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Article Title: **Thermal tolerance and preference are both consistent with the clinal distribution of house fly proto-Y chromosomes**

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Abstract

Selection pressures can vary within localized areas and across massive geographical scales. Temperature is one of the best studied ecologically variable abiotic factors that can affect selection pressures across multiple spatial scales. Organisms rely on physiological (thermal tolerance) and behavioral (thermal preference) mechanisms to thermoregulate in response to environmental temperature. In addition, spatial heterogeneity in temperatures can select for local adaptation in thermal tolerance, thermal preference, or both. However, the concordance between thermal tolerance and preference across genotypes and sexes within species and across populations is greatly understudied. The house fly, *Musca domestica*, is a well-suited system to examine how genotype and environment interact to affect thermal tolerance and preference. Across multiple continents, house fly males from higher latitudes tend to carry the male-determining gene on the Y chromosome, whereas those from lower latitudes usually have the male-determiner on the third chromosome. We tested whether these two male-determining chromosomes differentially affect thermal tolerance and preference as predicted by their geographical distributions. We identify effects of genotype and developmental temperature on male thermal tolerance and preference that are concordant with the natural distributions of the chromosomes, suggesting that temperature variation across the species range contributes to the maintenance of the polymorphism. In contrast, female thermal preference is bimodal and largely independent of congener male genotypes. These sexually dimorphic thermal preferences suggest that temperature-dependent mating dynamics within populations could further affect the distribution of the two chromosomes. Together, the differences in thermal tolerance and

preference across sexes and male genotypes suggest that different selection pressures may affect the frequencies of the male-determining chromosomes across different spatial scales.

Impact Statement

Genetic variation within species can be maintained by environmental factors that vary across the species' range, creating clinal distributions of alleles responsible for ecologically important traits. Some of the best examples of clinal distributions come from temperature-dependent phenotypes, such as thermal tolerance and preference. Although genotype and developmental temperature strongly affect physiological and behavioral traits in ectotherms, the correlation between these traits across genotypes and sexes within species is greatly understudied. We show that two different male-determining chromosomes found in natural populations of house fly affect both thermal tolerance and preference in a way that is concordant with their clinal distributions across latitudes. This provides strong evidence that temperature variation across the species range contributes to the maintenance of the polymorphism. Furthermore, we find evidence that thermal preference is sexually dimorphic, suggesting that temperature-dependent mating dynamics could further affect the distribution of genetic variation in this system. Therefore, at a macro-geographical scale, the differences in thermal tolerance and preference across male genotypes likely contributes to the maintenance of the cline. Within populations, differences in thermal preference likely affect sexual selection dynamics, which may further affect the frequencies of the chromosomes.

Introduction

Ecological variation across a species' range can select for local adaptation within populations, which can contribute to the maintenance of genetic variation by favoring different alleles across the range (Levene 1953; Felsenstein 1976; Hedrick et al. 1976; Kawecki and Ebert 2004). In addition, heterogeneous selection pressures that are distributed as a gradual continuum from one end of the species' range to another can create a cline of genetic variation responsible for phenotypes under selection (Slatkin 1973; Endler 1977). Some of the best examples of latitudinal clines come from temperature-dependent phenotypes (e.g., body size, developmental rate, and thermal tolerance) that have been well-documented in flies (Partridge et al. 1994; Eanes 1999; Robinson and Partridge 2001; Hoffmann et al. 2002). Moreover, heterogeneous selection pressures across a cline may affect males and females differently (Connallon 2015; Connallon et al. 2019), although the empirical evidence for such variation in sex-specific selection across geographic ranges is mixed (Delcourt et al. 2009; Delph et al. 2011; Allen et al. 2017; Lasne et al. 2018).

Thermal adaptation within populations and across a species range can occur via selection on physiological, anatomical, or behavioral traits. For example, north-south gradients in heat and cold tolerance have been observed in *Drosophila* (Hoffmann et al. 2002), suggesting physiological adaptation to thermal environments. In addition, ectotherms, such as flies, rely on behavioral mechanisms of thermoregulation by avoiding suboptimal temperatures in search of more optimal ones (Dillon et al. 2009), and thermal preference may be correlated with optimal thermal performance (Dawson 1975; Angilletta et al. 2002).

Concordance across genotypes between different thermal traits could reinforce the response to selection, whereas negative correlations could constrain adaptation (Etterson and Shaw 2001). However, it is not clear if physiological and behavioral thermal traits are genetically correlated within a species, between sexes, or across populations (Dawson 1975; Angilletta et al. 2002; Gilbert and Miles 2017). For example, experiments in *Drosophila subobscura* identified individual chromosomes that affected thermal tolerance or temperature preference, but no single chromosome affected both physiological and behavioral phenotypes (Dolgova et al. 2010; Rego et al. 2010; Castañeda et al. 2019). Furthermore, temperature-dependent traits can affect assortative mating and male reproductive success (Dolgin et al. 2006; Keller and Seehausen 2012), suggesting inter-sexual differences in thermoregulation could affect genetic variation within populations via sexual selection. These sex-specific selection pressures could also contribute to the maintenance of genetic variation via inter-sexual conflict or context-dependent selection (Kotiaho et al. 2001; Rostant et al. 2015; Meisel 2018). Despite the importance of inter-sexual differences, previous work did not test for differences in the genetic correlation of thermal traits between males and females.

We used a sex chromosome polymorphism in house fly, *Musca domestica*, to investigate the concordance of thermal tolerance and preference across clinally distributed male genotypes. House fly has a polygenic sex determination system, with a male-determining gene most commonly found on either the third chromosome (III^M) or what was historically refered to as the Y chromosome (Y^M) (Hamm et al. 2014; Sharma et al. 2017). Both III^M and Y^M are very young proto-Y chromosomes that are minimally differentiated from their homologous proto-X

93 chromosomes (Meisel et al. 2017; Son et al. 2019; Son and Meisel 2020). Y^M and III^M are
94 distributed along latitudinal clines on multiple continents in the Northern Hemisphere (Tomita
95 and Wada 1989; Hamm et al. 2005; Kozielska et al. 2008). Y^M is most frequently found at
96 northern latitudes, and III^M is more common at southern latitudes (Figure 1A). This distribution
97 suggests that the Y^M chromosome confers higher fitness in colder climates, and, conversely, III^M
98 confers higher fitness in hotter climates. Therefore, variation in temperature across the species
99 range may create heterogeneous selection pressures that maintain the proto-Y chromosome cline
100 in house fly. Consistent with this hypothesis, seasonality in temperature is the best predictor of
101 the frequencies of the proto-Y chromosomes across natural populations (Feldmeyer et al. 2008).
102 We tested the hypothesis that the Y^M chromosome confers cold-adaptive phenotypes and
103 III^M confers heat-adaptive phenotypes in house fly males, which would be consistent with their
104 latitudinal distributions (Tomita and Wada 1989; Hamm et al. 2005; Feldmeyer et al. 2008;
105 Kozielska et al. 2008). To those ends, we first evaluated if males carrying the III^M chromosome
106 (hereafter III^M males) have greater tolerance to extreme heat and if males carrying the Y^M
107 chromosome (Y^M males) have greater cold tolerance. Second, we tested if III^M males prefer
108 warmer temperatures than Y^M males, and if males and females differ in their thermal preference.
109 We performed all experiments using flies raised at multiple developmental temperatures because
110 thermal acclimation strongly affects temperature-dependent phenotypes in flies and other
111 ectotherms (Krstevska and Hoffmann 1994; Dillon et al. 2009). Together, we evaluated if thermal
112 preference and tolerance are aligned for sex-linked genetic variants, tested if this alignment could

maintain polygenic sex determination, and then discuss how these temperature-dependent phenotypes could affect the access of males to female mates.

Materials and methods

Fly strains and rearing

We performed our experiments using five nearly isogenic house fly strains, three with III^M males and two with Y^M males (Supplementary Methods). All five strains have a common genetic background from an inbred III^M strain that was produced from a mixture of flies collected across the United States (Scott et al. 1996; Hamm et al. 2005). Each of the three III^M strains carries a different III^M chromosome from a separate wild-derived line, and, likewise, the two Y^M strains carry different Y^M chromosomes.

We reared each strain at 18°C, 22°C, and 29°C for two generations in order to evaluate how thermal acclimation affects thermal tolerance (Chown and Terblanche 2006) and thermal preference (Krstevska and Hoffmann 1994; Dillon et al. 2009). Flies from each developmental temperature were assayed at equivalent physiological ages estimated by accumulated degree days (Barnard and Geden 1993; Wang et al. 2018). For our heat and cold tolerance assays, we used flies 22–50 total degree days after eclosion. For thermal preference assays, we used flies 96–115 total degree days after eclosion. Additional details and calculations are provided in the Supplementary Methods.

Thermal tolerance

We measured heat and cold tolerance in individual male and female flies. To measure heat tolerance, lightly anaesthetized individual flies were transferred to a 1.5 ml centrifuge tube that

was sealed with fabric. We placed the 1.5 ml tube in a heat block set to 53°C. This temperature was selected because it is the lowest at which heat tolerance could be measured in a reasonable period of time. The time at which a fly fell to the bottom of the tube and could not make its way back to the top of the tube was considered the knockdown time. To measure cold tolerance, lightly anaesthetized flies were transferred to a fabric-sealed 20 ml glass vial individually, and the vials were placed in a 4°C refrigerator with a transparent door. Knockdown occured when a fly fell on its back to the bottom of the vial. We gently tapped the assay vial every 2–3 minutes to ensure flies were active.

For both heat and cold tolerance assays, we performed an analysis of variance (ANOVA) using the lmer() function in the lme4 (v1.1) R package (Bates et al. 2015) to model the effect of genotype (G: Y^M vs III^M), developmental temperature (T: 18°C or 29°C), and their interaction on knockdown time (K):

$$K \sim G + T + G \times T + B + S,$$

with batch (B) and strain (S) treated as random effects. We also constructed another model excluding the interaction term:

$$K \sim G + T + B + S.$$

We then used a drop in deviance test to compare the fit of the models with and without the interaction term using the anova() function in R. We also compared heat and cold tolerance between males raised at 22°C and 29°C, using the same approaches as described above. Additional details are provided in the Supplementary Methods.

154 ***Thermal preference***

155 We measured thermal preference as the position of individual flies along a 17–37°C
156 thermal gradient (Figure S1), following a slightly modified version of previous protocols
157 (Anderson et al. 2013; Lynch et al. 2018). For each individual fly, we report mean thermal
158 preference (T_{pref}) as the average position during a 10 minute assay window (measured once per
159 minute). We also report thermal breadth, $T_{breadth}$ (Carrascal et al. 2016), as the coefficient of
160 variation of individual-level T_{pref} during the assay window. $T_{breadth}$ provides an estimate of how
161 individuals utilize thermal space within their environment (Slatyer et al. 2013). Choosier
162 individuals show a lower $T_{breadth}$ value and, thus, would be expected to occupy a narrower range of
163 temperatures within a given thermal habitat.

164 To determine the effects of developmental temperature (18°C, 22°C, and 29°C), genotype
165 (Y^M and III^M), and their interaction on mean T_{pref} across sexes, we created a mixed-effects model
166 using the lme4 package (v1.1) in R (Bates et al. 2015). Developmental temperature, genotype,
167 and their interaction were included as fixed effects, and strain, batch, and lane in the thermal
168 gradient (L) were included as random effects:

169 $$T_{pref} \sim G + T + G \times T + B + S + L.$$

170 We did the same for $T_{breadth}$. We then determined whether groups significantly differed in T_{pref} or
171 $T_{breadth}$ using Tukey contrasts with the multcomp package (v1.4) in R (Hothorn et al. 2008).
172 Within developmental temperature treatments, we used Bayesian information criterion (BIC)
173 scores from the mclust (v5.4.5) package in R (Scrucca et al. 2016) to determine whether the

174 distribution of individual measures of T_{pref} within a group are best explained by one or multiple
175 normal distributions.

176 **Results**

177 ***Thermal tolerance depends on developmental temperature and male genotype***

178 We measured extreme heat (53°C) and cold (4°C) tolerance as a readout of differences in
179 physiological thermal adaptation between Y^M and III^M house fly males. We observed the expected
180 effect of acclimation on both heat and cold tolerance (Chown and Terblanche 2006): flies raised
181 at 18°C tolerate cold longer than the flies raised at 29°C, and flies raised at 29°C tolerate heat
182 longer than flies raised at 18°C (Figure 1). We also find that Y^M males are more cold tolerant, and
183 III^M males are more heat tolerant, consistent with the latitudinal distributions of Y^M and III^M
184 males in nature (Tomita and Wada 1989; Hamm et al. 2005; Feldmeyer et al. 2008; Kozielska et
185 al. 2008). However, the effect of genotype on thermal tolerance depends on acclimation.
186 Specifically, a linear model with an interaction between genotype (Y^M or III^M) and developmental
187 temperature fits the cold tolerance data significantly better than a model without the interaction
188 term ($\chi^2_1 = 19.3$, $p = 1.1 \times 10^{-5}$). This provides evidence for a G×T effect on cold tolerance—Y^M
189 males are more cold tolerant than III^M males, but only if they are raised at 18°C (Figure 1B).
190 There is also a significant G×T interaction affecting heat tolerance ($\chi^2_1 = 4.71$, $p = 0.030$
191 comparing models with and without the interaction term): III^M males are more heat tolerant than
192 Y^M males, but only if raised at 29°C (Figure 1D).

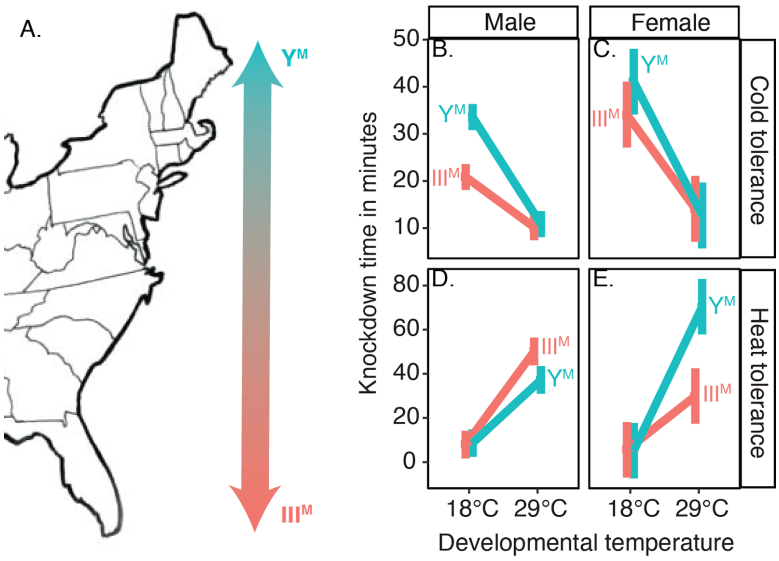


Figure 1 - Thermal tolerance in males and females. (**A**) Map of the eastern United States, showing the cline of Y^M (more common in the north) and III^M (more common in the south). (**B–E**) Graphs show the effect of developmental temperature on knockdown time at either 4°C (cold tolerance) or 53°C (heat tolerance) for Y^M (turquoise) and III^M (salmon) male flies. Proto-Y chromosomes for females reflect whether males from the strain carry the Y^M or III^M chromosome. Mean knockdown time is plotted for each combination of genotype and temperature. Error bars represent standard error.

We next attempted to identify a threshold temperature for the genotype-specific benefits of acclimation by comparing heat and cold tolerance of flies raised at 22°C and 29°C (instead of 18°C and 29°C). We did not observe a significant effect of the interaction between developmental temperature and male genotype on extreme cold tolerance ($\chi^2_1 = 0.947$, $p = 0.331$ comparing models with and without an interaction term) (Figure S2). We therefore hypothesize that there is a

threshold temperature between 18°C and 22°C, below which Y^M males experience a greater benefit of cold acclimation than III^M males. In contrast, there is a significant interaction between genotype and developmental temperature on heat tolerance when comparing males raised at 22°C and 29°C ($\chi^2_1 = 11.02$, $p = 9.0 \times 10^{-4}$ comparing models with and without the interaction term) (Figure S2). Therefore, the threshold for a genotype-specific benefit from heat acclimation lies between 22°C and 29°C.

We do not expect any difference in heat or cold tolerance across females from our different strains because all females have the same genotype, regardless of the male genotype in the strain. Indeed, a model with an interaction between developmental temperature and male genotype does not fit the female cold tolerance data better than a model without the interaction term ($\chi^2_1 = 1.46$, $p = 0.23$) (Figure 1C). There is a significant effect of developmental temperature on cold tolerance in females ($\chi^2_1 = 43.5$, $p = 4.3 \times 10^{-11}$ comparing a model with and without developmental temperature), demonstrating that females benefit from cold acclimation regardless of male genotype (Figure 1C). Surprisingly, there is a significant interaction between male genotype and developmental temperature on heat tolerance in females ($\chi^2_1 = 10.4$, $p = 0.0013$ comparing a model with and without the interaction term). In general, females raised at warmer temperatures are more heat tolerant (Figure 1E). However, the interaction of male genotype and developmental temperature is in the opposite direction from what would be expected based on the latitudinal distribution of Y^M and III^M: females from strains with Y^M males that are raised at 29°C are more heat tolerant than females from III^M strains raised at 29°C (Figure 1E). We thus conclude that the heat and cold tolerance differences between Y^M and III^M males are specific to

males and/or the proto-Y chromosomes (i.e., not genetic background) because we do not observe the same heat or cold tolerance differences in females from those strains (who do not carry the proto-Y chromosomes).

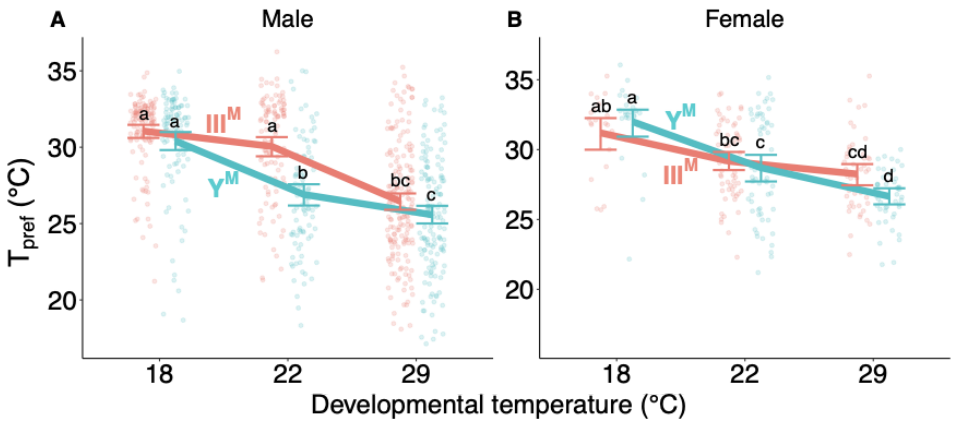


Figure 2 - Thermal preference (T_{pref}) of (**A**) male and (**B**) female house flies according to genotype (III^M = salmon points and line, Y^M = turquoise points and line) and developmental temperature. Each point depicts the mean thermal preference for an individual fly, with lines and error bars denoting means within groups and standard errors of the mean, respectively. Significant differences between groups are denoted by letters, with differing letters highlighting significantly different mean thermal preferences (Tukey's *post hoc* test, $p < 0.05$).

Thermal preference depends on developmental temperature and male genotype

We next tested if genotype and developmental temperature affect thermal preference (T_{pref}). First, we find that T_{pref} is inversely proportional to developmental temperature (Figure 2), with house flies that develop at a warmer temperature preferring cooler temperatures (and *vice versa*), regardless of sex (male: $F_{2, 742.7} = 138.4$, $p < 1.0 \times 10^{-5}$; female: $F_{2, 245.3} = 37.1$, $p = 1.19 \times$

240 10^{-4}; Figure 2). This is consistent with how developmental acclimation affects T_{pref} in *Drosophila*
241 (Dillon et al. 2009).

242 We also find that male proto-Y chromosome genotype (Y^M vs III^M) affects T_{pref} ($F_{1, 756.2}$=
243 44.5, $p < 1.0 \times 10^{-5}$). There is also a significant interaction effect between developmental
244 temperature and genotype on T_{pref} in males ($F_{2, 756.3} = 8.47$, $p = 2.31 \times 10^{-4}$, Figure 2A). Male T_{pref}
245 is similar across genotypes when they develop at either 18°C or 29°C. However, when reared at
246 22°C, III^M males prefer warmer temperatures than Y^M males (Tukey's *post hoc* test, $p < 0.001$).
247 This is consistent with III^M males being more common at lower latitudes (where average
248 temperatures are warmer), and Y^M males more common at higher latitudes (Tomita and Wada
249 1989; Hamm et al. 2005; Feldmeyer et al. 2008; Kozielska et al. 2008). We do not expect
250 differences in T_{pref} in females across strains because all females have the same genotype. Indeed,
251 the genotype of males in a strain (Y^M vs III^M) and the interaction between male genotype and
252 female developmental temperature showed no significant effect on T_{pref} in females (ANOVA, all p
253 > 0.1 in Figure 2B).

254 ***Thermal breadth depends on sex and thermal preference***

255 We used thermal breadth ($T_{breadth}$) as a measure of the specificity of T_{pref}. Male $T_{breadth}$ was
256 not significantly affected by either developmental temperature, genotype, or the interaction
257 between genotype and developmental temperature (ANOVA, all $p > 0.1$, Figure S3A). In contrast,
258 developmental temperature ($F_{2, 236.9} = 16.5$, $p < 1.0 \times 10^{-5}$), as well as the interaction between
259 developmental temperature and male genotype ($F_{2, 243.6} = 5.35$, $p = 0.005$), had significant effects
260 on $T_{breadth}$ in females. However, the significant interaction is of small effect, as females from

strains with differing male genotypes do not significantly differ in $T_{breadth}$ within any developmental temperature treatment (Figure S3B).

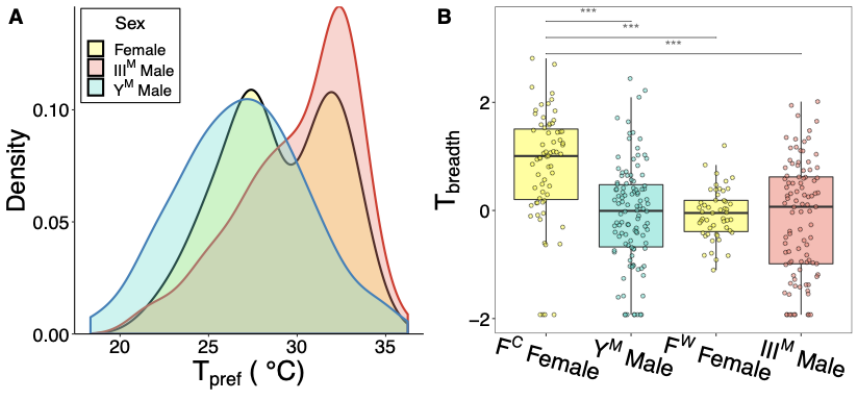


Figure 3 - Thermal breadth ($T_{breadth}$) depends on male genotype and sex. (**A**) Distribution of individual-level mean thermal preferences (T_{pref}) of III^M males, Y^M males, and pooled females that developed at 22°C. Y-axis represents relative density of data points and is analogous to frequency of data points for a given T_{pref} value. (**B**) $T_{breadth}$ of individuals raised at 22°C according to group (F^C = cold-preferring females, F^W = warm-preferring females). Boxplots denote median values and lower- and upper- quartiles. Asterisks denote significant differences in $T_{breadth}$ between groups (***: Tukey's *post hoc* test, $p < 0.01$).

The effect of developmental temperature on $T_{breadth}$ in females is driven by increased variance in T_{pref} when females develop at 22°C. The increased variance in female T_{pref} can be explained by a mixture of two normal distributions (Figure 3A, see Table S1 for statistics). This bimodal distribution is not a result of differences across strains because the same pattern was observed among females separately analyzed based on male genotype (Figure S4). In

comparison, a single normal distribution best fit Y^M male T_{pref} when developed at 22°C, and two normal distributions best explained the III^M male T_{pref} when developed at 22°C. Upon inspection, however, the two distributions representing III^M male T_{pref} likely correspond to the tail (mean of 28.7°C and large variance of 10.4°C) and peak (mean of 32.6°C and small variance of 0.4°C) of a single skewed distribution, which we are unable to detect using the mclust package we used to fit distributions to our data.

We used our model-based clustering analysis of T_{pref} to classify individuals that developed at 22°C into one of four groups: Y^M males (lower T_{pref}), III^M males (higher T_{pref}), females with cooler T_{pref} (F^C females, 59.3% of females tested), and females with warmer T_{pref} (F^W females, 40.7% of females tested). The mean T_{pref} of F^C females (26.90°C) is nearly equal to the mean T_{pref} of Y^M males (26.87°C; Figure 3A). Similarly, the mean T_{pref} of F^W females (32.2°C) is near the mode of the T_{pref} of III^M males (32.0–32.5°C; Figure 3A).

We further find that T_{pref} is predictive of $T_{breadth}$ for flies that develop at 22°C. We considered flies from our four T_{pref} groups (Y^M males, III^M males, F^C females, and F^W females), and we found a significant effect of group on $T_{breadth}$ ($F_{3, 32.9} = 9.40$, $p = 1.24 \times 10^{-4}$). Specifically, F^C females have significantly greater $T_{breadth}$ than all other groups (Tukey's *post hoc* test, all $p < 1.0 \times 10^{-5}$, Figure 3B). Therefore, if we consider $T_{breadth}$ as a measure of the strength of T_{pref}, adult house flies can be summarized by one of three phenotypes related to thermal behavior when developed at 22°C: a relatively strong preference for warm temperatures (III^M males and F^W females, which have high T_{pref} and low $T_{breadth}$), a strong preference for cooler temperatures (Y^M

295 males, with low T_{pref} and low $T_{breadth}$), and a relatively weak preference for cooler temperatures
296 (F^C females, with low T_{pref} and high $T_{breadth}$).

297 **Discussion**

298 We tested if thermal tolerance and preference depend on sex and male genotype in house
299 fly. We find that males carrying the Y^M chromosome (which is common in the northern end of the
300 species' range) are more cold tolerant and prefer colder temperatures. Conversely, males carrying
301 the III^M chromosome (which is common in the southern end of the species' range) are more heat
302 tolerant and prefer warmer temperatures. Our results are therefore consistent with the general
303 trend that temperate populations are typically more cold-tolerant than (sub-) tropical ones (Gibert
304 and Huey 2001; Hoffmann et al. 2002). Moreover, these differences in thermal tolerance and
305 preference depend on developmental temperature, and they are not observed in congener females
306 from the same strains (who do not carry the Y^M or III^M chromosome). However, females exhibit a
307 bimodal T_{pref}, with females from each of the two subgroups overlapping with one of the male
308 genotypes.

309 ***Thermal tolerance and preference depend on developmental temperature, genotype, and sex***

310 Our results demonstrate, to the best of our knowledge, the first documented example of
311 concordant temperature preference, cold tolerance, and heat tolerance across genotypes within a
312 species. We find that Y^M males both have greater cold tolerance and prefer colder temperatures,
313 whereas III^M males have greater heat tolerance and prefer warmer temperatures (Figures 1 and 2),
314 consistent with their latitudinal distributions (Tomita and Wada 1989; Hamm et al. 2005;
315 Feldmeyer et al. 2008; Kozielska et al. 2008). Previous work has identified concordant T_{pref} and

316 heat tolerance differences across species (Qu et al. 2011), or found no clear relationship between
317 thermal tolerance and preference across genotypes within species (Yang et al. 2008; Rego et al.
318 2010; Castañeda et al. 2019).

319 We observed strong effects of developmental temperature on both thermal tolerance and
320 preference that depend on both genotype and sex. Acclimation effects on heat and cold tolerance
321 (Figure 1) are well-documented for ectotherms, including flies and other insects (Bowler and
322 Terblanche 2008). An inverse relationship between developmental temperature and thermal
323 preference has also been observed in other flies (Dillon et al. 2009; Castañeda et al. 2013).
324 Behaviorally navigating towards compensatory temperatures could serve as a means of mitigating
325 the costs of thermally suboptimal development (i.e., too hot or too cold). The observed
326 relationships between thermal tolerance and developmental temperatures are unlikely to be the
327 result of natural selection during acclimation within our experiment for two reasons. First, there is
328 unlikely to be sufficient genetic variation in these inbred strains for selection to generate these
329 results within 2 generations. Second, prior attempts at selecting for thermal tolerance in house
330 flies resulted in negligible differences in tolerance across developmental temperatures (Geden et
331 al. 2019). We therefore conclude that the differences in thermal tolerance (and preference)
332 between Y^M and III^M males have evolved across the natural populations from which we sampled
333 the Y^M and III^M chromosomes.

334 There are important methodological implications for our observation that variation in
335 thermal preference across genotypes depends on developmental temperature. We only observe
336 warmer (colder) thermal preferences in III^M (Y^M) males when developed at 22°C; thermal

337 preference did not differ between male genotypes when raised at more extreme (18°C, 29°C)
338 temperatures (Figure 2A). Previous studies attempting to estimate genetic variance in thermal
339 preference within or among populations of *Drosophila* have had mixed results. While some
340 studies identified genetic variance among populations within species (Good 1993; Castañeda et
341 al. 2013), others did not detect substantial variance within (Krstevska and Hoffmann 1994) or
342 among species (MacLean et al. 2019). Our results show that the phenotypic presentation of
343 genetic variation for thermal preference can depend on the environmental conditions experienced,
344 which could explain why this variance was not detected in other experiments.

345 We identified a female-specific plasticity for thermal preference that does not map to
346 genotype. In females, we found that neither thermal tolerance nor thermal preference differ
347 predictably between strains where males carry different proto-Y chromosomes (Figures 1C, E and
348 2B). However, there is a bimodal thermal preference for females that develop at 22°C (Figure
349 3A), regardless of congener male genotype. In addition, females that had colder T_{pref} when
350 developed at 22°C also had a larger $T_{breadth}$ (Figure 3B). This suggests that, in nature, females with
351 colder temperature preferences may occupy a wider range of temperatures than females with
352 warmer temperature preferences. Because all females in our experiment are expected to have the
353 same genotype, we hypothesize that these differences in T_{pref} and $T_{breadth}$ are conferred by a plastic
354 response to some yet to be characterized factor (e.g., microclimates within larval rearing
355 containers). Alternatively, this plasticity could have a stochastic origin that is intrinsic to the
356 development of thermal preference (Honegger and de Bivort 2018; Jensen 2018).

357 The correlation between thermal preference and thermal breadth at 22°C is
358 female-specific: Y^M and III^M males have similar $T_{breadth}$ values when raised at 22°C despite their
359 differences in T_{pref}. Although general sex differences in thermal tolerance (Hoffmann et al. 2005)
360 and thermal preference (Krstevska and Hoffmann 1994) have been documented, this is the first
361 study, to our knowledge, to identify sex differences in the relationship between thermal
362 preference and thermal breadth. Our results suggest that male and female house flies exhibit
363 different thermoregulatory behavioral patterns which may further be influenced by genotype.
364 Directly identifying a sex-by-genotype-by-environment interaction is beyond the scope of this
365 study because sex and genotype are confounded in our experimental design (the females in our
366 experiment have a different genotype from either male, characterized by a lack of either the III^M
367 or Y^M chromosome). Nonetheless, the house fly is a tractable system for directly testing for
368 sex-specific genotype-by-environment interactions on thermoregulation. For example, future
369 work could test for sex-specific effects of Y^M and III^M by measuring phenotypes in females
370 carrying a proto-Y chromosome along with the epistatic female-determining *Md-tra*^{D} allele
371 (Hediger et al. 2010; Hamm et al. 2014).

372 ***Environmental heterogeneity and the maintenance of polygenic sex determination***

373 Sex determination pathways rapidly diverge across species, driving evolutionary turnover
374 of sex chromosomes (Bull 1983; Beukeboom and Perrin 2014). Polygenic sex determination
375 systems, in which more than one master sex determining locus segregate independently on
376 different chromosomes, have been observed in multiple animal species (Moore and Roberts
377 2013). Most population genetic models that attempt to explain the stable maintenance of

378 polygenic sex determination focus on sexually antagonstic effects of sex determining loci or
379 linked alleles on sex chromosomes (Rice 1986; van Doorn and Kirkpatrick 2007; Meisel et al.
380 2016). Less attention has been given to ecological factors that can maintain polygenic sex
381 determination (cf. Bateman and Anholt 2017).

382 Our results demonstrate how spatially variable ecological factors can maintain polygenic
383 sex determination. Specifically, thermal tolerance and preference phenotypes conferred by the Y^M
384 and III^M chromosomes (Figures 1 and 2) are consistent with the clinal and temperature-dependent
385 distributions of the Y^M and III^M chromosomes (Tomita and Wada 1989; Hamm et al. 2005;
386 Feldmeyer et al. 2008; Kozielska et al. 2008). Previous experiments identified multiple fitness
387 advantages conferred by the III^M chromosome over Y^M at warmer temperatures, including an
388 increase in frequency of III^M over generations in a laboratory population (Hamm et al. 2009).
389 However, these fitness differences can only explain the invasion or fixation of the III^M
390 chromosome, not the maintenance of the polymorphism. In contrast, differences in thermal
391 tolerance and preference could maintain proto-Y chromosome polymorphism across the species'
392 range, similar to how selection maintains other clinal variation (Slatkin 1973; Endler 1977).

393 The house fly system reveals how temperature variation can contribute to the maintenance
394 of polygenic sex determination independently of selection on the sex-determination pathway
395 itself. Temperature is an important contributor to the evolution of sex determination pathways in
396 vertebrates (Bull and Vogt 1979; Holleley et al. 2015). However, the effects of the house fly
397 proto-Y chromosomes on thermal tolerance and preference likely act independently of the sex
398 determination pathway because there are not differences in the expression of sex determination

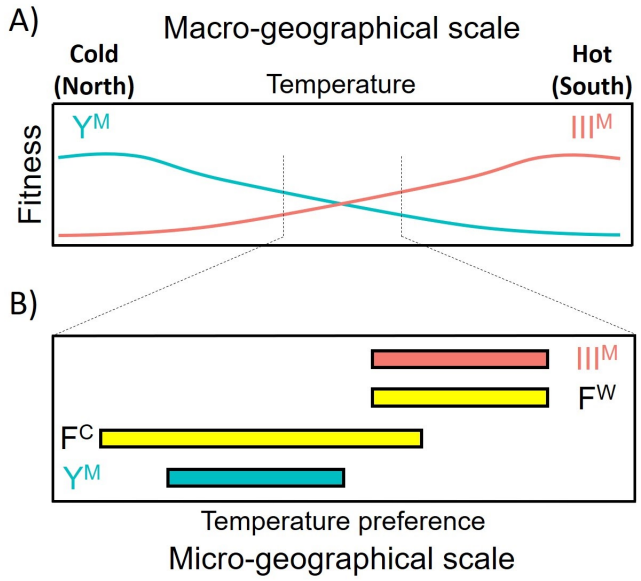
399 genes across house fly male genotypes raised at different temperatures in a way that is consistent
400 with their clinal distribution (Adhikari et al. n.d.). This suggests that the effects of the Y^M and III^M
401 chromosomes on thermal phenotypes is a result of alleles on proto-Y chromosomes that are
402 genetically linked to the male-determining locus, as opposed to the male-determiner itself.
403 Therefore, our results highlight how temperature can be important for the evolution of sex
404 determination independently of temperature-dependent activity of the sex determination pathway.
405 Future theoretical work should consider the effect of spatially heterogeneous selection pressures
406 on the maintenance of polygenic sex determination, similar to how temporal heterogeneity can
407 create fluctuating selection pressures that maintain polygenic sex determination (Bateman and
408 Anholt 2017).

409 ***Selection on thermal phenotypes may depend on geographical scale***

410 Our results suggest that selection on thermal traits differs between macro-geographic
411 species ranges and at a micro-geographical scale within populations. Thermal tolerance and
412 preference in male house flies depend on proto-Y chromosomes genotype in a way that is
413 consistent with the latitudinal distribution of the Y^M and III^M chromosomes (Figures 1 and 2).
414 This suggests that, at the macro-geographic scale, selection is operating on male physiology and
415 behavior to create or maintain the clinal distribution of Y^M and III^M (Figure 4A). In comparison,
416 at an intermediate developmental temperature (22°C), female thermal preference is bimodal for a
417 reason that we have yet to determine (Figure 3A). This raises the possibility that within
418 populations near the center of the cline, where Y^M and III^M both segregate (e.g., Hamm and Scott

419 2008; Meisel et al. 2016), sexual selection may favor males that can preferentially obtain access
420 to the two different female phenotypes.

421 III^M males may disproportionately benefit from differences in T_{pref} and $T_{breadth}$ between
422 males and females. F^C females that prefer colder temperatures have greater $T_{breadth}$ than warm
423 preferring F^W females and both male genotypes (Figure 3B), suggesting that F^C females occupy a
424 wider range of thermal habitats. Thus, III^M males may gain an advantage by having greater access
425 to F^W females, as well as occasional access to F^C females, in contrast to Y^M males who would
426 only be likely to encounter F^C females (Figure 4B). This raises the possibility that differences in
427 thermal preference across genotypes and sexes could affect the dynamics of sexual selection.



428 **Figure 4** - Selection on the III^M and Y^M chromosomes likely differs across geographic scales. (**A**)
429 At the macro-geographical scale, selection for thermal tolerance and/or thermal preference results
430 in the clinal distribution of the Y^M (turquoise) and III^M (salmon) chromosomes. (**B**) At

431 intermediate developmental temperatures, male genotypes (Y^M vs III^M) differ in thermal
432 preference, which may create asymmetrical mating opportunities because of variation in female
433 thermal preference and breadth (F^C vs F^W). The asymmetry of the overlap of males and females at
434 the intermediate developmental temperature could affect sexual selection in populations where
435 Y^M and III^M both segregate.

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Author Contributions

PD, KA, and RM conceived and designed the study. KA and JC collected, and KA analyzed, all thermal tolerance data. PD, RP, JT, and AM collected, and PD analyzed, all thermal preference data. PD, KA, and RM wrote the manuscript, and all authors reviewed the manuscript prior to submission.

Data Accessibility

All data files used for analyses described in this manuscript have been deposited in Dryad (doi:10.5061/dryad.n2z34tmvs). Raw video and image files from thermal preference assays are available from the authors upon request.

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