differences observed

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

348 between the populations sampled from the latitudinal extremes (Figure 4 C,D vs.
349 Figure 4 G,H).
350
351 *Starvation resistance.* Similar to the observed differences in various metrics
352 for body size, starvation resistance was also distinct between the *foxo* genotypes
353 and varied predictably with geography (Table 2, Figure 4). For both males and
354 females, the genotype homozygous for the high-latitude *foxo* allele was associated
355 with increased starvation tolerance, which may be associated with effects of the
356 alleles on body size and lipid content (e.g., Chippindale et al. 1996). A significant
357 amount of variance in starvation tolerance was also associated with cage effects for
358 both sets of inbred lines and culture replicate (Table 2). The effect size associated
359 with *foxo* genotype was approximately 10%, again similar to what was observed for
360 associated differences in body size. However, unlike the patterns observed for size
361 related traits, the differences between *foxo* genotypes appear to explain a small
362 amount of the variance in this trait among natural populations across the sampled
363 geographic range in the eastern U.S. (Figure 4A, H). These distinct patterns suggest
364 that differences in starvation tolerance are not determined exclusively by differences
365 in size.
366
367 *Development time.* Development time varied significantly across sets of lines
368 and replicates; in contrast to the other traits measured, development time was not
369 distinct between the high- and low-latitude *foxo* alleles (Table 2, Figure 4). Overall
370 these results suggest that there is no functional significance associated with these
371 naturally occurring *foxo* alleles with respect to development time. This is somewhat
372 congruent with data on Australian clines, where the relationship between body size

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

373 and development time is inconsistent across latitude (e.g., James et al 1995, 1997).
374 Development time was the most variable of the traits studied here, both with respect
375 to experimental replication and variation among natural as well as reconstituted
376 outbred populations.
377

378 4. DISCUSSION

379 In *D. melanogaster*, there is abundant evidence for local adaptation. Natural
380 populations exhibit rapid and predictable responses to environmental parameters
381 that vary with season, both in terms of phenotypic (Schmidt and Conde 2006;
382 Behrman et al. 2015; Rajpurohit et al. 2017; Behrman et al. 2018a; Behrman et al.
383 2018b; Rajpurohit et al. 2018) and allele frequency (Cogni et al. 2014; Bergland et al.
384 2014; Behrman et al. 2018a; Machado et al. 2018) change. Similarly, many fitness-
385 associated traits have been shown to vary predictably with latitude, often in parallel
386 across independent gradients (e.g., Oakeshott et al. 1982; Paaby et al. 2010; Yang
387 and Edery 2018). Latitudinal allele frequency clines at candidate loci (e.g., Schmidt
388 et al. 2000; Betancourt et al. 2001; Sezgin et al. 2005; Paaby et al. 2010; Cogni et al.
389 2017) are now placed in a genomic context in which tens of thousands of SNPs are
390 known to be clinal (e.g., Kolackzowski et al. 2011; Fabian et al. 2012; Bergland et al.
391 2016; Kapun et al. 2016). While it is clear that some allele frequency clines may be
392 generated by demography (Kao et al. 2015; Bergland et al. 2016), it is also clear that
393 at least some of the observed clines may be generated by spatially varying selection
394 (Schmidt et al. 2008; Svetec et al. 2016). Two of the associated, major questions
395 are: 1) how many, or what proportion of, allele frequency clines reflect spatially
396 varying selection and thus local adaptation; and 2) how are allele frequency and
397 phenotypic clines integrated? Alleles exist in a genomic context, and complex traits

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

398 are similarly correlated as well as affected by epistasis (Mackay 2014); it is
399 extremely unlikely that all allele and phenotypic clines are independent and reflect
400 selection on single variants or traits. However, the effect size of individual "adaptive
401 polymorphisms" and the architecture of local adaptation are, arguably, not well
402 resolved. There is a paucity of detailed, mechanistic and comprehensive
403 investigations as to the functional significance of segregating polymorphisms in
404 natural populations. It is clearly infeasible, as well as misguided, to assess the
405 functional impact of all polymorphisms across the genome. However, we suggest
406 that multiple, intersecting methodologies (e.g., direct mapping, expression analyses,
407 mutant analysis, patterns of variation in natural populations) can be used to identify a
408 subset of variants that may be examined for functional significance. Ideally,
409 investigation of a sufficient number of outliers could generate an empirical
410 distribution of genic or allelic effect sizes for specific fitness-associated traits. Such
411 investigations are essential in resolving the genetic architecture and dynamics of
412 local adaptation.

413 The *foxo* alleles we examine here are an example of a robust candidate
414 suitable for functional analysis. Genetic manipulations of the *foxo* gene have
415 revealed pronounced effects on lifespan, multiple aspects of stress tolerance
416 including starvation resistance, and growth phenotype (Jünger et al., 2003; Puig,
417 Marr, Ruhf, & Tjian, 2003; Kramer, Davidge, Lockyer, & Staveley, 2003; Hwangbo,
418 Gersham, Tu, Palmer, & Tatar, 2004; Giannakou et al. 2004; Kramer, Slade,
419 Staveley, 2008; Slack, Giannakou, Foley, Goss, & Partridge, 2011). These traits vary
420 with latitude in *D. melanogaster* (Coyne & Beecham 1987; James et al. 1995;
421 Schmidt et al. 2005); thus, the *foxo* gene is a logical candidate for determining
422 variation for these traits in natural populations (de Jong and Bochdonavits 2003).

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

423 However, the effects of natural variation have not been previously addressed. While
424 loss of function mutants are highly pleiotropic and have pronounced effects on life
425 history phenotypes, and the IIS/TOR pathway is in general extremely pleiotropic,
426 polymorphisms segregating in natural populations need not affect variance for all
427 traits related to gene function (e.g., Stern 2011).

428 Our results demonstrate that allelic variation at *foxo* affects body size and
429 starvation tolerance. We further show that these measures of size, thorax length and
430 wing area, as well as starvation tolerance, vary predictably with latitude in natural
431 populations of *D. melanogaster*. Furthermore, the high-latitude *foxo* allele is
432 associated with larger size and greater starvation resistance, whereas flies
433 homozygous for the low-latitude *foxo* allele are smaller and less tolerant. Thus, the
434 allelic effects also parallel patterns of variation in natural populations. This is distinct
435 from the countergradient patterns that have been previously observed for allelic
436 variants at the *Drosophila insulin receptor* (*InR*) (Paaby et al. 2014). The effect size
437 of the *foxo* alleles is seemingly large, particularly for body size: the difference
438 between high and low-latitude *foxo* alleles is approximately the same as the
439 observed size difference between flies sampled from Florida and Maine. This
440 suggests that allelic variation at *foxo* is a major contributor to variance in body size in
441 these populations. It remains to be determined whether *foxo* is a major effect locus
442 for size in other taxa.

443 Durmaz et al. (2018) demonstrate that these focal *foxo* variants also have
444 significant effects on viability, fat catabolism, and FOXO activity, as indicated by
445 differences in transcript abundance of a FOXO target (*InR*). These investigations
446 were done after an additional four generations of recombination, and parallel
447 differences across studies were observed for different measures of body size: wing

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

448 area, thorax length, the ratio of wing area:length, and femur length were all greater in
449 the populations homozygous for the high-latitude allele than in the recombinant
450 populations homozygous for the low-latitude allele. Differences between the high and
451 low-latitude *foxo* variants were largely consistent across two assayed temperatures
452 and two diets, although genotype by environment interactions were observed for
453 both starvation tolerance and lipid metabolism (Durmaz et al. 2018). While our
454 results for *foxo* allelic effects are consistent with those of Durmaz et al. (2018) for
455 measures of size, patterns of starvation tolerance for the high and low-latitude alleles
456 are opposite: in our data, populations with the high-latitude *foxo* allele were more
457 starvation tolerant, whereas in Durmaz et al. (2018) the low-latitude allele was
458 associated with increased tolerance. The discrepancy may be due to methodological
459 disparity, as the two assays are distinct; our method is associated with faster
460 mortality and may involve a greater degree of desiccation stress than the agar
461 method. Desiccation tolerance does vary with latitude in North American populations
462 (Rajpurohit et al. 2018), although the effects of *foxo* on desiccation tolerance are
463 unknown. Alternatively, the one observed difference across the two studies may be
464 associated with laboratory-specific microbiota, which is known to vary across labs
465 and culture conditions (Staubach et al. 2012) and has pronounced effects on *D.*
466 *melanogaster* life histories (Walters et al., in review).

467 Despite the parallels we observed between the assayed *foxo* variants and the
468 patterns in natural populations, we cannot conclude that it is these two focal SNPs
469 (positions) that themselves cause the observed differences in starvation tolerance
470 and size, or directly contribute to variance for these traits in natural populations. The
471 linkage disequilibrium present in the founding inbred lines (DGRP) would decay to
472 some extent by the eight generations of outcrossing among founder lines, but

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

473 remains pronounced; thus, without further characterization these SNPs are
474 interpreted as markers for the functionally significant allelic variation segregating at
475 this locus. Gene editing or similar techniques, in which the focal sites are
476 manipulated in multiple common genetic backgrounds, would be essential in directly
477 examining causality. Such investigations are the focus of future work.
478

479 5. CONCLUSION

480 We find that the marker allele at *foxo* has predictable effects on body size and
481 starvation tolerance; no effects of *foxo* alleles on development time were observed.
482 We also show that both starvation tolerance and body size exhibit pronounced
483 latitudinal clines in six sampled natural populations, whereas development time
484 exhibited no association with latitude. The assayed alleles at *foxo* explain a small
485 amount of the variance among natural populations for starvation tolerance, but
486 appear to be a major factor in the determination of variance in size. Our results
487 suggest a distinct genetic architecture for correlated fitness traits, and that allelic
488 variation at the *foxo* locus underlies, in part, patterns of local adaptation in natural
489 populations of this genetic model.
490

491 **ACKNOWLEDGEMENTS**

492 We thank members of the Flatt and Schmidt labs for their gracious assistance in all
493 aspects of this work. Financial support was provided by the National Institutes of
494 Health (R01GM100366 to P.S.), the U.S. National Science Foundation
495 (DEB0921307 to P.S.), the Austrian Science Foundation (FWF P21498-B11 to T.F.),
496 and the Swiss National Science Foundation (PP00P3_133641 to T.F.).
497

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

498 **DATA ACCESSIBILITY**

499 The raw phenotypic data are available from Dryad at: XXXXX.

500

501 **AUTHOR CONTRIBUTIONS**

502 P.S. and T.F. conceived the project. D.F. and M.K. identified the *foxo* SNPs and
503 performed genomic analyses. P.S., S.R., E.D. and T.F. designed the experiments.
504 N.B. and S.R. established populations and performed the experiments. P.S., N.B.,
505 E.D., M.K., S.R. and T.F. analyzed the data. N.B., T.F. and P.S. wrote the paper with
506 input from the other authors.

507

508 **ORCID**

509 *Nicolas Betancourt* https://orcid.org./0000-0002-8768-9829
510 *Paul Schmidt* https://orcid.org/0000-0002-8076-6705
511 *Subhash Rajpurohit* https://orcid.org/0000-0001-9149-391X
512 *Thomas Flatt* https://orcid.org/0000-0002-5990-1503
513 *Esra Durmaz* https://orcid.org/0000-0002-4345-2264
514 *Martin Kapun* https://orcid.org/0000-0002-3810-0504

515

516 **COMPETING INTERESTS**

517 The authors of this manuscript have declared no competing interests.

518

519

520

521

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

522

523 **LITERATURE CITED**
524

525 Arnegard, M. E., McGee, M. D., Matthews, B., Marchinko, K. B., Conte, G. L., Kabir,
526 S., … Schluter, D. (2014). Genetics of ecological divergence during
527 speciation. *Nature*, *511*(7509), 307–311. http://doi.org/10.1038/nature13301
528 Ashton KG. (2002). Patterns of within-species body size variation of birds: strong
529 evidence for Bergmann's rule. Global Ecol. Biogeogr. 11:505–523.
530 Azevedo, R.B.R., James, A.C., McCabe, J., & Partridge, L. (1998). Latitudinal
531 variation of wing: thorax size ratio and wing aspect ratio in *Drosophila*
532 *melanogaster*. *Evolution*, 52, 1353-1362. https://doi.org/10.1111/j.1558-
533 5646.1998.tb02017.x.
534 Barton N.H., A.M. Etheridge, A. Veber. (2017). The infinitesimal model: Definition,
535 derivation, and implications. Theoretical Population Biology 118 (2017) 50–73.
536 BEHRMAN, E. L., WATSON, S. S., O'BRIEN, K. R., HESCHEL, M. S., & SCHMIDT,
537 P. S. (2015). Seasonal variation in life history traits in
538 two *Drosophila* species. *Journal of Evolutionary Biology*, *28*(9), 1691–1704.
539 http://doi.org/10.1111/jeb.12690
540 Behrman, E. L., Howick, V. M., Kapun, M., Staubach, F., Bergland, A. O., Petrov, D.
541 A., … Schmidt, P. S. (2018). Rapid seasonal evolution in innate immunity of wild
542 *Drosophila melanogaster. Proceedings of the Royal Society of London B*, 285,
543 20172599. https://doi.org/10.1098/rspb.2017.2599
544 Berhman, E.L., Kawecki, T.J., & Schmidt, P. (2018). Natural variation in couch potato
545 mediates rapid evolution of learning and reproduction in natural populations of
546 *Drosophila melanogaster*. doi: https://doi.org/10.1101/288696
547 Bergland, A. O., Behrman, E. L., O'Brien, K. R., Schmidt, P. S., & Petrov, D. A.
548 (2014). Genomic evidence of rapid and stable adaptive oscillations over seasonal
549 time scales in *Drosophila*. *PLoS Genetics*, 10, e1004775.
550 https://doi.org/10.1371/journal.pgen.1004775
551 Bergland, A. O., Tobler, R., González, J., Schmidt, P., & Petrov, D. (2016).
552 Secondary contact and local adaptation contribute to genome-wide patterns of
553 clinal variation *in Drosophila melanogaster. Molecular Ecology*, 25, 1157-1174.
554 https://doi.org/10.1111/mec.13455
555 Blanckenhorn W. U. 2000. The evolution of body size: What keeps organisms small?
556 Q. Rev. Biol. 75, 385–407.
557 Blanckenhorn, W.U. and M. Demont 2004. Bergmann and converse bergmann
558 latitudinal clines in arthropods: two ends of a continuum? *Integr Comp Biol.*
559 *44(6):413-24.*
560 Bonnet T, Wandeler P, Camenisch G, Postma E (2017) Bigger Is Fitter? Quantitative
561 Genetic Decomposition of Selection Reveals an Adaptive Evolutionary Decline of
562 Body Mass in a Wild Rodent Population. PLoS Biol 15(1): e1002592.
563 https://doi.org/10.1371/journal.pbio.1002592
564 Boyle EA, YI Li, JK Pritchard. (2017). An expanded view of complex traits: from
565 polygenic to omnigenic. Cell 169 (7), 1177-1186.
566 Brown JH, Marquet PA, Taper ML. (1993). Evolution of body size: consequences of
567 an energetic definition of fitness. Am Nat.142(4):573-84. doi: 10.1086/285558.
568 Chakraborty, M., and J. D. Fry. (2016). Evidence that environmental heterogeneity
569 maintains a detoxifying enzyme polymorphism in *Drosophila melanogaster*. Current
570 Biology 26:219-223.

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

571 Cheviron, Z. A., G. C. Bachman, A. D. Connaty, G. B. McClelland, and J. F. Storz.
572 (2012). Regulatory changes contribute to the adaptive enhancement of
573 thermogenic capacity in high-altitude deer mice. Proceedings of the National
574 Academy of Sciences USA, 109: 8635-8640.
575 Chippindale A. K., Chu T. J. F. and Rose M. R. 1996. Complex trade-offs and the
576 evolution of starvation resistance in *Drosophila melanogaster*. Evolution 50, 753–
577 766.
578 Clancy DJ, Gems D, Harshman LG, Oldham S, Stocker H, Hafen E, Leevers SJ,
579 Partridge L. 2001. Extension of life-span by loss of CHICO, a Drosophila insulin
580 receptor substrate protein. Science 2001; 292:41-3.
581 Clemente F, Vogl C (2012) Unconstrained evolution in short introns? – An analysis
582 of genome-wide polymorphism and divergence data from *Drosophila*. *Journal of*
583 *Evolutionary Biology*, **25**, 1975–1990.
584 Cogni R, Kuczynski C, Koury S, Lavington E, Behrman EL, O'Brien KR, Schmidt PS,
585 Eanes WF. (2014).The intensity of selection acting on the couch potato gene--
586 spatial-temporal variation in a diapause cline. Evolution 68(2):538-48. doi:
587 10.1111/evo.12291.
588 Cogni, R., Kuczynski, K., Koury, S., Lavington, E., Behrman, E. L., O'Brien, K. R., …
589 Eanes, W. F. (2017). On the Long-term Stability of Clines in Some Metabolic
590 Genes in *Drosophila melanogaster*. *Scientific Reports*, *7*, 42766.
591 http://doi.org/10.1038/srep42766
592 Colombani J, Bianchini L, Layalle S, Pondeville E, Dauphin-Villemant C,
593 Antoniewski C, Carré C, Noselli S, Léopold P. (2005). Antagonistic actions of
594 ecdysone and insulins determine final size in Drosophila. Science 310(5748):667-
595 70.
596 Colosimo PF, Hosemann KE, Balabhadra S, Villarreal G Jr, Dickson M, Grimwood
597 J, Schmutz J, Myers RM, Schluter D, Kingsley DM. (2005). Widespread parallel
598 evolution in sticklebacks by repeated fixation of Ectodysplasin alleles. Science.
599 307(5717):1928-33.
600 Corbett-Detig RB, Cardeno C, Langley CH (2012) Sequence-based detection and
601 breakpoint assembly of polymorphic inversions. *Genetics*, **192**, 131–137.
602 Comeault AA, Flaxman SM, Riesch R, Curran E, Soria-Carrasco V, Gompert Z,
603 Farkas TE, Muschick M, Parchman TL, Schwander T, Slate J, Nosil P. (2015).
604 Selection on a genetic polymorphism counteracts ecological speciation in a stick
605 insect. Curr Biol. 25(15):1975-81. doi: 10.1016/j.cub.2015.05.058.
606 Coyne J. A. and Beecham E. 1987. Heritability of two morphological characters
607 within and among natural populations of Drosophila melanogaster. Genetics 117:
608 727–737.
609 David J. R. and Capy P. 1988. Genetic variation of Drosophila melanogaster natural
610 populations. Trends Genet. 4, 106–111.
611 de Jong G. and Bochdanovits Z. 2003 Latitudinal clines in Drosophila melanogaster:
612 body size, allozyme frequencies, inversion frequencies, and the insulin-signalling
613 pathway. J. Genet. 82, 207–223
614 Djawdan M., Chippindale A. K., Rose M. R. and Bradley T. J. 1998. Metabolic
615 reserves and evolved stress resistance in Drosophila melanogaster. Physiol. Zool.
616 71, 584–594.
617 Durmaz, E., Rajpurohit, S., Betancourt, N., Fabian, D.K., Kapun, M., Schmidt, P., &
618 Flatt, T. (2018). A clinal polymorphism in the insulin signaling transcription factor
619 foxo contributes to life history adaptation in Drosophila. Molecular Ecology, in
620 review.

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

621 Fabian DK, Kapun M, Nolte V, Kofler R, Schmidt PS, Schlotterer C, and Flatt T.
622 2012. Genome-wide patterns of latitudinal differentiation among populations of
623 Drosophila melanogaster from North America. Molecular Ecology (2012). Blackwell
624 Publishing Ltd.
625 Fabian, D. K., Lack, J. B., Mathur, V., Schlötterer, C., Schmidt, P. S., Pool, J. E., …
626 Flatt, T. (2015). Spatially varying selection shapes life history clines among
627 populations of *Drosophila melanogaster* from sub-Saharan Africa. *Journal of*
628 *Evolutionary Biology*, 28, 826-840. https://doi.org/10.1111/jeb.12607
629 Fielenbach, N., & Antebi, A. (2008). *C. elegans* dauer formation and the molecular
630 basis of plasticity. *Genes & Development*, 22, 2149-2165.
631 https://doi.org/10.1101/gad.1701508
632 Garofalo R. S. 2002. Genetic analysis of insulin signaling in Drosophila. Trends
633 Endocrinol. Metab. 13, 156–162.
634 Giannakou ME, Goss M, Juenger MA, Hafen E, Leevers SJ, Partridge L. 2004.
635 Long-lived Drosophila with overexpressed dFOXO in adult fat body. Science 2004;
636 305:361.
637 Giannakou ME, Partridge L. 2007. Role of insulin-like signalling in Drosophila
638 lifespan. Trends in Biochemical Science, 32, 180–188.
639 Gilchrist, A.S., Azevedo, R.B.R., Partridge, L., & O'Higgins, P. (2000). Adaptation
640 and constraint in the evolution of *Drosophila melanogaster* wing shape. *Evolution &*
641 *Development*, 2, 114–124. https://doi.org/10.1046/j.1525-142x.2000.00041.x
642 Harshman L. G., Hoffmann A. A. and Clark A. G. 1999. Selection for starvation
643 resistance in Drosophila melanogaster: physiological correlates, enzyme activities
644 and multiple stress responses. J. Evol. Biol. 12, 370–379.
645 Hoffmann A. A., Hallas R., Sinclair C. and Mitrovski P. 2001. Levels of variation in
646 stress resistance in Drosophila among strains, local populations and geographic
647 regions: Patterns for desiccation, starvation, cold resistance, and associated traits.
648 Evolution 55, 1621–1630.
649 Huey, R.B., G.W. Gilchrist, M.L. Carson, D. Berrigan and L. Serra. (2000). Rapid
650 evolution of a geographic cline in size in an introduced fly. Science 287: 308-309.
651 Hwangbo, D. S., Gersham, B., Tu, M.-P., Palmer, M., & Tatar, M. (2004). *Drosophila*
652 dFOXO controls lifespan and regulates insulin signalling in brain and fat body.
653 *Nature*, 429, 562-566. https://doi.org/10.1038/nature02549
654 James A. C. and Partridge L. 1995. Thermal evolution of rate of larval development
655 in Drosophila melanogaster in laboratory and field populations. J. Evol. Biol. 8,
656 315–330.
657 James A. C., Azevedo R. B. R. and Partridge L. 1995. Cellular basis and
658 developmental timing in a size cline of Drosophila melanogaster. Genetics 140,
659 659–666.
660 James A. C., Azevedo R. B. R. and Partridge L. 1997. Genetic and environmental
661 responses to temperature of Drosophila melanogaster from a latitudinal cline.
662 Genetics 146, 881–890.
663 Jones MR, Mills LS, Alves PC, Callahan CM, Alves JM, Lafferty DJR, Jiggins FM,
664 Jensen JD, Melo-Ferreira J, Good JM. (2018). Adaptive introgression underlies
665 polymorphic seasonal camouflage in snowshoe hares. Science. 360(6395):1355-
666 1358. doi: 10.1126/science.aar5273.
667 Kao, J. Y., Zubair, A., Salomon, M. P., Nuzhdin, S. V., & Campo, D. (2015).
668 Population genomic analysis uncovers African and European admixture in
669 *Drosophila melanogaster* populations from the south-eastern United States and

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

670 Caribbean Islands. *Molecular Ecology*, 24, 1499-1509.
671 https://doi.org/10.1111/mec.13137
672 Kapun M, Fabian DK, Goudet J, Flatt T (2016) Genomic Evidence for Adaptive
673 Inversion Clines in *Drosophila melanogaster*. *Molecular Biology and Evolution*, **33**,
674 1317–1336.
675 Karan D., Dahiya N., Munjal A. K., Gibert P., Moreteau B., Parkash R. and David J.
676 R. 1998. Desiccation and starvation tolerance of adult Drosophila: Opposite
677 latitudinal clines in natural populations of three different species. Evolution 52,
678 825–831.
679 Kolaczkowski, B., Kern, A. D., Holloway, A. K., & Begun, D. J. (2011). Genomic
680 differentiation between temperate and tropical Australian populations of *Drosophila*
681 *melanogaster*. *Genetics*, 187, 245–260.
682 https://doi.org/10.1534/genetics.110.123059
683 Kramer, J. M., Davidge, J. T., Lockyer, J. M., & Staveley, B. E. (2003). Expression of
684 *Drosophila* FOXO regulates growth and can phenocopy starvation. *BMC*
685 *Developmental Biology*, 3, 5. https://dx.doi.org/10.1186%2F1471-213X-3-5
686 Kramer, J. M., Slade, J. D., & Staveley, B. E. (2008). *foxo* is required for resistance
687 to amino acid starvation in Drosophila. *Genome*, 51, 668-672.
688 https://doi.org/10.1139/G08-047
689 Lack JB, Monette MJ, Johanning EJ, Sprengelmeyer QD, Pool JE (2016)
690 Decanalization of wing development accompanied the evolution of large wings in
691 high altitude *Drosophila. Proc Natl Acad Sci USA* 113:1014-1019.
692 Langley CH, Stevens K, Cardeno C *et al.* (2012) Genomic variation in natural
693 populations of *Drosophila melanogaster*. *Genetics*, **192**, 533–598.
694 Laurie, CC and LF Stam. (1988). Quantitative analysis of RNA produced by Slow
695 and Fast alleles of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 85: 5161-
696 5165.
697 Lamichhaney S, Han F, Berglund J, Wang C, Almen MS, Webster MT, et al. (2016).
698 A beak size locus in Darwins finches facilitated character displacement during a
699 drought. Science. 352(6284):470±474. doi: 10.1126/science.aad8786 PMID:
700 27102486
701 Lewontin, R. C. (1974). *The Genetic Basis of Evolutionary Change.* New York, USA:
702 Columbia University Press.
703 Libina, N., Berman, J. R., & Kenyon, C. (2003). Tissue-specific activities of *C.*
704 *elegans* DAF-16 in the regulation of lifespan. *Cell*, 115, 489-502.
705 https://doi.org/10.1016/S0092-8674(03)00889-4
706 Machado, H. E., Bergland, A. O., Taylor, R., Tilk, S., Behrman, E. L., Dyer, K., …
707 Petrov, D. (2018). Broad geographic sampling reveals predictable and pervasive
708 seasonal adaptation in *Drosophila. bioRxiv* 337543. https://doi.org/10.1101/337543
709 Mackay, T. F. C. (2014). Epistasis and Quantitative Traits: Using Model Organisms
710 to Study Gene-Gene Interactions. *Nature Reviews. Genetics*, *15*(1), 22–33.
711 http://doi.org/10.1038/nrg3627
712 Mackay TF, Richards S, Stone EA, Barbadilla A, Ayroles JF, Zhu D, Casillas S, Han
713 Y, Magwire MM, Cridland JM, Richardson MF, Anholt RRH, Barrón M,
714 Bess C, Blankenburg KP, Carbone MA, Castellano D, Chaboub L, Duncan L,
715 Harris Z, Javaid M, Jayaseelan JC, Jhangiani SN, Jordan KW, Lara F. 2012. The
716 Drosophila melanogaster Genetic Reference Panel. Nature 482, 173–178.
717 Mattila, J., Bremer, A., Ahonen, L., Kostiainen, R., & Puig, O. (2009). *Drosophila*
718 FoxO Regulates Organism Size and Stress Resistance through an Adenylate

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

719 Cyclase. *Molecular and Cellular Biology*, 29, 5357-5365.
720 https://doi.org/10.1128/MCB.00302-09
721 Manceau M, Domingues VS, Mallarino R, Hoekstra HE. The developmental role of
722 Agouti in color pattern evolution. Science 2011;331:1062-5.
723 McKown AD, Klápště J, Guy RD, Geraldes A, Porth I, Hannemann J, Friedmann M,
724 Muchero W, Tuskan GA, Ehlting J, Cronk QC, El-Kassaby YA, Mansfield SD,
725 Douglas CJ. (2014). Genome-wide association implicates numerous genes
726 underlying ecological trait variation in natural populations of *Populus trichocarpa*.
727 New Phytol. 203(2):535-53. doi: 10.1111/nph.12815.
728 Noach E. J. K., de Jong G. and Scharloo W. 1996. Phenotypic plasticity in
729 morphological traits in two populations of Drosophila melanogaster. J. Evol. Biol. 9,
730 831–844.
731 Oakeshott JG, Gibson JB, Anderson PR, Knibb WR, Anderson DG, Chambers GK.
732 (1982). Alcohol dehydrogenase and glycerol-3-phosphate dehydrogenase clines in
733 Drosophila melanogaster on different continents. Evolution 36(1):86-96.
734 doi:10.1111/j.1558-5646.1982.tb05013.x.
735 Oldham S. and Hafen E. 2003. Insulin/IGF and target of rapa- mycin signaling: a
736 TOR de force in growth control. Trends Cell Biol. 13, 79–85.
737 Paaby AB, Schmidt PS. 2008. Functional significance of allelic variation at
738 methuselah, an aging gene in Drosophila. PLoS ONE, 3, e1987.
739 Paaby, A. B., & Schmidt, P. S. (2009). Dissecting the genetics of longevity in
740 *Drosophila melanogaster. Fly*, 3, 1-10. https://doi.org/10.4161/fly.3.1.7771
741 Paaby, A. B., Bergland, A. O., Behrman, E. L., & Schmidt, P. S. (2014). A highly
742 pleiotropic amino acid polymorphism in the *Drosophila* insulin receptor contributes
743 to life-history adaptation. *Evolution*, 68, 3395–3409.
744 https://doi.org/10.1111/evo.12546
745 Paaby, A. B., Blacket, M. J., Hoffmann, A. A., & Schmidt, P. S. (2010). Identification
746 of a candidate adaptive polymorphism for *Drosophila* life history by parallel
747 independent clines on two continents. *Molecular Ecology*, 19, 760-774.
748 https://doi.org/10.1111/j.1365-294X.2009.04508.x
749 Partridge L, Coyne JA. (1997). Bergmann's rule in ectotherms: is it adaptive?
750 Evolution. 51:632–635.
751 Parsch J, Novozhilov S, Saminadin-Peter SS, Wong KM, Andolfatto P (2010) On the
752 utility of short intron sequences as a reference for the detection of positive and
753 negative selection in *Drosophila*. *Molecular Biology and Evolution*, **27**, 1226–1234.
754 R Development Core Team (2009) R: A Language and Environment for Statistical
755 Computing. *R-project.org*.
756 Rajpurohit, S., Hanus, R., Vrkoslav, V., Behrman, E. L., Bergland, A. O., Petrov, D.,
757 … Schmidt, P. S. (2017). Adaptive dynamics of cuticular hydrocarbons
758 in *Drosophila*. *Journal of Evolutionary Biology*, *30*(1), 66–80.
759 http://doi.org/10.1111/jeb.12988
760 Rajpurohit S, Gefen E, Bergland AO, Petrov DA, Gibbs AG, Schmidt PS. (2018).
761 Spatiotemporal dynamics and genome-wide association analysis of desiccation
762 tolerance in *Drosophila melanogaster.* Mol Ecol. 27(17):3525-3540. doi:
763 10.1111/mec.14814.
764 Reinhardt, J. A., Kolaczkowski, B., Jones, C. D., Begun, D. J., & Kern, A. D. (2014).
765 Parallel Geographic Variation in *Drosophila melanogaster*. *Genetics*, 197, 361-373.
766 https://doi.org/10.1534/genetics.114.161463
767 Robinson S. J. W., Zwaan B. and Partridge L. 2000. Starvation resistance and adult
768 body composition in a latitudinal cline of Drosophila melanogaster. Evolution 54,

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

769 1819–1824.
770 Robinson S. J. W. and Partridge L. 2001. Temperature and cli- nal variation in larval
771 growth efficiency in Drosophila melanogaster. J. Evol. Biol. 14, 14–21.
772 Rockman, M. V. (2012). The QTN program and the alleles that matter for evolution:
773 all that's gold does not glitter. *Evolution*, 66, 1-17. https://doi.org/10.1111/j.1558-
774 5646.2011.01486.x
775 Roff DA (2007). A centennial celebration for quantitative genetics. Evolution.
776 61(5):1017±1032. doi: 10.1111/j.1558-5646.2007.00100.x PMID: 17492957
777 Saucedo L. J. and Edgar B. A. 2002. Why size matters: altering cell size. Curr. Opin.
778 Genet. Dev. 12, 565–571.
779 Savolainen O, Lascoux M, Merilä J. (2013). Ecological genomics of local adaptation.
780 Nat Rev Genet. 14(11):807-20. doi: 10.1038/nrg3522.
781 Schmidt, P. S., & Conde, D. R. (2006). Environmental Heterogeneity and the
782 Maintenance of Genetic Variation for Reproductive Diapause in *Drosophila*
783 *melanogaster*. *Evolution*, 60, 1602. https://doi.org/10.1111/j.0014-
784 3820.2006.tb00505.x
785 Schmidt, P. S., & Paaby, A. B. (2008). Reproductive Diapause and Life-History
786 Clines in North American Populations of *Drosophila melanogaster*. *Evolution*, 62,
787 1204-1215. https://doi.org/10.1111/j.1558-5646.2008.00351.x
788 Schmidt, P. S., Duvernell, D. D., & Eanes, W. F. (2000). Adaptive evolution of a
789 candidate gene for aging in *Drosophila*. *Proceedings of the National Academy of*
790 *Sciences*, 97, 10861–10865. https://dx.doi.org/10.1073%2Fpnas.190338897
791 Schmidt, P. S., Matzkin, L., Ippolito, M., & Eanes, W. F. (2005). Geographic Variation
792 in Diapause Incidence, Life-History Traits, and Climatic Adaptation in *Drosophila*
793 *melanogaster*. *Evolution*, 59, 1721-1732. https://doi.org/10.1111/j.0014-
794 3820.2005.tb01821.x
795 Schmidt, P. S., Paaby, A. B., & Heschel, M. S. (2005). Genetic variance for diapause
796 expression and associated life histories in *Drosophila melanogaster*. *Evolution*, 59,
797 2616-2625. https://doi.org/10.1111/j.0014-3820.2005.tb00974.x
798 Schmidt, P. S., Zhu, C-T., Das, J., Batavia, M., Yang, L., & Eanes, W. F. (2008). An
799 amino acid polymorphism in the *couch potato* gene forms the basis for climatic
800 adaptation in *Drosophila melanogaster*. *Proceedings of the National Academy of*
801 *Sciences USA*, 105, 16207-16211. https://doi.org/10.1073/pnas.0805485105
802 Sezgin, E., Duvernell, D. D., Matzkin, L. M., Duan, Y., Zhu, C.-T., Verrelli, B. C., &
803 Eanes, W. F. (2004). Single-Locus Latitudinal Clines and Their Relationship to
804 Temperate Adaptation in Metabolic Genes and Derived Alleles in *Drosophila*
805 *melanogaster*. *Genetics*, *168*(2), 923–931.
806 http://doi.org/10.1534/genetics.104.027649
807 Sim C, Kang DS, Kim S, Bai X, and Denlinger D. 2015. Identification of FOXO
808 targets that generate diverse features of the diapause phenotype in the mosquito
809 *Culex pipiens.* PNAS. March 24, 2015 | vol. 112 | no. 12 | 3811–3816
810 Staubach, F., Baines, J. F., Künzel, S., Bik, E. M., & Petrov, D. A. (2013). Host
811 Species and Environmental Effects on Bacterial Communities Associated
812 with *Drosophila* in the Laboratory and in the Natural Environment. *PLoS ONE*, *8*(8),
813 e70749. http://doi.org/10.1371/journal.pone.0070749
814 Stern, D. L., (2011). Evolution, development, & the predictable genome. Roberts &
815 Co. Publishers. https://doi.org/10.1086/663915
816 Stillwell, R.C. (2010). Oikos. 119(9): 1387–1390. doi: 10.1111/j.1600-
817 0706.2010.18670.x

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

818 Stillwell RC, Morse GE, Fox CW. (2007). Geographic variation in body size and
819 sexual size dimorphism of a seed-feeding beetle.*Am Nat. 170(3):358-69.*
820 Sutter, N. B., Bustamante, C. D., Chase, K., Gray, M. M., Zhao, K., Zhu, L., …
821 Ostrander, E. A. (2007). A Single *IGF1* Allele Is a Major Determinant of Small Size
822 in Dogs. *Science (New York, N.Y.)*, *316*(5821), 112–115.
823 http://doi.org/10.1126/science.1137045
824 Svetec, N., Cridland, J. M., Zhao, L., & Begun, D. J. (2016). The Adaptive
825 Significance of Natural Genetic Variation in the DNA Damage Response
826 of *Drosophila melanogaster*. *PLoS Genetics*, *12*(3), e1005869.
827 http://doi.org/10.1371/journal.pgen.1005869
828 Tatar M, Kopelman A, Epstein D, Tu M-P, Yin C-M, Garofalo RS. 2001. A mutant
829 Drosophila insulin receptor homolog that extends life-span and impairs
830 neuroendocrine function. Science 2001; 292:107-10
831 Tatar M, Yin C. (2011). Slow aging during insect reproductive diapause: Why butter-
832 flies, grasshoppers and flies are like worms. Exp Gerontol 36(4-6):723–738.
833 Umina, PA, AR Weeks, MR Kearney, SW McKechnie and AA Hoffmann. (2005). A
834 rapid shift in a classic clinal pattern in Drosophila reflecting climate change.
835 Science 308(5722): 691-693.
836 van't Hof, A.E., Campagne, P., Rigden, D.J., Yung, C.J., Lingley, J., Quail, M.A.,
837 Hall, N., Darby, A.C., and Saccheri, I.J. (2016). The industrial melanism mutation in
838 British peppered moths is a transposable element. Nature 534, 102–105.
839 Walters, AW, Matthews, MK, Hughes, RC, Malcolm, J, Rudman, SR, Newell, PD,
840 Douglas, A, Schmidt, P, & Chaston, JM. 2018. The microbiota influences the
841 Drosophila melanogaster life history strategy. PLoS Biology, in review.
842 Weir BS, Cockerham CC (1984) Estimating *F*-Statistics for the Analysis of
843 Population Structure. *Evolution*, **38**, 1358–1370.
844 Wellenreuther M, Hansson B. (2016). Detecting Polygenic Evolution: Problems,
845 Pitfalls, and Promises. Trends Genet. 32(3):1±10. doi: 10.1016/j.tig.2015.12.004
846 Yang, Y. and I. Edery. (2018). Parallel clinal variation in the mid-day siesta of
847 *Drosophila melanogaster* implicates continent-specific targets of natural selection.
848 PloS Genetics. https://doi.org/10.1371/journal.pgen.1007612
849 Zwaan B. J., Azevedo R. B. R., James A. C., van't Land J. and Partridge L. 2000.
850 Cellular basis of wing size variation in Drosophila melanogaster: a comparison of
851 latitudinal clines on two continents. Heredity 84, 338–347.
852
853
854
855
856
857
858

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Table 1. Analyses of variance for the assayed phenotypic traits among natural populations.

	Starvation Tolerance							
	Females				Males			
	DF	DFDen	F	*p*	DF	DFDen	F	*p*
Population	5	116.6	11.0359	***	5	114.1	5.6347	***

	Development Time							
	Females				Males			
	DF	DFDEn	F	*p*	DF	DFDen	F	*p*
Population	5	133.3	6.7468	***	5	132.1	6.4188	***
Block	2	649.8	69.6903	***	2	539.6	75.5155	***

	Wing Area							
	Females				Males			
	DF	DFDen	F	*p*	DF	DFDen	F	*p*
Population	5	136.8	14.15	***	5	136.4	11.537	***
Block	2	386.6	2.719		2	394.9	4.707	**

	Thorax Length							
	Females				Males			
	DF	DFDen	F	*p*	DF	DFDen	F	*p*
Population	5	135.4	4.712	***	5	140.9	3.2804	**
Block	2	363.6	0.0459		2	365.8	1.9564	

	Wing Area / Thorax Length							
	Females				Males			
	DF	DFDen	F	*p*	DF	DFDen	F	*p*
Population	5	135.6	13.914	***	5	137.7	11.45	***
Block	2	365.3	3.687	*	2	372.1	3.272	*

* $p<0.05$
** $p<0.01$
*** $p<0.0001$

859

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Table 2. Analyses of variation for the effects of the high and low latitude *foxo* alleles on phenotype.

Starvation

	Females				Males				
	DF	SS	F	*p*	Source	DF	SS	F	*p*
Allele	1	1128.838	8.381	**	Allele	1	1039.233	8.142	**
Set [Allele]	2	2535.857	9.414	***	Set [Allele]	2	1988.758	7.791	***
Cage [Allele, Set]	4	2492.854	4.627	**	Cage [Allele, Set]	4	1202.370	2.355	

Development Time

	Females				Males				
	DF	SS	F	*p*	Source	DF	SS	F	*p*
Allele	1	57.118	0.670		Allele	1	111.796	3.233	
Set [Allele]	2	416.404	2.443		Set [Allele]	2	307.582	4.447	*
Cage [Allele, Set]	4	2400.365	7.041	***	Cage [Allele, Set]	4	413.108	2.987	*

Wing Area

	Females				Males				
	DF	SS	F	*p*	Source	DF	SS	F	*p*
Allele	1	1.95E+11	57.7364	***	Allele	1	8.69E+10	41.351	***
Set [Allele]	2	1.12E+11	16.4958	***	Set [Allele]	2	4.30E+10	10.229	***
Cage [Allele, Set]	4	2.46E+11	18.1487	***	Cage [Allele, Set]	4	1.01E+11	11.997	***

Thorax Length

	Females				Males				
	DF	SS	F	*p*	Source	DF	SS	F	*p*
Allele	1	8185.111	8.167	**	Allele	1	5689.745	9.974	**
Set [Allele]	2	11580.028	5.777	**	Set [Allele]	2	5644.897	4.948	**
Cage [Allele, Set]	4	14914.627	3.721	**	Cage [Allele, Set]	4	9119.648	3.997	**

Wing Area / Thorax Length

	Females				Males				
	DF	SS	F	*p*	Source	DF	SS	F	*p*
Allele	1	97159.11	39.827	***	Allele	1	52392.037	34.190	***
Set [Allele]	2	38974.63	7.988	***	Set [Allele]	2	19500.916	6.363	**
Cage [Allele, Set]	4	152264.86	15.604	***	Cage [Allele, Set]	4	85498.41	13.949	***

860 * $p<0.05$ ** $p<0.01$ *** $p<0.0001$

861
862
863
864
865

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

866
Figure legends

867 **Figure 1.** Allele frequency changes for *foxo*-associated SNPs in 10 populations
868 sampled along the North American east coast. Both plots show allele frequency
869 differences conditioned to increase from south to north, with frequencies in Florida
870 being set to zero. Panel A shows allele frequency changes for all SNPs according to
871 their genomic position. Here, the *foxo* candidate SNPs are highlighted by two vertical
872 black lines (solid: 3R: 9,892,517; dashed: 3R: 9,894,449; *Drosophila melanogaster*
873 reference genome v6). Panel B shows how allele frequencies change with latitude.
874 The two *foxo* candidate SNPs are highlighted in red (solid: 3R: 9,892,517; dashed:
875 3R: 9,894,449).
876
877 **Figure 2**. Empirical cumulative density functions (ECDF; total area = 1) calculated
878 from the distribution of $-\log_{10}$(p-values) for generalized linear models that test for
879 associations between allele frequencies and latitude in 20,000 neutrally evolving
880 SNPs (Panels A, B) and 1,372 non-candidate SNPs located inside or within 2 kbp
881 distance to *foxo.* The vertical dashed lines indicate the significance values of the two
882 candidate SNPs 3R: 9,892,517 (Panels A, C) and 3R: 9,894,449 (Panels B, D). The
883 grey areas limited by the dashed line indicate the percentiles of neutral or non-
884 candidate *foxo* SNPs with significance values larger than the candidates.
885
886 **Figure 3.** F_{ST} Manhattan plots for the biological replicates A and B, constructed from
887 independent sets of inbred lines from the DGRP panel. All SNPs associated with
888 *foxo* are highlighted in red. These analyses show that only the two focal SNPs are
889 fixed for alternative alleles in the low- and high-latitude population cages of sets A
890 and B.

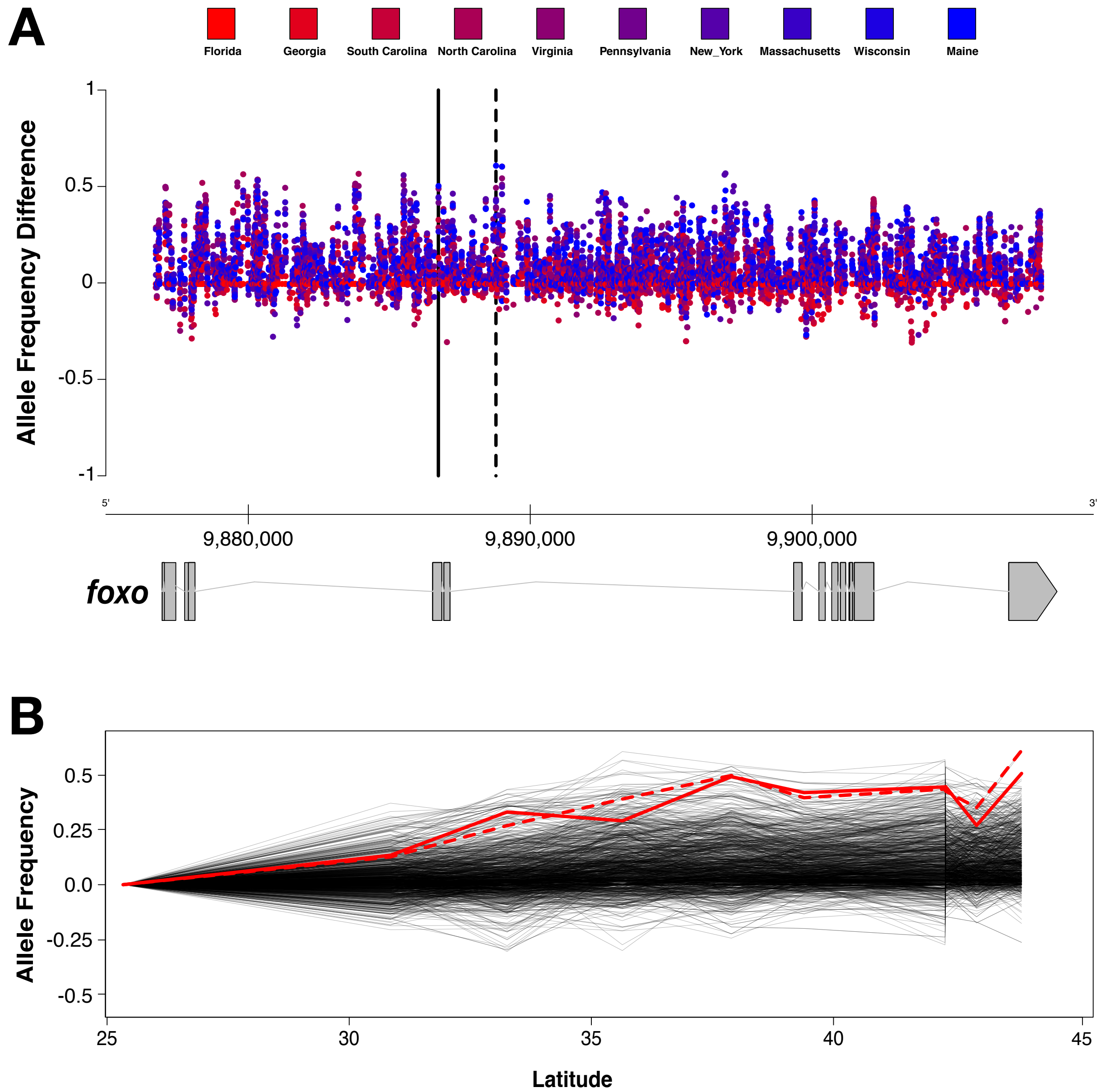
bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

891

892 **Figure 4**. Phenotypic analysis of natural populations collected across the latitudinal
893 gradient in the eastern U.S. (A-D) and the homozygous high- and low-latitude foxo
894 genotypes (E-H). In all panels, females are depicted by filled symbols and males by
895 open symbols. Starvation tolerance increases with increasing latitude (A); similarly,
896 the high-latitude *foxo* allele is associated with increased starvation resistance (E).
897 Development time does not vary predictably with latitude (B), and is also equivalent
898 between *foxo* alleles (F). Wing area (C) and the ratio of wing area to thorax length
899 (D) exhibit a positive latitudinal cline in the sampled populations; these patterns of
900 size variation in the natural populations are mirrored in both magnitude and direction
901 by the observed differences in size parameters between the low and high-latitude
902 foxo alleles (G, H).
903
904

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

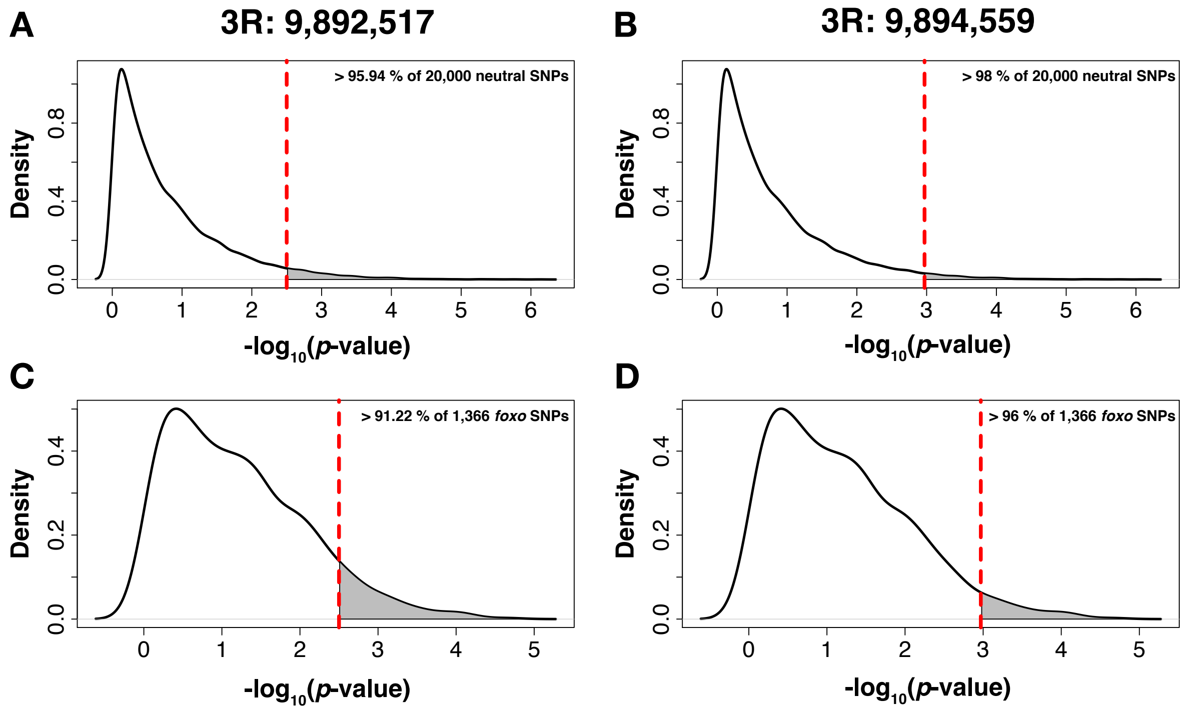
905
906 Figure 1.
907
908



909
910
911
912

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

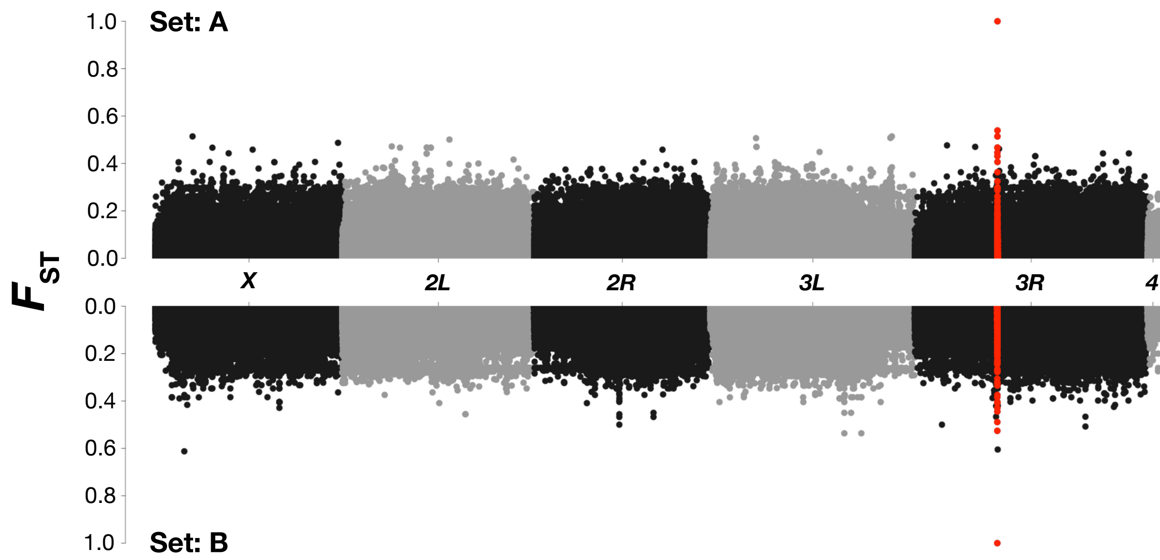
913
914 Figure 2.
915
916
917



918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

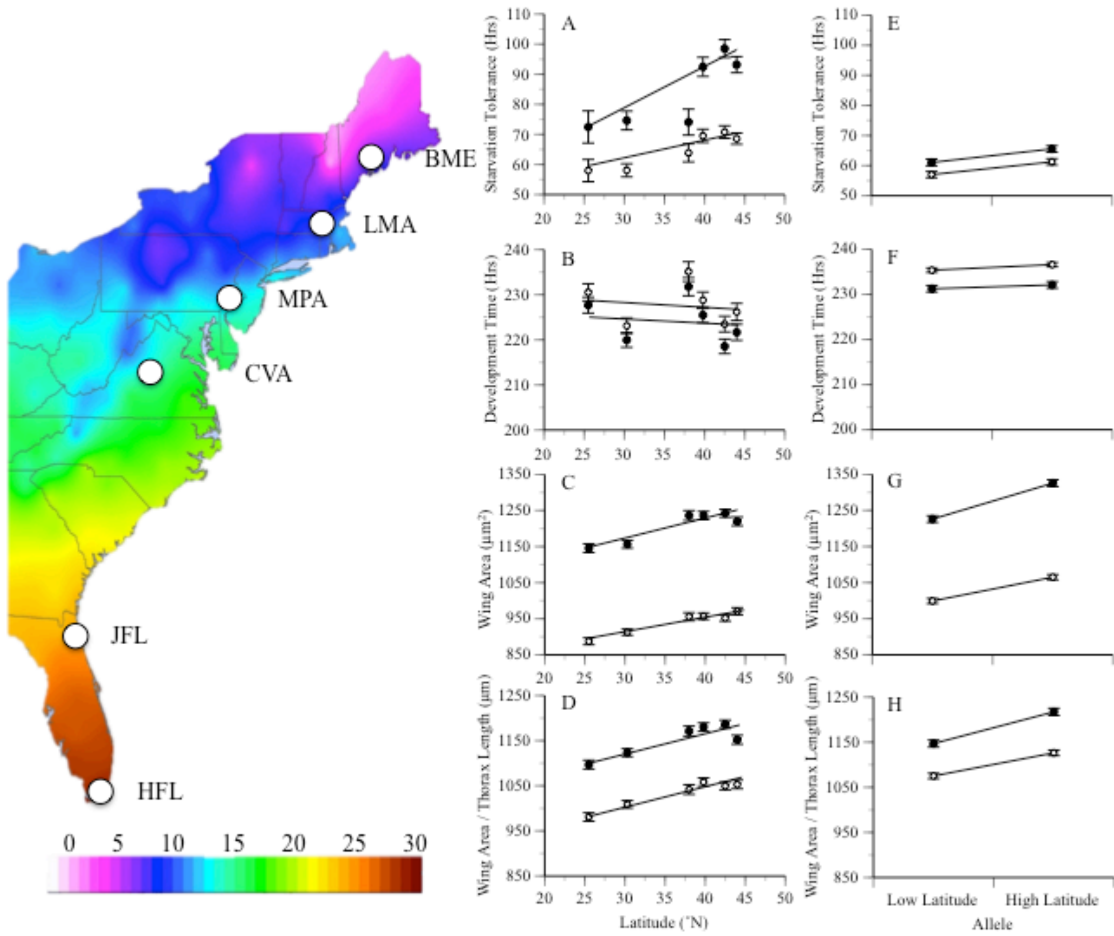
944
945 Figure 3.
946
947



948
949
950
951
952

bioRxiv preprint doi: https://doi.org/10.1101/471565; this version posted November 16, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

953
954
955 Figure 4.
956
957
958
959
960
961
962
963



964
965