Article

Phenotypic plasticity as a function of genetic polymorphism: thermal dominance reversal in *Drosophila* species with contrasting melanism

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Authors contribution

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Conflicts of interest

The authors declare no conflict of interest.

Data availability statement

All data associated with this study are provided in a Figshare Folder and will be released upon publication.

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Abstract

Phenotypic plasticity is often seen as an alternative adaptive strategy to genetic polymorphism, especially in response to rapid environmental changes. Indeed, a link between plasticity and heterozygosity, *i.e.* the measure of polymorphism, has previously been dismissed. Here, we compare the thermal plasticity of abdominal pigmentation in eight *Drosophila* species, four belonging to the *melanogaster* species group and four to the *montium* group. Despite a conserved developmental pathway for melanin synthesis, the genetic architecture of its variation has significantly evolved, being polygenic in most species (like *D. melanogaster*) and Mendelian or invariable in others. By investigating the thermal plasticity of this trait in species with distinct architectures, we show the degree of plasticity to strongly associate with heterozygosity. Plasticity was resurrected in hybrids between species with no plastic responses but with contrasting melanism, and was higher in heterozygotes in species with simple Mendelian polymorphism. In plastic cases, pigmentation dominance is reversed depending on the developmental temperature. We propose simple genetic models with empirical molecular support to explain this link between phenotypic plasticity and genetic polymorphism. The relationship between these two phenomena, and the impact of each on the evolution of the other, may be more relevant than it is currently appreciated.

Keywords

Overdominance; genetic architecture; reaction norm; heterozygosity; dominance reversal.

68 **Introduction**

69 Phenotypic plasticity is the ability of a genotype to produce different phenotypes under
70 distinct environmental conditions (Bradshaw 1965; Scheiner 1993; Pigliucci 2001; West-
71 Eberhard 2003; Sultan 2021). When optimal phenotypic values are generated in the different
72 environments, plasticity can facilitate adaptation even in the absence of genetic variation
73 (Ghalambor et al. 2015). This becomes particularly important in cases where the population
74 loses a significant portion of genetic polymorphism, such as after a founder bottleneck during
75 colonization of a new environment, or when the environment change is faster than the rate of
76 accumulation of new adaptive mutations, the so-called 'buying time' role of plasticity
77 (Diamond and Martin 2021). Phenotypic plasticity and genetic polymorphism might be
78 considered as distinct alternative adaptive strategies (e.g., Wennersten and Forsman 2012).

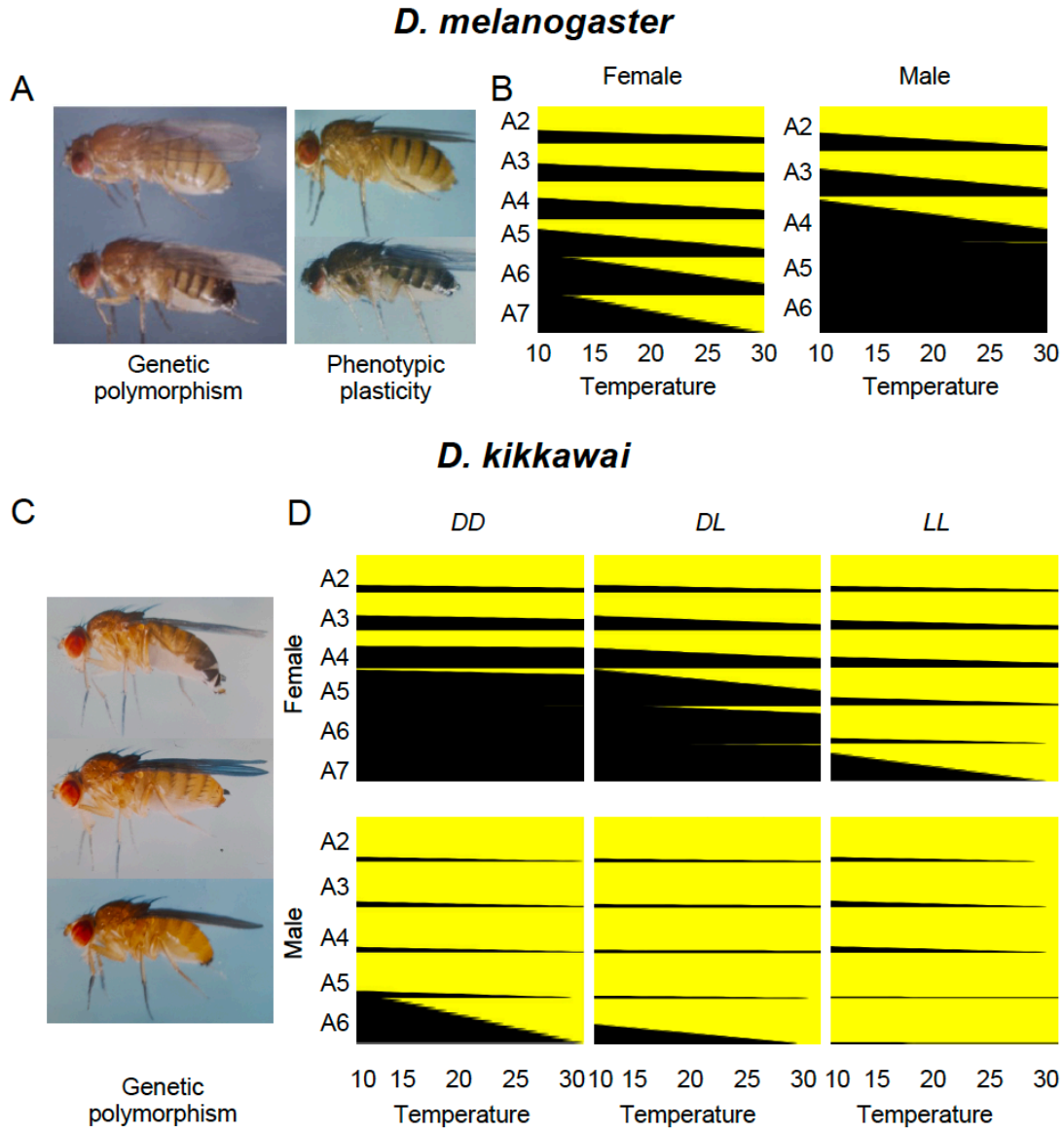
79 Despite the definition of plasticity as a property of a genotype, it is usually studied for
80 complex phenotypic traits whose underlying genotypes are not fully known (e.g., flowering
81 time in *Arabidopsis*, growth in yeasts, etc.) (Goldstein and Ehrenreich 2021; Kovuri et al. 2023).
82 Genotypes do not only differ in the degree of their phenotypic plasticity (e.g., the slope of the
83 reaction norm) but also in their degree of genetic variability, with heterozygous genotypes in
84 non-haploid organisms having more alleles than hemi- or homozygotes. Among the early
85 genetic models of phenotypic plasticity, the Overdominance model explicitly related plasticity
86 to genotypic diversity (Scheiner 1993). The model postulated that plasticity is a function of
87 homozygosity, with the heterozygous genotype considered to be the most advantageous
88 because it is the most robust, *i.e.* the least plastic. This assumption reflected the view of
89 plasticity at the mid-20th century as a developmental nuisance (Lerner 1954; Bradshaw 2006).
90 Indeed, some of the earliest empirical examples of the overdominance model came from studies
91 of wing morphology in *Drosophila melanogaster*, where two mutants showed the highest
92 temperature-sensitivity when found in the homozygous state, but their heterozygote

recapitulated the wildtype thermal response, *i.e.* it was less plastic than either mutant (Harnly and Harnly 1936; Schmalhausen 1949). Gillespie and Turelli (1989) extended this view to a multi-locus quantitative genetics model and concluded that stabilizing selection on more environmentally robust heterozygotes will maintain genetic variation in populations. Pigliucci (1992) developed a one-locus, two-allele classical population genetics model for testing the evolution of plasticity under overdominance and concurred with Gillespie and Turelli's (1989) multilocus result. He concluded that plasticity becomes "increasingly disadvantageous when the system approaches a perfect equilibrium between the probabilities of occurrence of the two environments."

A direct link between heterozygosity and robustness was not always evident from the empirical literature, and overdominance was disfavored relative to other genetic models such as pleiotropy, *i.e.* plasticity is a function of the expression of a gene, and epistasis, *i.e.* plasticity is a function of interactions between loci. Scheiner (1993) concluded that "plasticity is not a function of heterozygosity", and Pigliucci (2005), a decade later, stated that "heterozygosity ha[d] little, if anything, to do with the genetic basis of plasticity." A search using "heterozygo*" in a recent 400-page long book on *Phenotypic Plasticity and Evolution* (Pfennig 2021) yielded no result. However, almost none of the studies cited by Scheiner (1993) and Pigliucci (2005) to reach their conclusions has addressed the link between heterozygosity and plasticity in the most optimal setting similar to Pigliucci's (1992) theoretical model, *i.e.* comparing the reaction norms of a phenotype with a simple, well-characterized genotypic basis. Conspicuous color polymorphisms are ideally suited for these experiments, because they often involve a simple genetic architecture and color synthesis in many species results from a well conserved gene network (Orteu and Jiggins 2020). In several lineages of moths, simple Mendelian polymorphisms have evolved in response to the industrial revolution, with the melanic wing allele being dominant in some species and recessive in others (Ford 1945). The genetic basis of

118 this industrial melanism has recently been identified to be a conserved micro-RNA close to the
119 locus of the transcription factor *Cortex* (Tian et al. 2024). Remarkably, several butterfly species
120 also show a seasonal color polyphenism, with cold season morphs being darker. van der Burg
121 et al. (2020) showed that mutations at the same locus containing *Cortex*, and consequently the
122 linked micro-RNA, underlie this polyphenism in *Junonia caenia*. Plasticity in simple color
123 polymorphism systems was also observed in *Harmonia* ladybugs morphs (Michie et al. 2010).
124 Much of our knowledge about the genetic and developmental basis of melanin synthesis
125 comes from studies in *Drosophila*. Pigmentation differences between body segments, sexes,
126 geographical populations, seasons, and species are often due to *cis*- and *trans*-regulatory
127 modifications of melanin synthesis genes, such as *tan (t)*, *yellow (y)* or *ebony (e)*, and their
128 transcription factors, such as *bric-à-brac (bab)*, *POU-domain-motif-3 (pdm3)* or *Abdominal-B*
129 *(Abd-B)* (Massey and Wittkopp 2016; Hughes et al. 2023). Wild-caught flies from the same
130 locality usually differ in their degree of pigmentation (Figure 1 A; Bastide et al. 2013, 2016;
131 Dembeck et al. 2015; Endler et al. 2016; Castro et al. 2018), but flies collected from cold high
132 altitudes, latitudes, and seasons tend to be dark, even when their progeny is maintained under
133 standard thermal conditions (Telonis-Scott et al. 2011; Bastide et al. 2014, 2016; Takahashi et
134 al. 2014; Negoua et al. 2019; Berardi et al. 2024). Similarly, when full-sib flies issued from the
135 same isofemale line are grown under different temperatures, a negative reaction norm is
136 detected, with flies grown under low temperatures being darker (Figure 1; (Gibert et al. 1996,
137 1998, 2000; Chakir et al. 2002; Pétavy et al. 2002; Gibert et al. 2004b, 2009; Pétavy et al. 2018;
138 Lafuente et al. 2021, 2024). Thermal plasticity in *D. melanogaster* is due to the higher
139 expression of the *t* and *y* genes in colder temperatures (Gibert et al. 2016, 2017b). A natural
140 variation in the transcription factor *bab* underlying color differences also showed distinct
141 responses to developmental temperature through repression of *t* and *y* in hotter environments
142 (Castro et al. 2018). This parallelism between genetic polymorphism in the wild and phenotypic

143 plasticity in the lab is strongly suggestive for an adaptive role for darker pigmentation in
144 thermoregulation. Indeed, drosophilids with darker cuticle have higher body temperature (Freoa
145 et al. 2023).



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147 **Figure 1** – Pigmentation variation in *Drosophila melanogaster* and *D. kikkawai*. A) Variation
148 in abdominal pigmentation in *D. melanogaster* can be due to genetic polymorphism (left) or
149 thermal phenotypic plasticity (right). B) Contour plots showing pigmentation thermal plasticity
150 in *D. melanogaster* females (left) and males (right) at each abdominal segment (A2-A7). C)
151 Variation in abdominal pigmentation in *D. kikkawai* is caused by a single locus with two alleles
152 dark (*D*) and light (*L*) whose effect is more pronounced on females (upper *DD* and middle *LL*
153 photos) than males (lower photo). D) Contour plots showing pigmentation thermal plasticity in
154 *D. kikkawai* females (above) and males (below) for the three genotypes *DD*, *DL*, and *LL*. For
155 each abdominal segment, a reaction norm was inferred as a linear regression slope using mean
156 values available published in Gibert et al. (1999, 2000). Photos are from Jean R. David's
157 archive.

158 The genetic architecture of pigmentation variation has dramatically evolved between
159 drosophilid species. Whereas female abdominal pigmentation variation is continuous and
160 polygenic in *D. melanogaster* as well as most species, it is discrete and monogenic in multiple
161 species (Payant 1986; Yassin et al. 2016a,b). Female-limited Mendelian melanism has evolved
162 in *D. erecta* of the *melanogaster* group and in 22 species of the *montium* group belonging to
163 five distinct subgroups (Yassin et al. 2016b; Yassin 2018). Of the 16 *montium* species where
164 the genetics was studied, the dark allele was dominant in 13 species (Yassin et al. 2016b; Prigent
165 et al. 2020). The phenotypic plasticity of abdominal pigmentation was investigated by Gibert
166 et al. (1999) in one species, namely *D. kikkawai*, where females have two morphs and males
167 have a light abdomen. The authors found major effect of genotype, segment, sex, and
168 temperature (Figure 1B). In some other species, pigmentation is nearly fixed with very slight
169 variability even between the sexes. This is the case of the two sister species, *D. santomea* and
170 *D. yakuba*, where both males and females' posterior abdomens are invariably light or dark,
171 respectively (Lachaise et al. 2000; Jeong et al. 2008; David et al. 2022).
172 In this paper, we compared the level of pigmentation phenotypic plasticity in eight
173 species with distinct genetic architectures grown under five thermal regimes. To this end, we
174 focused on the effect of heterozygosity, as it can be seen in heterozygous genotypes of five
175 species with monogenic architectures as well as in species hybrids (*i.e.* the ultimate viable
176 heterozygotes). Our results support a positive link between plasticity and the degree of genetic
177 polymorphism that was not previously assumed.
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Materials and Methods

Strains used and fly husbandry

We studied five cases where for each the thermal phenotypic plasticity can be compared between homozygous dark and light lines and their reciprocal hybrids. Four cases considered within-species monogenic Mendelian color polymorphism with one (*D. erecta*) from the *melanogaster* species group and three cases (*D. jambulina*, *D. burlai*, and *D.* cf. *bocqueti*) from its sister clade, the *montium* species group. For each of these species homozygous dark and light lines for the color causing allele were established through selection from a polymorphic mass culture (Table 1). The monogenic basis of female pigmentation in these species are known (Yassin et al. 2016a,b; Prigent et al. 2020). In two species (*D. erecta* and *D. burlai*), the dark allele is dominant, although the causative locus is different being the X-linked *tan* in *D. erecta* and the autosomal *pdm3* in *D. burlai* (Yassin et al. 2016a,b). In the two other species (*D. jambulina* and *D.* cf. *bocqueti*), the light allele is dominant (Watanabe et al. 1982; Prigent et al. 2020), but the causative locus is still not mapped although color variation associates with polymorphism in *pdm3* in the two species (Fukutomi et al. 2025). The fifth case involved hybridization between the two sister species *D. santomea* and *D. yakuba* of the *melanogaster* group, whose posterior segments are either monomorphically light or dark, respectively (Table 1). The pigmentation difference between these two species is controlled by mutations in at least six loci (Liu et al. 2019; David et al. 2022). All strains were kept in a rearing room at 21°C with 12:12 dark:light cycles on a food medium composed of 10 g agar, 101 g sugar, 25 g yeast, 100 g corn flour, 6.9 g nipagin, and 50 mL ethanol per 1 L. We also obtained published data of the thermal plasticity response of *D. melanogaster* (Gibert et al. 2000), the nomitypical species of the *melanogaster* group with polygenic basis of pigmentation variation, and the monogenic *D. kikkawai* (Gibert et al. 1999) for comparison purposes (Table 1).

208 Table 1. *Drosophila* strains analyzed in this study.

Group	Subgroup	Complex	Species	Color variation	Strain
melano-gaster	*melano-gaster*	*melano-gaster*	*melanogaster*	Complex	Bordeaux, France (Gibert et al. 2000)
melano-gaster	*melano-gaster*	*yakuba*	*yakuba mayottensis*	Nearly absent	Mayotte, France, 2013 (J. R. David, V. Debat, N. Gidaszewski)
melano-gaster	*melano-gaster*	*yakuba*	*santomea*	Nearly absent	Sao Tomé, Sao Tomé & Principe, 1998 (D. Lachaise)
melano-gaster	*melano-gaster*	*erecta*	*erecta*	Monogenic	La Lopé Forest, Gabon, 1998 (D. Lachaise)
montium	*kikkawai*	*kikkawai*	*kikkawai*	Monogenic	Chandigarh, India (Gibert et al. 1999)
montium	*seguyi*	*jambulina*	*jambulina*	Monogenic	New Delhi, India, 2014 (J. R. David, S. Mohanty)
montium	*seguyi*	*bocqueti*	*burlai*	Monogenic	Mt Oku, Cameroon, 2008 (M. Veuille)
montium	*seguyi*	*bocqueti*	cf. *bocqueti*	Monogenic	Sao Tomé, Sao Tomé & Principe, 2015 (J. R. David, M. Lang)

209

210 *Pigmentation scoring*

211 For monogenic species, we followed the nomenclature given in Gibert et al.'s (1999)
212 paper, referring to the light and dark alleles as *L* and *D*, respectively, and to the three genotypes
213 as *LL*, *LD* or *DL*, and *DD*. For heterozygotes, the allele from the mother is given first depending
214 on the cross. For each genotype, pigmentation was scored on >4 days old 10 males and 10
215 females as in Yassin et al. (2022) following the pigmentation score scale proposed by David et
216 al. (1990). This pigmentation score visually quantifies the proportion of the pigmented part of
217 an abdominal segment when viewed on the lateral side of a fly under a stereoscope and it goes
218 from 0 (no pigmentation) to 10 (fully pigmented). It is therefore different from other
219 pigmentation scores in *Drosophila* that depend on the intensity of melanism or the length of the
220 pigmented stripe quantified after numerical photographing (e.g., Bastide et al. 2014; Lafuente

221 et al. 2021). Scorings were made by the same observer (the first author) between 2013 and 2019
222 (but mostly between March 2016 and April 2017) to limit observation bias. Data were manually
223 entered on a printed sheet with a template table for morphometric data and then the means for
224 each segment were calculated in a separate sheet. All sheets are archived. Scorings were made
225 by the same observer to limit observation bias between 2013 and 2019 (but mostly between
226 March 2016 and April 2017). Abdomens of some flies at extreme temperatures (see below)
227 were dissected and mounted on microscopic slides and photomicrographed (cf. data associated
228 with this study). In each case, pigmentation was scored on six and five abdominal segments in
229 females and males respectively (denoted A2-7 with F and M for females and males,
230 respectively).

231 Flies were grown at five temperatures going from 12°C to 31°C with 3-4° increments.
232 For most cases, the five categories were 14, 17, 21, 25, and 28°C, but a few cases were scored
233 at 19 (*D. jambulina DD*), 29 (*D.* cf. *bocqueti DL*), and 30°C (*D. erecta DD*, *DL*, *LD*, and *LL*).
234 Mortality during development was not assessed. For *D. melanogaster* and *D. kikkawai*, only
235 mean values per genotype were retrieved from the literature for seven (12, 14, 17, 21, 25, 28,
236 and 31°C) and four (12, 17, 25, and 30°C) temperature degrees, respectively (Gibert et al. 1999,
237 2000). For three genotypes < 10 flies per sex were available at the time of observation (namely,
238 *D.* cf. *bocqueti LL* males at 21°C ($N = 2$), *D. jambulina DD* females and males at 25°C ($N = 5$).
239 Consequently, temperature was coded either as a degree or as a class depending on the statistical
240 test used (see below), with the five classes being: cold (12-14°C), mildly cold (17-19°C), mild
241 (21°C), mildly hot (25°C), and hot (28-31°C). In total, pigmentation was scored for nearly 8,703
242 abdominal segments over 1,582 flies for the four species with monogenic melanism. For the
243 hybridization experiment between *D. santomea* and *D. yakuba*, only the sheet recording the
244 means for each genotype per sex per temperature was retrieved for the five temperatures.
245 However, photomicrographs of abdominal segments for the four genotypes (parental strains

246 and reciprocal crosses) at three temperatures (14, 21, and 28°C) are provided in the data
247 associated with this study. Analyses on the whole set of temperatures for this experiment were
248 therefore limited to the mean values.
249
250 *Statistical analyses*
251 All statistical analyses were conducted using R (R Core Team 2023). As a serially
252 homologous trait, abdominal pigmentation can be tested either as in an integrative (*i.e.* the
253 overall pigment production) or a modular way (*i.e.* pigmentation patterns across segments).
254 Indeed, pigmentation in all segments is produced by the same melanin synthesis genes and their
255 variants that each individual carries. Previous works in *D. melanogaster* and *D. kikkawai* have
256 shown a substantial degree of correlation especially among adjacent segments (David et al.
257 1990; Gibert et al. 1999, 2000). However, pigmentation variation associates with different
258 genetic variants in different abdominal segment in *D. melanogaster* measured on the same
259 isogenic lines (Dembeck et al. 2015), and the reaction norms of the thermal plasticity are
260 different even among adjacent abdominal segments in *D. simulans* (Gibert et al. 1998). To test
261 for the independence between the abdominal segments we conducted correlation analyses on
262 the whole individual-based dataset using the Corrplot package in R (Wei and Simko 2024), and
263 found indeed substantial degrees of correlations which were stronger among adjacent segments
264 (see Results). Consequently, we conducted our further analyses either on each segment
265 separately or using the sum of all segments per an individual depending on the test.
266 We first conducted a multi-factor Analysis of Variance (ANOVA) on the individual-
267 based dataset for the four species with monogenic melanism to test of the effect of each factor
268 (species, genotype, sex, segment, and temperature) as well as of their interactions on
269 pigmentation variation. We estimated the effect of these factors as the proportion of variance
270 explained (η^2) using the effectsize package (Ben-Shachar et al. 2020) in R. To test for the effect

271 of reciprocal crosses, we conducted the same analysis only on the *DL* and *LD* genotypes.
272 Because no significant difference was found between these genotypes, they were pooled into a
273 single F_1 heterozygous category for subsequent analyses. For a balanced ANOVA, equal
274 number of segments were used for each sex. To do so in *D. kikkawai*, Gibert et al. (1999)
275 excluded the most posterior female abdominal segment, *i.e.* FA7. However, subsequent
276 analyses showed that this segment is among the most variable within and between Drosophila
277 species (Bastide et al. 2013; Yassin et al. 2016a,b). Consequently, we excluded here the most
278 anterior female segment, *i.e.* A2, which usually shows less variation and strong correlation with
279 other anterior segments (see Results). Similarly, temperature classes rather than temperature
280 degrees were used to test the effect of temperature in a balanced way across species and
281 genotypes.

282 Previous analyses have shown the shape of the thermal reaction norms to differ between
283 segments of the same isofemale lines. For example, segments 5, 6, and 7 in *D. simulans* females
284 have quadratic, linear, and sigmoid (logistic) norms, respectively, although all show the same
285 response of higher melanism in colder environments (Gibert et al. 1998). Because our main
286 focus in this paper is to compare the level of plasticity between heterozygotes and homozygotes
287 rather than the diversity of the shape of the reaction norms, linear regressions of pigmentation
288 scores over temperature degrees were conducted using the lm function in R for every segment
289 as well as for the sum of all segments, and the slope of the regression line (*b*) was considered
290 as a proxy for the degree of plasticity. Regression analyses were conducted on the whole
291 individual-based dataset rather than on averages for the measurements generated by this study,
292 although for the data retrieved from the literature for *D. melanogaster* and *D. kikkawai*, and for
293 the hybridization experiment, only averages were available.

294 To visualize the patterns of segment-specific diversity for each genotype per sex per
295 species we used a customized perl script to translate the linear regression equations generated

296 above into a pigmentation pattern. Each segment was divided into a grid of 100 centiles so that
297 a pigmentation score of 2.2 for a segment means that the 22 most posterior centiles of that
298 segment have a pigmentation score of 10, whereas the 78 most anterior centiles have a score of
299 0. A 2D contour map was then drawn using the Plotly package in R (Sievert 2020). For the
300 overall pigmentation score (*i.e.* sum of all segments), 2D plots were drawn for each sex per
301 species using the plot function in R. Genetic dominance was inferred as the difference between
302 the slope of pooled heterozygotes (*DL* and *LD*) and the slope of the pooled homozygotes (*DD*
303 and *LL*), with dominance reversal being witnessed at the intersection between the two slopes
304 within the range of vital temperatures between 10 and 30°C. To test if heterozygotes had
305 different plasticity than homozygotes, we ranked the slope of each of the three genotypes from
306 the highest to the lowest as 3, 2, and 1 for each species and conducted a non-parametric
307 Wilcoxon test on the relation between rank and genotype.

308 For the hybridization experiment between *D. santomea* and *D. yakuba*, only mean scores
309 for each genotype per sex per temperature were available (see above). We first conducted a
310 multi-factor ANOVA on these values, after excluding female A2 and considering the five
311 temperature classes as for the species with the monogenic melanism. We also conducted
312 ANOVA on only the hybrid genotypes to test for the effect of reciprocal crosses on the whole
313 dataset. To quantify and visualize plasticity at each segment as well as on the total pigmentation
314 scores we conducted linear regression analyses on the mean values as above. We note that for
315 species with monogenic melanism, regression slopes strongly correlated between the whole
316 datasets and the means ($r = 0.997$, $P < 2.2 \times 10^{-16}$; Student's t $P = 0.871$), although the standard
317 error of the slope value was significantly reduced in individual-based analyses ($r = 0.849$, $P <$
318 2.2×10^{-16}; Student's t $P = 1.671 \times 10^{21}$). Consequently, the slope reported for the mean values
319 of this experiment correctly reflects the plasticity response.
320

Results

Pigmentation thermal plasticity varies with species, sex, segment, and genotype in species with monogenic melanism

ANOVA on the whole dataset of the four species with monogenic melanism indicated a strong effect of all factors and their interactions (Table 2). However, the most significant effect was due to segment position (explaining 30% of the variance), followed by temperature and genotype (5%), then species (2%) and sex (1%). To test for the effect of reciprocal crosses, we conducted the same ANOVA on the data set of only heterozygous genotypes (*LD* and *DL*). We found that the genotype had no effect at all, whether individually or in interaction with other factors. For simplicity, we pooled data for heterozygous individuals issued from reciprocal crosses into a single genotypic group (F_1). When we conduct the analysis using the complete genotypic dataset (*i.e.* with pooled *LD* and *DL*), the ANOVA results showed the significant effect of all factors and their interactions.

Table 2. Analysis of variance (ANOVA) for pigmentation variation in four species with monogenic melanism. *D* and *L* refers to dark and light alleles, respectively. Df = degree of freedom, GEN = genotype, SEG = segment position, SPE = species, and TCL = temperature class. *** $P < 0.001$.

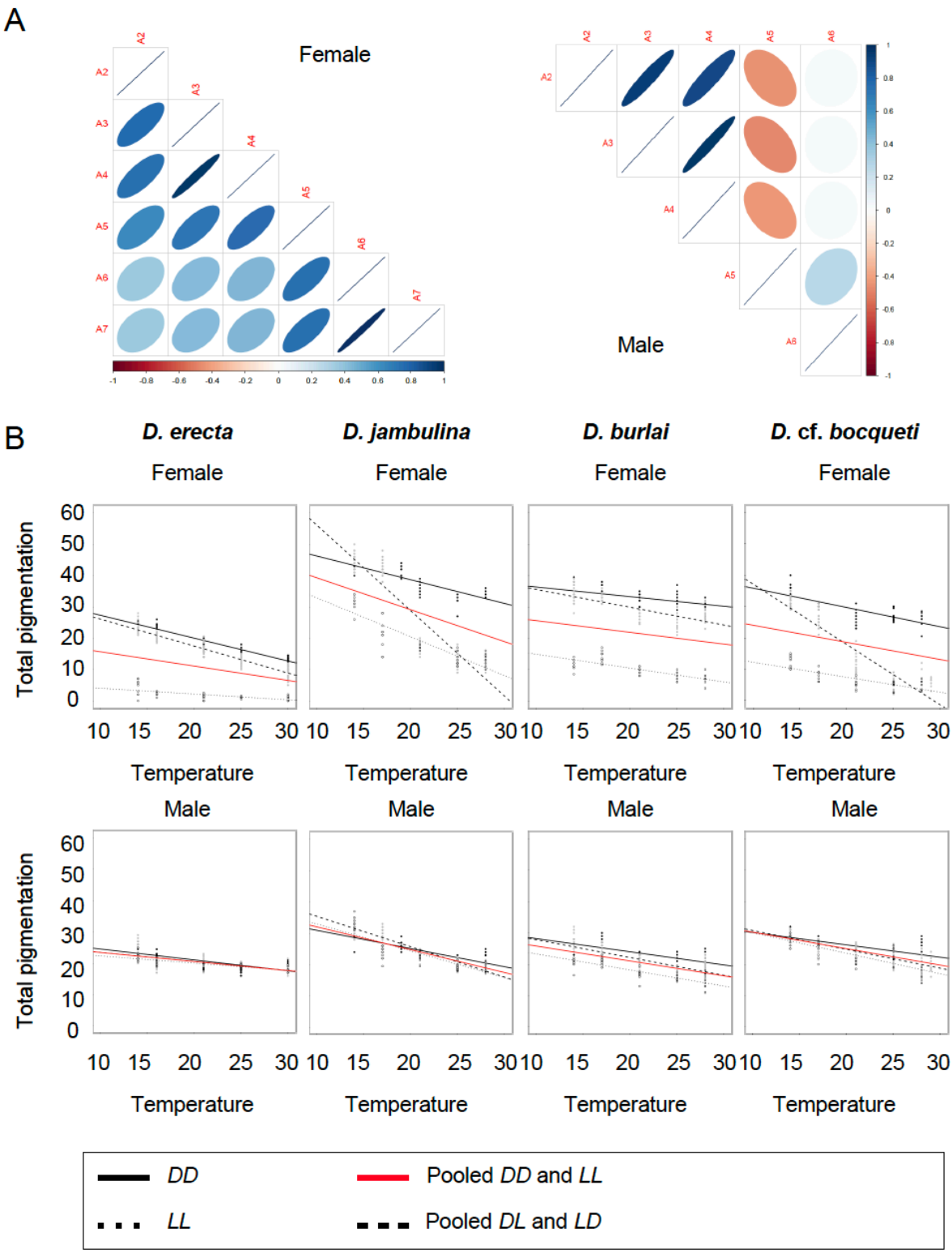
Genotypes	*DD, DL, LD, LL*			*DL, LD*			*DD, F_1, LL*		
Factor	Df	F	η^2	Df	F	Eta2	Df	F	η^2
SPE	3	222.438***	0.02	3	104.487***	0.02	3	222.897***	0.02
GEN	3	546.944***	0.05	1	0.433	<0.01	2	821.871***	0.05
TCL	1	1459.131***	0.05	1	1183.554***	0.08	1	1462.142***	0.05
SEX	1	337.125***	0.01	1	87.979***	<0.01	1	337.820***	0.01
SEG	1	9325.489***	0.30	1	5121.901***	0.36	1	9344.735***	0.30
SPE:GEN	9	21.791***	<0.01	3	0.328	<0.01	6	32.573***	<0.01
SPE:TCL	3	70.243***	<0.01	3	79.879***	0.02	3	70.388***	<0.01
GEN:TCL	3	55.130***	<0.01	1	3.112	<0.01	2	81.154***	<0.01
SPE:SEX	3	156.685***	0.02	3	115.028***	0.02	3	157.008***	0.02

GEN:SEX	3	306.050***	0.03	1	0.107	<0.01	2	459.963***	0.03
TCL:SEX	1	96.736***	<0.01	1	178.999***	0.01	1	96.936***	<0.01
SPE:SEG	3	736.924***	0.07	3	503.505***	0.11	3	738.445***	0.07
GEN:SEG	3	518.281***	0.05	1	0.781	<0.01	2	778.596***	0.05
TCL:SEG	1	45.414***	<0.01	1	119.222***	<0.01	1	45.508***	<0.01
SEX:SEG	1	1118.545***	0.04	1	308.347***	0.02	1	1120.853***	0.04
SPE:GEN:TCL	9	11.695***	<0.01	3	1.447	<0.01	6	16.783***	<0.01
SPE:GEN:SEX	9	13.581***	<0.01	3	0.138	<0.01	6	20.337***	<0.01
SPE:TCL:SEX	3	20.594***	<0.01	3	32.869***	<0.01	3	20.636***	<0.01
GEN:TCL:SEX	3	34.231***	<0.01	1	0.056	<0.01	2	51.421***	<0.01
SPE:GEN:SEG	9	22.254***	<0.01	3	0.051	<0.01	6	33.421***	<0.01
SPE:TCL:SEG	3	10.443***	<0.01	3	26.690***	<0.01	3	10.464***	<0.01
GEN:TCL:SEG	3	30.370***	<0.01	1	0.079	<0.01	2	45.606***	<0.01
SPE:SEX:SEG	3	55.643***	<0.01	3	96.490***	0.02	3	55.758***	<0.01
GEN:SEX:SEG	3	347.385***	0.03	1	1.411	<0.01	2	521.377***	0.03
TCL:SEX:SEG	1	115.968***	<0.01	1	227.193***	0.02	1	116.208***	<0.01
SPE:GEN:TCL:SEX	9	6.391***	<0.01	3	0.085	<0.01	6	9.559***	<0.01
SPE:GEN:TCL:SEG	9	9.518***	<0.01	3	0.412	<0.01	6	14.079***	<0.01
SPE:GEN:SEX:SEG	9	20.399***	<0.01	3	0.212	<0.01	6	30.545***	<0.01
SPE:TCL:SEX:SEG	3	25.856***	<0.01	3	49.527***	0.01	3	25.910***	<0.01
GEN:TCL:SEX:SEG	3	45.620***	<0.01	1	0.207	<0.01	2	68.457***	<0.01
SPE:GEN:TCL:SEX:SEG	9	11.697***	<0.01	3	0.097	<0.01	6	17.529***	<0.01
Residuals	7780			3936			7812		

Higher plasticity of overall pigmentation in heterozygous genotypes in species with monogenic melanism

Despite the strong effect of body segmentation on pigmentation diversity between species, sexes, and genotypes, previous studies have reported strong correlation in pigmentation between adjacent segments (David et al. 1990; Gibert et al. 1999, 2000). We tested this hypothesis using individual data for the four species and indeed found substantial degrees of correlations, especially among adjacent segments, indicating the non-independence of segment-

347 based effect (Figure 2A). Consequently, to exclude such non-independence, we summarized
348 pigmentation variation as sum scores across segments per individual. A multi-factor ANOVA
349 on the sum pigmentation scores showed a significant effect of all factors and their interactions,
350 with the strongest effect being of temperature (explaining 21% of the variance), followed by
351 genotype (18%) and species (11%). To visualize the effect of these three factors, we plotted
352 sum pigmentation scores of each genotype per species over temperature. In all cases, a
353 significant negative linear relation was found, with pigmentation increases in colder
354 environments (Figure 2B). Adjusted R^2 ranged from 0.339 to 0.899, indicating that some
355 relationships may better be explained by more complex non-linear regressions. However, for
356 the sake of comparisons across species and genotypes, we used the slope of the linear regression
357 of each genotype as a proxy for the degree of phenotypic plasticity. Species greatly differed in
358 these slopes (averaged over three genotypes and two sexes), being highest in *D. jambulina*
359 (mean b=-1.189±0.617), followed by *D.* cf. *bocqueti* (b=-0.781±0.329), *D. burlai* (b=-
360 0.470±0.010), and *D. erecta* (b=-0.441±0.080). For each species, we ranked the three genotypes
361 from the most plastic (*i.e.* strongest slope) to the least plastic, and found that heterozygosity
362 strongly correlated with higher plasticity (Wilcoxon's W = 16, P = 0.002).
363



364

365 **Figure 2** – Correlation between abdominal segments and plasticity of total pigmentation in
366 species with monogenic melanism. A) Correlation between pigmentation scores of abdominal
367 segment in females (left) and males (right) showing stronger correlation between adjacent
368 segments. B) Linear regression of total pigmentation (sum of segment scores) over
369 developmental temperature in homozygous dark (*DD*) and light (*LL*) genotypes and pooled
370 heterozygotes (*DD* and *LL*). Dominance can be inferred as the difference between the slope of
371 the pooled heterozygotes (black dashed) and that of the pooled homozygotes (red solid).

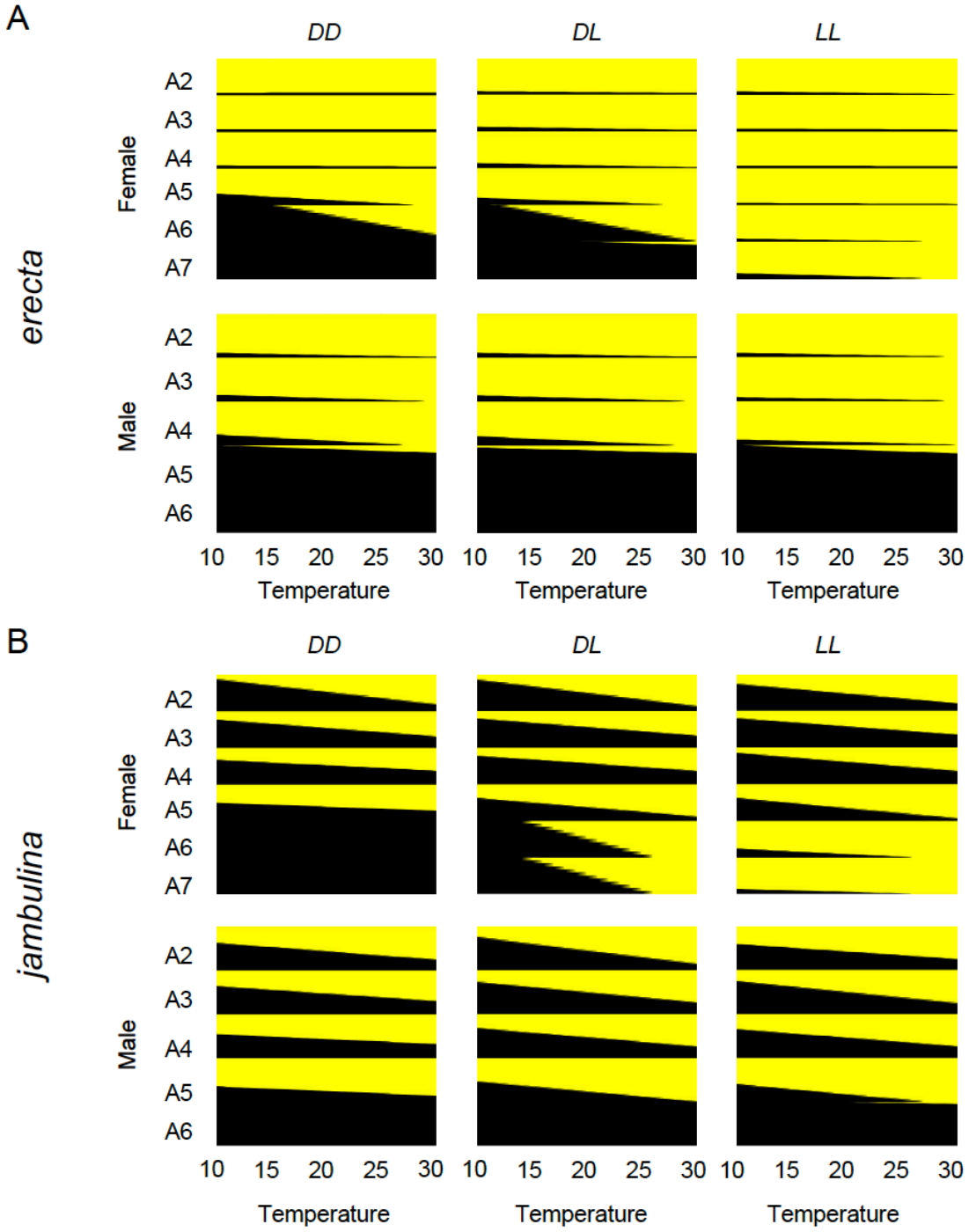
372 *Higher plasticity of heterozygotes associates with dominance reversal*

373 The stronger slope of the heterozygote relative to the homozygotes indicate that
374 dominance between the dark *D* and light *L* alleles likely changes with developmental
375 temperature. To evaluate this effect, we inferred the slope of pooled homozygous genotypes,
376 which is a proxy of the mid-parent values across temperatures. We found indeed that the slope
377 of the heterozygotes approaches this mid-parent slope in almost all cases at higher temperature,
378 indicating a reduction of the dominance of the *D* allele with increasing temperature. The
379 heterozygotes' slope intercrossed that of the mid-parent at 17°C in *D.* cf. *bocqueti* males, 19°C
380 in *D.* cf. *bocqueti* females, 20°C in *D. jambulina* females, 25°C in *D. jambulina* males and 30°C
381 in *D. burlai* males, indicating a reversal of dominance within the vital temperature range
382 between 10 and 30°C. In two cases, *D. erecta* and *D. burlai* females the intercrossing was
383 expected at >30°C, *i.e.* the *D* allele is dominant throughout the vital range although with
384 decreasing dominance. In *D. erecta* males, the slope superimposed that of the mid-parent
385 throughout the temperature range, indicating a non-plastic codominance of the *D* and *L* alleles.
386 Visual depiction of the regression slope at each segment showed differences between the
387 segments especially towards the posterior of the abdomen (Figures 3 and 4). The reversal of
388 dominance in *Drosophila jambulina* is interesting, since the dominance relationship in this
389 species is ambiguous. Parkash and Sharma (1978) found dark dominance for an Indian
390 population, whereas Watanabe et al. (1982) found light dominance also for Indian populations
391 and concluded that dominance in *D. jambulina* was "a great problem existing in a simple genetic
392 phenomenon". Our results suggest that Parkash and Sharma (1978) and Watanabe et al. (1982)
393 might have conducted their experiments at different temperatures, hence reaching contradictory
394 results. For *D.* cf. *bocqueti*, Prigent et al. (2020) reported that the light allele was dominant in
395 the Sao Tomé strains that we analyze here. Our results indicate that this statement is
396 temperature-dependent. However, because both *D. jambulina* and *D.* cf. *bocqueti*, are tropical,

397 predominantly living in environments with an average temperature ≥21°C, the light allele may

398 be considered the dominant one in nature.

399



400

401 **Figure 3** – Thermal phenotypic plasticity of pigmentation on successive abdominal segments
402 in *D. erecta* (A) and *D. jambulina* (B). For each panel, contour plots show pigmentation thermal
403 plasticity (proportion of black stripe) in females (above) and males (below) for the three
404 genotypes *DD*, *DL*, and *LL*. For each abdominal segment, a reaction norm was inferred as a
405 linear regression slope using individual values.

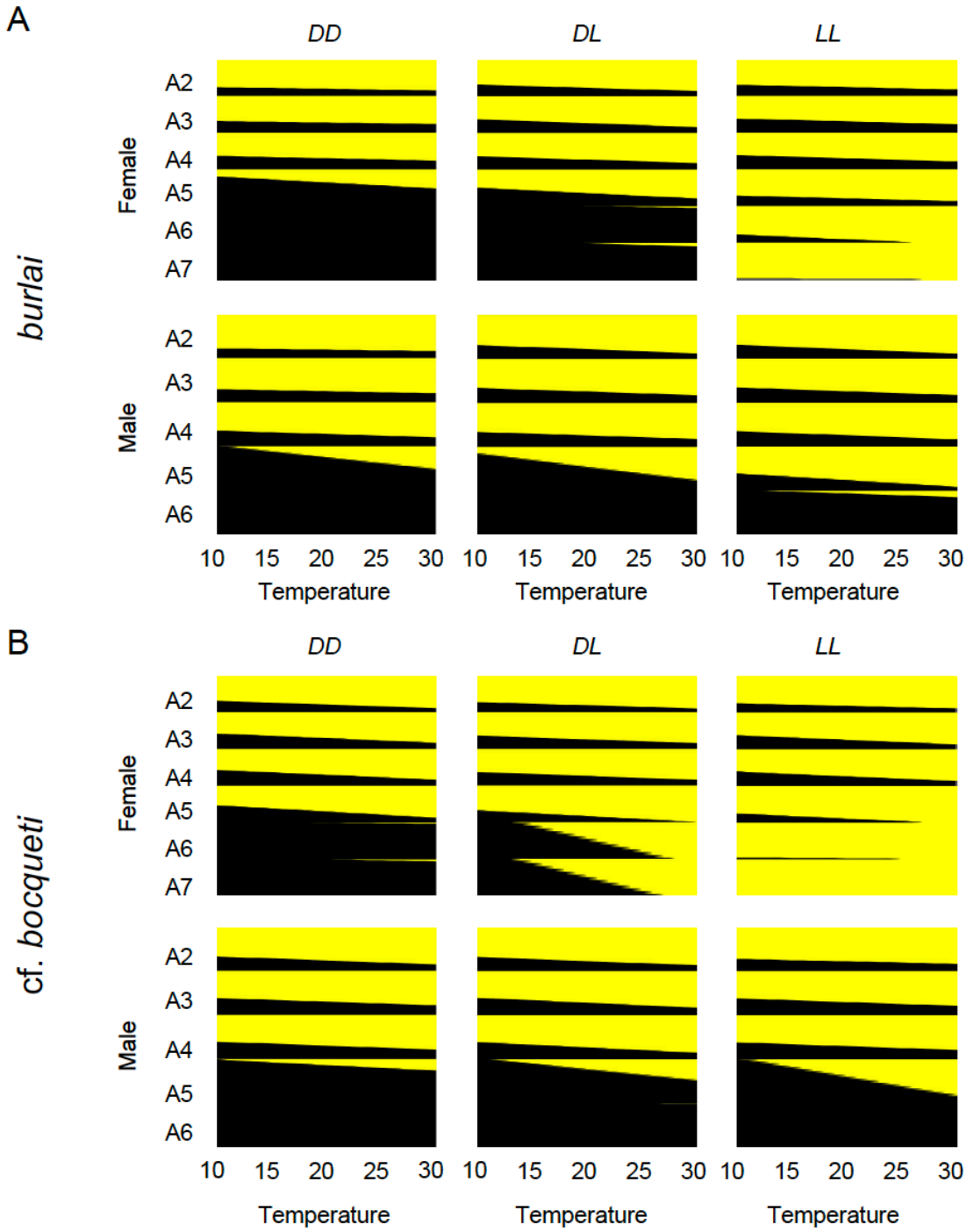


Figure 4 – Thermal phenotypic plasticity of pigmentation on successive abdominal segments in *D. burlai* (A) and *D.* cf. *bocqueti* (B). For each panel, contour plots show pigmentation thermal plasticity (proportion of black stripe) in females (above) and males (below) for the three genotypes *DD*, *DL*, and *LL*. For each abdominal segment, a reaction norm was inferred as a linear regression slope using individual values.

415 *Plasticity increases in hybrids of* D. santomea *and* D. yakuba

416 Multi-factor ANOVA showed all factors but sex to have a significant effect on
417 abdominal pigmentation in *D. santomea*, *D. yakuba*, and their hybrids, with the genotype being
418 the most important factor (accounting for nearly 39% of the variance), followed by the segment
419 position (25%), and temperature (4%) (Table 3). Indeed, the two parental species are known to
420 show very little sexual dimorphism, but very different pigmentation especially on the posterior
421 segments, with *D. yakuba* and *D. santomea* being nearly completely dark and light, respectively
422 (David et al. 2022; Yassin et al. 2022). Contrary to species with monogenic melanism, multi-
423 factor ANOVA on the heterozygotes from the reciprocal crosses showed a significant effect of
424 the genotype (Table 3).

425

426 Table 3. Analysis of variance (ANOVA) for pigmentation variation in *D. santomea* (*LL*), *D.*
427 *yakuba* (*DD*), and their hybrids. *D* and *L* refers to dark (*D. yakuba*) and light (*D. santomea*)
428 alleles, respectively. Df = degree of freedom, GEN = genotype, SEG = segment position, and
429 TCL = temperature class. ** $P < 0.01$; *** $P < 0.001$.

Experiment	*DD, DL, LD, LL*			*DL, LD*		
Factor	Df	F	η^2	Df	F	η^2
GEN	3	230.863***	0.39	1	43.272***	0.08
TCL	1	64.244***	0.04	1	65.130***	0.12
SEX	1	2.511	1.41e-03	1	3.352.	5.97e-03
SEG	1	450.899***	0.25	1	238.814***	0.43
GEN:TCL	3	7.945***	0.01	1	0.132	2.35e-04
GEN:SEX	3	10.601***	0.02	1	23.579***	0.04
TCL:SEX	1	0.001	3.04e-07	1	0.321	5.71e-04
GEN:SEG	3	73.875***	0.12	1	16.177***	0.03
TCL:SEG	1	22.129***	0.01	1	35.368***	0.06
SEX:SEG	1	13.212***	7.42e-03	1	14.030***	0.02
GEN:TCL:SEX	3	0.683	1.15e-03	1	0.970	1.73e-03
GEN:TCL:SEG	3	7.495***	0.01	1	0.835	1.49e-03
GEN:SEX:SEG	3	5.626**	9.48e-03	1	9.489**	0.02
TCL:SEX:SEG	1	2.662	1.50e-03	1	3.439.	6.13e-03
GEN:TCL :SEX:SEG	3	1.686	2.84e-03	1	2.550	4.54e-03
Residuals	208			104		

430

431 Total pigmentation (summed over all segments) showed significant thermal plasticity in
432 both sexes in *D. yakuba* (females: $b = -0.365 \pm 0.039$, $P < 0.001$; males: $b = -0.513 \pm 0.057$, P
433 < 0.001) but only and to a lesser degree in *D. santomea* females (females: $b = -0.093 \pm 0.020$,
434 $P < 0.01$ males: $b = -0.145 \pm 0.057$, $P = 0.065$) (Figure 5). The slope of the linear regression
435 was higher in hybrids, approaching -1 in females (*DL*: $b = -1.277 \pm 0.155$, $P < 0.01$; *LD*: $b = -$
436 0.941 ± 0.092, $P < 0.001$) and males (*DL*: $b = -0.866 \pm 0.161$, $P < 0.01$; *LD*: $b = -1.020 \pm 0.096$,
437 $P < 0.001$) (Figure 5). As for species with monogenic melanism, the slope of the heterozygotes
438 approached or crossed that of the mid-parents indicating a reversal of dominance. In females of
439 reciprocal crosses, the intercrossing occurred at intermediated temperatures (17-20°C). In
440 males, on the other hand, the slope of the heterozygotes approached that of the pooled
441 homozygotes but in opposite sides. For the *san x yak (LD)* males, the *santomea* light phenotype
442 was dominant throughout the range, approaching codominance at cold temperatures. For the
443 *yak x san (DL)* males, the opposite was found with the *yakuba* dark phenotype was dominant
444 throughout the range approaching codominance at hot temperatures. This indicates a strong
445 effect of the X chromosome, which carries two melanin-synthesis genes with loss-of-function
446 mutations in *D. santomea* (Liu et al. 2019; David et al. 2022; see Discussion).

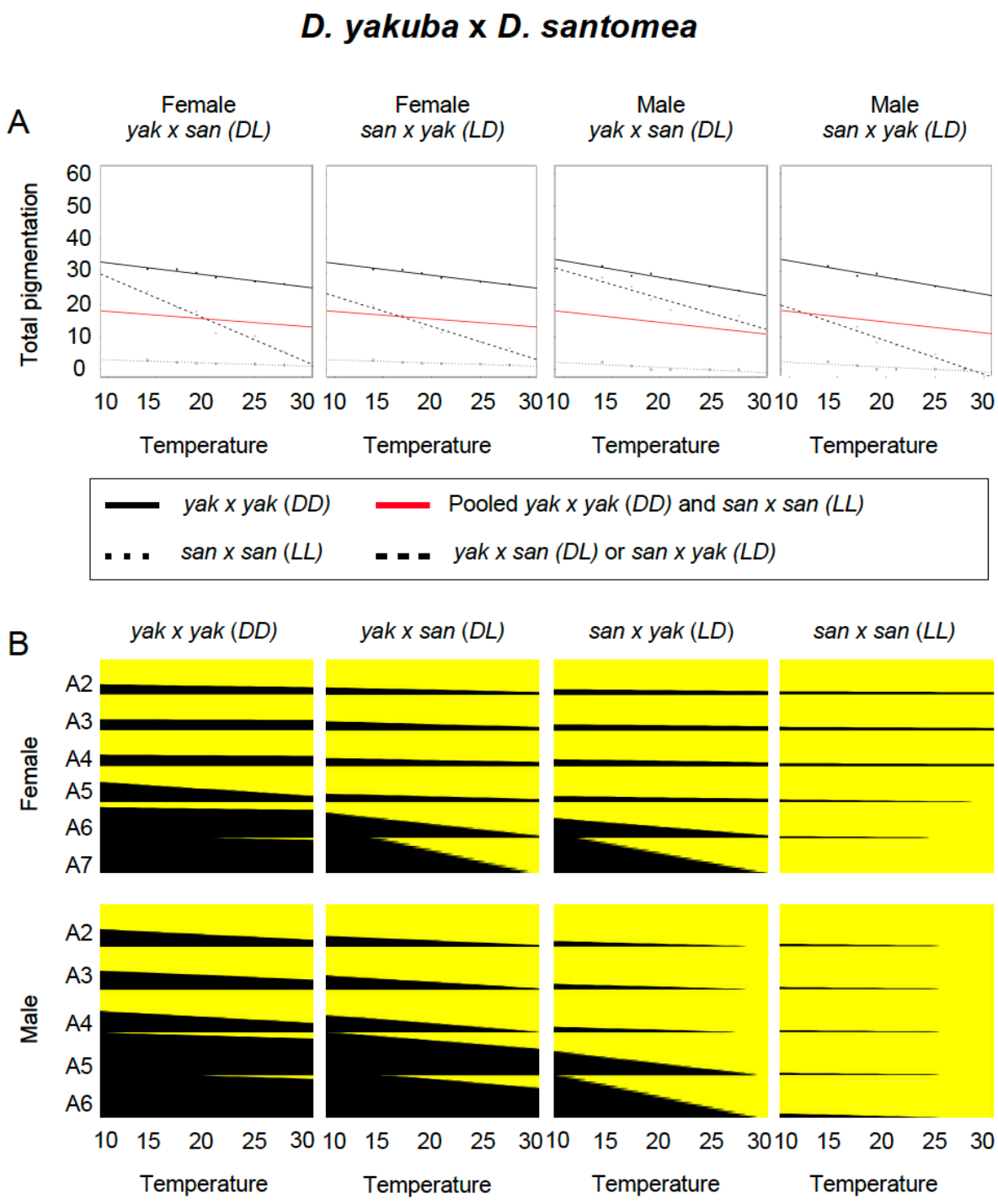
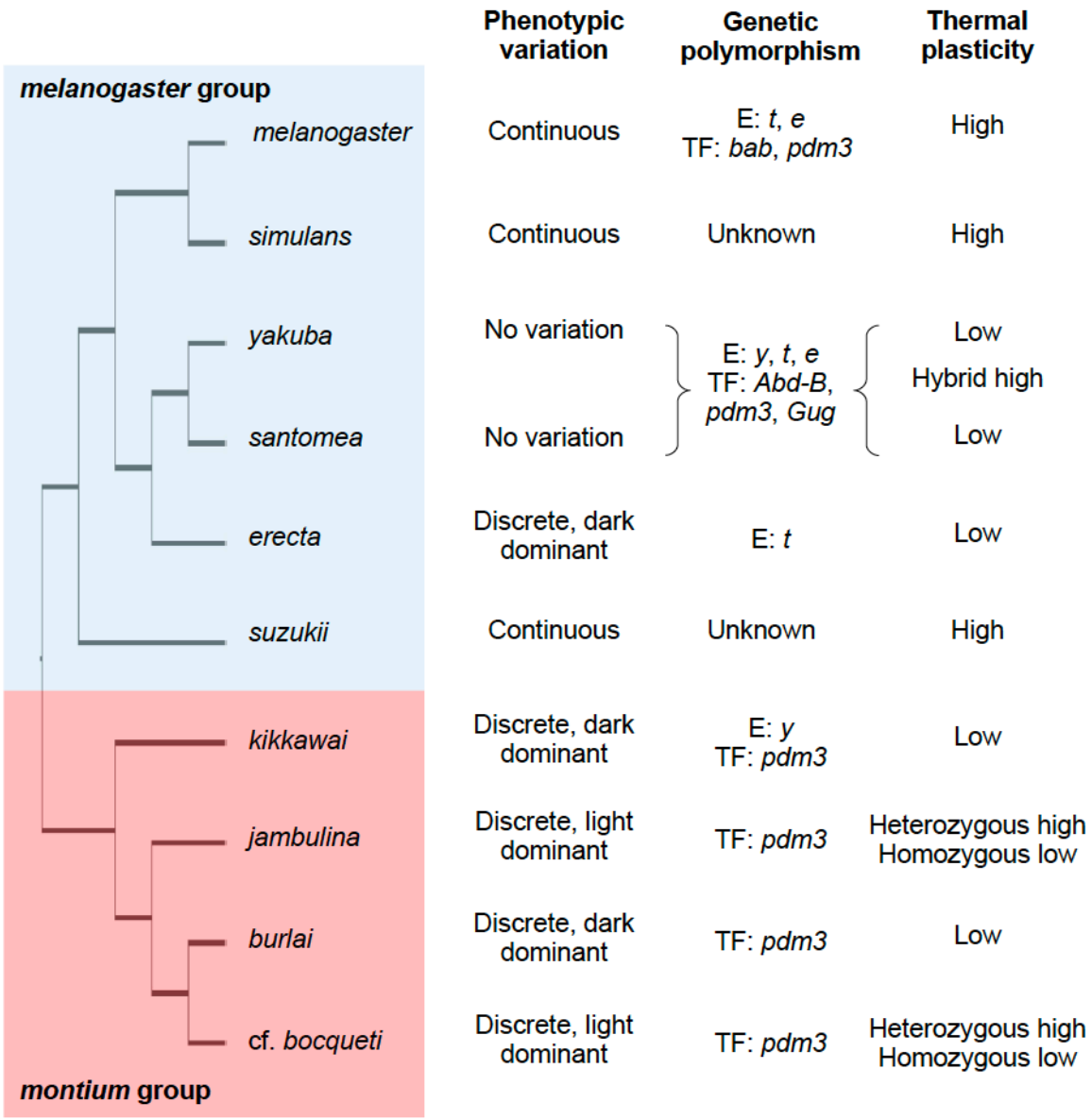


Figure 5 – Thermal phenotypic plasticity of abdominal pigmentation in *D. yakuba*, *D. santomea*, and their hybrids. A) Linear regression of total pigmentation (sum of segment scores) over developmental temperature in homozygous dark (*DD*) and light (*LL*) genotypes and pooled heterozygotes (*DD* and *LL*). Dominance can be inferred as the difference between the slope of the pooled heterozygotes (black dashed) and that of the pooled homozygotes (red solid). B) Plasticity patterns at each abdominal segment. For each panel, contour plots show pigmentation thermal plasticity (proportion of black stripe) in females (above) and males (below) for the three genotypes *DD*, *DL*, and *LL*. For each abdominal segment, a reaction norm was inferred as a linear regression slope using mean values (see text).

458 As indicated from the ANOVA analyses, segment position had very important effect on
459 pigmentation, especially in comparing reciprocal crosses where it explained 43% of the
460 variance (Table 3). This is perfectly seen in the visual depiction for each segment, where
461 differences in pigmentation plasticity were more pronounced in posterior segments (Figure 5).
462
463
464 **Discussion**
465 *The molecular basis of the association between plasticity and heterozygosity in* Drosophila
466 Although it has long been recognized that genetic variation of plasticity exists, the nature
467 of this variation remains elusive. Early works suggested three non-mutually exclusive
468 mechanisms, namely overdominance, pleiotropy, and epistasis (Scheiner 1993). Of the three
469 models, only overdominance made an explicit prediction about the relationship between
470 plasticity and heterozygosity. The lack of strong evidence that heterozygous genotypes were
471 more robust has led to the dismissal of the overdominance model, and current studies on the
472 molecular basis of plasticity either consider the epigenetic and transcriptomic landscapes (*i.e.*
473 the pleiotropy model) or the shape of regulatory networks (*i.e.* the epistasis model) (Goldstein
474 and Ehrenreich 2021; Chevin et al. 2022). Our analysis of abdominal pigmentation in multiple
475 Drosophila species supports an association between plasticity and heterozygosity but in a
476 positive rather than negative way, hence revisiting the classical overdominance model.
477 However, as indicated by Scheiner (1993), the three models are not mutually-exclusive, and we
478 will illustrate how our results can agree with the different models depending on the species and
479 the sex considered.
480 In its simplest form, a gene regulatory network consists of a transcription factor
481 activating or suppressing an effector gene. Of the five monogenic cases studied here, the
482 underlying locus involves an effector gene, *t*, in one species (*D. erecta*) (Yassin et al. 2016a),

483 and a transcription factor, *pdm3*, in four species (*D. kikkawai*, *D. jambulina*, *D. burlai* and *D.*
484 cf. *bocqueti*) (Yassin et al. 2016b; Fukutomi et al. 2025) (Figure 6). Loss-of-function regulatory
485 mutations of *t* reduce plasticity in *D. erecta* recessive light homozygotes (Figure 2B), and only
486 slightly affect the degree of dominance, *i.e.* plasticity of the functional allele is nearly
487 codominant near the upper thermal limit when both dark heterozygotes and homozygotes
488 become lighter (Figure 2B). Indeed, the *t* regulatory element which is involved in female color
489 variation in both *D. erecta* and *D. melanogaster* (Bastide et al. 2013; Endler et al. 2016; Gibert
490 et al. 2017a) partly affects pigmentation plasticity in the later species (Gibert et al. 2016). On
491 the other hand, mutations in the transcription factor *pdm3* show more complex relationships
492 (Figures 2B). First, the plasticity of both homozygotes shows almost similar slopes (but
493 different means). Second, dominance relationship differed between species, even among sister
494 species such as *D. burlai* and *D.* cf. *bocqueti*. In two species (*D. kikkawai* and *D. burlai*), the
495 dark allele is dominant throughout the temperature range, whereas in the two other species (*D.*
496 *jambulina* and *D.* cf. *bocqueti*), a strong dominance reversal was observed in females.
497 Polymorphism in *pdm3* associates with female abdominal pigmentation variation in European
498 and African populations of *D. melanogaster* (Endler et al. 2016). Mutations in this gene also
499 underlie pigmentation evolution between the sister species *D. yakuba* and *D. santomea* with
500 contrasting melanism (Liu et al. 2019; David et al. 2022). Whereas pigmentation plasticity in
501 these two species is low, their reciprocal hybrids, which are heterozygous for *pdm3* interspecific
502 differences along with other genes, are highly plastic (Figure 2A; Figure 5B). This may indicate
503 that the relation between heterozygosity and plasticity may differ according to the type of the
504 underlying gene.

505



506

507 **Figure 6** – Evolution of the genetic architecture of pigmentation variation and its thermal
508 plasticity in the *melanogaster* and *montium* species groups. E = effector melanin-synthesis
509 gene; TF = transcription factor. Phylogenetic relationships are after Suvorov et al. (2022).
510

511 Besides, the types of the mutations in the alleles of the polymorphic gene may also affect
512 the degree of the plastic response. Indeed, plasticity was highest in the heterozygotes of the two
513 *montium* group species, *D. jambulina* and *D.* cf. *bocqueti*, where the light allele was dominant
514 under optimal developmental temperature regimes, *i.e.* ≥21°C. The reversal of dominance
515 according to the temperature, the main reason of the steepness of the reaction norm, is

516 associated with the evolution of dominance between the *pdm3* alleles in these species. In *D.*
517 *melanogaster*, the wildtype light allele is dominant (Tichy et al. 2008), but RNAi lines targeting
518 different parts of this gene yields contrasting results, either increasing pigmentation (mostly in
519 females) or decreasing it (mostly in males) (Rogers et al. 2014). Consequently, it is possible
520 that the *pdm3* mutations underlying the dark allele in *montium* species where it is dominant,
521 such as in *D. kikkawai* and *D. burlai*, are of the dominant negative type, which are common for
522 transcription factors (Gerasimavicius et al. 2022). Such transcription factors usually have
523 multiple isoforms, one of them has the DNA binding domain but lacks polymerase or other
524 protein binding domains that are needed to express a gene, whereas another isoform has both
525 DNA and protein binding domains. Consequently, the isoforms have opposite effects on the
526 trait. In the heterozygote, the isoform lacking the protein binding domain competes with the
527 isoform bearing this domain, hence altering its function. On the other hand, mutations in species
528 with the light allele being dominant, like *D. jambulina* and *D. cf. bocqueti*, may be of the
529 classical loss-of-function type that may reduce but do not completely eliminate the effect of the
530 wild-type light allele. Further molecular dissection of the underlying alleles is required to
531 understand the evolution of dominance reversal at *pdm3*.

532 The effect of mutations in both modifier and effector genes, *i.e.* the epistatic model of
533 plasticity, is best illustrated in *D. kikkawai* and the hybrids between *D. santomea* and *D. yakuba*.
534 In *D. kikkawai*, the effector *yellow (y)* gene lacks a conserved *cis*-regulatory element leading to
535 the loss of body pigmentation (Jeong et al. 2008). Because this gene is X-linked, a dosage effect
536 may occur between the sexes. Plasticity in males additively increased with the number of copies
537 of the dominant dark *pdm3* allele, which is an indirect regulator of *y* (Liu et al. 2019). Putatively
538 negative dominant mutations in this gene are expected to activate *y*, but the effect may depend
539 on the number of copies of *y*, being higher in females. Similarly, whereas hybrid males
540 mothered by *D. santomea*, *i.e.* those carrying defective X-linked melanin synthesis genes *t* and

541 *y*, had similar plasticity slopes to hybrid females. But this slope was halved in hybrid males
542 mothered by *D. yakuba*, with functional *y* and *t*. Therefore, plasticity is affected by the
543 functionality of the polymorphism at multiple genes, as predicted by the epistasis model,
544 although the degree of plasticity was higher in fully heterozygous background.
545
546 *The association between plasticity and polymorphism may be more general than currently*
547 *appreciated*
548 We have restricted our analyses to eight species belonging to two species groups of the
549 subgenus *Sophophora*. Despite the differences in their reaction norms, both groups contain
550 species with sexually dimorphic melanism, and a single gene with antagonistic sexual effect
551 may underlie the association between plasticity and heterozygosity, hence reducing the
552 independence of our observations. Future analyses should investigate pigmentation thermal
553 plasticity in non-*Sophophora* fly species lacking sexual dimorphism, with or without Mendelian
554 polymorphism (e.g., Brisson et al. 2005; Rocha et al. 2009; Wittkopp et al. 2009). Beyond
555 *Drosophila*, color polymorphism abounds in nature (Orteu and Jiggins 2020; Sapir et al. 2021).
556 Investigation of the diversity in reaction norms in species with heterozygous and homozygous
557 color genotypes, especially in these with a hierarchy of dominance (e.g., Durand et al. 2014; Le
558 Poul et al. 2014), should shed light on the generality of the relationship between heterozygosity
559 and the degree of plasticity.
560 Most quantitative traits for which phenotypic plasticity was studied in *Drosophila*, such
561 as body size, wing shape, bristle number, ovariole number or life-history traits, are highly
562 polygenic with often a redundant genetic basis (David et al. 2004; Gibert et al. 2004a; Mackay
563 and Lyman 2005; Lobell et al. 2017; Lafuente et al. 2018; Pitchers et al. 2019). Identifying the
564 underlying genotypes of these traits to test for the effect of heterozygosity may be difficult. An
565 alternative to the 'forward genetics' approach that we leveraged here, is to use a 'reverse

566 genetics' analysis, wherein the transcription level of polymorphic genes can be analyzed in
567 homozygous vs. heterozygous states in different environments, even in the lack of knowledge
568 about the phenotypic consequences of the genotypes. One such significant example comes from
569 a study by Chen et al. (2015) who showed that 62% of genes with thermal plastic response in
570 *D. melanogaster* (*i.e.* ~51% of total genes) swapped allelic dominance in heterozygotes
571 between cold and hot environments. Future similar transcriptomic analyses are therefore highly
572 needed to test the generality of these patterns, especially these using hybrids between species.
573
574 *The evolutionary consequences of links between phenotypic plasticity and polymorphism*
575 A link between phenotypic plasticity and polymorphism can provide simple
576 explanations on how plasticity evolves. In fact, plasticity can be lost through any mechanism
577 reducing genetic heterozygosity such as inbreeding or directional selection. Indeed, the 'flexible
578 stem hypothesis' postulates that from a plastic ancestor, descendants with fixed phenotypes
579 from the ancestral phenotypic spectrum can evolve (West-Eberhard 2003; Gibert 2017). A
580 likely example from our data is the case of *D. santomea* and *D. yakuba*. According to the
581 phylogeny given in Figure 6, it is likely that the ancestor of these species was highly plastic
582 given the high plasticity of *D. melanogaster* (Gibert et al. 2000) and *D. suzukii* (Colinet and
583 Kustre 2025). Pigmentation difference between *D. yakuba* and *D. santomea* is due to mutations
584 in at least six loci (Liu et al. 2019; David et al. 2022), but it is unlikely that all these mutations
585 have arisen simultaneously. This means that a substantial degree of heterozygosity segregated
586 in the ancestor of these species, and consequently this ancestor has gradually lost its plasticity
587 as more and more alleles became fixed in each lineage. Indeed, pigmentation plays a role in the
588 reproductive isolation between these two species (David et al. 2022), and a hybrid zone on the
589 African island of Sao Tomé between these two species exists (Lachaise et al. 2000; Llopart et

590 al. 2005; Turissini and Matute 2017). A plastic hybrid is disadvantaged and the 'flexible stem
591 hypothesis' may in essence reflects a simple model of 'underdominance' of the heterozygote.
592 Conversely, phenotypic plasticity can be gained by any mechanism that increases
593 genetic variability and heterozygosity in a population, such as mutations, outbreeding (e.g.,
594 gene flow and introgression), and balancing selection. Indeed, any new mutation in a di- or
595 polyploid organism first arises in the heterozygous state, and it has been argued that a
596 heterozygous advantage is essential for the spread of new mutations and counteracting the loss
597 by random genetic drift (Sellis et al. 2011). In cases where plasticity is adaptive, the higher
598 plasticity of the heterozygote can facilitate the spread of this mutation. Such adaptive plasticity
599 gain model is in essence an 'overdominance' model. An important consequence of the
600 overdominance model is that plasticity could be a mechanism to maintain genetic diversity, *i.e.*
601 rather than 'buying time' for genetic adaptation to occur, plasticity may be a major source for
602 'saving time' by maintaining standing genetic variation, such as in the case of introduced
603 species or multivoltine species inhabiting a heterogeneous spatial or seasonal environment
604 (Gulisija et al. 2016; Wittmann et al. 2017; Promy et al. 2023). Further theoretical and empirical
605 investigations of the link between heterozygosity and plasticity will definitively improve our
606 understanding of the evolution of both.
607
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