

1 Frequencies of house fly proto-Y chromosomes across populations are
2 predicted by temperature heterogeneity within populations

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22 Abstract

23 Sex chromosomes often differ between closely related species and can even be polymorphic
24 within populations. Species with polygenic sex determination segregate for multiple different sex
25 determining loci within populations, making them uniquely informative of the selection pressures
26 that drive the evolution of sex chromosomes. The house fly (*Musca domestica*) is a model
27 species for studying polygenic sex determination because male determining genes have been
28 identified on all six of the chromosomes, which means that any chromosome can be a “proto-Y”
29 chromosome. In addition, chromosome IV can carry a female-determining locus, making it a W
30 chromosome. The different proto-Y chromosomes are distributed along latitudinal clines on
31 multiple continents, their distributions can be explained by seasonality in temperature, and they
32 have temperature-dependent effects on physiological and behavioral traits. It is not clear,
33 however, how the clinal distributions interact with the effect of seasonality on the frequencies of
34 house fly proto-Y chromosomes across populations. To address this question, we measured the
35 frequencies of house fly Y and W chromosomes across nine populations in the United States of
36 America. We confirmed the clinal distribution along the eastern coast of North America, but it is
37 limited to the eastern coast. In contrast, annual mean daily temperature range is significantly
38 correlated with proto-Y chromosome frequencies across the entire continent. Our results
39 therefore suggest that temperature heterogeneity can explain the distributions of house fly
40 proto-Y chromosomes in a way that does not depend on the cline. These results contribute to
41 our understanding of how ecological factors affect sex chromosome evolution.

42 Introduction

43 Sex chromosomes and sex determining genes often differ between even closely related
44 species (Bachtrog et al. 2014; Beukeboom and Perrin 2014). Evolutionary changes in sex
45 chromosomes typically occur via one of two methods (Abbott et al. 2017). First, an autosome
46 can fuse to a sex chromosome, turning the autosome into a neo-sex chromosome. Second, an
47 autosome can acquire a new master sex determining locus, turning the autosome into a
48 proto-sex chromosome (and allowing the ancestral sex chromosome to “revert” to an
49 autosome). Sex-specific selection pressures are thought to be important for the invasion and
50 fixation of both neo- and proto-sex chromosomes because sex-linkage can resolve inter-sexual
51 conflicts (van Doorn and Kirkpatrick 2007; Roberts et al. 2009; van Doorn 2014; Mank et al.
52 2014). In addition, if sex ratios are distorted from their evolutionary stable equilibrium, a new sex
53 determiner on a proto-sex chromosome can be favored if it increases the frequency of the sex
54 that is below its equilibrium value (Bull 1983; Werren and Beukeboom 1998). Notably, ecological
55 factors can modulate the effects of these sex-specific selection pressures, but the extent to
56 which ecological selection pressures drive sex chromosome evolution are not yet resolved
57 (Meisel 2022).

58 We used the house fly (*Musca domestica*) as a model species to explore how ecological
59 factors affect sex chromosome evolution. House fly is well-suited for this purpose because it has
60 a highly polymorphic multifactorial sex determination system (Hamm et al. 2015). Male
61 determining factors have been genetically mapped to all six of the house fly chromosome pairs,
62 and a single gene (*Mdmd*) has been implicated as the male-determiner on at least four of the six
63 chromosomes (Sharma et al. 2017). Each of these *Mdmd*-bearing chromosomes is a young
64 “proto-Y” chromosome (Meisel et al. 2017; Son and Meisel 2021). Nearly every male house fly
65 in North America carries one or both of the two most abundant proto-Y chromosomes, the Y
66 chromosome (Y^M) and third chromosome (III^M), and males with other proto-Y chromosomes are
67 rarely found (Hamm et al. 2015). Y^M and III^M form latitudinal clines in Europe, Japan, and North
68 America, with Y^M most common in northern populations and III^M predominating in the south
69 (Franco et al. 1982; Denholm et al. 1986; Tomita and Wada 1989; Hamm et al. 2005).
70 Consistent with this geographical distribution, the Y^M chromosome confers greater tolerance to
71 extreme cold and preference for cooler temperatures, while III^M confers improved tolerance to
72 extreme heat and preference for warmer temperatures (Delclos et al. 2021). In addition, higher
73 Y^M frequency is associated with locations with low seasonality of temperatures, or small
74 differences between minimum and maximum values of monthly high and low temperatures
75 (Feldmeyer et al. 2008). Moreover, in some house fly populations, males can carry multiple
76 proto-Y chromosomes (e.g., both Y^M and III^M , or homozygous for III^M), which could create
77 male-biased sex ratios and selection in favor of a female-determining factor (Eshel 1975; Bull
78 and Charnov 1977; Bulmer and Bull 1982). Indeed, such a female-determiner exists in house fly
79 populations, in the form of a dominant allele of the house fly ortholog of *transformer* (*Md-tra^D*),
80 which causes embryos to develop into females even if they carry multiple male-determining
81 chromosomes (Hediger et al. 2010). The frequency of *Md-tra^D* is correlated with the frequency of
82 males with multiple male-determining chromosomes across populations, suggesting that there is
83 selection for balanced sex-ratios (Meisel et al. 2016).

84 We aimed to test if the frequencies of Y^M , III^M , and *Md-tra^D* across North American
85 populations could be explained by climatic variables. Previous studies linking the frequencies of

86 male-determining chromosomes to climatic variation in North America have been limited to a
87 latitudinal cline along the eastern coast (Hamm et al. 2005; Hamm and Scott 2008), and only
88 Japanese and African populations were sampled to study how *Md-tra^D* frequencies vary across
89 climates (Feldmeyer et al. 2008). However, the frequencies of Y^M , III^M and *Md-tra^D* vary in
90 non-clinal patterns across regions of North America outside the eastern coast (McDonald et al.
91 1975; Meisel et al. 2016), suggesting that different climatic variables may predict their
92 distribution outside the cline. To address this question, we genotyped male and female house
93 flies from nine different locations across the United States of America, and we tested if the
94 frequencies of Y^M , III^M and *Md-tra^D* were correlated with a variety of climatic variables.

95 **Materials and Methods**

96 *House fly collections*

97 House flies were collected from dairy farms, poultry farms, and other locations where
98 flies are present in nine different populations across the United States of America (Supplemental
99 Table S1). Collections were performed in May–June 2021 using sweep nets. Flies were allowed
100 to lay eggs in laboratories near the collection sites. Resulting pupae were then shipped to
101 Cornell University (Ithaca, NY), where colonies from each collection site were established.
102 Pupae from each of those colonies were then shipped to the University of Houston (Houston,
103 TX) within 4 generations of establishing the laboratory colonies. Those pupae were raised into
104 adults in laboratory conditions (22°C) at the University of Houston, and the emerging adults
105 were frozen for genotyping.

106 *DNA extraction and genotyping*

107 DNA was extracted from individual frozen house fly heads using the hot sodium
108 hydroxide and tris, HotSHOT, protocol (Truett et al. 2000). We performed PCR to test for the
109 presence of Y^M using the A12CMF1 and A12CMR1 primer pair (Hamm et al. 2009). We were
110 unable to design a PCR primer pair that could reliably identify the III^M chromosome. We used
111 the GM2IIIF1 and GM2IIIR2 primer pair as a positive control to confirm successful DNA
112 amplification from males (Hamm et al. 2009). We tested for *Md-tra^D* in females using a primer
113 pair that amplifies a region of exon 3 of the *Md-tra* gene containing the diagnostic deletion
114 (Hediger et al. 2010; Meisel et al. 2016). We tested for the presence of *Mdmd* in females using
115 the Mdmd_F1 and Mdmd_R4 primer pair (Sharma et al. 2017).

116 Each male was genotyped for the presence of Y^M . From these data, we determined the
117 frequencies of males with Y^M and males without Y^M (Figure 1A). The latter group (males without
118 Y^M) presumably consists of III^M males, but some males without Y^M may also carry III^M . We used
119 population genetic simulations (see below) to estimate the frequency of III^M along with the
120 frequencies of all possible male genotypes in each population (Figure 1B).

121 Each female was genotyped for the presence of *Md-tra^D*, *Mdmd*, and Y^M . For each
122 population, we determined the frequencies of: females without *Md-tra^D*; females with *Md-tra^D* but
123 not *Mdmd*; females with *Md-tra^D* and Y^M ; and females with *Md-tra^D* and *Mdmd* but not Y^M

124 (Figure 1A). We also used these data to determine the frequencies of *Md-tra^D*, *Mdmd* in
125 females, and *Mdmd* in *Md-tra^D* females.

126 *Population genetic simulations to determine genotype frequencies*

127 We used population genetic simulations to predict the frequencies of 18 possible sex
128 chromosome genotypes in each sampled population (Figure 1B). Our PCR genotyping method
129 is unable to detect the III^M chromosome nor is it able to diagnose specific genotypes, which
130 means our genotype data are incomplete. There are a total of 18 possible genotypes (8 male
131 and 10 female) when considering all possible combinations of Y^M, III^M, and *Md-tra^D* (Meisel
132 2021). To help overcome the deficiency in our genotyping protocol, we performed population
133 genetic simulations in order to identify genotype frequencies that would produce the frequencies
134 of Y^M and *Md-tra^D* observed in our data (Figure 1C). Our simulations used the same recursion
135 equations that we have previously used to model randomly mating house fly populations,
136 without selection (Meisel et al. 2016). We performed separate simulations for each of the nine
137 sampled populations in order to estimate the frequencies of Y^M, III^M, *Md-tra^D*, and each genotype
138 in each population.

139 In the simulations, we calculated the frequency of a chromosome (Y^M or III^M) or allele
140 (*Md-tra^D*) as follows. The frequency of Y^M was calculated as the number of Y^M chromosomes in
141 a population divided by the sum of the number of Y^M and X chromosomes in that population.
142 The frequency of the III^M chromosome was calculated as the number of third chromosomes with
143 *Mdmd* (i.e., III^M) divided by the total number of third chromosomes. The frequency of *Md-tra^D*
144 was calculated as the number of *Md-tra^D* alleles divided by the total number of *Md-tra* genes.
145 The frequencies of Y^M and III^M can take values between 0 and 1, while the *Md-tra^D* frequency
146 can take values between 0 and 0.25 (because it is a W chromosome).

147 We started each simulation with estimates of the frequencies of Y^M, III^M, and *Md-tra^D*
148 from our PCR assay. The initial frequency of Y^M (f_{YM}) was estimated as half of the frequency of
149 males carrying a Y^M chromosome. This initial frequency assumes that all males carrying Y^M also
150 carry an X chromosome, and all copies of Y^M are found in heterozygous individuals. The initial
151 frequency of III^M (f_{IIIM}) was estimated as $\frac{1}{2} - f_{YM}$, which assumes all males without Y^M carry
152 one copy of III^M. Both of these calculations assume that f_{YM} and f_{IIIM} are equal in males and
153 females. The initial frequency of *Md-tra^D* (f_{traD}) was estimated as one quarter of the number of
154 females carrying *Md-tra^D*, which requires no assumptions about genotypes because each
155 female carrying *Md-tra^D* must be heterozygous and males cannot carry *Md-tra^D*.

156 From the initial estimated frequencies of Y^M, III^M, and *Md-tra^D*, we calculated initial
157 frequencies of each of the 18 possible genotypes. We first calculated the initial frequencies of
158 each single chromosome genotype assuming random mating. For example, the frequency of the
159 X/X genotype was estimated as $(1 - f_{YM})^2$; the X/Y^M frequency was estimated as
160 $2f_{YM}(1 - f_{YM})$; and the Y^M/Y^M frequency was estimated as f_{YM}^2 . Similar calculations were
161 performed to estimate the frequencies of the third chromosome genotypes. The frequency of the
162 *Md-tra^D*/*Md-tra⁺* genotype was estimated as $f_{traD}(1 - f_{traD})$, and the frequency of the

163 *Md-tra⁺/Md-tra⁺* genotype was estimated as $(1 - f_{traD})^2$. The initial frequencies of the 18
164 multi-chromosome genotypes were then calculated using the product of each single
165 chromosome genotype, and each of the 18 frequencies were divided by the sum to obtain a
166 new estimate that summed to one.

167 We next performed simulations for 10 generations of random mating using those initial
168 genotype frequencies and previously developed recursion equations (Meisel et al. 2016) to
169 determine the equilibrium frequencies of each chromosome and genotype, given the initial
170 genotype frequencies. We compared the resulting values of f_{YM} and f_{traD} after 10 generations
171 with the observed values measured in the respective natural population. We tested if the
172 simulated values were within 0.002 of the observed values (Figure 1C). If the simulated
173 frequency of a chromosome was less than the observed frequency, we increased the initial
174 frequency and repeated the simulation. Conversely, if the simulated frequency was greater than
175 the observed frequency, we decreased the initial frequency and repeated the simulation. We
176 repeated this process until the simulated frequencies of f_{YM} and f_{traD} matched the observed
177 frequencies within 0.002. We used these simulated frequencies at equilibrium (i.e., after 10
178 generations) as estimates of the chromosome and genotype frequencies in downstream
179 analyses.

180

(A) PCR assay for: <ul style="list-style-type: none">• Y^M in males• <i>Md-tra^D</i> in females• <i>Mdmd</i> in females• Y^M in females	(B) Simulations of populations with Y^M , III^M , and <i>Md-tra^D</i> (C) Test if simulated frequencies of Y^M and <i>Md-tra^D</i> are within 0.002 of observed values <ul style="list-style-type: none">(i) If not, adjust starting frequencies and repeat step (B)(ii) If yes, use simulated frequencies of Y^M, III^M, and <i>Md-tra^D</i> as estimates of frequencies in each population
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181

182 **Figure 1.** Approach to estimate the frequencies of Y^M , III^M , and *Md-tra^D* in each of the nine sampled
183 populations. **A.** PCR was used to determine the frequencies of proto-sex chromosomes and sex
184 determining loci/alleles. **B.** Simulations were performed to determine equilibrium frequencies of proto-sex
185 chromosomes in randomly mating populations. **C.** If those equilibrium frequencies deviated from the
186 observed frequencies (i), then the starting frequencies were adjusted and the simulations repeated. If the
187 equilibrium frequencies were similar to the observed frequencies in a population (ii), then the equilibrium
188 frequencies were used as estimates of the frequencies of Y^M , III^M , and *Md-tra^D* in a population.

189 *Climate data*

190 We tested if climatic features were associated with the frequencies of sex chromosomes
191 and genotypes across the sampled populations. To do so, we obtained weather data from the
192 nearest NOAA station to the collection site measured between 1991–2020 (Table 1;
193 Supplemental Table S1). From these data, we extracted many of the same features as a
194 previous analysis comparing the frequencies of house fly sex chromosomes and climatic data
195 (Feldmeyer et al. 2008), and we used annual precipitation (Precip) instead of humidity
196 measurements. We additionally calculated mean temperatures for only summer months
197 (May–July) because that was when the house flies in our collections were sampled. All
198 temperatures were provided in Fahrenheit, and we converted the values to Celcius for analysis.

199 We performed two separate analyses to test if climate features were associated with the
200 frequencies of Y^M , III^M , $Md-tra^D$, males with multiple proto-Y chromosomes, and males with both
201 Y^M and III^M . First, we used the `prcomp()` function in R to perform a principal component analysis
202 (PCA) on the annual climate data (with variables scaled to have unit variance), excluding the
203 measurements that only sampled summer months (May–June). We then tested if each principal
204 component (PC) is correlated or associated with Y^M or III^M frequencies across populations. We
205 also calculated pairwise rank-order (Spearman) correlations between chromosome frequencies
206 and individual climate features.

207

208

209 **Table 1.** Climate features analyzed

Name	Description
T_{mean}	Annual mean average daily temperature
T_{min}	Annual mean minimum daily temperature
T_{max}	Annual mean maximum daily temperature
Daily _{TR}	Annual mean daily temperature range
Precip	Annual precipitation
T_{active}	Mean temperature of warmest month
Season ₁	Coefficient of variation of average monthly temperatures
Season ₂	Average of difference between highest and lowest monthly maximum temperature and difference between highest and lowest monthly minimum temperature
Summer _{mean}	Mean average monthly temperature during May-July
Summer _{max}	Mean maximum monthly temperature during May-July
Summer _{min}	Mean minimum monthly temperature during May-July

210

211

212 Results

213 We used PCR assays to determine the frequencies of male house flies carrying the Y^M
214 chromosome across nine locations (populations) sampled in 2021 in the United State of
215 America (Figure 2A). We also used PCR to determine the frequencies of female house flies
216 carrying $Md-tra^D$, $Mdmd$, and Y^M in the same nine populations (Figure 2A). We then performed
217 population genetic simulations to identify frequencies of Y^M , III^M , and $Md-tra^D$ in each population
218 that could produce the observed frequencies we observed in our PCR assays (Figure 1B;
219 Supplemental Figures S1-S9). The III^M chromosome was at the highest frequency in two of the
220 three southernmost populations (CA and FL). In the FL population, males were predicted to be
221 almost entirely III^M , and there were very few $Md-tra^D$ females. In contrast, the northernmost
222 population (PA) was predicted to have almost entirely Y^M males. In addition, there was a positive
223 correlation between the predicted frequencies of males with multiple male-determining
224 chromosomes (Y^M and/or III^M) and females carrying $Md-tra^D$ ($r^2 = 0.975$, $p = 4.5 \times 10^{-7}$;
225 Figure 2C).

226 We compared our estimates of the frequencies of Y^M , III^M , $Md-tra^D$, and four sex
227 chromosome genotypes in the CA and NC populations with previous measurements in nearby
228 populations from California and North Carolina (Table 2). A population was sampled from Chino,
229 CA in 1982 and 2014 (Meisel et al. 2016), ~50 km from our CA collection site (sampled in 2021).
230 There was a high frequency of $Md-tra^D$ in both populations. However, the Chino population had
231 a higher frequency of Y^M chromosomes, while the CA population we sampled in 2021 had a
232 higher III^M frequency. In contrast, we observed similar frequencies of Y^M , III^M , and $Md-tra^D$ in the
233 NC population we sampled in 2021 and the populations sampled in 2002, 2006, and 2007. All of
234 the NC populations were sampled in close proximity within Wake County.

235 We tested for associations between climatic variables and the frequencies of sex
236 chromosomes across the nine populations we sampled. To those ends, we first performed a
237 principal component analysis (PCA) using eight climate features measured across the nine
238 populations. The first three PCs explain >98% of the variance in the data (Supplemental Table
239 S2), with PC1 and PC2 explaining nearly 90% of variance (Figure 3A). PC1 captured variation
240 in seasonality (Season₁ and Season₂), along with minimum, maximum, and average
241 temperatures (T_{mean} , T_{min} , T_{max} , and T_{active}) across populations. PC2 captured variation in
242 precipitation (Precip) and annual mean daily temperature range (Daily_{TR}) across populations.
243 We tested for correlations between PC1 or PC2 and the frequencies of sex chromosomes
244 across populations. Only PC2 and Y^M frequency had a significant correlation, with Y^M frequency
245 decreasing as PC2 values increased ($\rho = -0.767$, $p = 0.0214$). We additionally constructed linear
246 models in which we tested if PC1, PC2, and their interaction predicted the frequencies of Y^M or
247 III^M . The only significant relationship in these models was between PC2 and III^M frequency
248 ($F = 8.372$, $p = 0.034$). Therefore, there is evidence that Y^M and III^M frequencies across
249 populations were associated with PC2, which captured variation in precipitation and daily
250 temperature range.

251 We observed similar patterns when we calculated pairwise correlations between climatic
252 features and sex chromosome frequencies (Supplemental Table S3). The only significant
253 correlations were between the frequencies of Y^M or III^M and the annual mean daily temperature
254 range (Figure 3C). Specifically, the frequency of Y^M was negatively correlated with the daily

255 temperature range ($\rho = -0.883$, $p = 0.003$), and the frequency of III^M was positively correlated
 256 with the daily temperature range ($\rho = 0.783$, $p = 0.017$). These results provide consistent
 257 evidence that daily temperature range is associated with proto-Y chromosome frequencies.

258 The CA population appeared to be an outlier in many respects, which could have driven
 259 some of the patterns we observed. For example, the CA population had higher frequencies of
 260 III^M chromosomes, males with multiple male determiners, and females with *Md-tra^D*, when
 261 compared to all other populations (Figure 2). In addition, the CA site was an outlier along PC2
 262 because it had a higher daily temperature range and lower precipitation than the other
 263 populations (Figure 3). When we excluded the CA site from our climate PCA, we observed
 264 similar loadings of the climate variables: PC1 explained 71.69% of the variance and captured
 265 variation in seasonality and minimum/maximum temperature, while PC2 explained 22.13% of
 266 variance and captured variation in daily temperature range and precipitation (Supplemental
 267 Figure S10). We also observed a significant negative correlation between daily temperature
 268 range and Y^M frequency ($\rho = -0.881$, $p = 0.007$) when the CA population was excluded.
 269 Therefore, the relationships between climate features and proto-Y chromosome frequencies
 270 was not driven solely by the CA population.

271

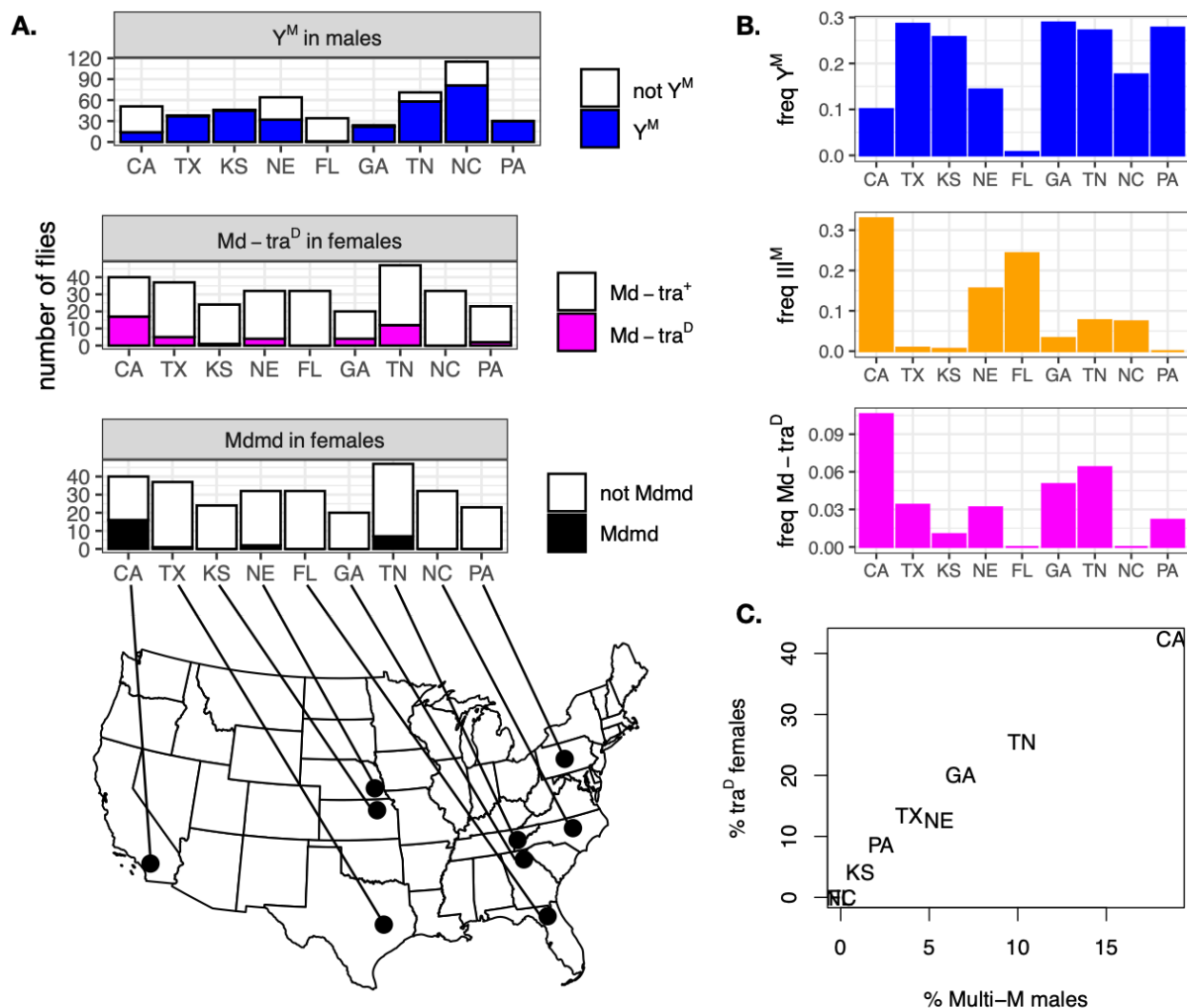
272 **Table 2.**

		Percent in Population (%)						
Genotype/ allele	Sex	Chino 1982 ^a	Chino 2014 ^a	CA 2021	NC 2002 ^b	NC 2006 ^c	NC 2007 ^c	NC 2021
X/Y ^M ; III/III	male	68.0	76.0	16.2	77.7	77.8	95.3	70.4
X/X; III ^M /III	male	0.0	8.0	65.2	20.0	19.4	2.3	29.6
X/Y ^M ; III ^M /III	male	12.0	4.0	8.8	2.3	1.4	0.0	0.0
Y ^M /Y ^M ; III/III	male	-	12.0	0.4	0.0	1.4	2.3	0.0
Y ^M	male	-	92.0	27.4	80.0	80.6	97.6	70.4
III ^M	male	-	12.0	83.4	22.3	20.8	2.3	29.6
<i>Md-tra^D</i>	female	-	33.6	42.4	5.9	-	4.2	0.0

273 a) Meisel et al. (2016)

274 b) Hamm et al. (2005)

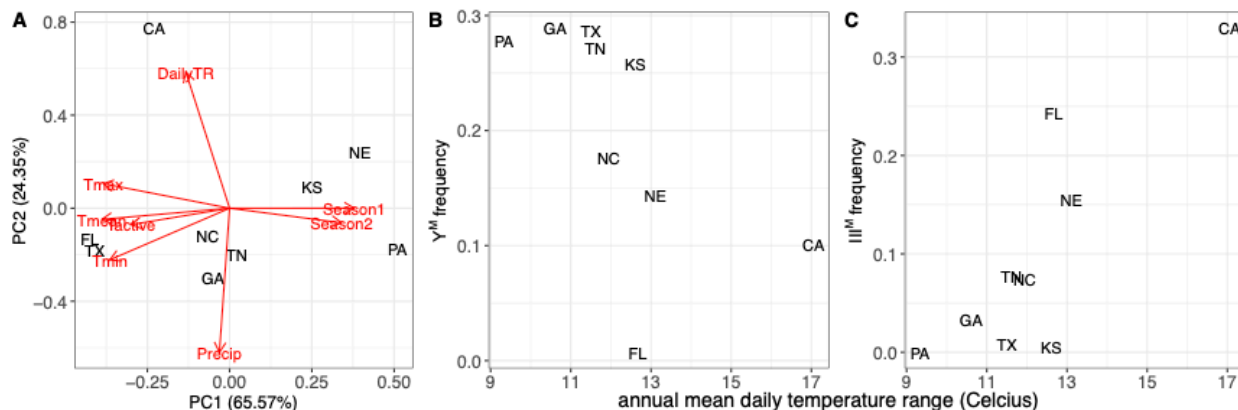
275 c) Hamm and Scott (2008)



276

277 **Figure 2.** Observed and inferred frequencies of sex chromosomes, determiners, and alleles across nine
 278 populations. **A.** The number of males genotyped with Y^M (blue bars), females with Md-tra^D (magenta
 279 bars), and females with Mdmd (black bars) from each of nine populations are plotted. The number of flies
 280 without each chromosome, allele, or gene are shown in white bars. The sampling locations for each
 281 population are indicated by dots on the map. **B.** The estimated frequencies of Y^M, III^M, and Md-tra^D in each
 282 of the nine populations are plotted. Estimated frequencies are from population genetics simulations which
 283 produced observed frequencies shown in panel A. **C.** The relationship between the percent of females
 284 carrying Md-tra^D and the percent of males with multiple male-determining chromosomes (Y^M and/or III^M)
 285 are plotted for the nine populations based on estimated genotype frequencies.

286



287

288 **Figure 3.** Associations between climate features and proto-Y chromosome frequencies across the nine
289 sampled populations. Each population is represented by the two letter abbreviation of the state from
290 which it was collected. **A.** Populations are plotted according to the first two principal components (PCs)
291 based on climate features. The loadings of each climate feature are indicated by labeled red vectors.
292 Vector labels are described in Table 1. **B-C.** The relationships between the predicted frequency of Y^M or
293 III^M and the annual mean daily temperature range are plotted for each population.

294 Discussion

295 We observed substantial variation in the frequencies of Y^M , III^M , and $Md-tra^D$ across
296 populations of house flies in North America (Figure 2). Along the eastern coast, Y^M was most
297 common in the north (PA), III^M was most common in the south (FL), and both Y^M and III^M were
298 found in the central (NC) population, consistent with the previously documented cline (Hamm et
299 al. 2005). However, moving west, we found that the clinal distribution eroded, and latitude was
300 not associated with the frequencies of Y^M and III^M . For example, the GA, TN, NE, and CA
301 populations all had moderate to high frequencies of III^M , Y^M , and $Md-tra^D$, without any
302 relationship to latitude. In addition, the presence of all three chromosomes/alleles in CA is
303 consistent with previous observations (Meisel et al. 2016).

304 We used population genetic simulation models to estimate the frequencies of Y^M , III^M ,
305 $Md-tra^D$, and all 18 genotypes in each population based on PCR assays for the presence of Y^M ,
306 $Mdmd$, and $Md-tra^D$ from individual flies (Figure 1). Our PCR assays likely do not measure
307 allele, chromosome, and genotype frequencies as accurately as more direct genotyping assays
308 that were used in prior studies (e.g., Hamm et al. 2005; Feldmeyer et al. 2008; Meisel et al.
309 2016). In addition, we sampled flies after multiple generations of lab breeding, which could lead
310 to deviations from the frequencies in natural populations. Nonetheless, we believe that our
311 estimates of allele, chromosome, and genotype frequencies were sufficiently accurate for the
312 analyses we performed. First, our estimated frequencies of Y^M , III^M , and $Md-tra^D$ are largely
313 concordant with prior estimates from the same county in North Carolina (Table 2). Second,
314 previous work found that Y^M is more common than III^M in Texas (McDonald et al. 1975),
315 consistent with our results (Figure 2). Furthermore, we predicted a positive correlation between
316 the frequencies of $Md-tra^D$ and males with multiple male-determining chromosomes (Figure 2C),
317 as expected if Y^M , III^M , and $Md-tra^D$ frequencies are under selection to maintain balanced
318 sex-ratios (Meisel et al. 2016).

319 Despite the concordance with prior results, there are discrepancies between the
320 frequencies we predicted and those previously observed in California. The CA population we
321 sampled was estimated to have a high frequency of III^M (Figure 2B), but previous collections
322 from a nearby population found that Y^M was at a higher frequency than III^M (Meisel et al. 2016).
323 This discrepancy between our observations and prior measurements from California can likely
324 be explained by a >50 km distance between our site and the previous sampling locations.
325 Similar differences in the frequencies of house fly male-determining chromosomes have been
326 observed over relatively short distances in Japan and Spain (Tomita and Wada 1989; Li et al.
327 2022). Therefore, small-scale variations in Y^M and III^M frequencies appear to be a global
328 phenomenon, in addition to the large-scale variation observed across the entire continent
329 (Figure 2).

330 Our results contribute to the body of evidence that climatic factors affect the frequencies
331 of Y^M and III^M in natural populations of house fly. In addition to the clinal distribution we
332 confirmed in eastern North America (Figure 2), we also detected consistent associations
333 between Y^M or III^M frequencies and the annual mean daily temperature range, Daily_{TR} (Figure 3).
334 This climate metric captures the extent of temperature heterogeneity within days, averaged over
335 the entire year. We predicted that Y^M was at the highest frequency when daily temperature
336 heterogeneity was lowest, and III^M frequency was higher with more daily temperature
337 heterogeneity. The same general patterns held when the CA population, which had extreme
338 values relative to other populations, was excluded.

339 Our results differ from a prior observation that the frequencies of non-Y^M,
340 male-determining chromosomes (e.g., III^M) in Africa and Europe were higher when seasonality
341 in temperature was highest (Feldmeyer et al. 2008). Feldmeyer et al. (2008) measured
342 seasonality as the difference between the minimum and maximum values of the monthly
343 minimum and maximum temperatures. We found this measure of seasonality was orthogonal to
344 daily temperature range (Figure 3A) and not significantly correlated with III^M or Y^M frequency
345 (Supplemental Table S3). However, both seasonality and daily temperature range are measures
346 of temperature heterogeneity across time, suggesting that temperature variation more generally
347 may be an important selection pressure that affects proto-Y chromosome frequencies across
348 house fly populations.

349 There is growing evidence that ecological factors contribute to sex chromosome
350 evolution (Meisel 2022). Our results contribute to evidence that temperature variation across the
351 species range predicts the frequencies of house fly proto-Y chromosomes (Franco et al. 1982;
352 Denholm et al. 1986; Tomita and Wada 1989; Hamm et al. 2005; Feldmeyer et al. 2008). In
353 addition, the proto-Y chromosomes affect thermal traits in ways that are consistent with their
354 clinal distributions (Delclos et al. 2021). These temperature-dependent phenotypic effects likely
355 create variation in the fitness effects of the proto-Y chromosomes, which in turn allow for the
356 maintenance of multiple male-determining loci across populations. This is a special case of local
357 adaptation maintaining genetic variation across populations (Wadgyman et al. 2022). It may also
358 be possible for these differences in fitness effects to promote divergence between populations
359 and subsequent speciation. These links between ecological adaptation and proto-Y
360 chromosomes could therefore provide a mechanism to explain the disproportionate effects of
361 sex chromosomes on speciation (Payseur et al. 2018).

362 It remains unclear if or how ecological selection pressures that affect sex chromosome
363 evolution are related to sex-specific selection pressures that are predicted to be important for
364 sex chromosome evolution. The house fly proto-Y chromosomes are disproportionately found in
365 males relative to females, but they can also be carried by females who have an *Md-tra^D* allele.
366 Population genetic models predict that the proto-Y chromosomes could have male-beneficial,
367 female detrimental sexually antagonistic fitness effects, which could contribute to the
368 maintenance of the polymorphism within populations (Meisel et al. 2016; Meisel 2021).
369 However, there is no direct evidence for sexually antagonistic effects of the proto-Y
370 chromosomes, let alone sexual antagonism that depends on temperature or any other
371 ecological factor. Future work is therefore needed to evaluate if the well-documented
372 temperature-dependent fitness effects of house fly proto-Y chromosomes have any relationship
373 to their hypothesized sexually antagonistic effects. Such evidence would provide an important
374 link between the effects of sexual antagonism and ecological variation on sex chromosome
375 evolution.

376

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381

382 Supplemental Material

383 Supplemental Table S1

384 Collection sites for house flies and closest NOAA station

state	city	NOAA station ¹
CA	Moreno Valley	March Air Force Base
FL	Alachua	Gainesville Regional Airport
GA	Gillsville	Gilmer Airport
KS	Manhattan	Manhattan
NC	Raleigh	Raleigh Durham International Airport
NE	Lincoln	Lincoln Municipal Airport
PA	State College	State College
TN	Walland	Knoxville McGhee Tyson Airport
TX	Bryan	College Station

385

386 1. Closest NOAA station from which weather data were downloaded

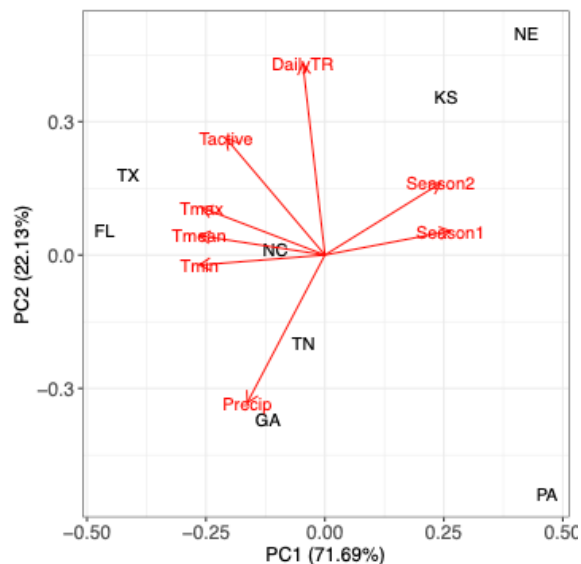
387 (<https://www.ncei.noaa.gov/access/us-climate-normals/>)

388

389

390 **Supplemental Figures S1–S9.** Simulation results to predict chromosome and genotype frequencies in
391 each of the nine sampled populations. Graphs show the frequencies of males carrying III^M (orange M),
392 males carrying Y^M (blue Y), females carrying *Md-tra*^D (magenta D), and males (black m) across ten
393 generations of the simulation. Dashed blue and magenta lines show the observed frequencies of males
394 carrying Y^M and females carrying *Md-tra*^D, respectively. Only the results of the final simulation that
395 accurately predicted the observed chromosome frequencies are shown. Code to generate graphs from
396 intermediate simulations is provided in the Supplemental Material.

397



398

399 **Supplemental Figure S10.** Principal component analysis (PCA) of climate features across sampling
400 locations. Populations are plotted according to the first two principal components (PCs) based on climate
401 features. The loadings of each climate feature are indicated by labeled red vectors. Vector labels are
402 described in Table 1. Each population is represented by the two letter abbreviation of the state from which
403 it was collected.

404

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