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Quantitative genetics of speciation: additive and non-additive genetic differentiation between *Drosophila madeirensis* and *Drosophila subobscura*

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Abstract The role of dominance and epistasis in population divergence has been an issue of much debate ever since the neoDarwinian synthesis. One of the best ways to dissect the several genetic components affecting the genetic architecture of populations is line cross analysis. Here we present a study comparing generation means of several life history-traits in two closely related *Drosophila* species: *Drosophila subobscura*, *D. madeirensis* as well as their F_1 and F_2 hybrids. This study aims to determine the relative contributions of additive and non-additive genetic parameters to the differentiation of life-history traits between these two species. The results indicate that both negative dominance and epistatic effects are very important in the differentiation of most traits. We end with considerations about the relevance of these findings for the understanding of the role of non-additive effects in speciation.

Keywords Speciation · Generation means · Hybrid breakdown · Dominance · Epistasis · *Drosophila madeirensis* · *Drosophila subobscura*

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Introduction

Population differentiation is a central issue in evolutionary biology. Fisher and Wright, two fundamental contributors to the neoDarwinian synthesis, disagreed on the processes underlying the evolution of natural populations. Specifically, they disagreed on the role that additive and non-additive genetic factors play in population differentiation. According to Fisher selection acts primarily on individual loci, and non-additive effects have little evolutionary importance (Fisher 1930). On the other hand, Wright's shifting balance theory of evolution relies on epistatic gene action (Wright 1977) and the formation of coadapted gene complexes is fundamental in his model (Fenster et al. 1997). In spite of the considerable theoretical and empirical developments in this area, the controversy is far from solved (e.g. Coyne et al. 1997, 2000; Wade and Goodnight, 1998; Goodnight and Wade 2000; Gravilets 2004). One of the motives is the paucity of empirical studies that test the role of epistasis in the evolution of fitness related traits (Barton and Turelli 1989; Whitlock et al. 1995; Fenster et al. 1997).

Non-additive gene action has been commonly associated with population differentiation (Lynch and Walsh 1998). Dominance effects are relatively abundant in the literature and are frequently expressed as heterosis (e.g., Bieri and Kawecki 2003; Edmands 1999; Facon et al. 2005; Fenster and Galloway 2000; but see Teotónio et al. 2004 for evidences of negative dominance). Comparatively, evidence of epistasis is scarcer, not very consistent and comes mainly from intraspecific studies (Blows and Sokolowski 1995; Starmer et al. 1998; Gilchrist and Partridge 1999; Fenster and Galloway 2000; Carrol et al. 2001, 2003; Bieri and

Kawecki 2003; Fox et al. 2004; Teotónio et al. 2004; Bradshaw et al. 2005). Intraspecific hybrids between species can express outbreeding depression or hybrid breakdown—having lower fitness than the parental species (Waser and Price 1989, 1994; Brown 1991; Burton 1990; Leberg 1993; Fenster and Galloway 2000; Templeton 1981; Coyne and Orr 1989, 1997). Hybrid breakdown is generally attributable to the disruption of favourable gene interactions that have evolved independently in the two parental types and is expected to occur in more differentiated populations or species, whether it is partly a cause or just a consequence of the reproductive isolation, as mentioned in the Dobzhansky-Muller model (see Fenster et al. 1997; Johnson 2002; Gavrilets 2004).

Line-cross analysis is a powerful way to dissect the relative contributions of additive and non-additive genetic effects to population differentiation (e.g., Mather and Jinks 1982; Lynch 1991; Lynch and Walsh 1998; Kearsey and Pooni 1996). However, to properly dissect these effects it is necessary to compare several hybrid generations (e.g., F_1, F_2 hybrids and/or backcrosses with the parentals, see Mather and Jinks 1982). The scarcity of evidences for epistasis is in part due to these demanding designs (e.g., difficulties in obtaining hybrids of more than one generation) and to a low statistical power to detect these effects (cf. Lynch and Walsh 1998). In spite of all the inherent difficulties, evidence for epistasis in studies involving different species has been found both in plants (e.g., Macnair and Cumbs 1989; Fritz et al. 2003), and animals (e.g., Breeuwer and Werren 1995; Hatfield 1997). Species that hybridize successfully for several generations are thus a valuable material to explore in these issues. Such is the case of the species pair *Drosophila madeirensis*–*Drosophila subobscura*.

Drosophila madeirensis Monclús and *D. subobscura* Collin are two closely related species that coexist on Madeira Island, the former being endemic. The estimated time of divergence between both species is 0.6–1.0 Myr ago (Ramos-Onsins et al. 1998). However, they are not completely isolated reproductively, as some crosses produce fertile hybrid females and sterile males in both directions (Khadem and Krimbas 1991, 1993, 1997; Papaceit, San Antonio and Prevosti 1991), F_1 hybrids being easier to obtain when *D. madeirensis* is the maternal species. Crossing *D. madeirensis* females with *D. subobscura* males yields progeny with a 1:1 sex ratio, but the reciprocal cross tends to be male biased (Khadem and Krimbas 1991, 1993). However, in our particular case, it was possible to produce fertile male hybrids in both directions, and F_2 progeny could be obtained, though the *D. subobscura* females–*D. madeirensis* males direction proved to be much harder, basically due to the extremely male biased sex ratio in the F_1 hybrids (Rego et al. 2006).

In this study we investigated the genetic basis of evolutionary divergence of several fecundity related traits and survival between *D. madeirensis* and *D. subobscura* by comparing the mean values of several generations: parental, F_1 and F_2 hybrids. By testing several genetic models we were able to infer which genetic effects, additive, and non-additive (dominance, epistasis and maternal) may be contributing to the differentiation between these two species.

Materials and methods

Population stocks and crosses

The *D. madeirensis* and *D. subobscura* base stocks were derived from a sample of wild flies collected at Ribeiro Frio (Madeira Island; for details see Rego et al. 2006). Laboratory populations of both species were set up in April 2001 and split into three replicates (m_1, m_2, and m_3 for *D. madeirensis*; s_1, s_2, and s_3 for *D. subobscura*) at generation 3. All replicated populations were kept on a discrete generation (of 30 days), controlled larval and adult densities regime at 18°C on a 12:12 light:dark period (see Matos et al. 2000; Matos et al. 2002). The number of breeding adults per population was typically around 1,000 flies, never dropping below 400. The assays in the present study were made after 23 generations of adaptation to laboratory conditions.

For each pair of replicated populations reciprocal F_1 hybrids were obtained by mass crossing 250 virgin females and 250 virgin males. The mass crosses ♀♀ *D. madeirensis* (m_i; $i = 1, 2, 3$) × ♂♂ *D. subobscura* (s_i) gave the series $F_1 \cdot \mathrm{m}_1\mathrm{s}_1$, $F_1 \cdot \mathrm{m}_2\mathrm{s}_2$, and $F_1 \cdot \mathrm{m}_3\mathrm{s}_3$ (i.e., the maternal species is always indicated first); and the mass crosses ♂♂ *D. subobscura* × ♀♀ *D. madeirensis* the series $F_1 \cdot \mathrm{s}_1\mathrm{m}_1$, $F_1 \cdot \mathrm{s}_2\mathrm{m}_2$, and $F_1 \cdot \mathrm{s}_3\mathrm{m}_3$. All $F_1 \cdot \mathrm{m_i s_i}$ produced F_2 progeny when hybrid females and males were mass-crossed (hereafter referred to as $F_2 \cdot \mathrm{m}_1\mathrm{s}_1$, $F_2 \cdot \mathrm{m}_2\mathrm{s}_2$, and $F_2 \cdot \mathrm{m}_3\mathrm{s}_3$, respectively); however, only the crosses involving individuals from $F_1 \cdot \mathrm{s}_2\mathrm{m}_2$ produced enough F_2 hybrids (i.e., $F_2 \cdot \mathrm{s}_2\mathrm{m}_2$) as to be included in the present study. The reason was that F_1 hybrids were harder to obtain when *D. subobscura* was the maternal species and, in addition, the sex ratio was greatly male biased. All generations (parental, F_1 and F_2) were assayed synchronously, which involved the formation of F_1 hybrids on two separate occasions: the first to produce the F_2 generation and the second to obtain the F_1

individuals for the assays. All fly handling was done at room temperature (22–24°C) using CO_2 anaesthesia when necessary.

Assays of fitness traits

We measured age of first reproduction, early and peak fecundity, and female survival from a total of 12 individual couples of virgin flies from each replicated parental, F_1, and F_2 populations. Each couple was placed in a vial containing 1 ml of Drosophila medium less than 4 hours after eclosion. During two weeks the flies were transferred daily to new vials and the eggs laid by each female were counted. Age of first reproduction was measured as the number of days until a female laid her first egg since emergence, early fecundity as the number of eggs laid during the first week, peak fecundity as the number of eggs laid during the second week, and survival as the number of days the female remained alive during the fecundity assays (i.e., the upper bound for survival was two weeks).

Age of first reproduction was estimated conditional to the female not dying before the first egg appeared and, therefore, we discarded a few females that died before the third day since emergence. Similarly, early fecundity was estimated conditional to the female being alive at the end of the first week, and peak fecundity conditional to being alive on the last day of the assay (day 14).

Analysis of generation means

To properly estimate several composite genetic parameters using Mather and Jinks' coefficients (1982)—specifically the several types of digenic interactions—both types of F_2 hybrids and backcrosses are needed (Mather and Jinks 1982; Kearsey and Pooni 1996; Lynch and Walsh 1998). Since we do not have data from backcrosses we only tested here for the presence of the composite additive effect $[a]$ (i.e., the sum of individual effects of loci with both alleles derived from the same parental species); the composite dominance effect $[d]$ (the sum of individual effects of loci with alleles derived from the two species); a composite epistasis effect $[e]$, which includes here the epistatic terms describing additive × additive, additive × dominance, and dominance × dominance epistatic interactions; and maternal effects $[m]$.

The first estimation is the genetic difference between the two species, obtained by comparing their means: $[a] = \bar{m}_i - \bar{s}_i; i = 1, 2, 3$. Conformity with a purely additive model means that F_1 hybrids would be at the midpoint from the parental values, which can be tested as:

$$[\Delta_d] = \left(\overline{F_1 \cdot m_i s_i} + \overline{F_1 \cdot s_i m_i}\right) - \left(\overline{m_i} + \bar{s}_i\right); i = 1, 2, 3;$$

i.e., as the difference between the average trait in F_1 hybrids to that in the parental species. Following a similar reasoning, the conformity to the additive-dominance model can be tested as:

$$[\Delta_e] = 2\left(\overline{F_2 \cdot m_i s_i} + \overline{F_2 \cdot s_i m_i}\right) - \left(\overline{F_1 \cdot m_i s_i} + \overline{F_1 \cdot s_i m_i}\right) - \left(\overline{m_i} + \bar{s}_i\right); i = 1, 2, 3.$$

Since only one $F_2 \cdot s_i m_i$ replicate was available (i.e., $F_2 \cdot s_2 m_2$), we used a slightly modified version of Δ_e to test for epistasis (see below). Finally, the difference in mean phenotypes of daughters from the two reciprocal F_1 crosses allows testing for maternal effects.

Statistical analyses were performed by means of two-way mixed ANOVAs, with generation as fixed and replicate as random factors. Statistical significance of each composite effect was tested via orthogonal contrasts between the corresponding means (each comparison or contrast has one degree of freedom). Table 1 gives the contrast coefficients we used. The generation × replicate interaction terms provided the appropriate error terms, thus avoiding the heteroscedasticity problem due to the higher within-family variance in the F_2 generation (Mather and Jinks 1982).

Results

Averages for the fitness traits assayed are plotted in Fig. 1, and statistical analyses are shown in Table 2. It is worth noting that replicated crosses performed quite similarly as non-significant differences were generally detected for the 'replicate' effect. This suggests that using only one replicate for F_2 hybrids when *D. subobscura* was the maternal species (i.e., $F_2 \cdot s_2 m_2$) does not introduce a substantial bias in the analysis.

The only fitness trait that was noticeably different between *Drosophila subobscura* and *D. madeirensis* was fecundity; with *D. subobscura* laying substantially more eggs in both fecundity periods (early fecundity: $[\hat{a}] = -39.0$; peak fecundity: $[\hat{a}] = -40.3$; caret denotes "an estimator of").

The F_1 hybrids from both cross directions only differed between them in survival ($[\hat{m}] = 1.6$), $P < 0.01$), clearly indicating maternal effects for this trait but not for fecundity-related traits. Cytoplasmic gene(s) in *D. subobscura* seem to play an important role in decreasing survival of F_1. s_i m_i hybrids (Fig. 1).

Table 1 Contrast coefficients for the four composite genetic parameters. [*a*]– additive effects, [*d*]–dominance effects, [*e*]–epistatic effects [*m*] – maternal effects

	D. madeirensis			*D. subobscura*			♀♀ *D. madeirensis* × ♂♂ *D. subobscura*			♀♀ *D. subobscura* × ♂♂ *D. madeirensis*			♀♀ $F_1 \cdot m_i s_i \times$ ♂♂ $F_1 \cdot m_i s$			♀♀ $F_1 \cdot s_2 m_2 \times$ ♂♂ $F_1 \cdot s_2 m_2$
	m_1	m_2	m_3	s_1	s_2	s_3	$F_1 \cdot m_1 s_1$	$F_1 \cdot m_2 s_2$	$F_1 \cdot m_3 s_3$	$F_1 \cdot s_1 m_1$	$F_1 \cdot s_2 m_2$	$F_1 \cdot s_3 m_3$	$F_2 \cdot m_1 s_1$	$F_2 \cdot m_2 s_2$	$F_2 \cdot m_3 s_3$	$F_2 \cdot s_2 m_2$
[*a*]	1	1	1	–1	–1	–1	0	0	0	0	0	0	0	0	0	0
[*d*]	–1	–1	–1	–1	–1	–1	1	1	1	1	1	1	0	0	0	0
[*e*]	–1	–1	–1	–1	–1	–1	–1	–1	–1	–1	–1	–1	3	3	3	3
[*m*]	0	0	0	0	0	0	1	1	1	–1	–1	–1	0	0	0	0

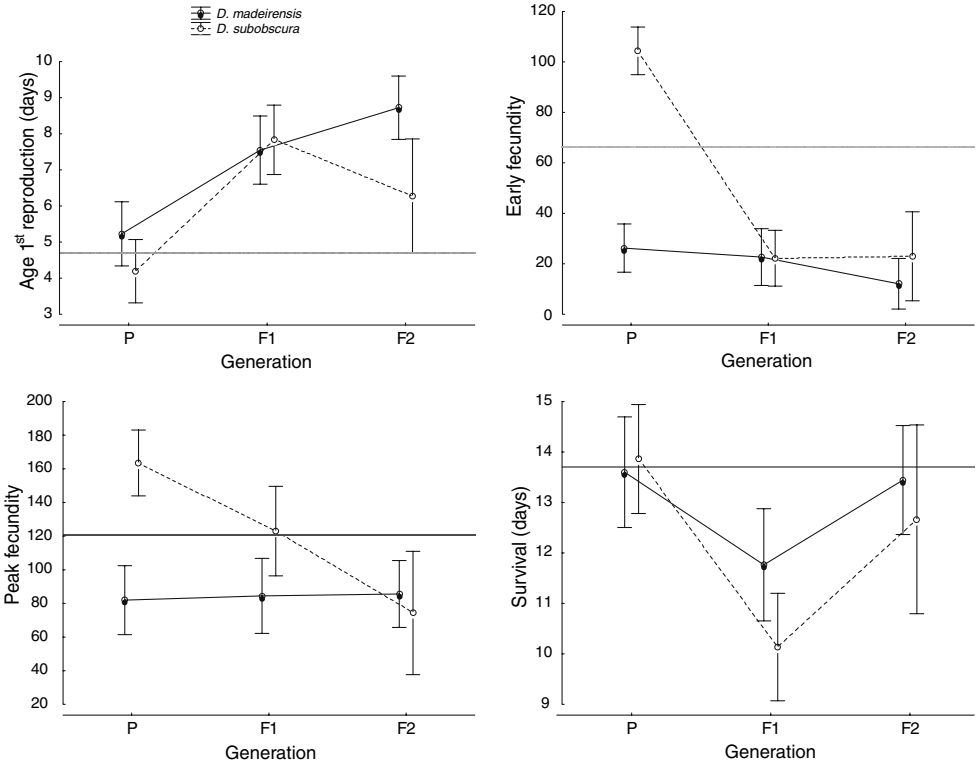

Fig. 1 Generation means for the parental species *D. madeirensis* and *D. subobscura*, and their F_1 and F_2 hybrids from both reciprocal crosses, for all analysed traits: age of first reproduction, early fecundity, peak fecundity and survival. Full dots: *D. madeirensis* and $F_1$and F_2 hybrids with this species as maternal species; empty dots: *D. subobscura* and $F_1$and F_2 hybrids with *D. subobscura* as maternal species. Lines connect the dots of the same maternal direction. Standard errors and a line indicating the mid-parent value for each trait are also given

Though F_1 hybrids had lower survival, F_2 hybrids presented similar values to the parental species.

Overall, the results indicated that F_1 hybrids performed worse than the mid-parent (age first reproduction: $\left[\hat{d}\right] = 3.0$; early fecundity: $\left[\hat{d}\right] = -42.6$; peak fecundity: $\left[\hat{d}\right] = -19.8$; survival: $\left[\hat{d}\right] = -2.8$). However, when compared to the maternal species it was clear that the significant drop in early fecundity was mainly relative to *D. subobscura* (Fig. 1) since the F_1 hybrids performed more or less alike *D. madeirensis* (i.e., dominance for fecundity was toward *D. madeirensis*): the average dominance $[\hat{d}]/[\hat{a}]$ was equal to 1.1.

Peak fecundity was the only fitness trait where a simple additive genetic model was adequate (Table 2). For all other traits epistasis was statistically significant, despite difficulties to quantify it with sample sizes as small as these here. Using the contrast coefficients for [*e*] in Table 1 to measure epistasis, the resulting values were as follows. Age of first reproduction: $[\hat{e}] = 22.8$; early fecundity: $[\hat{e}] = -348.3$; peak fecundity: $[\hat{e}] = -355.1$; survival: $[\hat{e}] = 11.0$. The figures always point in the direction of F_2 progeny being less fit than the parental species and/or F_1 hybrids (Fig. 1).

Of the several parameters tested, [*d*] was the most consistent. Dominance effects were highly significant in three of the four analysed traits (Table 2). This indicates that dominance effects may play an important role in the differentiation of life-history traits between *D. madeirensis* and *D. subobscura*. Epistatic effects [*e*] seem also to be very important, as their presence was detected in all traits with the exception of peak fecundity.

Table 2 ANOVAs for the fitness traits assayed (age at first reproduction, early fecundity, peak fecundity, and survival) measured for six generations (parental species *D. madeirensis* and *D. subobscura*, two F_1 hybrids, and two F_2 hybrids) with up to three replicated populations each. Composite genetic parameters were tested from orthogonal linear contrasts (see Table 1). The denominator used to calculate F-values for main effects and contrasts is the corresponding replicate × generation interaction

	Source of variation	df	SS	MS	F
Age of first reproduction	Replicate(R)	2	29.4	14.7	1.24
	Generation(G)	5	509.6	101.9	8.56**
	[*a*]	1	18.8	18.8	1.58
	[*d*]	1	292.2	292.2	24.50**
	[*e*]	1	125.0	125.0	10.49*
	[*m*]	1	0.1	0.1	0.01
	R × G	8	95.4	11.9	1.76§
	Error	163	1106.5	6.8	
Early fec.	Replicate	2	648.0	324.0	0.20
	Generation (G)	5	201672.5	40334.5	24.44***
	[*a*]	1	108073.2	108073.2	65.57***
	[*d*]	1	55835.5	55835.5	33.88***
	[*e*]	1	27738.8	27738.8	16.83**
	[*m*]	1	11.7	11.7	0.01
	R × G	8	13185.5	1648.2	1.95§
	Error	155	139079.7	799.3	
Peak fec.	Replicate	2	9403.7	4701.8	0.57
	Generation	5	179479.1	35895.8	4.26*
	[*a*]	1	108333.3	108333.3	12.86**
	[*d*]	1	10438.3	10438.3	1.24
	[*e*]	1	27181.9	27181.9	3.23
	[*m*]	1	15975.5	15975.5	1.90
	R × G	8	67372.3	8421.5	2.71**
	Error	141	621682.1	3814.0	
Survival	Replicate	2	20.1	10.0	3.54§
	Generation(G)	5	371.2	74.2	26.38***
	[*a*]	1	1.2	1.2	0.42
	[*d*]	1	276.9	276.9	97.95***
	[*e*]	1	30.1	30.1	10.63*
	[*m*]	1	46.0	46.0	16.26**
	R × G	8	22.6	2.8	0.25
	Error	174	1785.6	11.0	

Note: Analyses were carried out in STATISTICA V6, with Type III sums of squares.

§ $0.10 > P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Discussion

Genetic differentiation between *Drosophila subobscura* and *Drosophila madeirensis*

There is a clear genetic differentiation in life-history traits between *Drosophila subobscura* and *Drosophila madeirensis*. This differentiation involves additive and non-additive effects, the latter appearing in most traits analysed. Both dominance and epistasis are involved, and outbreeding depression is expressed since the first hybrid generation.

Drosophila subobscura generally showed a higher performance compared to *Drosophila madeirensis* for all life history traits. This difference was highly significant for early and peak fecundity, though not for age of first reproduction and survival. However, our failure to detect significant differences between the two species in the last two traits does not mean that they are not genetically different. In fact the detection of significant dominance effects for both traits suggests that the parental species are in fact genetically different, because dominance is strongly affected by the heterozygosity of the genes for which the species differ (Kearsey and Pooni 1996).

Epistatic interactions were also frequently involved. Peak fecundity was the only trait where epistasis was not detected. A misleading effect can come from the presence of maternal effects, which can be confounded with epistasis (Kearsey and Pooni 1996). However, in our particular case, survival was the only trait where maternal effects were detected, which renders unlikely that our general finding of epistasis are only due to these effects.

For both fecundity traits, the estimated [*e*] and [*d*] values were negative. This suggests that both dominance effects between the two species and dis-

ruption of gene combinations within each species lead to a reduction of fitness in hybrids. Survival also presented a drop of performance in F_1 hybrids, corresponding to a negative [d], though, somewhat surprisingly F_2 hybrids presented an improvement, getting close to the mean parental values, corresponding to a positive [e].

Though we have been interpreting [d] and [e] values as indicating dominance and epistasis, respectively, the actual scenario is a bit more complicated than that. According with Kearsey and Pooni (1996), [d] is affected by several genetic parameters, of which only one is dominance. Specifically in our estimates [d] = 2[D] + 2[DD] (considering maternal effects irrelevant), where D stands for dominance and DD for dominance-by-dominance digenic composite effects. Similarly, [e] is equal to –2[aa]– [DD] (in the absence of maternal effects), where [aa] stands for additive-by-additive composite effects.

By comparing these two parameters we can try to infer the particular importance of the several genetic effects involved. If only dominance-by-dominance composite effects were involved, we would expect [e] to be similar to [d]/2. None of the comparisons suggests such a simple scenario. In fact, [d]/2 was smaller than [e] in absolute values and of the same sign for early fecundity and age of first reproduction. This, together with the values presented by the several generations (see Fig. 1) does not allow us to exclude any of the potential contributions of dominance and of the two epistatic effects. As for survival, combining the information of [e] (positive), [d] (negative) and Fig. 1 suggests the presence of dominance and digenic dominance-by-dominance epistasis (since F_2 is close to the parentals, not expected by additive epistasis). In this particular case a more complex model including additive epistasis is not needed.

Comparisons with other studies

Evidence for epistasis by means of line cross analysis are relatively scarce in the literature, both due to the demanding designs and low statistical power (see Lynch and Walsh 1998). Nevertheless, some indications of epistatic effects have been obtained with this method (e.g., Macnair and Cumbs 1989; Breeuwer and Werren 1995; Hatfield 1997; Starmer et al. 1998; Fritz et al. 2003). Other methodologies applied to studies on population differentiation look promising to test for epistasis, and non-additive effects in general, such as QTL analysis (e.g. Li et al. 1997a, b; Orr and Irving 2001). Though general methodological difficulties also applies to QTL analysis (Tanksley 1993; Orr 2001), recent developments in this area have improved the ability to detect these effects (e.g. Baierl et al. 2006; Blanc et al. 2006).

Line cross analysis in intraspecific crosses are more abundant and give contrasting results in the genetic effects detected, both between and within studies (e.g., Edmands 1999; Bieri and Kawecki 2003; Teotónio et al. 2004). Teotónio et al. (2004), studying highly differentiated *D. melanogaster* populations, found little evidence of epistasis. These authors compared two selective regimes with their respective controls, one regime selected for increased starvation resistance and the other for accelerated development. They found that the only trait that revealed epistasis was male starvation resistance, curiously in the regime selecting for accelerated development, less differentiated for starvation resistance. On the other hand, our interspecific study presents several suggestions of epistatic effects, both in fecundity related traits and survival. The discrepancy in finding epistatic effects, both between studies and traits, could be generally related with the degree of differentiation presented by the populations in each trait, particularly considering studies involving populations from the same species (Edmands 1999; Bieri and Kawecki 2003; Teotónio et al. 2004) vs. the interspecific analysis in our case. Nevertheless, there is no simple rule, as the study of Teotónio et al. (2004) illustrates. Lair et al. (1997) suggested that additive effects may be more important in the early stages of divergence, whereas differences due to epistasis arise after longer periods of isolation. However, differentiation due to epistatic effects can arise very quickly (100 generations) during population divergence (e.g. Carrol et al. 2001, 2003; but see Teotónio et al. 2004 for contrasting results). It seems thus that there is no simple rule allowing generalizations from the results. There is also some evidence that the genetic basis of differentiation may vary according to the trait analysed (e.g., Crnokrak and Roff 1995; Orr 2001; Carroll et al. 2003). For instance, Orr (2001) in a review of studies on the genetics of species differences found that hybrid sterility and inviability involve more frequently epistasis and recessivity than other species differences. These several factors may explain discrepancies of results among studies.

Does non-additivity play a role in speciation and maintenance of specific diversity?

The presence of negative dominance and epistasis effects in the differentiation of our species, does not allow us to infer that these interactions were a cause of speciation (Coyne 1992; Fenster et al. 1997). Accord-

ing to the Dobzhansky-Muller model maladaptive genotypes only appear in the hybrids of well differentiated populations and not in the ancestral populations, previous to genetic differentiation. If this is the case, then epistasis will not promote, at least directly, evolutionary divergence, as defended in a Wrightanian scenario; it will only be a consequence of this process (see Fenster et al. 1997; Johnson 2002). The same reasoning can be applied to negative dominance effects as the ones also obtained in this study. Having said this, the finding of negative epistasis and dominance in the differentiation between species is relevant for the discussions about the role of such genetic effects on speciation. There is now growing evidence that gene interaction may play an important role in speciation (e.g. Aspi 2000; Wade 2002). It is likely that epistasis is also responsible, at least in part, for fostering the evolution of mechanisms causing reproductive isolation, preventing the formation of maladapted gene combinations in the hybrids (Whitlock et al. 1995; Turelli and Orr 2000; Orr 2001, Wade 2002). Negative dominance, as we found in this work, may lead to similar evolutionary scenarios. Curiously, the literature focus much more on epistasis (see Orr 2001).

The major finding of our work is the detection of significant negative dominance and epistatic effects, contributing to the differentiation in life history traits between *Drosophila madeirensis* and *Drosophila subobscura*. This type of genetic differentiation may have contributed to the speciation event per se and/or to the reinforcement of genetic and evolutionary barriers that maintain these species. As more and more empirical data appear similar to ours, we will hopefully be able to answer the ultimate question: what is a cause and what is a consequence of the speciation event? For now, speciation remains ''the mystery of mysteries'' as Darwin had already called it.

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