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3
Limited genetic variation for male mating success reveals low evolutionary potential for thermal plasticity in *Drosophila melanogaster*

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28 **Abstract**

29 Populations respond to novel environmental challenges either through genetic changes,
30 through adaptive phenotypic plasticity for the traits in question, or by a combination of these
31 factors. Here, we investigated the evolutionary potential of phenotypic plasticity for male
32 mating success, locomotory ability, and heating rate (a physiological performance trait) in the
33 fruitfly *Drosophila melanogaster*, using isogenic male lines from the Drosophila Reference
34 Genome Panel (DGRP) and hemi-clonal males. We quantified thermal reaction norms of how
35 male mating success changed in relation to a temperate gradient, ranging from cold (18 °C)
36 via optimal (24 °C) to hot and stressful environments (either 30 °C or 36 °C). We found
37 significant differences in male mating success and locomotory performance between different
38 lines, as well as significant main effects of temperature, but no significant genotype-by-
39 environment interactions (GEI:s). A statistical power analysis revealed that the variance
40 explained by GEI:s for thermal plasticity using this sample size is likely to be modest or very
41 small, and represent only 4% of the total variation in male mating success. The lack of strong
42 GEI:s for these two behavioral traits contrast with the presence of significant GEI:s for male
43 heating rate, as measured by thermal imaging (infrared camera technology). These results
44 suggest that sexual selection through male mating success is not likely to be efficient in
45 mediating evolutionary rescue through changed plasticity in response to changing
46 temperatures.

47 **Keywords:**

48 additive genetic variation, fruit fly, infrared camera, sexual selection, insects, thermal
49 imaging, thermal performance, thermal plasticity

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54 **Introduction**

55 Populations can respond to environmental challenges either evolutionarily through changes in
56 allele frequencies or through adjustments in phenotypic plasticity (Schlichting and Pigliccui
57 1998). Phenotypic plasticity is the capacity of a single genotype to change its phenotype under
58 different environmental conditions (Bradshaw 1965; Roff 1997; Pigliucci 2001). Phenotypic
59 plasticity can increase a population's mean fitness across several environments, and plasticity
60 might increase niche space and geographical range (Ayrinhac et al. 2004; Manenti *et al*. 2015;
61 Mather and Schmidt 2017). Responding plastically to changing environmental conditions is,
62 however, a short-term survival strategy for a population, as there are costs and limits to
63 plasticity (Lande 2014; Murren et al. 2015; Sgro et al. 2016), which can limit the potential to
64 respond to sustained environmental change (Gonzalez et al. 2013).

65

66 Adaptive evolution of phenotypic plasticity requires not only that a population responds to
67 changing environmental conditions, but the different genotypes must also differ in how they
68 respond to these changing environmental conditions (Lande 2009; 2014; Chevin et al. 2010).
69 A population must thus harbor enough standing genetic variation in environmental reaction
70 norms to respond to sustained selection pressures driven by environmental change and
71 thereby evolve adaptive phenotypic plasticity (Schlichting and Pigliucci 1998; Gonzalez et al.
72 2013). The presence of genetic variation in phenotypic plasticity is recorded by the presence
73 of significant genotype-by-environment interactions (GEI:s)(Schlichting and Pigliucci 1998;
74 Lande 2009). If a population lacks such genetic variation in reaction norm slopes,
75 microevolutionary changes in plasticity will be prevented (Sisodia & Singh 2010; Husby *et al*.
76 2010).

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78 Hansen and Houle (2004) argued that the vast majority phenotypic traits have large amounts
79 additive genetic variation, even when such traits show evidence of long term stasis in the
80 fossil record or in extant populations. However, and in contrast to this view, there are some
81 documented empirical examples where genetic variation for physiological traits has been
82 demonstrated to be low enough to act as an evolutionary limit (Blows & Hoffmann 2005). If a
83 population is invariant in its evolutionary response to sustained environmental change, any
84 change outside of a critical range should rapidly lead to population decline and ultimately
85 extinction (Charmantier *et al*. 2008; Visser 2008; Chevin *et al*. 2010).

86

87 Thermal plasticity is a form of phenotypic plasticity that is particularly important for
88 ectotherms, which have limited ability to buffer themselves against external temperature
89 changes (Angilletta *et al*. 2002; Angiletta 2009). Many ectotherm species might already be
90 close to their upper physiological thermal limits (Addo-Bediako *et al*. 2000; Deutsch *et al*.
91 2008; Kellermann *et al*. 2012). In particular, small insects and other ectotherms may lack the
92 ability to buffer themselves against external temperatures altogether (Stevenson 1985). This
93 might be reflected as canalization (i.e. low genetic variance in thermal reaction norms)
94 resulting in low thermal plasticity (Angilletta *et al*. 2002; Charmantier *et al*. 2008). In general,
95 we know relatively little about the amount of genetic variation in thermal plasticity in natural
96 populations. Moreover, most previous studies on thermal adaptation and thermal plasticity in
97 insects and other ectotherms focus on how temperature affects survival and hence the
98 implications for natural selection. In contrast, the consequences of temperature challenges for
99 sexual selection (e.g. how mating rates and mating success is affected by temperature and
100 thermal plasticity) has seldom been a focus for empirical investigations (see Olsson et al.
101 2011 and Taylor et al. 2015 for exceptions). Only recently has temperature also been linked
102 to several aspects of sexual selection and speciation in ectotherms. Examples of such recent

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103 studies exploring the link between thermal adaptation and sexual selection include how
104 melanin-based dark colouration affects body temperatures within local populations and across
105 latitudinal clines (Punzalan et al. 2008; Svensson and Waller 2013), how different
106 microclimatic environments reduces immigrant male viability (Gosden et al. 2015), how
107 mating rates might be temperature-dependent (Olsson et al. 2011; Taylor et al. 2015) and a
108 recent finding that postzygotic isolation evolves more rapidly between various species of
109 *Drosophila* in hot tropical areas, compared to cooler temperate areas (Yukilevich 2013).

110

111 Isogenic *Drosophila melanogaster* lines offer an excellent opportunity to investigate genetic
112 variation in thermal reaction norms. The Drosophila Genetic Reference Panel (DGRP) is a
113 public resource consisting of more than 200 inbred lines derived from a population in Raleigh,
114 North Carolina (Mackay *et al*. 2012). Since the lines are isogenic, any phenotypic differences
115 between these lines can be attributed to genetic effects, provided that they are raised and kept
116 under identical conditions. Here we investigate and quantify the amount of genetic variation
117 in thermal reaction norms of 30 DGRP lines, using a hemiclonal experimental approach
118 (Abbott & Morrow 2011), and also by comparing these different DGRP-lines directly with
119 each other. These DGRP lines should be representative sample and a snapshot of naturally
120 segregating genetic variation in the local populations from which they were derived (Mackay
121 *et al*. 2012) and have also been previously used them in a study on sexual selection on wing
122 interference patterns (WIPs)(Katayama et al. 2014).

123

124 A necessary condition for the evolution of adaptive phenotypic plasticity in a novel thermal
125 environment is that the population harbors enough standing genetic variation in the trait of
126 interest (Chevin et al. 2010). A relatively low amount of genetic variation would indicate that

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127 the population is unlikely to respond evolutionarily to these novel thermal conditions. Here
128 we investigate how two behavioral traits important to male fitness – male locomotion and
129 mating success – are influenced by varying thermal conditions and whether these two fitness-
130 related traits show any evidence for genetic variation in plasticity. We used male locomotor
131 activity as a measure of performance as this trait is likely to be associated with fitness because
132 of its links with reproductive success, dispersal, predator avoidance, and foraging (Gilchrist,
133 1996; Roberts et al., 2003; Long & Rice, 2007; Latimer et al. 2011). We complemented these
134 analyses of behavioral performance traits with an analysis of a physiological trait – heating
135 rate – using the technique of thermal imaging ("infrared camera") on a subset of these DGRP-
136 lines. Heating rate is also likely to covary with physiological performance and mating success,
137 especially in ectotherms. Thermal imaging is a technique by which body temperatures of both
138 endotherms and ectotherms can be quantified, under laboratory, semi-natural, and natural
139 field conditions (Tattersall et al 2009; Tattersall and Cadena 2010; Symonds and Tattersall
140 2010; Svensson and Waller 2013).

141

142 Methods

143 *Drosophila melanogaster* sources

144 Isogenic lines used in this experiment were obtained from the Drosophila melanogaster
145 Genetic Reference Panel (DGRP) of the Bloomington Stock Centre (Mackay *et al*. 2012),
146 which were created after 20 generations of full sibling inbreeding of the stock inbred fly
147 populations (Mackay et al. 2012). Wild type (LHm) flies were originally obtained from
148 Edward H. Morrow (EHM), University of Sussex, Falmer, UK, and maintained in Lund since
149 2012 in the laboratory of J. Abbott. These LHm flies originated from 400 flies collected by L

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150 Harshman in central California in 1991 and they have been maintained since that time by L
151 Harshman (1991–1995), WR Rice (1995–2004) and EHM (2004–present) (Carter et al. 2009).
152

153 **Producing hemiclonal males from DGRP-lines**

154 A total of 16 male DGRP flies for each isogenic line were crossed with 16 wildtype virgin
155 LHm females (in a total of 992 vials). The outbred male offspring were then used for the
156 mating and locomotion assays. We refer to these outbred male flies as hemi-clones, following
157 previous terminology (Abbott and Morrow 2011). This outbreeding procedure was conducted
158 to reduce any inbreeding effects on mating behaviour that could potentially remain among the
159 DGRP-lines (Huang et al. 2012). Moreover, by comparing male mating success in the DGRP
160 background vs. mating success in a hemi-clonal background, we were also able to reduce the
161 effects of non-heritable genetic variation across the different genetic backgrounds. Additive
162 genetic variance in male mating success is expected to produce a significant correlation in
163 male mating success between the DGRP-lines and the corresponding male genotype in the
164 hemi-clonal background. Conversely, a weak correlation between male mating success in
165 these different genetic backgrounds would imply low additive genetic variance for this trait
166 and might also indicate a large non-additive effects, arising from e.g. epistatic genetic
167 variance (Meffert et al. 2002) or dominance variation (Merilä & Sheldon 1999). All flies were
168 cultured in a 20mm medium of cornmeal, yeast, and molasses and kept at 25°C in an
169 incubator, on a light-dark cycle of 12hrs:12hrs.

170 *DGRP-lines and hemiclonal experiments*

171 Two complementary groups of flies were used: 1) 32 pure isogenic DGRP lines, and 2) 30
172 hemi-clonal DGRP lines (Mackay et al. 2012). Throughout this paper, we refer to these
173 separate experimental fly categories as DGRP-lines and hemiclonal lines, respectively. For

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174 the 32 pure DGRP-lines, the mating assay was performed in three test temperatures: 18°C
175 (cold), 24°C (optimal) and 30°C (hot). We performed three replicates for each line and
176 temperature treatments for these pure DGRP-lines for a total of 265 vials.

177 In a first pilot study using hemiclonal males, we selected 10 DGRP lines for outbreeding to
178 produce 10 groups of hemiclonal males. These 10 DGRP lines were specifically selected for
179 outbreeding because they showed variable patterns in their mating rates to different
180 temperatures in the initial mating assay with the 32 pure DGRP-lines. In general, however, the
181 individual patterns we observed in the pure DGRP lines were only weakly related to the
182 patterns in the outbred lines (Fig. 4) (Huang et al. 2012). Hemi-clones were produced by
183 mating 992 vials of 16 DGRP males with 16 virgin females from the outbred LH_M population
184 (Chippindale et al. 2001). Male offspring from these crosses will therefore have one set of
185 DGRP autosomes and the Y in a random LH_M background. For three test temperatures, 18°C
186 (cold), 24°C (optimal) and 30°C (hot), we performed both mating and locomotion assays.

187

188 We followed up the first pilot hemiclonal study with a second study, where we used 30
189 isogenic lines (DGRP) to produce a new set of 30 hemiclonal groups of males. In this follow-
190 up study, we performed mating and locomotion assays at 18°C (cold), 24°C (optimal), 30°C
191 (hot) and we also added one additional temperature treatment at 36°C (extremely hot) (Trotta
192 *et al*. 2006). This temperature range is similar to what has been used in other studies (Latimer
193 et al. 2011). We performed six replicates for each line and temperature treatment in this assay,
194 although the total number of replicates will be slightly greater for those lines in which the
195 pilot study was also included. We pooled the results from first hemiclonal study with the
196 second to increase statistical power, while accounting for the effect of experimental sessions
197 as a block in our statistical analyses.

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198 **Mating assays**

199 Each line was anaesthetised with CO_2 gas. Seven males per vial were collected and placed in
200 separate 25 x 95 mm vials (with fly medium). Seven LHm virgin females were also collected
201 per vial, and placed in separate vials. Vials of males and females were kept at 25°C overnight,
202 to allow recovery from anaesthesia. For the experiment, vials containing hemiclonal males
203 (one vial per line), and vials of LHm females were placed in an incubator at the test
204 temperature and allowed to acclimatise for 30 minutes. After this time, the flies from one
205 female vial were transferred without anaesthesia ("flipped") into a male vial. Vials with males
206 and females were shaken lightly to avoid early mating while the other vials were being
207 combined. The vials, now with 14 flies in total, were placed back in the incubator at the test
208 temperature for 1 hour as the mating assay was conducted. One vial for each of our clonal
209 lines (hemi or pure) was placed in the incubator at a time. For each of the test temperatures,
210 the number of copulating pairs was used a measure of male mating success. The number of
211 mating males in each vial could thus vary from 0 to 7, and it was recorded every 10 minutes
212 (or 15 minutes for the pure lines) over a one hour period. Here, we are measuring a mating
213 rate, which also captures any variation in the latency to mate. All observations were recorded
214 each day between 9.00 and 17.00hrs. We randomized the time of day for mating observations
215 within each line.

216 **Locomotion assay**

217 After 30 minute period of incubation, and before the flies from the male and female vials were
218 combined, the male vials were tapped to cause all the males to fall to the bottom of the food
219 vial. We then recorded the time required for the fastest male fly to walk up the side of the vial
220 from the bottom of the food to the top of the vial plug (95 mm). This was repeated three times
221 for each line and we took the average value per vial (Gibert *et al*. 2001).

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222 **Heating rate assay**

223 Using a thermal imaging camera (NEC Avio Infrared Technologies H2640) and macro lens
224 (NEC Avio Infrared Technologies TH92-486), we recorded the heating rate of 10 DGRP lines
225 (see supplement for information about the specific DGRP lines used). Fly individuals from
226 each line were cooled in a climate chamber at 5°C for 3 minutes before being removed from
227 the chamber and allowed to heat up to room temperature (approximately 23°C) in a petri dish.
228 Thermal images were taken every 5 seconds for around 30 seconds or when all the flies had
229 left the petri dish. Two experimental blocks of each line were performed. We recorded how
230 body temperature changed for each line over this time period by analyzing these thermal
231 images using the software provided by NEC Avio (see Svensson and Waller 2013 for more
232 methodological details).

233 **Statistical analyses**

234 All statistical analysis in this paper were conducted in R (R Development Core Team 2008).
235 R-code for all the analyses are uploaded to Dryad, and details are provided in the
236 Supplementary Material. To analyze the heating rates of 10 chosen DGRP lines, we
237 performed an analysis of variance with temperature at each time point as the dependent
238 variable. The following model was used: Temp = Time + Line + Block + Time*Line +
239 Time*Block + Temp*Line*Block.

240

241 For the hemi-clones, we performed a two-way analysis of variance (ANOVA) with the
242 number of matings at 10 minutes as the dependent variable, with line, temperature, and their
243 interactions as dependent factors. In this analysis, line and temperature were both treated as
244 categorical factors. In a follow-up analysis of these hemi-clonal males, we instead treated
245 temperature as continuous variable, both as a simple linear term and as a quadratic term and

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246 their two-way interactions with line (i.e. mating rate = Temp + Line + Temp*Line + $Temp^2$
247 + Line*$Temp^2$). We chose to analyse temperature as both a continuous and categorical
248 variable because both analyses have useful interpretative value. In the analysis of the pure
249 DGRP-lines, we performed a similar two-way analysis of variance (ANOVA) with the
250 number of matings at 15 minutes as the dependent variable and line, temperature and their
251 interaction as independent dependent factors. In this analysis, line and temperature were also
252 both treated as categorical factors.

253

254 We additionally performed a repeated measures analysis on the hemiclonal lines using the R-
255 package 'nlme' (Pinheiro et al. 2016). We used the number of matings as the dependent
256 variable, and experimental temperature category (18, 24, 30, 36 °C as different levels), line
257 (the 30 hemi-clonal lines as different levels), and time (10, 20, 30, 40, 50, 60 minutes) were
258 treated as fixed effects in this model. Vial and experimental block were treated as nested a
259 random effects. This allowed us to control for the non-independence of the repeated mating
260 counts over each vial.

261 The two-way interaction between line and temperature treatment in these tests, should reflect
262 the magnitude and possible statistical significance of genotype-by-environment interaction
263 with respect to thermal plasticity for male mating rate. Significant line-by-temperature
264 interactions would thus indicate the presence of genetic variation in the thermal reaction
265 norms of males belonging to different DGRP- and hemi-clones respond in terms of their
266 mating success at different temperatures.

267 Finally, we performed a power analysis simulation to quantify the minimum amount of
268 genetic variation in thermal reaction norms that we would be able to detect an effect in the 30
269 hemi-clonal lines, and using a given sample size (R-code for this simulation will be provided

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270 on Dryad). To analysis variation in heating rate, we performed a two-way analysis of variance
271 (ANOVA) with the average temperature at each time point as the dependent variable and line,
272 time, and their interactions as dependent factors, while also controlling for a block effect. An
273 analysis modelling mating rate as a proportion is presented in the Supplementary Material
274 (Table S1).

275

276 **Results**

277 We found significant variation in mating rates and locomotory performance among lines,
278 using both the hemi-clonal and the pure DGRP lines (Tables 1-4, S1, S2, S3; Fig. 1). As
279 expected, all lines of both hemi-clonal and pure DGRP experimental categories responded
280 plastically to the different temperature treatments (Fig. 2). Male mating rates and locomotory
281 performance were significantly affected by temperature (Fig 2).

282

283 For all of our statistical analyses, and for neither the hemi-clonal nor the pure DGRP assays,
284 we did not find any statistically significant interaction between line and temperature, that
285 would be indicative of GEI:s (Fig. 1; Tables 1-3, S1, S2, S3). Moreover, we were not able to
286 find any evidence for a statistically significant interaction between line and the squared
287 temperature component (Table 3), i.e. neither the slopes nor the curvatures of the thermal
288 response curves differed significantly between lines (Fig. 1).

289

290 Visual inspection of the thermal performance curves for male mating success in Fig. 1
291 revealed that 24 out of the 30 hemi-clonal lines peaked at intermediate temperatures (24 °C or
292 30 °C), whereas only 6 lines had maximal mating success in the cold (18 °C) or extremely hot

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293 treatments (36 °C). For the DGRP-lines, 17 peaked in male mating success at intermediate
294 temperature (24 °C), 9 at cold temperature (18 °C) and the remaining four at 30 °C (note that
295 the DGRP-lines were not evaluated under the extreme treatment (36 °C; see Fig. 1). However,
296 note that the relationship between male mating success and temperature was often quite flat in
297 the range between 18 and 24 °C, after which it dropped (Fig. 1). Taken together, these results
298 suggest a genetically quite invariant intermediate temperature optimum and more or less flat
299 fitness peak around 24 °C (Fig. 2). Thus, we found no evidence for any statistically significant
300 difference between genotypes in the location of this fitness optimum and neither any evidence
301 for different slopes of the thermal response curves or their curvatures (Table 3).

302

303 Using our statistical power simulation we were able to put a minimum bound on the genetic
304 variation in thermal plasticity (variation in the effect of our Line x Temp interaction) (Fig. 3).
305 The main conclusion from these simulations is that our statistical power is high enough under
306 realistic parameter values, meaning that we should have detected a large GEI:s if they had
307 existed (Fig. 3). The magnitude of the GEI we recorded in these experiments can explain at
308 most 4% of variation in mating rate. For instance, our statistical power approaches one with
309 an effect size of 0.03 (treating temperature as a continuous variable) and 0.15 (treating
310 temperature as a categorical variable)(Fig. 3). Finally, at 18° and 30° C there was no
311 detectable correlations between the mating rate of the hemi-clones and our DGRP lines. At
312 24° C, there was a slightly stronger and significant correlation between the mating rates of the
313 DGRP and hemi-clonal males (Fig. 4).

314

315 In the analysis of heating rates using thermal imaging, we found significant variation among
316 the 10 DGRP-lines we investigated, and a significant Line x Time interaction (Fig. 5; Table

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317 5). This shows that for these 10 DGRP-lines, the slopes of the thermal reaction norms
318 differed, although the effect was significant only in the first experimental block (Fig. 5; Table
319 5).

320

321 **Discussion**

322 Evolutionary change requires genetic variation in the traits under selection (Blows &
323 Hoffmann 2005). This basic requirement for evolutionary change also applies to a trait like
324 thermal plasticity, and the evolution of thermal plasticity will require genetic variation in the
325 slopes of thermal reaction norms. In this study, we have found significant genetic variation in
326 male mating rates, using two different approaches: a pure-clonal approach (using a subset of
327 un-manipulated DGRP-lines bred by Mackay et al. 2012) and a hemi-clonal approach (Abbott
328 and Morrow 2011), where these DGRP-lines were introduced into an outbred LH_M
329 background (Fig. 1). Using these two complementary experimental approaches, we found no
330 statistically significant variation in the reaction norm of male mating rate to temperature in the
331 different lines (Figs. 1, 3). This shows that although all these lines altered their mating rates in
332 relation to temperature (i.e. phenotypic plasticity; Fig. 1, Table 1-4) the changes were parallel
333 and all lines responded in a similar manner. This implies that genetic variation in thermal
334 plasticity for male mating success is low (Fig. 1), explaining at most 4 % of the variation (Fig.
335 3). Similar conclusions apply to male locomotory performance (Fig. 2), where we also did not
336 find any evidence for significant GEI (Table 4).

337

338 The lack of strong GEI:s for these two behavioural traits contrasts our findings of a significant
339 GEI for male heating rate, a physiological performance trait, measured using thermal imaging
340 (Fig. 5), where we did find evidence for GEI (Table 5). Heating rate is an important fitness

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341 trait for ectotherms because it might covary with ability to adjust to natural ambient
342 temperatures. It also measures the capacity of an individual to buffer itself against the
343 ambient environmental temperatures. The use of thermal imaging is a very powerful tool to
344 quantify genetic and phenotypic variation of a physiological trait like heating rate, as done in
345 previous studies of non-model organisms (Tattersall et al 2009; Tattersall and Cadena 2010;
346 Symonds and Tattersall 2010; Svensson and Waller 2013). The fact that the significant GEI
347 for thermal reaction norm slopes in these heating rates have no counterpart in the behavioural
348 assays of mating rates and locomotory assays (Figs. 1-2; Tables 1-4) might be biologically
349 important. Behavioural traits and life-history traits are further downstream than the
350 physiological traits are from the genes that govern phenotypic traits (Price and Schluter 1991),
351 hence genetic variation on these grounds expected to be lower for such higher-level traits,
352 such as mating rate and locomotory performance.

353

354 Overall, and across all lines, male mating success was maximal at 24 °C (DGRP-lines) or at
355 either 24 °C or 30 °C (Fig. 2), with a few exceptions (Fig. 1). This suggests that the thermal
356 optimum for male mating success falls well within the normal temperature range *Drosophila*
357 *melanogaster* will experience in North Carolina in the wild (Annual high temperature: 21.5°,
358 Annual low temperature: 9.3°, Average temperature: 14.9° C (Daly 2000)), where these
359 DGRP-lines originated (Mackay et al. 2012). Thus, although our results suggests limited
360 evolutionary potential for thermal plasticity with respect to male mating success, they are
361 consistent with males being locally adapted with respect to their local temperature regime,
362 consistent with a previous study on *Drosophila melanogaster* (Dolgin et al. 2006, Latimer et
363 al. 2011).

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364 These DGRP lines were derived from field-caught flies and variation among these lines
365 should reflect naturally segregating genetic variation in the source population (Mackay *et al*.
366 2012). Using a statistical power simulation (Fig. 3), we were able to put a minimum bound on
367 the amount of variation in reaction norms in our clonal lines. The line effect had a standard
368 deviation of < 0.5, which implies that variation in mating rate was at a maximum less than
369 0.01 matings for any given temperature. Comparing the slopes of the reaction norms from
370 optimal temperature condition (24° C) to extreme temperature condition (36° C), we conclude
371 that the standard deviation in slopes is likely < 0.01. This effect covers only about 4% of the
372 total variation in male mating success (Line = 11%, Temp = 58%, Block = 21%, Line:Temp =
373 4%, Residual = 5%). This means that lines varied less than around ±0.008 matings (in 10 min)
374 per 1°C (Fig. 3). Whether this low amount of variation in thermal reaction norms would allow
375 for evolution of adaptive phenotypic plasticity is an open question and depends on several
376 other ecological and evolutionary factors, including population size, the strength of selection,
377 the rate of environmental change, generation time, and intrinsic rate of increase (Hoffmann
378 2010; Chevin *et al*. 2010). However, we note that the effect sizes in these power calculations
379 are minimum effect sizes, and the true amount of genetic variation in thermal plasticity might
380 be considerably lower.

381

382 One concern is that mating rate is the product of the behaviour of multiple individuals
383 interacting, so it is not only the male's behaviour that matters, but also the female's
384 preference. For instance, LHm females prefer males from some of the DGRP-lines more than
385 males from other DGRP-lines. A second concern is that it is the additive genetic variation that
386 determines the evolutionary potential of a population, yet the hemi-clones still share the
387 same half-genome, so any epistatic effects arising from interactions between chromosomes
388 within the DGRP half will also be included. Our experimental design is for these reasons

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389 conservative with respect to our ability to detect significant GEI:s, since the line-effects will
390 partly also include non-additive effects. Additionally, there might exist variation in latency to
391 mate after a disturbance between lines, and this might be of some concern. However, any
392 differences in willingness to mate after disturbance will be captured by the line effect in our
393 statistical analyses. This means that lines might differ not only mating rate per se but also in
394 their willingness to mate at each temperature. This variation in willingness to mate after a
395 disturbance would not interfere with detecting GEI:s in mating rate, unless there was an
396 interaction between the latency to mate and temperature treatment.

397

398 For the hemi-clonal lines, epistatic effects would not be included by the line factor, since our
399 starting iso-genetic lines (30) were homozygous at all loci. Thus, epistatic interactions with
400 the outbred (LHm) genetic background would not be included in the line effect, and would
401 become part of the error variance. Such epistatic genetic variance would represent hidden
402 genetic variation that we were not able to detect with our hemi-clonal lines (Huang *et al.*
403 2012; Mackay 2014). Epistatic variance of traits related to mating success, such as courtship,
404 have been demonstrated for other species of flies (Meffert et al. 2002). In these previous
405 studies it has been shown that such epistatic variance can be converted to additive genetic
406 variance following population bottlenecks (Meffert et al. 2002). From a theoretical viewpoint,
407 traits that are closely related to fitness such as male mating success (a major fitness
408 component) are expected to show low additive genetic variance, due to the depleting effects
409 of strong directional sexual selection (Rowe & Houle 1996). Directional sexual selection
410 should therefore expect reduce the additive genetic variance fraction for male mating success,
411 resulting in a relatively higher fraction of the remaining genetic variation being non-additive,
412 reflecting either epistatic (Meffert et al. 2002) or dominance variance (Merilä & Sheldon
413 2002). Such non-additive genetic variance for male mating success could potentially explain

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414 the low concordance between male mating success in the DGRP-lines and the hemi-clonal
415 lines (Fig. 4). This interpretation of high epistatic variance for male mating success in these
416 DGRP-lines would be consistent with previous studies of these DGRP-lines, where high
417 epistatic variance was found for a number of other fitness-related traits, including cold
418 tolerance (measured as chill coma recovery) (Huang et al. 2012).

419

420 Our results have some implications for the prospects of evolutionary rescue through the
421 evolution of adaptive phenotypic plasticity (Chevin et al. 2013) and by sexual selection
422 (Candolin & Heuschele 2008). Previous laboratory experiments on several species of
423 *Drosophila* (Holland 2002; Rundle et al. 2006) and seed beetles *Callosubruchus maculatus*
424 (Martinossi-Allibert et al. 2016) have found at most weak or at best mixed support for sexual
425 selection improving the rate of evolution of local adaptation to novel stressful environments,
426 such as thermally challenging environments. The results in the present study add to this small
427 but growing body of literature, and indicate that thermal plasticity for male mating success is
428 unlikely to evolve, as the genetic variation in thermal reaction norms is limited (Fig. 3). Some
429 theoretical models suggest that sexual selection could improve environmental adaptation at
430 low demographic costs, due to purging of deleterious alleles in males (Agrawal 2001; Siller
431 2001; Whitlock & Agrawal 2009). However, the results in this and other experimental studies
432 (cited above) give only weak support to these models. Thus, our results provide only limited
433 support to the hypothesis that sexual selection could act as an evolutionary rescuer of
434 populations experiencing rapid environmental change (Candolin & Heuschele 2008).

435

436 Our results also agree with other research in this area showing that evolutionary responses to
437 novel and challenging thermal conditions may be constrained (Bennett & Lenski 1993;

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438 Kellermann *et al*. 2009; Mitchell & Hoffmann 2010; Hoffmann 2010; Kelly *et al*. 2012; Kelly
439 *et al*. 2013; Kristensen *et al*. 2015). In particular, ectotherms, particularly those living in
440 tropical areas that already experience temperatures close to their upper thermal tolerance
441 limits might have a reduced capacity to adapt to higher temperatures via evolutionary means
442 (Araujo *et al*. 2013; Kristensen *et al*. 2015) (Fig 2). Insights from studies of niche
443 conservatism also suggests that it might be difficult for many species to evolve new
444 physiological limits (Parsons 1982; Kimura & Beppu 1993; Wiens & Graham 2005;
445 Kellermann *et al*. 2009; Hoffmann 2010). For example, the invasive species *Drosophilia*
446 *subobscura* has a range in the Americas that is restricted to climates that are similar to those
447 found in its native range in Europe (Prevosti *et al*. 1988; Gilchrist *et al*. 2008; Huey & Pascual
448 2009; Hoffmann 2010). However, some natural *Drosophila* populations have adapted to their
449 local climates (Trotta *et al*. 2006). For instance, warm-adapted populations in India have
450 higher levels of desiccation resistance and melanism compared with cold-adapted populations
451 (Parkash *et al*. 2008). Similarly, populations in Africa are more viable at warm temperatures
452 than temperate populations (Bouletreau-Merle *et al*. 2003; David *et al*. 2004; David *et al*.
453 2005; Hoffmann 2010). Other laboratory studies have shown that populations might have the
454 capacity to adapt to temperature via evolutionary adaptation (Hoffmann 2010; Latimer et al.
455 2011; Mukuka *et al*. 2010; Hoffmann *et al*. 2013; van Heerwaarden and Sgro 2013;
456 Blackburn *et al*. 2014; van Heerwaarden at al. 2016). For example, in some laboratory
457 experiments, *Drosophilia* have been successfully selected for increased survival after cold and
458 heat shocks (Bubliy & Loeschcke 2005). Finally, it might be that the expression of additive
459 genetic variation for heat tolerance is contingent on developmental temperature (van
460 Heerwaarden at al. 2016). For instance, two rainforest *Drosophila* species exhibited
461 significant levels of additive genetic variation when raised in warmer environments, but not
462 when raised at 25°C (van Heerwaarden at al. 2016).

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463 **Conclusions**

464 The capacity of natural populations of *Drosophila melanogaster* to adapt evolutionarily (i.e.
465 via changing the genetic composition of the population) to novel thermal conditions through
466 the evolution of adaptive phenotypic plasticity remains an open question. In this study, we
467 were not able to detect any significant genetic variation in thermal reaction norms to different
468 thermal environments in either the DGRP-lines or the hemi-clonal lines. However, we were
469 able to put a lower bound on the amount of genetic variance in thermal plasticity. Isogenic
470 lines such as these DGRP-lines in combination with hemi-clonal approaches offer
471 opportunities to quantify genetic variation in phenotypes where the underlying genotypes are
472 known and line-differences can attributed to genetic differences without the need for complex
473 breeding designs (Abbott & Morrow 2011). Such isogenic lines could also be raised in
474 different temperatures in the future, which might reveal that significant genetic variation
475 exists, but this variation might depend on temperatures experienced during the larval
476 development stage (van Heerwaarden at al. 2016). It is possible that GEI:s might be more
477 pronounced among juveniles, and that strong selection during these earlier life stages might
478 have reduced additive genetic variance in thermal reaction norms that could be detected
479 during the adult stage (cf. Martinossi-Allibert et al. 2016). In the present study, we were not
480 able to detect any significant genetic variation in thermal plasticity in adult male mating
481 success or locomotion.

482 **Figure Legends**

483

484 **Fig 1.** Thermal reaction norms of male mating success estimated for of each pure DGRP
485 (black) and hemi-clonal (empty) DGRP-line in relation to experimental temperature
486 treatments. Numbers above each sub-panel refer to the DGRP-lines described in Mackay et al.

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487 (2012). Although temperature affects mating rates in all lines and often peaks at intermediate
488 temperatures, there is no evidence for any genotype-by-environment interaction with respect
489 to temperature, i.e. the mating success of the different lines are similarly affected across
490 environmental treatments. Mating rate is the number of copulations that were recorded in a
491 vial after 10 minutes (for the hemiclones) and after 15 minutes (for the pure clones). The
492 mating assay was only performed for some lines with either only the hemi-clones (303, 486)
493 or only the pure lines (305, 315). Confidence intervals (95%) are shown as vertical lines.

494

495 **Fig 2.** Temperature-related male performance for the hemi-clonal males showed as male
496 locomotory performance (left panel) and male mating rate (right panel). Males from different
497 lines vary in mean mating rates and locomotory performance (intercepts), but variation
498 between lines in thermal reaction norms (slopes) is low (Fig. 1). Hence, there is no evidence
499 for significant genetic variation in the thermal reaction norms (Fig. 1). Each data point
500 represents the mean performance of each line at each temperature treatment.

501

502 **Fig 3.** Power plots to detect significant GEI:s (reflected as Line*Temp interactions) for male
503 mating success. Temperature is either treated as continuous variable (left plot) or as a discrete
504 experimental factor (right plot). Comparing the slopes of the reaction norm (left panel) from
505 optimal temperature to extreme (hemiclonal lines: 24° to 36° C; pure DGRP-lines: 24° to 30°
506 C), we found that the standard deviation in slope is likely to be < 0.01 for the hemi-clonal
507 lines and < 0.025 for the pure DGRP-lines. This means that lines varied in less ±0.008
508 matings (per 10 min) per 1°C. Treating temperature as discrete (right panel), the standard
509 deviation of this Line*Temp effect is most likely < 0.07 for the hemi-clonal lines and < 0.12
510 for the pure clonal lines. This means that the variation in mating rate is maximally < 0.01

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511 matings for a given temperature. These are minimum effect sizes, and the true variation in
512 thermal plasticity is likely to be lower. R-code for these power simulations are provided on
513 Dryad. Each data point represents 1000 simulations (hemi-clonal: n = 7 and pure-clonal: n = 3
514 replicates per line n = 30).

515

516 **Fig. 4.** Correlations between mating rates (at 10 min and 15 between our hemi-clonal and
517 DGRP lines, measured at three different temperatures (measured at 18 ^{o}C (“cold” = white), 24
518 ^{o}C (“optimal” = gray) and 30 ^{o}C (“hot” = black). We studied a total of 28 Lines per
519 temperature treatment, i.e. the total number of datapoints in this graph is 84). The “random”
520 Lhm genetic background in our hemi-clonal lines likely has a large effect on mating rate,
521 since the mating rates the DGRP-lines, put either in to hemi-clones or measured as pure
522 clones show only weak non-significant relationships (All: r=0.12, *P*=0.24, N=84, 18C: r
523 =0.08, *P* = 0.68, N=29; 24C: r= 0.37, *P*=0.05, N=29; 30C: r= 0.10, *P*=0.61, N=29). The lack
524 of strong significant correlations between the mating rates of the different lines in different
525 genetic backgrounds indicates low additive genetic variance in male mating success and
526 implies strong epistatic effects on mating rate (Huang et al. 2012), as our hemi-clonal and
527 pure-clonal lines will only share the additive effects of their genotypes.

528

529 **Fig 5. A)** Heating rates of 10 DGRP lines after exposer to a cool shock 5°C for 3 minutes and
530 then allowed to recover at room temperature for 30 seconds. Two experimental blocks were
531 performed of these same 10 DGRP lines. Thermal images were taken every 5 seconds.
532 Heating rate was significantly variable between lines in block 1 but not in block 2 (Table 5).

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538 **Figures and Tables**

539 **Table 1.** Analysis of variance (ANOVA) for the mating rate in hemi-clonal males. Line
540 (N=30) and temperature (N=4; 18,24,30,36 °C) were both treated as categorical factors. We
541 used the first mating observation at 10 minutes to avoid double counting matings at the next
542 observation time 20 minutes. Here, a significant Line x Temp interaction would be indicative
543 of a GEI and reveal significant genetic variation (greater than zero) in plasticity in our hemi-
544 clonal males. Block is a categorical factor (N=2), which controls for differences in the two
545 experimental runs. See table S1 in the supplementary material and a model wherein mating
546 rate is treated as a proportion.

Term	DF	SSE	MSE	F-value	P-value	Significance
Line	29	4.54	0.16	2.37	<0.0001	****
Temp	3	2.48	0.83	12.51	<0.0001	****
Block	1	0.3	0.3	4.52	0.034	*
Line x Temp	87	4.88	0.06	0.85	0.83	ns
Residuals	870	57.38	0.07			

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556 **Table 2.** Analysis of variance (ANOVA) for the mating rate of the DGRP males. Line (N=32)
557 and temperature (N=3; 18,24,30 °C) were both treated as categorical factors. We used the first
558 observation time at 15 minutes to avoid double counting matings at the next observation time
559 30 minutes. A significant Line x Temp interaction would be indicative of a GEI.

Term	DF	SSE	MSE	F-value	P-value	Significance
Line	31	5.22	0.17	3.55	<0.0001	****
Temp	2	1.31	0.66	13.82	<0.0001	****
Line x Temp	58	2.35	0.04	0.86	0.752	ns
Residuals	172	8.16	0.05			

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573 **Table 3.** Analysis of covariance (ANOVA) for the mating rate hemi-clonal males. Here Line
574 (N=30) is treated as a categorical factor and temperature is treated as a continuous variable.
575 The quadratic effect of temperature ($Temp^2$) allows the model to detect any curvature in the
576 reaction of mating rate to temperature. We used the first observation time at 10 minutes to
577 avoid double counting matings at the next observation time 20 minutes. Here, a significant
578 Line x Temp interaction would be indicative of a GEI, and indicate significant genetic
579 variation (greater than zero) in plasticity in our hemi-clonal males. Block is a categorical
580 factor (N=2), which controls for differences in the two experimental runs.

Term	DF	SSE	MSE	F-value	P-value	Significance
Line	29	4.54	0.16	2.41	<0.0001	****
Temp	1	0.02	0.02	0.34	0.557	ns
$Temp^2$	1	2.39	2.39	36.81	<0.0001	****
Block	1	0.31	0.31	4.82	0.028	*
Line x Temp	29	2.27	0.08	1.2	0.212	ns
Line x $Temp^2$	29	1.67	0.06	0.89	0.641	ns
Residuals	900	58.38	0.06			

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591 **Table 4.** Analysis of locomotion performance in the hemi-clonal lines (N=30). Locomotion
592 is the speed (mm/seconds) of the fastest hemiclonal male (7 per vial) to walk up the side of
593 the vial (repeated 3 times for each line) at the different experimental temperatures. We
594 performed a two-way analysis of variance (ANOVA) with locomotion time the as the
595 dependent variable and line, temperature and their interaction as independent dependent
596 factors.

Term	DF	SSE	MSE	F-value	P-value	Significance
Line	29	4197.69	144.75	1.76	0.009	**
Temp	3	7449.08	2483.03	30.22	<0.0001	****
Line x Temp	87	5691.16	65.42	0.8	0.907	ns
Residuals	592	48635.14	82.15			

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611 **Table 5.** Analysis of variance (ANOVA) for the heating rate of DGRP males. 2 replicates of
612 Lines (N=10). Time is measured in seconds. A thermal image was taken at each time point.
613 Here, a significant Line x Time interaction would be indicative of significant variation in
614 heating rate between lines. However, a significant Time x Line x Block effect complicates
615 this interpretation. Block is a categorical factor (N=2), which controls for differences in the
616 two experimental runs.

Term	DF	SSE	MSE	F-value	P-value	Significance
Time	1	135.14	135.14	290.3	<0.0001	***
Line	9	42.62	4.74	10.17	<0.0001	***
Block	1	35.44	35.44	76.13	<0.0001	***
Time x Line	9	9.46	1.05	2.26	0.024	*
Time x Block	1	3.94	3.94	8.47	0.004	**
Line x Block	9	16.48	1.83	3.93	0.0002	***
Time x Line x Block	9	8.47	0.94	2.02	0.04	*
Residuals	95	44.22	0.47			

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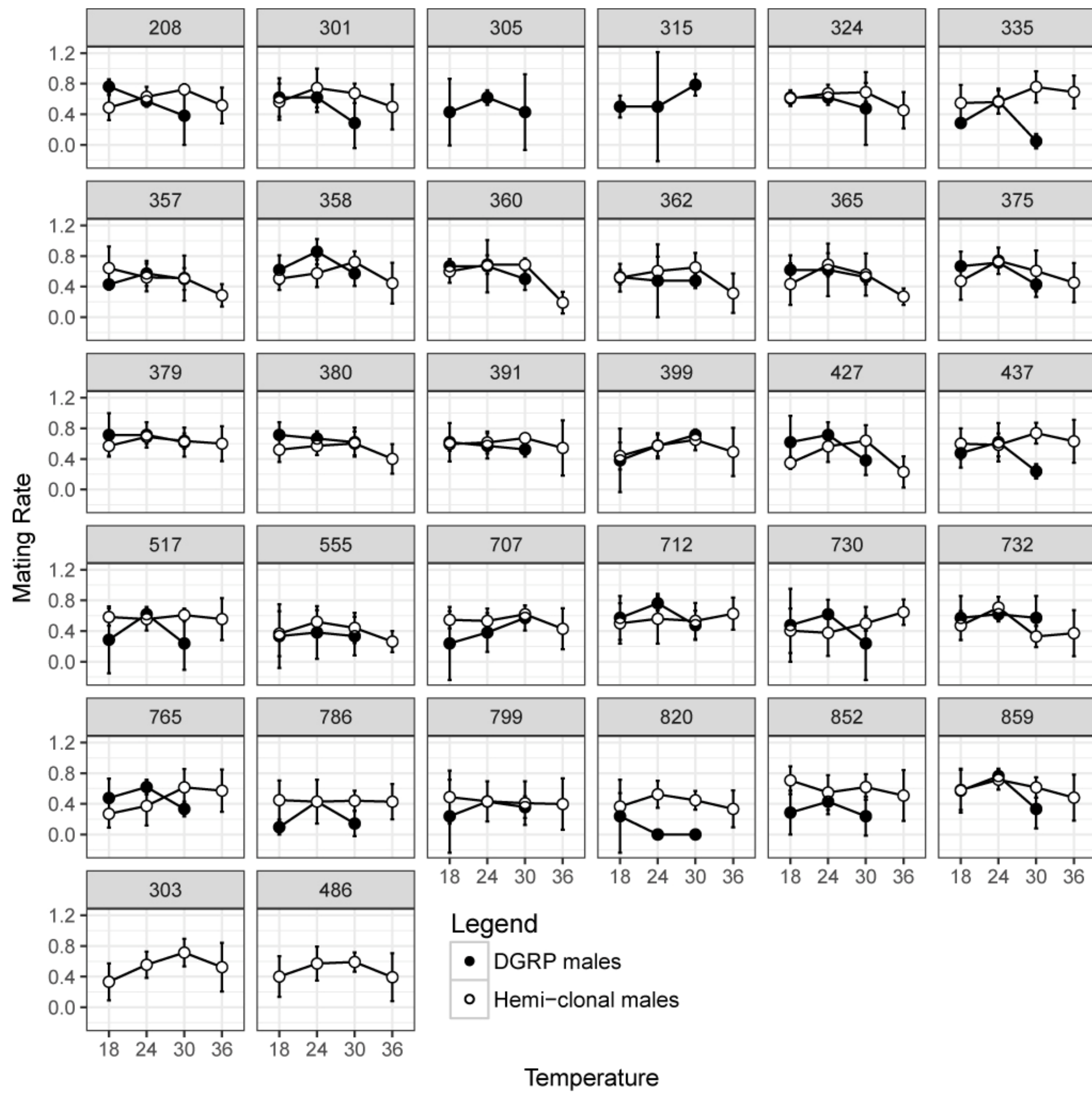

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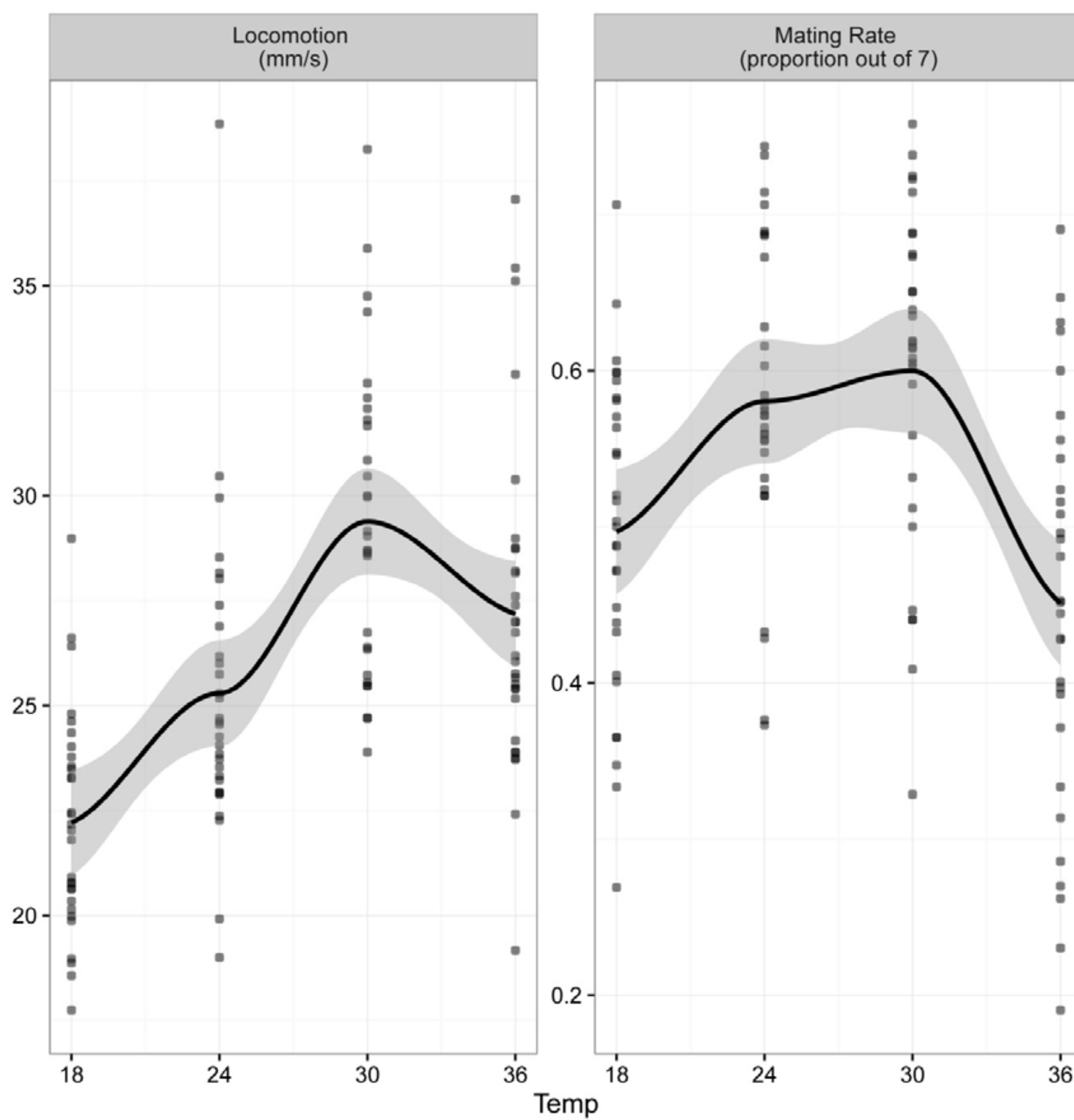

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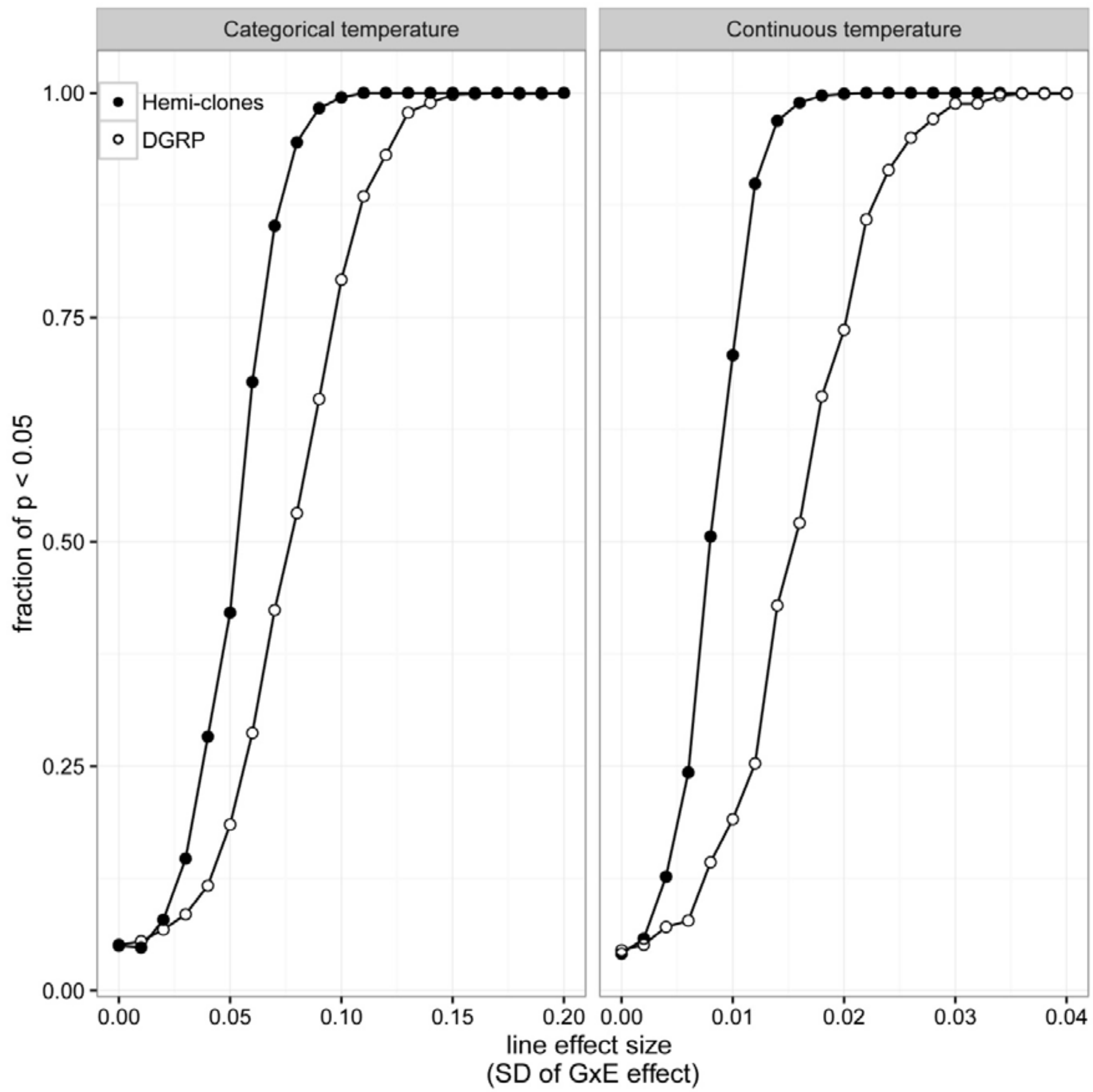

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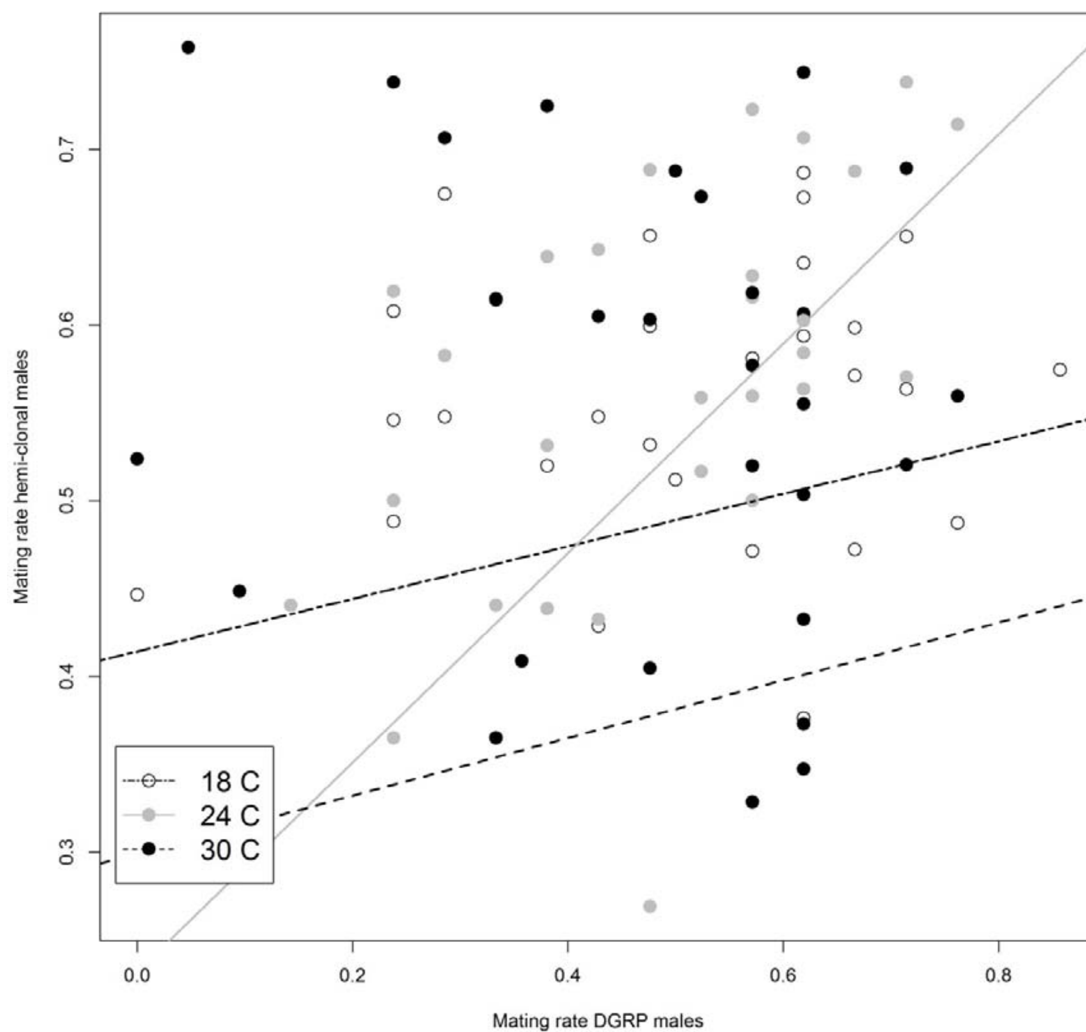

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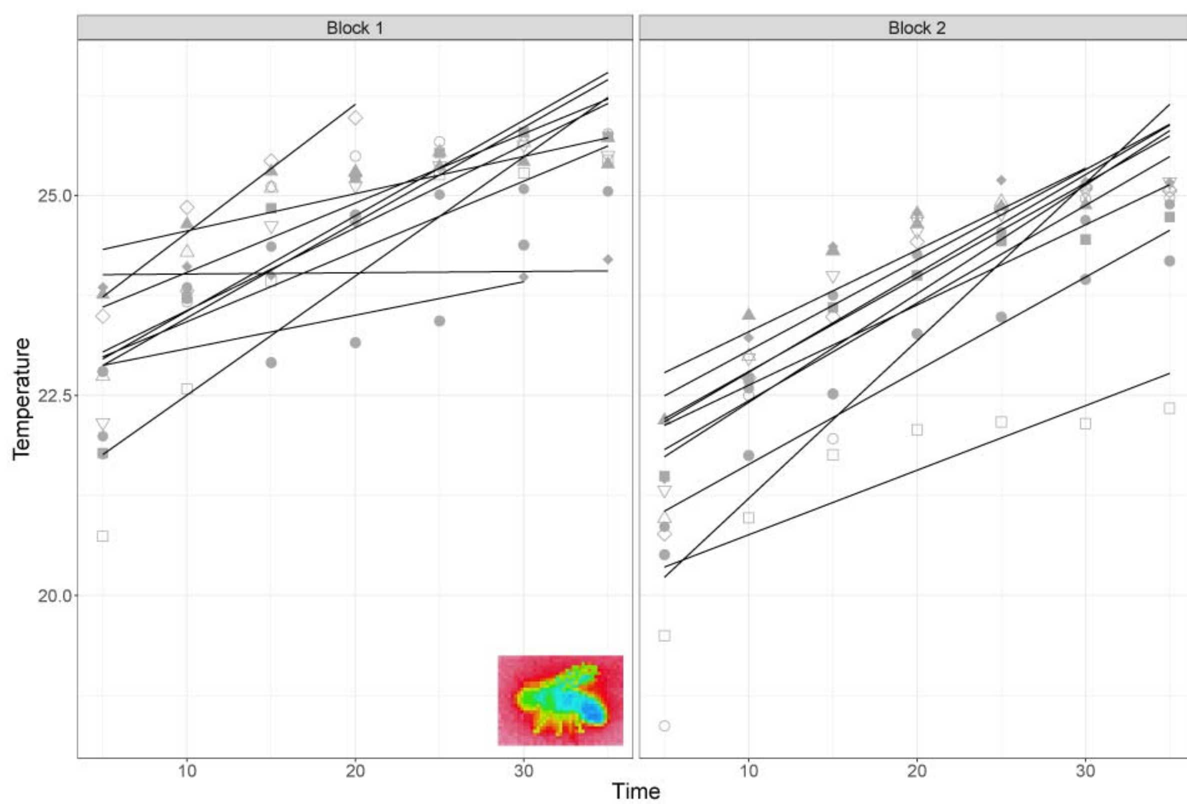

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