Article

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

Jean R. David[1,2*], Béatrice Denis[1], Pascaline Francelle[2], Alicia Lemaire[1], Aparup Das[3], Sujata Mohanty[4], Patricia Gibert[5], Amir Yassin[1,2**]

[1]Laboratoire Évolution, Génomes, Comportement et Écologie, CNRS, IRD, Université Paris-Saclay – Institut Diversité, Écologie et Évolution (IDEEV), 91190 Gif-sur-Yvette, France.

[2]Institut Systématique Evolution Biodiversité (ISYEB) CNRS, MNHN, Sorbonne Université, EPHE, Université des Antilles, 57 rue Cuvier, CP 50, 75005 Paris, France.

[3]ICMR-National Institute of Research in Tribal Health, Jabalpur, Madhya Pradesh, India.

[4]Department of Biotechnology, Jaypee Institute of Information Technology, Sector 62, Noida, Uttar Pradesh-201309, India.

[5]Université Claude Bernard Lyon 1, Laboratoire de Biométrie et Biologie Evolutive, CNRS, UMR5558, Villeurbanne, Rhônes-Alpes, 69100, France.

Running title: Phenotypic plasticity and heterozygosity

*Deceased in 2021.

****Corresponding author:** amir.yassin@universite-paris-saclay.fr

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 basic measure of polymorphism, has previously been dismissed. Here, we investigate 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.

Introduction

Phenotypic plasticity is the ability of a genotype to produce different phenotypes under distinct environmental conditions (Bradshaw 1965; Scheiner 1993; Pigliucci 2001; West-Eberhard 2003; Sultan 2021). Plasticity produces responses that are often summarized in terms of a reaction norm (Woltereck 1909; Dupont et al. 2024). When such a response generates the optimal phenotypic values in the different environments, plasticity can facilitate adaptation even in the absence of genetic variation (Ghalambor et al. 2015). This becomes particularly important in cases where the population loses a significant portion of genetic polymorphism, such as after a founder bottleneck during colonization of a new environment, or when the environment change is faster than the rate of accumulation of new adaptive mutations, the so-called 'buying time' role of plasticity (Diamond and Martin 2021). Consequently, several authors have considered phenotypic plasticity and genetic polymorphism as distinct alternative adaptive strategies (e.g., Wennersten and Forsman 2012).

Despite the definition of plasticity as a property of a genotype, it is usually studied for complex phenotypic traits whose underlying genotypes are not fully known (e.g., flowering time in *Arabidopsis*, growth in yeasts, etc.) (Goldstein and Ehrenreich 2021; Kovuri et al. 2023). Genotypes do not only differ in the degree of their phenotypic plasticity (e.g., the slope of the reaction norm) but also in their degree of genetic variability, with heterozygous genotypes in non-haploid organisms having more alleles than hemi- or homozygotes. Among the early genetic models of phenotypic plasticity, the Overdominance model explicitly related plasticity to genotypic diversity (Scheiner 1993). The model postulated that plasticity is a function of homozygosity, with the heterozygous genotype considered to be the most advantageous because it is the most robust, *i.e.* the least plastic. This assumption reflected the view of

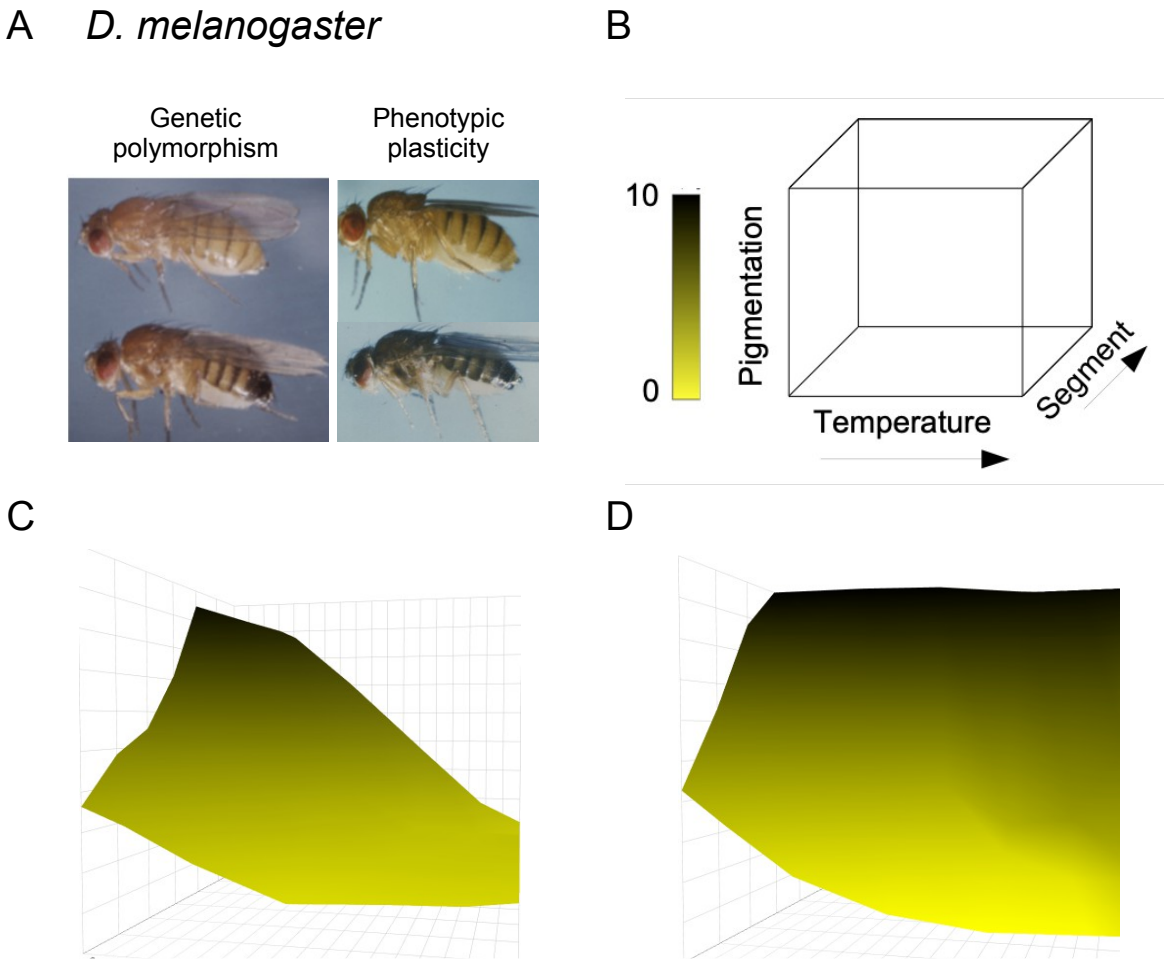
68 plasticity at the mid-20th century as a developmental nuisance (Lerner 1954; Bradshaw 2006).
69 Indeed, some of the earliest empirical examples of the overdominance model came from
70 studies of wing morphology in *Drosophila melanogaster*, where two mutants showed the
71 highest temperature-sensitivity when found in the homozygous state, but their heterozygote
72 recapitulated the wildtype thermal response, *i.e.* it was less plastic than either mutant (Harnly
73 and Harnly 1936; Schmalhausen 1949). Gillespie and Turelli (1989) extended this view to a
74 multi-locus quantitative genetics model and concluded that stabilizing selection on more
75 environmentally robust heterozygotes will maintain genetic variation in populations. Pigliucci
76 (1992) developed a one-locus, two-allele classical population genetics model for testing the
77 evolution of plasticity under overdominance and concurred with Gillespie and Turelli's (1989)
78 multilocus result. He concluded that plasticity becomes "increasingly disadvantageous when
79 the system approaches a perfect equilibrium between the probabilities of occurrence of the
80 two environments."

81 A direct link between heterozygosity and robustness was not always evident from the
82 empirical literature, and overdominance was disfavored relative to other genetic models such
83 as pleiotropy, *i.e.* plasticity is a function of the expression of a gene, and epistasis, *i.e.* plasticity
84 is a function of interactions between loci. Scheiner (1993) concluded that "plasticity is not a
85 function of heterozygosity", and Pigliucci (2005), a decade later, stated that "heterozygosity
86 ha[d] little, if anything, to do with the genetic basis of plasticity." A search using "heterozygo*"
87 in a recent 400-page long book on *Phenotypic Plasticity and Evolution* (Pfennig 2021) yielded
88 no result. However, almost none of the studies cited by Scheiner (1993) and Pigliucci (2005) to
89 reach their conclusions has addressed the link between heterozygosity and plasticity in the
90 most optimal setting similar to Pigliucci's (1992) theoretical model, *i.e.* comparing the reaction
91 norms of a phenotype with a simple, well-characterized genotypic basis. Conspicuous color

92 polymorphisms are ideally suited for these experiments, because they often involve a simple
93 genetic architecture and color synthesis in many species results from a well conserved gene
94 network (Orteu and Jiggins 2020). This is particularly true for melanism. In several lineages of
95 butterflies, simple Mendelian polymorphisms have evolved in response to the industrial
96 revolution, with the melanic wing allele being dominant in some species and recessive in
97 others (Ford 1945). The genetic basis of this industrial melanism has recently been identified
98 to be a conserved micro-RNA close to the locus of the transcription factor *Cortex* (Tian et al.
99 2024). Remarkably, several butterfly species also show a seasonal melanic polyphenism, with
100 cold season morphs being darker. van der Burg et al. (2020) showed that mutations at the
101 same locus containing *Cortex*, and consequently the linked micro-RNA, underlie this
102 polyphenism in *Junonia caenia*. Plasticity in simple color polymorphism systems was also
103 observed in *Harmonia* ladybugs morphs (Michie et al. 2010).

104 Much of our knowledge about the genetic and developmental basis of melanin
105 synthesis comes from studies in *Drosophila*. Pigmentation differences between body
106 segments, sexes, geographical populations, seasons, and species are often due to *cis*- and
107 *trans*-regulatory modifications of melanin synthesis genes, such as *tan (t)*, *yellow (y)* or *ebony*
108 *(e)*, and their transcription factors, such as *bric-à-brac (bab)*, *POU-domain-motif-3 (pdm3)* or
109 *Abdominal-B (Abd-B)* (Massey and Wittkopp 2016; Hughes et al. 2023). Wild-caught flies from
110 the same locality usually differ in their degree of pigmentation (Figure 1 A; Bastide et al. 2013,
111 2016; Dembeck et al. 2015; Endler et al. 2016; Castro et al. 2018), but flies collected from cold
112 high altitudes, latitudes and seasons tend to be dark, even when their progeny is maintained
113 under standard thermal conditions (Telonis-Scott et al. 2011; Bastide et al. 2014, 2016;
114 Takahashi et al. 2014; Negoua et al. 2019; Berardi et al. 2024). Similarly, when full-sib flies
115 issued from the same isofemale line are grown under different temperatures, a negative

reaction norm is detected, with flies grown under low temperatures being darker (Figure 1; (Gibert et al. 1996, 1998, 2000, 2004b, 2009; Chakir et al. 2002; Pétavy et al. 2002, 2018; Lafuente et al. 2021, 2024). Thermal plasticity in *D. melanogaster* is due to the higher expression of the *t* and *y* genes in colder temperatures (Gibert et al. 2016, 2017b). A natural variation in the transcription factor *bab* underlying color differences also showed distinct responses to developmental temperature through repression of *t* and *y* in hotter environments (Castro et al. 2018). This parallelism between genetic polymorphism in the wild and phenotypic plasticity in the lab is strongly suggestive for an adaptive role for darker pigmentation in thermoregulation. Indeed, drosophilids with darker cuticle have higher body temperature (Freoa et al. 2023).



FA6	-0.40±0.03***
FA7	-0.58±0.06***

MA5	-0.03±0.00**
MA6	0.00±0.00

Figure 1 – Pigmentation variation in *Drosophila melanogaster*. A) Variation in abdominal pigmentation can be due to genetic polymorphism (left) or thermal phenotypic plasticity (right) (Photos from Jean R. David's archive). B) Three-dimensional analysis of pigmentation variation across growth temperatures and abdominal segments. C) Pigmentation thermal plasticity in females. D) Pigmentation thermal plasticity in males. Reaction norm slopes for the last two abdominal segments (A6 and A7 in females (F) and A5 and A6 in males (M)) are given for each sex. (Data from Gibert et al. 2000).

The genetic architecture of pigmentation variation has dramatically evolved between drosophilid species. Whereas female abdominal pigmentation variation is continuous and polygenic in *D. melanogaster* as well as most species, it is discrete and monogenic in multiple

138 species (Payant 1986; Yassin et al. 2016a,b). In some species, pigmentation is nearly fixed with
139 very slight variability even between the sexes. This is the case of the two sister species, *D.*
140 *santomea* and *D. yakuba*, where both males and females' posterior abdomens are invariably
141 light or dark, respectively (Lachaise et al. 2000; Jeong et al. 2008; David et al. 2022). We aim
142 here at quantifying the level of pigmentation phenotypic plasticity in eight species with distinct
143 genetic architectures grown under six thermal regimes. To this end, we focused on the effect
144 of heterozygosity, as it can be seen in species hybrids (*i.e.* the ultimate viable heterozygotes)
145 and heterozygous genotypes of five species with monogenic architectures. Our results support
146 a positive link between plasticity and the degree of genetic polymorphism that was not
147 previously assumed.
148
149 **Materials and Methods**
150 We studied five cases where for each the thermal phenotypic plasticity can be
151 compared between homozygous dark and light lines and their reciprocal hybrids. Four cases
152 considered within-species monogenic Mendelian color polymorphism with one (*D. erecta*)
153 from the *melanogaster* species group and three cases (*D. jambulina*, *D. burlai* and *D.* cf.
154 *bocqueti*) from its sister clade, the *montium* species group. For each of these species
155 homozygous dark and light lines were established through selection from a polymorphic mass
156 culture (Table 1). The monogenic basis of female pigmentation in these species are known
157 (Yassin et al. 2016a,b; Prigent et al. 2020). In two species (*D. erecta* and *D. burlai*), the dark
158 allele is dominant (Yassin et al. 2016a,b), whereas in the two other species (*D. jambulina* and
159 *D.* cf. *bocqueti*), the light allele is dominant (Watanabe et al. 1982; Prigent et al. 2020). The
160 fifth case involved hybridization between the two sister species *D. santomea* and *D. yakuba* of
161 the *melanogaster* group (Table 1). We also obtained published data of the thermal plasticity

162 response of *D. melanogaster* (Gibert et al. 2000), the nomitypical species of the *melanogaster*
163 group with polygenic basis of pigmentation variation, and the monogenic *D. kikkawai* (Gibert
164 et al. 1999) for comparison purposes (Table 1).
165
166 Table 1. *Drosophila* strains analyzed in this study.

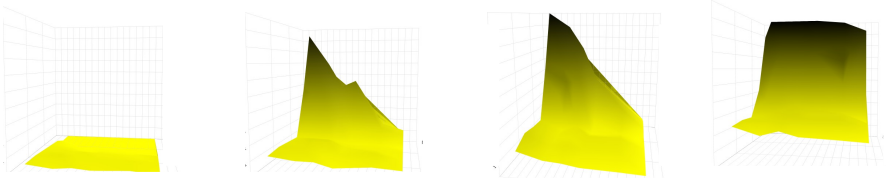
Group	Subgroup	Complex	Species	Strain
melano-gaster	*melano-gaster*	*melano-gaster*	*melanogaster*	Bordeaux, France (Gibert et al. 2000)
melano-gaster	*melano-gaster*	*yakuba*	*yakuba*	Kunde, Cameroon, 1967 (L. Tsacas)
melano-gaster	*melano-gaster*	*yakuba*	*santomea*	Sao Tomé, Sao Tomé & Principe, 1998 (D. Lachaise)
melano-gaster	*melano-gaster*	*erecta*	*erecta*	La Lopé Forest, Gabon, 1998 (D. Lachaise)
montium	*kikkawai*	*kikkawai*	*kikkawai*	Chandigarh, India (Gibert et al. 1999)
montium	*seguyi*	*jambulina*	*jambulina*	New Delhi, India, 2014 (J. R. David, S. Mohanty)
montium	*seguyi*	*bocqueti*	*burlai*	Mt Oku, Cameroon, 2008 (M. Veuille)
montium	*seguyi*	*bocqueti*	cf. *bocqueti*	Sao Tomé, Sao Tomé & Principe, 2015 (J. R. David, M. Lang)

167
168 For each case, pigmentation was scored on >4 days old 10 males and 10 females as in
169 Yassin et al. (2022) following the pigmentation score scale proposed by David et al. (1990).
170 Flies were grown at five temperatures going from 14°C to 28°C with 3° increments. In each
171 case, pigmentation was scored on six and five abdominal segments in females and males
172 respectively (denoted A2-7 with F and M for females and males, respectively). All statistical
173 analyses were conducted using R (R Core Team 2023), with 3D plots obtained using the Plotly
174 package in R (Sievert 2020). For the sake of simplicity, the degree of phenotypic plasticity (*i.e.*
175 the shape of the reaction norm) was summarized in terms of the slope of a linear regression.
176 For monogenic species, we followed the nomenclature given in Gibert et al.'s (1999) paper,

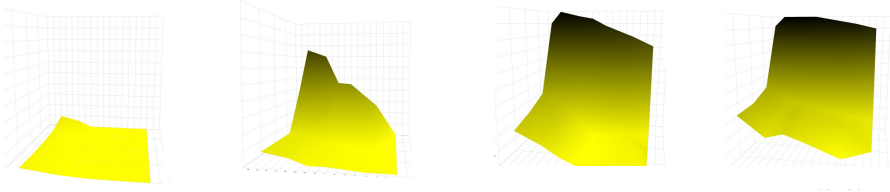
177 referring to the light and dark alleles as *L* and *D*, respectively, and to the three genotypes as *L*
178 *x L*, *L x D* or *D x L*, and *D x D*. For heterozygotes, the allele from the mother is given first
179 depending on the cross.
180
181 **Results**
182 *Ancestral plasticity resurrection in* D. santomea *and* D. yakuba *hybrids*
183 Abdominal pigmentation is the most discriminating quantitative trait among the nine
184 species of the *melanogaster* subgroup (Yassin et al. 2022). The thermal plasticity of this trait
185 was examined only in two sibling species, *D. melanogaster* and *D. simulans*, and showed quasi-
186 parallel reaction norms (Pétavy et al. 2002). In females of both species, the phenotypic
187 plasticity between cold and warm environments increases towards most posterior abdominal
188 segments, with the pigmentation of A7 being the most plastic (Figure 1C). In males, on the
189 other hand, this pattern is restricted to the first three abdominal segments (A2, A3 and A4),
190 whereas the last two segments (A5 and A6) are completely dark with almost no plastic
191 response (Figure 1D). Both species belong to the *melanogaster* complex, but similar patterns
192 of pigmentation plasticity was observed in *D. suzukii* (Colinet and Kustre 2025), an outgroup
193 species belonging to another subgroup of the *melanogaster* group, indicating that plasticity is
194 likely ancestral.
195 *Drosophila santomea* and *D. yakuba* belong to the *yakuba* complex of the
196 *melanogaster* subgroup. The two sister species are monomorphically either light (*D.*
197 *santomea*) or black (*D. yakuba*) and reciprocal crosses produce hybrid fertile females and
198 sterile males with intermediate pigmentation phenotypes in both (Llopart et al. 2005; Liu et
199 al. 2019; David et al. 2022). At least two X-linked and four autosomal genes underlie the
200 pigmentation differences between these species (Liu et al. 2019; David et al. 2022). We found

201 the two species to lack plasticity (Figure 2A). However, in reciprocal crosses, plasticity was
202 generated in both sexes in a pattern similar to that of *D. melanogaster* females, *i.e.* darker
203 phenotypes in cold temperature (Figure 2A). The degree of plasticity, as measured by the slope
204 of the reaction norms of the two last abdominal segments, did not differ between reciprocal
205 hybrid females, since these females share the same heterozygous genotypes for both the X-
206 linked and autosomal genes. In reciprocal hybrid males, on the other hand, males mothered
207 by *D. santomea*, *i.e.* those carrying loss-of-function mutations in the X-linked pigmentation
208 genes *y* and *t*, had a steeper slope than males mothered by *D. yakuba* with functional copies
209 of these genes. In females and males mothered by *D. santomea*, dominance is completely
210 reversed with pigmentation of hybrid flies resembling *D. yakuba* in cold temperature and *D.*
211 *santomea* in hot temperature.
212
213 *Loss-of-function mutations at* t *leads to loss of plasticity in* D. erecta *recessive homozygotes*
214 Of the six loci underlying pigmentation difference between *D. yakuba* and *D. santomea*,
215 only one, the X-linked *t*, underlies female-limited Mendelian color dimorphism in *D. erecta*
216 (Yassin et al. 2016a), a species of the *erecta* complex of the *melanogaster* subgroup. We found
217 that the loss-of-function mutations in a likely female-specific suppressor of this gene likely led
218 to no thermal plasticity in the recessive homozygous light line (Figure 2B). However, these
219 mutations are completely recessive in heterozygous females, where similar thermal slopes to
220 that of the homozygous dark females were found. Regardless of the genotype, males showed
221 no plastic response. So, plasticity does not correlate with heterozygosity in *D. erecta*, but
222 rather, to a low extent, with a functional *t* gene.
223
224

A *D. santomea*, *D. yakuba* and their hybrids

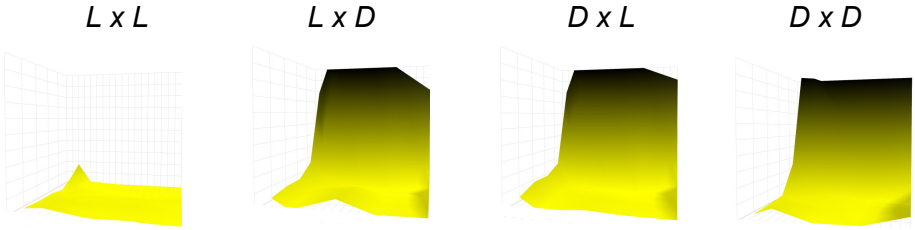


FA6	-0.02±0.01	-0.24±0.07*	-0.32±0.09*	-0.04±0.08
FA7	0.00±0.00	-0.56±0.07**	-0.67±0.06***	-0.07±0.03

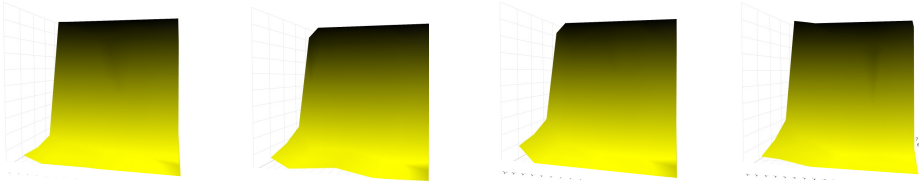


MA5	-0.02±0.01	-0.29±0.04**	-0.20±0.01***	-0.07±0.03
MA6	-0.06±0.02	-0.53±0.05***	-0.21±0.02***	-0.08±0.01**

B *D. erecta*



FA6	-0.04±0.03	-0.57±0.09**	-0.54±0.07**	-0.55±0.06**
FA7	-0.08±0.05	-0.11±0.05	-0.06±0.03	0.00±0.00



MA5	-0.09±0.05	-0.10±0.06	-0.03±0.07	-0.09±0.08
MA6	0.00±0.00	0.00±0.00	0.0±0.00	0.00±0.00

Figure 2 – Thermal phenotypic plasticity of pigmentation on successive abdominal segments in species of the *melanogaster* group. A) *Drosophila santomea (san)*, *D. yakuba (yak)* and their reciprocal hybrids. B) *D. erecta*. For each cross, the genotype of the mother is given first. *L* and *D* for light and dark alleles, respectively. Reaction norm slopes for the last two abdominal segments (A6 and A7 in females and A5 and A6 in males) are given for each sex and genotype. The 3D plot axes are as in Figure 1B.

233 *Phenotypic plasticity correlates with heterozygosity in females of species of the* montium *group*
234 *with monogenic variation and dark dominant allele*

235 Female-limited Mendelian melanism, similar to that found in *D. erecta*, has evolved in
236 22 *montium* group species belonging to five distinct subgroups (Yassin et al. 2016b; Yassin
237 2018). Of the 15 species where the genetics was studied, the dark allele was dominant in 13
238 species (Yassin et al. 2016b). In four species with dark-dominant allele namely *D. serrata*
239 (*serrata* subgroup), *D. kikkawai* and *D. leontia* (*kikkawai* subgroup) and *D. burlai* (*seguyi*
240 subgroup), the polymorphism maps to a single locus, the autosomal transcription factor *pdm3*
241 (Yassin et al. 2016b). In these species, males have a light abdomen in the three former species,
242 which is characteristic to their respective subgroups, whereas males have a dark abdomen in
243 *D. burlai*, which is common in the *seguyi* subgroup to which it belongs (Yassin et al. 2016b;
244 Yassin 2018). The loss of male pigmentation in *D. kikkawai* is, at least partially, due to *cis*-
245 regulatory mutations in the X-linked melanin synthesis gene *y* (Jeong et al. 2006).

246 Gibert et al. (1999) compared the thermal plasticity of abdominal pigmentation
247 between homozygous light and dark *D. kikkawai* lines and their progeny from reciprocal
248 crosses. For females, heterozygotes had nearly twice the slope of either homozygous line in
249 A6, whereas in males, plasticity increases with the number of copies of the dark *pdm3* allele
250 for both A5 and A6 (Figure 3A). In *D. burlai*, female heterozygotes had stronger plasticity than
251 homozygotes only for A7, whereas no difference was found at either A5 and A6 in males, with
252 most of the genotypes being plastic (except A6 in homozygous dark males) (Figure 3B).

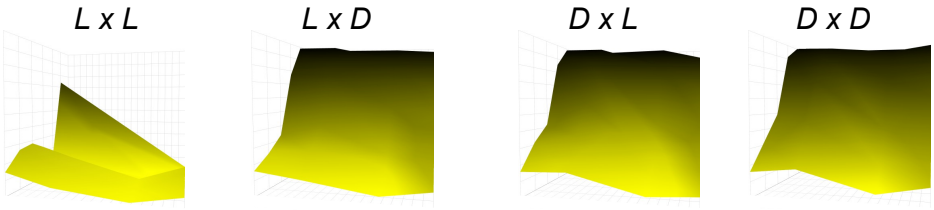
253

254

255

256

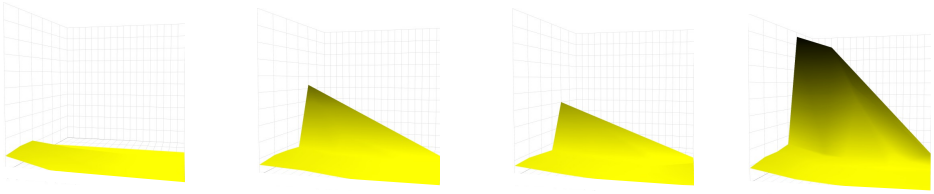
A *D. kikkawai*



Female

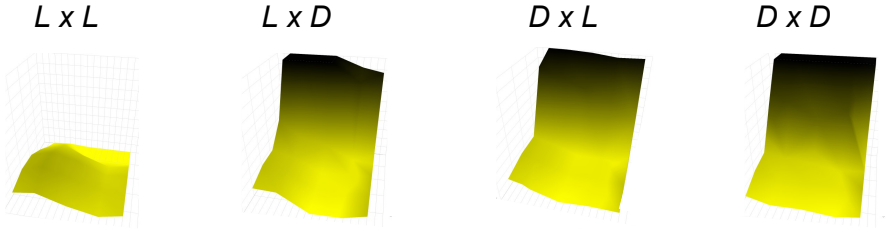
FA5	-0.09±0.05	-0.18±0.04*	-0.28±0.09	-0.07±0.07
FA6	-0.07±0.03	-0.11±0.02*	-0.12±0.02*	-0.01±0.02
FA7	-0.39±0.11	-0.02±0.00	-0.03±0.02	-0.01±0.00

Male



MA4	-0.07±0.01*	-0.04±0.01*	-0.01±0.02	-0.08±0.02*
MA5	-0.01±0.00*	-0.03±0.01	-0.03±0.01*	-0.08±0.02*
MA6	0.00±0.00	-0.27±0.11	-0.23±0.05*	-0.58±0.15

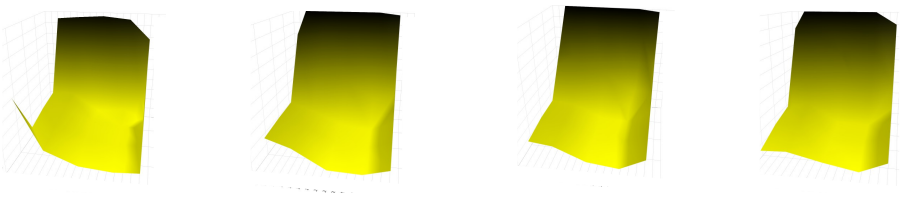
B *D. burlai*



Female

FA6	-0.11±0.02***	-0.08±0.02***	-0.01±0.00*	0.00±0.00
FA7	-0.01±0.01	-0.13±0.01***	-0.05±0.01***	0.00±0.00

Male



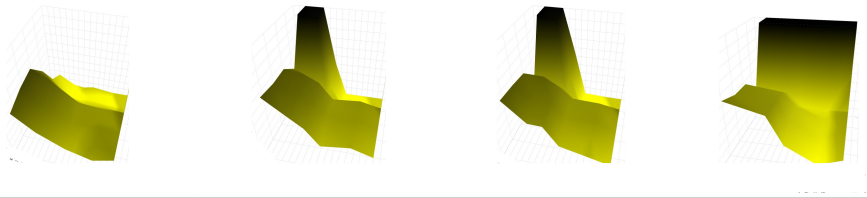
MA5	-0.14±0.01***	-0.35±0.04***	-0.24±0.03***	-0.27±0.03***
MA6	-0.10±0.02***	-0.01±0.00**	-0.01±0.01*	0.00±0.00

Figure 3 – Thermal phenotypic plasticity of pigmentation on successive abdominal segments in monogenic species of the *montium* group where the dark allele is dominant. A) *Drosophila kikkawai* (data from (Gibert et al. 1999). B) *D. burlai*. For each cross, the genotype of the mother is given first. *L* and *D* for light and dark alleles, respectively. Reaction norm slopes for the last two abdominal segments (A6 and A7 in females and A5 and A6 in males) are given for each sex and genotype. The 3D plot axes are as in Figure 1B.

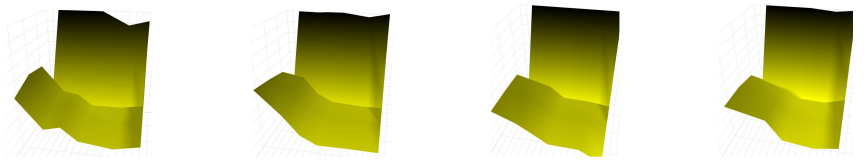
Phenotypic plasticity reaches its maximum in female heterozygotes of species of the montium *group with monogenic variation and light dominant allele*

Drosophila jambulina is one of three species of the *montium* group with female Mendelian melanism wherein the light allele was reported to be dominant (Yassin et al. 2016b; Prigent et al. 2020). However, this dominance relationship is ambiguous since Parkash and Sharma (1978) found dark dominance for an Indian population, whereas Watanabe et al. (1982) found light dominance also for Indian populations and concluded that dominance in *D. jambulina* was "a great problem existing in a simple genetic phenomenon". We analyzed the phenotypic plasticity of homozygous light and dark female strains along with their reciprocal heterozygotes. The female posterior abdominal pigmentation of both homozygous lines completely lacked a plastic response (Figure 4A). The heterozygous females on the other hand showed the steepest plastic response with a slope ≥0.80 for both A6 and A7. Indeed, at temperatures ≥21°C, the light allele is dominant, but at temperatures <21°C the dark allele is dominant. It is thus possible that Parkash and Sharma (1978) and Watanabe et al. (1982) have conducted their experiments at different temperatures reaching contradictory results. *D. jambulina*, like *D. burlai*, belongs to the *seguyi* subgroup, with males having dark posterior abdomen (Yassin 2018). Like *D. burlai*, A5 was plastic for nearly all genotypes (except homozygous light), whereas A6 showed weak plasticity.

A *D. jambulina*

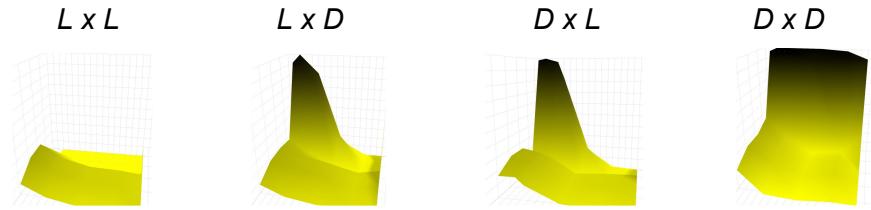


FA6	-0.02±0.04	-0.80±0.06***	-0.92±0.05***	0.00±0.00
FA7	-0.02±0.04	-0.84±0.06***	-0.95±0.05***	0.00±0.00

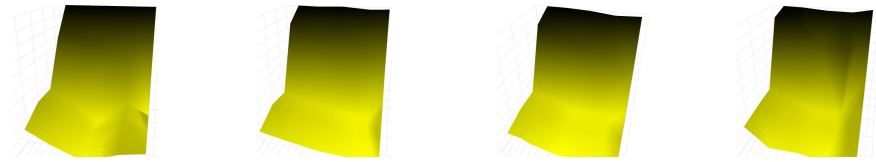


MA5	-0.12±0.02	-0.25±0.02***	-0.18±0.02***	-0.11±0.02***
MA6	-0.07±0.01***	-0.01±0.00*	0.00±0.00	0.00±0.00

B *D.* cf. *bocqueti*



FA6	0.00±0.00	-0.73±0.04***	-0.69±0.04***	-0.03±0.01***
FA7	0.00±0.00	-0.79±0.06***	-0.74±0.05***	-0.06±0.02***



MA5	-0.57±0.08***	-0.23±0.03***	-0.29±0.02***	-0.13±0.02***
MA6	0.00±0.00	-0.01±0.00	-0.02±0.01**	-0.01±0.01

Figure 4 – Thermal phenotypic plasticity of pigmentation on successive abdominal segments in monogenic species of the *montium* group where the light allele is dominant. A) *Drosophila jambulina*. B) *D.* cf. *bocqueti*. For each cross, the genotype of the mother is given first. *L* and *D* for light and dark alleles, respectively. Reaction norm slopes for the last two abdominal segments (A6 and A7 in females and A5 and A6 in males) are given for each sex and genotype. The 3D plot axes are as in Figure 1B.

293 Prigent et al. (2020) reported a third case of a *montium* species with dominant light
294 allele in the Sao Tomé strains that we analyze here as *D.* cf. *bocqueti*. Like *D. burlai* and *D.*
295 *jambulina*, *D.* cf. *bocqueti* belongs to the *seguyi* subgroup, but it is closely related to *D. burlai*,
296 with whom and along a third species, *D. chauvacae*, they constitute the Afrotropical *bocqueti*
297 species complex within the *seguyi* subgroup (Yassin 2018). We crossed *D.* cf. *bocqueti* and *D.*
298 *burlai* and reciprocal crosses yielded inviable progeny, hence supporting the close relationship
299 between them and their proper specific status despite their great morphological similarity.
300 Unlike *D. burlai*, where the dark allele is constantly dominant, we found strong dominance
301 reversal in heterozygotes similar to that found in the distantly-related *D. jambulina*; the light
302 allele was dominant at temperatures ≥21°C and the dark allele is dominant at <21°C (Figure
303 4B). Like *D. jambulina*, pigmentation of female heterozygotes has a very steep slope ≥0.70,
304 whereas the slope of the homozygotes never exceeded 0.06 for both A6 and A7. Similar to *D.*
305 *jambulina* also, the posterior abdomen of males showed a weak plasticity, with the strongest
306 slope found for the homozygous light genotype but for A5 rather than for A6. Because both
307 species are tropical, predominantly living in environments with an average temperature ≥21°C,
308 the light allele may be considered the dominant one.
309
310 *Excess heterozygosity among the most plastic abdominal segments*
311 The distribution of the total 272 reaction norms that we estimated for all segments,
312 sexes, strains and species has a near zero mean ~-0.13, meaning that most segments have low
313 plasticity and are developmentally robust (Figure 5A). However, the distribution is strongly
314 negatively skewed as expected from the general pattern of low pigmentation under high
315 temperatures. Nearly 10% of the reaction norms fall below -0.25, and these correspond to the
316 most plastic segments. This pool of strongly plastic segments was not enriched for species (chi-

317 squared P = 0.080) or sex (chi-squared P = 0.258). However, there was a bias for posterior
318 segments (A5-7) (chi-squared $P = 4.2 \times 10^{-5}$). Most importantly, 80% of the most plastic
319 segments were found in heterozygous genotypes (chi-squared $P = 6.4 \times 10^{-4}$), with the opposite
320 trend, *i.e.* excess homozygotes, in the pool of most robust segments with slopes above the 0.9
321 quantile (chi-squared P = 0.001) (Figure 5A).

322 All cases where a heterozygous genotype had a strong plasticity showed a reversal of
323 dominance, *i.e.* the phenotype of the heterozygote (F1, averaged over reciprocal crosses)
324 tended towards the dark homozygous genotype in cold temperatures (the blue curve of F1 –
325 *D x D* in Figure 5B), and towards the light homozygous genotype in hot temperatures (the red
326 curve of *L x L* – F1 in Figure 5B). The intersection between the two curves corresponds to the
327 temperature at which dominance is reversed, as well as the phenotypic value of the
328 codominance at that temperature, *i.e.* the point at which the heterozygote is at mid-distance
329 between both homozygotes. From these curves, showed for the last two abdominal segments
330 of each sex in Figure 5B, dominance reversal occurred for both males and females in the case
331 of the hybrids between *D. santomea* and *D. yakuba*, only in females in *D. erecta*, *D. jambulina*
332 and *D.* cf. *bocqueti*, and only in males in *D. burlai*. No intersection was found in *D. kikkawai*,
333 but an intersection at extreme temperature in the female at A5 was found. *D. kikkawai* is the
334 only species showing reversed slopes depending on the sex. The phenotype of the male
335 heterozygote approaches the dark genotype at high temperatures, where they both become
336 light, whereas the phenotype of the female heterozygote approaches the light genotype at
337 low temperatures, where they both become dark.

338

339

340

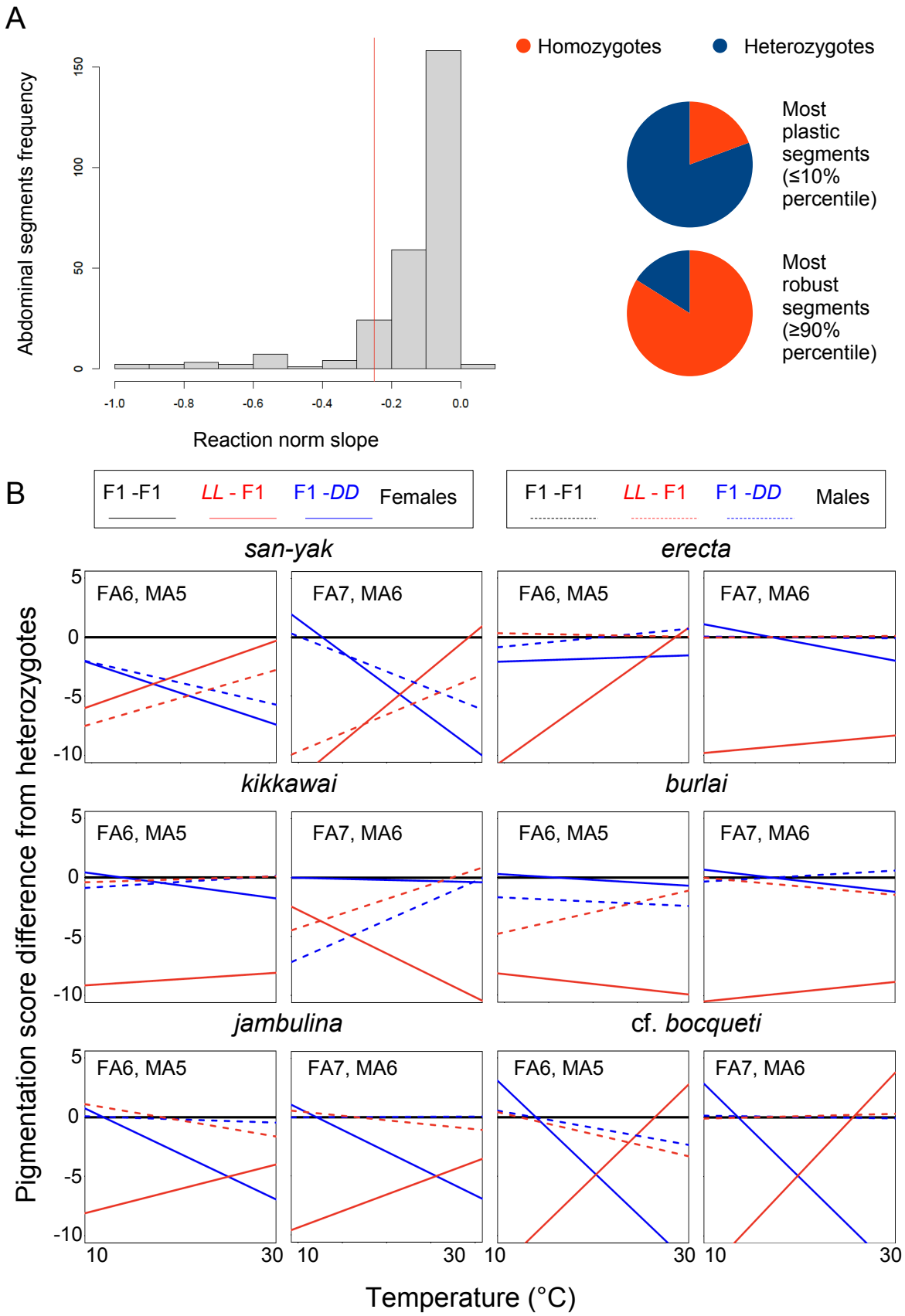


Figure 5 – Thermal phenotypic plasticity associates with heterozygosity. A) Histogram of the reaction norm slopes of 272 abdominal segments analyzed across all species. The red line

indicates the 0.1 quantile (values below which plasticity is high). The pie charts reflect the proportions of the heterozygous and homozygous genotypes among the most plastic (≤0.1 quantile) and the most robust (≥0.9 quantile) abdominal segments. B) Patterns of temperature-dependent dominance reversal in the six analyzed cases. F1 is averaged across reciprocal crosses. *LL* and *DD* are the light and dark homozygous genotypes, respectively. For each case the penultimate and ultimate abdominal segments are represented for both sexes on the left and right, respectively. Female slopes are represented as solid lines and male slopes as dashed lines. The graphs demonstrate the pigmentation difference between the heterozygous genotype (F1), and both homozygotes. The value of the heterozygote is presented as 0, *i.e.* F1 – F1, whereas the differences from the homozygotes are presented as *LL* – F1 and *DD* – F1, which should produce negative values as long as dominance is incomplete. Note that in species with dominance reversal, the *LL* – F1 difference tends to approach the value of the heterozygote in high temperatures, whereas the F1 – *DD* difference tends to approach the value of the heterozygote in cold temperatures. The point of intersection between *LL* – F1 and *DD* – F1 indicates the temperature at which dominance is reversed.

Discussion

The molecular basis of the association between plasticity and heterozygosity in Drosophila

Although it has long been recognized that genetic variation of plasticity exists, the nature of this variation remains elusive. Early works suggested three non-mutually exclusive mechanisms, namely overdominance, pleiotropy, and epistasis (Scheiner 1993). Of the three models, only overdominance made an explicit prediction about the relationship between plasticity and heterozygosity. The lack of strong evidence that heterozygous genotypes were more robust has led to the dismissal of the overdominance model, and current studies on the molecular basis of plasticity either consider the epigenetic and transcriptomic landscapes (*i.e.* the pleiotropy model) or the shape of regulatory networks (*i.e.* the epistasis model) (Goldstein and Ehrenreich 2021; Chevin et al. 2022). Our analysis of 272 reaction norms of a serially homologous trait supports an association between plasticity and heterozygosity but in a positive rather than negative way, hence revisiting the classical overdominance model.

In its simplest form, a gene regulatory network consists of a transcription factor activating or suppressing an effector gene. Of the five monogenic cases studied here, the

underlying locus involves an effector gene, *t*, in one species (*D. erecta*) (Yassin et al. 2016a), and a transcription factor, *pdm3*, in four species (*D. kikkawai*, *D. jambulina*, *D. burlai* and *D.* cf. *bocqueti*) (Yassin et al. 2016b; Fukutomi et al. 2025) (Figure 6). Loss-of-function regulatory mutations of *t* reduce plasticity in *D. erecta* recessive light homozygotes (Figure 2B), and only slightly affect the degree of dominance, *i.e.* plasticity of the functional allele is nearly completely dominant with a reversal of dominance near the upper thermal limit when both dark heterozygotes and homozygotes become light (Figure 5B). Indeed, the *t* regulatory element which is involved in female color variation in both *D. erecta* and *D. melanogaster* (Bastide et al. 2013; Endler et al. 2016; Gibert et al. 2017a) partly affects pigmentation plasticity in the later species (Gibert et al. 2016). On the other hand, mutations in the transcription factor *pdm3* increases plasticity when present in a heterozygous state in the four species of the *montium* group. Polymorphism in *pdm3* associates with female abdominal pigmentation variation in European and African populations of *D. melanogaster* (Endler et al. 2016). Mutations in this gene also underlie pigmentation evolution between the sister species *D. yakuba* and *D. santomea* with contrasting melanism (Liu et al. 2019; David et al. 2022). Whereas pigmentation in these two species is not plastic, their reciprocal hybrids, which are heterozygous for *pdm3* interspecific differences along with other genes, are highly plastic (Figure 2A; Figure 5B). This may indicate that the relation between heterozygosity and plasticity may mainly depend on the position of the polymorphic gene in the genetic network, *i.e.* being more pronounced if the gene is a transcription factor rather than an effector gene.

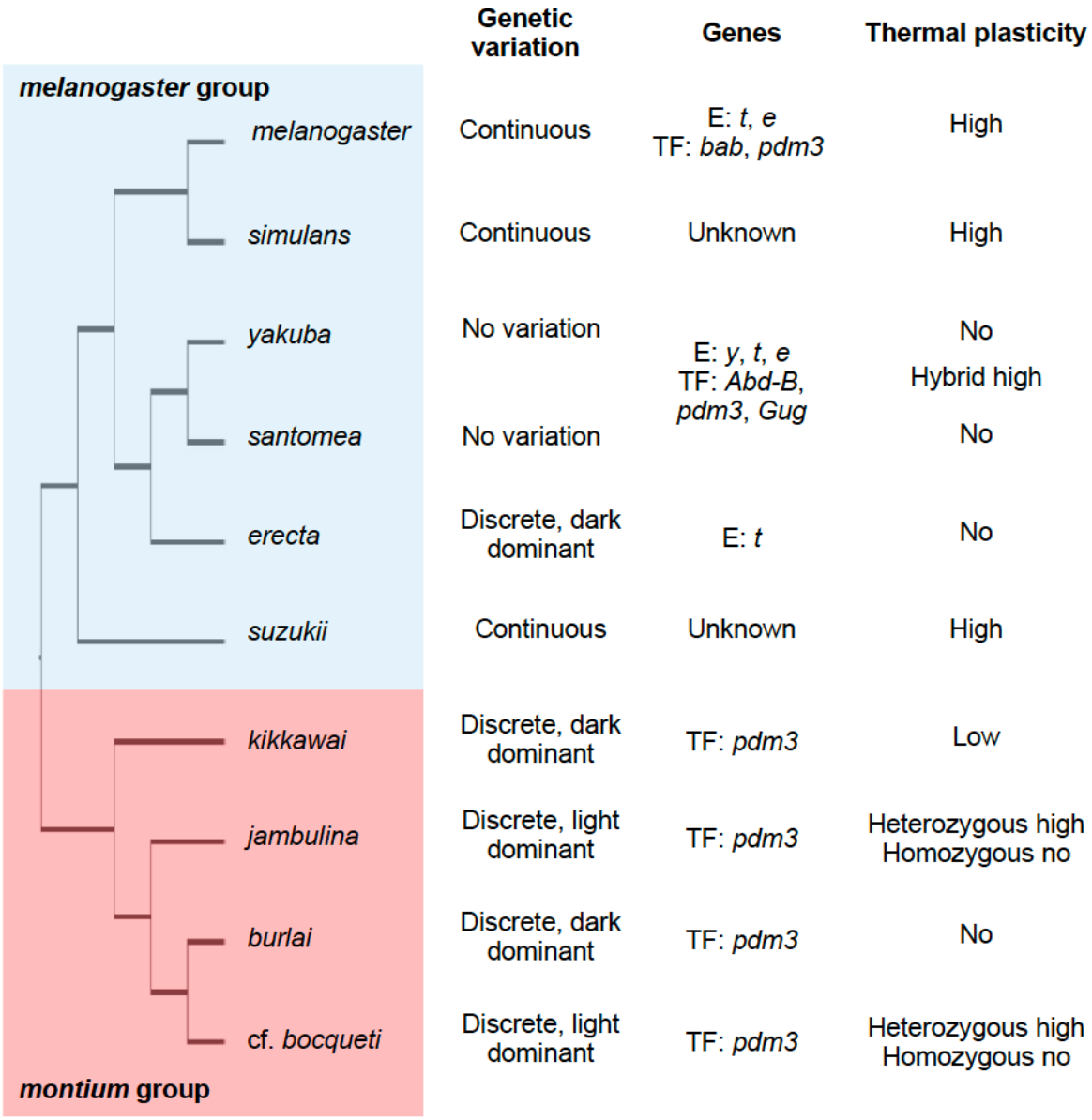


Figure 6 – Evolution of the genetic architecture of pigmentation variation and its thermal plasticity in the *melanogaster* and *montium* species groups. E = effector melanin-synthesis gene; TF = transcription factor. Phylogenetic relationships are after Suvorov et al. (2022).

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

409 norm, is associated with the evolution of dominance between the *pdm3* alleles in these
410 species. In *D. melanogaster*, the wildtype light allele is dominant (Tichy et al. 2008), but RNAi
411 lines targeting different parts of this gene yields contrasting results, either increasing
412 pigmentation (mostly in females) or decreasing it (mostly in males) (Rogers et al. 2014).
413 Consequently, it is possible that the *pdm3* mutations underlying the dark allele in *montium*
414 species where it is dominant, such as in *D. kikkawai* and *D. burlai*, are of the dominant negative
415 type, which are common for transcription factors (Gerasimavicius et al. 2022). *Pdm3* has
416 multiple isoforms in *D. melanogaster* and if one of them has the DNA binding domain but lacks
417 polymerase or other protein binding domains that are needed to express a gene, whereas
418 another isoform has both DNA and protein binding domains, the isoforms will have opposite
419 effects on pigmentation. In the heterozygote, the isoform lacking the protein binding domain
420 will compete with the isoform bearing this domain, hence altering its function. On the other
421 hand, mutations in species with the light allele being dominant, like *D. jambulina* and *D. cf.*
422 *bocqueti*, may be of the classical loss-of-function type that may reduce but do not completely
423 eliminate the effect of the wild-type light allele. Further molecular dissection of the underlying
424 alleles is required to understand the evolution of dominance reversal at *pdm3*.

425 The effect of mutations in both modifier and effector genes on plasticity is best
426 illustrated in *D. kikkawai* and the hybrids between *D. santomea* and *D. yakuba*. In *D. kikkawai*,
427 the effector *yellow (y)* gene lacks a conserved *cis*-regulatory element leading to the loss of
428 body pigmentation (Jeong et al. 2008). Because this gene is X-linked, a dosage effect may occur
429 between the sexes. Plasticity in males additively increased with the number of copies of the
430 dominant dark *pdm3* allele, which is an indirect regulator of *y* (Liu et al. 2019). Putatively
431 negative dominant mutations in this gene are expected to activate *y*, but the effect may
432 depend on the number of copies of *y*, being higher in females. Similarly, whereas hybrid males

433 mothered by *D. santomea*, *i.e.* those carrying defective X-linked melanin synthesis genes *t* and
434 *y* had similar plasticity slopes to hybrid females, but this slope was halved in hybrid males
435 mothered by *D. yakuba*, with functional *y* and *t*. Therefore, the degree of plasticity is affected
436 by the functionality of the polymorphism at the underlying genes.
437
438 *The association between plasticity and polymorphism may be more general than currently*
439 *appreciated*
440 We have restricted our analyses to eight species belonging to two species groups of the
441 subgenus *Sophophora*. Despite the differences in their reaction norms, both groups contain
442 species with sexually dimorphic melanism, and a single gene with antagonistic sexual effect
443 may underlie the association between plasticity and heterozygosity, hence reducing the
444 independence of our observations. Future analyses should investigate pigmentation thermal
445 plasticity in non-*Sophophora* fly species lacking sexual dimorphism, with or without Mendelian
446 polymorphism (e.g., Brisson et al. 2005; Rocha et al. 2009; Wittkopp et al. 2009). Beyond
447 *Drosophila*, color polymorphism abounds in nature (Orteu and Jiggins 2020; Sapir et al. 2021).
448 Investigation of the diversity in reaction norms in species with heterozygous and homozygous
449 color genotypes, especially in these with a hierarchy of dominance (e.g., Durand et al. 2014;
450 Le Poul et al. 2014), should shed light on the generality of the relationship between
451 heterozygosity and the degree of plasticity.
452 Most quantitative traits for which phenotypic plasticity was studied in *Drosophila*, such
453 as body size, wing shape, bristle number, ovariole number or life-history traits, are highly
454 polygenic with often a redundant genetic basis (David et al. 2004; Gibert et al. 2004a; Mackay
455 and Lyman 2005; Lobell et al. 2017; Lafuente et al. 2018; Pitchers et al. 2019). Identifying the
456 underlying genotypes of these traits to test for the effect of heterozygosity may be difficult. An

457 alternative to the 'forward genetics' approach that we leveraged here, is to use a 'reverse
458 genetics' analysis, wherein the transcription level of polymorphic genes can be analyzed in
459 homozygous vs. heterozygous states in different environments, even in the lack of knowledge
460 about the phenotypic consequences of the genotypes. One such significant example comes
461 from a study by Chen et al. (2015) who showed that 62% of genes with thermal plastic
462 response in *D. melanogaster* (*i.e.* ~51% of total genes) swapped allelic dominance in
463 heterozygotes between cold and hot environments. Future similar transcriptomic analyses are
464 therefore highly needed to test the generality of these patterns, especially these using hybrids
465 between species.
466
467 *Dominance reversal is a major mechanism for higher plasticity in heterozygotes*
468 Major insight on the genome-wide ubiquity of dominance reversal was recently
469 demonstrated by Grieshop and Arnqvist (2018). These authors compared fitness, a polygenic
470 trait by nature, between the sexes in crosses between inbred lines of seed beetles and found
471 a strong dominance reversal between the sexes. Grieshop et al. (2024) reviewed other
472 examples including environment-, trait- and sex-specific dominance reversal. Such examples
473 are ideal models to test the link between plasticity and heterozygosity, especially in the context
474 when sexual antagonism, *i.e.* distinct fitness optima for each sex, may interfere with
475 environment-specific dominance reversal.
476 We note here that in our analysis, a sexual dimorphism in thermal plastic response was
477 observed. In *D. burlai*, females are less plastic for all genotypes, whereas the opposite is true
478 for *D. melanogaster*. In *D. jambulina* and *D. cf. bocqueti*, homozygous genotypes were more
479 plastic in males than in females, whereas heterozygous genotypes were more plastic in
480 females. The strongest example was in *D. kikkawai*, wherein the dominance of the dark *pdm3*

481 allele reversed between the sexes (in the heterozygotes) with higher plasticity in opposite
482 homozygous genotypes (Gibert et al. 1999) (Figure 3A). Indeed, the sexual dimorphism in *D.*
483 *melanogaster* is caused by the action of the transcription factor *bab* which suppresses melanin
484 synthesis genes in females (Kopp et al. 2000; Rogers et al. 2014; Hughes et al. 2023). This same
485 gene is also responsible for the further suppression of pigmentation in females grown at high
486 temperature (Castro et al. 2018). Because *bab* is repressed in dark *D. burlai* females and
487 expressed in light *D. bocqueti* females (Kopp et al. 2000), and *bab* is a direct regulator of *y*, it
488 is possible that *bab* sexual expression and plasticity are regulated by *pdm3*. These observations
489 highlight the complexity underlying the link between heterozygosity and plasticity, and the
490 necessity for future theoretical modeling of this link to account for possible interactions
491 between the environment and the sex.

492 To the best of our knowledge, the only simple population genetics model, *i.e.* a single
493 locus with two alleles, for the evolution of plasticity was developed by Pigliucci (1992). He
494 modeled fitness as a phenotype and considered the heterozygous genotype to have equal
495 fitness in two environments, *i.e.* a robust fitness, in contrast to homozygous genotypes that
496 had symmetrically opposite plastic fitness. However, one should distinguish the difference
497 between developmental plasticity, *i.e.* the genotype-phenotype map, from fitness plasticity. In
498 fact, Pigliucci's (1992) model of complete dominance agrees with a model of developmental
499 dominance reversal, which can occur if the heterozygote switches its phenotype to that of
500 each homozygous genotype in the environment where the phenotype is beneficial.
501 Overdominance will follow if the homozygotes cannot switch their phenotype between the
502 environments, *i.e.* a robust genotype has a plastic fitness, whereas a plastic genotype has a
503 robust fitness. This will lead to fitness relationships similar to the ones that we present in
504 Figure 5B (compare to Figure 1 in Pigliucci 1992). The realization of this distinction between

505 developmental and fitness plasticity leads us to the exact opposite interpretations of Pigliucci's
506 (1992) model: plasticity, not robustness, should prevail when the system approaches
507 equilibrium between the probabilities of occurrences of the different environments.
508
509 *The evolutionary consequences of links between phenotypic plasticity and polymorphism*
510 A link between phenotypic plasticity and polymorphism can provide simple
511 explanations on how plasticity evolves. In fact, plasticity can be lost through any mechanism
512 reducing genetic heterozygosity such as inbreeding or directional selection. Indeed, the
513 'flexible stem hypothesis' postulates that from a plastic ancestor, descendants with fixed
514 phenotypes from the ancestral phenotypic spectrum can evolve (West-Eberhard 2003; Gibert
515 2017). A likely example from our data is the case of *D. santomea* and *D. yakuba*. Pigmentation
516 difference between these two species is due to mutations in at least six loci (Liu et al. 2019;
517 David et al. 2022), but it is unlikely that all these mutations have arisen simultaneously. This
518 means that a substantial degree of heterozygosity segregated in the ancestor of these species,
519 and consequently this ancestor has gradually lost its plasticity as more and more alleles
520 became fixed in each lineage. Indeed, pigmentation plays a role in the reproductive isolation
521 between these two species (David et al. 2022), and a hybrid zone on the African island of Sao
522 Tomé between these two species exists (Lachaise et al. 2000; Llopart et al. 2005; Turissini and
523 Matute 2017). A plastic hybrid is disadvantaged and the 'flexible stem hypothesis' may in
524 essence reflects a simple model of 'underdominance' of the heterozygote.
525 Conversely, phenotypic plasticity can be gained by any mechanism that increases
526 genetic variability and heterozygosity in a population, such as mutations, outbreeding (e.g.,
527 gene flow and introgression), and balancing selection. Indeed, any new mutation in a di- or
528 polyploid organism first arises in the heterozygous state, and it has been argued that a

529 heterozygous advantage is essential for the spread of new mutations and counteracting the
530 loss by random genetic drift (Sellis et al. 2011). In cases where plasticity is adaptive, the higher
531 plasticity of the heterozygote can facilitate the spread of this mutation. Such adaptive plasticity
532 gain model is in essence an 'overdominance' model. An important consequence of the
533 overdominance model is that plasticity could be a mechanism to maintain genetic diversity,
534 *i.e.* rather than 'buying time' for genetic adaptation to occur, plasticity may be a major source
535 for 'saving time' by maintaining standing genetic variation, such as in the case of introduced
536 species or multivoltine species inhabiting a heterogeneous spatial or seasonal environment
537 (Gulisija et al. 2016; Wittmann et al. 2017; Promy et al. 2023). Further theoretical and empirical
538 investigations of the link between heterozygosity and plasticity will definitively improve our
539 understanding of the evolution of both.
540

541 **Acknowledgments**

542 The authors thank Artyom Kopp for comments on the manuscript and for sharing with Yuichi
543 Fukutomi the association between female-limited color dimorphism and polymorphism at the
544 *pdm3* locus in *D. jambulina* and *D.* cf. *bocqueti*.
545

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