RESEARCH ARTICLE

Quantitative genetics of functional characters in *Drosophila melanogaster* populations subjected to laboratory selection

HENRIQUE TEOTÓNIO[1,2]*, MARGARIDA MATOS[3] and MICHAEL R. ROSE[1]

[1]*Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697-2525, USA*
[2]*Present address: Centro de Biologia do Desenvolvimento, Instituto Gulbenkian de Ciência, Rua da Quinta Grande 6, 2780-156 Oeiras, Portugal*
[3]*Centro de Biologia Ambiental, Dep. Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Campo Grande Lisboa, Portugal*

Abstract

What are the genetics of phenotypes other than fitness, in outbred populations? To answer this question, the quantitative-genetic basis of divergence was characterized for outbred *Drosophila melanogaster* populations that had previously undergone selection to enhance characters related to fitness. Line-cross analysis using first-generation and second-generation hybrids from reciprocal crosses was conducted for two types of cross, each replicated fivefold. One type of cross was between representatives of the ancestral population, a set of five populations maintained for several hundred generations on a two-week discrete-generation life cycle and a set of five populations adapted to starvation stress. The other type of cross was between the same set of ancestral-representative populations and another set of five populations selected for accelerated development from egg to egg. Developmental time from egg to eclosion, starvation resistance, dry body weight and fecundity at day 14 from egg were fit to regression models estimating single-locus additive and dominant effects, maternal and paternal effects, and digenic additive and dominance epistatic effects. Additive genetic variation explained most of the differences between populations, with additive maternal and cytoplasmic effects also commonly found. Both within-locus and between-locus dominance effects were inferred in some cases, as well as one instance of additive epistasis. Some of these effects may have been caused by linkage disequilibrium. We conclude with a brief discussion concerning the relationship of the genetics of population differentiation to adaptation.

[Teotónio H., Matos M. and Rose M. R. 2004 Quantitative genetics of functional characters in *Drosophila melanogaster* populations subjected to laboratory selection. *J. Genet*. **83**, 265–277]

Introduction

The genetic basis of phenotypic differentiation among populations and among species is an important topic in evolutionary biology research. Knowing the relative contributions of additive and nonadditive gene effects, such as dominance and epistasis, to phenotypic differences in functional characters is important, because these characters are subject to natural selection and as such give the opportunity to understand evolutionary history (Lynch 1991; Waser and Price 1994; Lynch and Walsh 1998; Merilä and Sheldon 1999). Also, and perhaps more significantly, determining the genetic basis of phenotypic diversity has been regarded as the first step to understanding the role that additive and nonadditive gene action may play in future evolution. In particular, nonadditivity for fitness is thought to generate rugged adaptive landscapes establishing a relationship between fitness and genetic composition that might constrain the trajectories of evolving populations (Wright 1931, 1982; Whitlock *et al*. 1995; Coyne *et al*. 1997; Wade and Goodnight 1998; Wolf *et al*. 2000; also see Simpson 1953; Barton and Turelli 1987; Turelli

*For correspondence. E-mail: teotonio@igc.gulbenkian.pt.

Keywords. line-cross analysis; epistasis; dominance; adaptation; experimental evolution; reverse evolution; life-history evolution; quantitative genetics; *Drosophila*.

and Barton 1994 for character based adaptive landscapes). Ultimately, differences in the distribution of genetic effects determine the response to natural selection and thus diversity within and among species. Few populations and few characters have had their genetic architecture empirically probed under contexts where evolutionary history and environmental conditions are known or controlled (see Wright 1977; Coyne *et al*. 1997; Lynch and Walsh 1998; Wolf *et al*. 2000).

Here we describe the genetics of differences in morphological, physiological and life-history characters among two sets of *Drosophila melanogaster* populations, ultimately derived from a common ancestry, but subjected to diversifying selection for either increased starvation resistance or short life cycles. This is accomplished by fitting a quantitative-genetics model to the mean character values of the parental and F_1 and F_2 hybrid generations, with the hybrids being produced by crosses between diverged populations and populations that had been maintained in the ancestral selection environment throughout their laboratory history. Assays were performed using conditions resembling the ancestral environment. The quantitative-genetic analysis explicitly models the composite effects of single and digenic additive and dominance effects, as well as maternal and paternal genetic effects. With this analysis we are able to estimate the distributions of genetic effects that result from the exclusive action of laboratory natural selection on ancestral genetic variability. Since the laboratory evolutionary history of the populations studied is well known, we conclude with a brief discussion of the relationship between the genetic structure of characters related to fitness, and fitness itself, particularly the implications that this relationship might have for the study of reverse evolution.

Materials and methods

Experimental populations

The evolutionary history of the populations used here has been previously described (Teotónio and Rose 2000; Teotónio *et al*. 2002; figure 1 here). Briefly, all populations are descendants from the same wild ancestor introduced into the laboratory in 1975 and maintained under controlled conditions for more than 100 generations (Rose 1984). In 1980, five replicate populations ($B_{1–5}$) were derived from this ancestral population and maintained in the same ancestral environment. This environment is characterized by two-week discrete generations, rearing at 25°C, constant light and high relative humidity on banana-molasses food, favouring increased fecundity for a short period of time (up to two hours), under conditions of high adult density (Teotónio *et al*. 2002) after growth at moderate larval crowding (50–100 larvae). Also in 1980, another group of populations ($O_{1–5}$) was derived from the same ancestral population, and was selected for increased lifespan and reproduction at old ages (Rose 1984). In 1989, the $SO_{1–5}$ populations were derived from the O populations by selection for increased starvation resistance, their generation time being between 3 and 4 weeks (Rose *et al*. 1992). The corresponding fed control populations were named $CO_{1–5}$ populations. Lastly, in 1992, from these CO populations another five populations were derived and selected for accelerated development and early fertility, and were called $ACO_{1–5}$ populations (Chippindale *et al*. 1997). All populations were maintained at high population sizes without ever being hybridized. Census sizes at the time of reproduction were at least 1000, even for stress-selected populations. With these population numbers, inbreeding and loss of heterozygosity should be limited over the course of laboratory adaptation (Hutchinson and Rose 1991; Rose and Matos 2004).

Experimental crosses

Two types of crosses were performed in the present study, each replicated fivefold, one set between the B populations and the ACO populations (the B × ACO crosses) and the other set between B and SO populations (the B × SO crosses). When the B × ACO crosses were performed, the B populations had undergone 460 generations, while the ACO populations had undergone 230 generations in their selective environment. When the B × SO crosses were conducted, the B populations had undergone 480

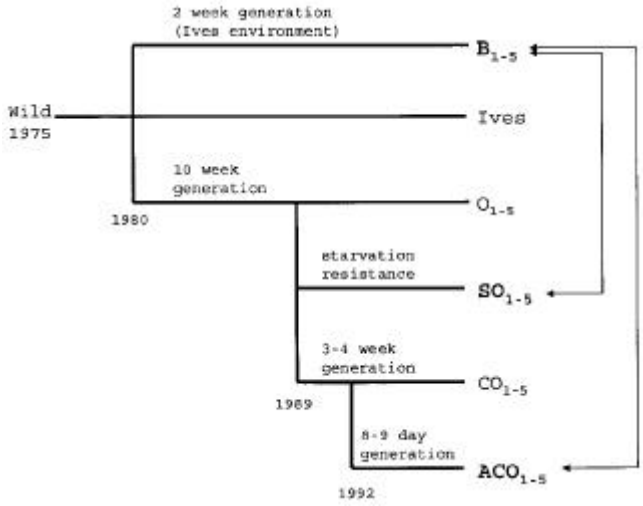

Figure 1. Phylogeny of laboratory selection history of the populations used in this study. All selection treatments are replicated as five independent populations (subscripts). Branch length does not depict evolutionary time; only year of derivation is indicated together with a brief description of selection treatments. The two types of crosses performed among the populations used in the present study are indicated by arrowed connectors. For further details, see Materials and methods.

generations, and the SO populations had undergone 125 generations in their respective environments.

Two reciprocal first-generation and second-generation hybrids were derived from each pair of parental population groups (either B and ACO or B and SO) (see table 1). For each type of cross, five replicate crosses were established from the correspondingly numbered replicate populations from each group; for example B_1 was crossed with either SO_1 or ACO_1, B_2 was crossed with SO_2 or ACO_2, etc. However, there is no correspondence of ancestry arising from these subscripts: the numbering systems are different in the two types of populations used in each cross. Reciprocal crosses were done for each replicate cross between a B population and an SO or ACO population, resulting in a total of 30 lines for each type of cross (B × SO or B × ACO): 10 parental, 10 (5 + 5R) F_1 and 10 (5 + 5R) F_2. The parental populations were maintained for two full generations in a common environment, while the formation of the F_1 and F_2 hybrids was carried out on a staggered basis, in order to assay all generations simultaneously. This design allowed us to reduce parental and grandparental environmental effects specific to each evolutionary treatment (selection regime), which could be confounded with genetic effects. The derivation of each hybrid line involved at least 450 virgin females, with males in excess, for each reciprocal replicate cross.

Population assay protocols

Once all experimental-line generations were obtained, they were maintained for 3 to 4 days in population cages with abundant food. On the day of the assay egg collection, flies were allowed to lay for 2 to 4 hours and exactly 60 eggs per assay vial were collected.

***Developmental time*:** Eight vials of eggs were set up per parental population, 16 vials per F_1 hybrid population, and 32 vials per F_2 hybrid population. Different sample sizes for each generation were used to reduce statistical differences among generation variances (Lynch and Walsh 1998). The vials were randomly placed in incubators for each of the replicate crosses. Flies emerging within 6-hour intervals were hand-transferred into holding vials, sexed and counted. A total of approximately 60,000 flies were measured for both types of crosses.

***Starvation resistance*:** At day 14 after egg collection, and after growth and maturity under the ancestral environment, four flies of the same sex were placed into the assay vial with no food but high humidity (Teotónio *et al*. 2002). Ten such vials were separately set up per gender and per parental population, 20 vials per gender for each of the F_1 hybrids and 40 vials per gender for each F_2 hybrid. Mortality was scored in 6-hour intervals for a total of approximately 55,000 flies.

***Dry body weight*:** Adult flies were collected at day 14 after egg collection and placed in an oven at 60°C overnight. After this period of drying, they were divided into samples of 10 flies (eight to 10 samples per gender and per population) and then weighed to the nearest 0.001 mg on a Cahn electrobalance. Approximately 6000 flies were measured.

***Early-life fecundity*:** After 14 days of standard ancestral assay rearing, 20 females and 20 males from each assay vial were transferred into individual vials containing normal maintenance food. Flies were allowed to lay eggs for one hour, after which they were discarded (Teotónio *et al*. 2002). Eight vials were set up per parental population, 16 vials for each F_1 hybrid, and 32 vials for each F_2 hybrid, for a total of more than 1100 vials. Each vial count was taken as the mean from the counts made by two different individuals (error rate < 5%).

Analysis of generation means with stepwise regression

Variation among the line-generation means was fit to a multiple regression model that predicts the generation means expected from the composite linear action of several genetic effects (Mather and Jinks 1982; Kearsey and Pooni 1996; see also Lynch and Walsh 1998). The parameter

Table 1. Regression coefficients of expected generation means used for estimation of digenic genetic models.

	Population mean	Additive	Dominance	Epistasis		Maternal genetic		Cytoplasmic	Y-linkage
Generation	*m*	[*a*]	[*d*]	[*aa*]	[*dd*]	[*am*]	[*dm*]	[*c*]	[*Y*]
Parental B	1	1	0	1	0	1	0	1	1
Parental ACO or parental SO	1	– 1	0	1	0	– 1	0	– 1	– 1
F_1 (female B × male ACO; or female B × male SO)	1	0	1	0	1	1	0	1	– 1
F_{1R} (female ACO × male B; or female SO × male B)	1	0	1	0	1	– 1	0	– 1	1
F_2 (F_1 × F_1)	1	0	0.5	0	0.25	0	1	1	– 1
F_{2R} (F_{1R} × F_{1R})	1	0	0.5	0	0.25	0	1	– 1	1

coefficients used are shown in table 1, where the intercept *m* indicates the general cross mean corresponding to the expected mean of an F_∞ hybrid, [*a*] indicates composite additive effects, [*d*] composite dominance effects, [*aa*] digenic additive-by-additive composite epistatic effects, [*dd*] digenic dominance-by-dominance effects, [*am*] maternal additive, [*dm*] maternal dominance, [*c*] cytoplasmic effects, and [*Y*] the composite Y-chromosome effects for male data.

Standardized data were calculated as the difference of each line-generation mean from the mean of all line generations within the same replicate cross, thereby reducing heterogeneity effects (genetic or environmental or both) among replicate crosses of the same type. These standardized data from all five replicate crosses were then used to estimate a single genetic model for each character for each gender separately, so that a total of 30 line-generation means were available for regression analysis for each character in each type of cross. All characters were analysed using an additive scale. Starvation resistance was also analysed using a multiplicative scale because it may be exponentially related to somatic maintenance. Results from log-transformed starvation resistance are however qualitatively similar to those using an additive scale (analyses of transformed data not shown).

The statistical models were first estimated by introducing the parameters (the predictor variables) one at a time into an existing model, starting only with the imposed general *m* population mean (the intercept) and without the a priori determination of the order of introduction of the remaining parameters. At each forward step, the parameter that was found to reduce the model's residual sum of squares the most was retained, until the addition of new parameters did not lead to an improvement, as measured by the significance of entering a new parameter conditioned to an *F* ratio of 0.25. Backward stepwise procedures were then employed. Three different statistics were used at this point to accept the model just found by forward procedures, or one with progressively fewer parameters. The final model was chosen based on the highest adjusted R^2, the smallest Mallow's C_p, and the smallest Ellner and Turchin V_2 (see Mueller and Joshi 2000, p. 87–88). These statistics evaluate the predictive power of the models taking into account the effects of overfitting the data with too many parameters. Usually, backward procedures eliminated one or at most two parameters from the model found by forward techniques. All stepwise fitting was done using weighted least-squares regression, weights being defined as the ratio between sample size (number of individuals or vials) and sampling variance. Once the final model was accepted, the significance of each individual parameter's difference from zero was tested using a two-tailed Student's *t* test. Power analysis was conducted as well for the *t* test for each parameter, adjusted for the total number of parameters introduced in the accepted model (Neter *et al*. 1990). All analyses were performed with JMP (SAS Institute Inc. 2000) 4.0 software.

By taking all replicate generation means to estimate a single genetic model for each character, we are describing the genetic structure that results from the deterministic action of laboratory selection under those conditions. The effects of genetic variation among replicate populations under the same selection environment, due perhaps to different mutation histories, genetic drift, variable selection coefficients, or a combination of these, are incorporated into the regression error terms. To study the peculiarities of among-replicate variation, the among-line variances would be needed. This would be empirically cumbersome, because a moderate degree of accuracy in the estimates of the among-line variances would involve a large increase in the number of replicate experiments (cf. Phillips *et al*. 2001). Nevertheless, analyses of covariance (ANCOVA) were performed with the final fitted accepted parameters as covariates, and replicate cross as a block effect, to get some empirical feel for the heterogeneity of genetic effects among the five replicate crosses within each type of cross.

Analysis of generation means with scaling tests

Certain linear relationships between the line-generation means can be used to estimate composite genetic effects, as well as a general nonconcordance with an additive or additive-dominance model (Mather and Jinks 1982; Lynch 1991; Lynch and Walsh 1998). For example, the mean value of the F_2 generation is expected to be equal to the average between the F_1 and the midparent value under an additive and dominance model. Given their statistical simplicity, we used both the *C* and *D* parameters of Mather and Jinks (1982) to complement the stepwise-regression analysis.

The *C* scaling parameter is equal to $4[0.5(F_2 + F_{2R})] - 2[0.5(F_1 + F_{1R})] - P_1 - P_2$, where P_1 and P_2 are the mean character values of the parental populations, F_1 and F_{1R} the mean character values of the F_1 populations resulting from reciprocal crosses, and F_2 and F_{2R} the mean character values of the F_2 populations. According to the model of table 1, *C* is equal to $-2[aa] - [dd] + 4[dm]$; note that the terms [*am*], [*c*] and [*Y*] cancel out when taking the average of mean character values in hybrid reciprocals. Thus, negative values of *C* occur when additive epistasis, dominance epistasis, or both, together outweigh maternal dominance effects. In particular, negative *C* values indicate whether such epistasis has arisen within the parental populations as a result of their evolutionary divergence (Lynch 1991; Waser and Price 1994). On the other hand, in the absence of dominance maternal or epistatic effects, a positive *C* value shows outbreeding enhancement, additive epistatic effects within parental populations than actually diminishing character values.

The other parameter estimated in our crosses is the *D* scaling parameter, which also gives an indication of outbreeding enhancement. The parameter *D* is defined as $2[0.5(F_1 + F_{1R})] - P_1 - P_2$ (Mather and Jinks 1982), which is equal to $2[d] + [dd] - 2[aa]$ (table 1). Thus, if within-loci or between-loci dominance effects, or both, outweigh additive epistasis, *D* will be different from zero and a deviation in character value towards one of the parentals due to dominance is inferred.

The comparison of *C* and *D* scaling parameters will also indicate the importance of additive epistasis relative to dominance epistasis when both maternal dominance and within-locus dominance can be controlled for or safely ignored. Both *C* and *D* were calculated for each of the five replicates of the B × ACO and B × SO crosses. These scaling parameter estimates were then averaged over replicates for each character, and differences from zero were tested using a two-tailed Student's *t* test, with the error being defined as the among-replicate cross error. The *C* and *D* parameters were compared by a two-tailed replicate paired *t* test. No correction for multiple comparisons was made since each parameter estimate questions results of different genetic effects or their interaction, although *C* and *D* parameter estimation for each character and type of cross are not independent of some of the same genetic effects.

Results

The results—line-cross generation means—for all characters of the B × ACO and the B × SO crosses are presented in figures 2 and 3, respectively. The stepwise-regression analyses estimating the genetic effects for each character and for each type of cross are shown in table 2, for standardized data. In most cases, these analyses lead to models with three or four significant parameters, with single-locus additive effects almost always incorporated first into the models, followed by dominance, epistasis or maternal effects. The single-locus additive effects were generally larger than all other effects in the regression models. Table 2 shows all parameters that significantly explain line-generation variation in the initial model fitting, even if some of them are not significant as assessed by the later *t* tests. In six of the models, the last parameter to be introduced by stepwise regression was found not to be significant by *t* test; these were always maternal or Y-chromosome genetic effects. All models showed a very good fit, as judged by relatively high adjusted R^2 values, with the exception of the model fit to data on B × ACO starvation resistance.

Besides strong single-locus additivity, the general patterns observed in table 2 are that single-locus dominance effects are common, being estimated in five out of 14 models. Dominance effects are particularly strong when biased in the direction of dominance of the ancestral B phenotypes. Also, digenic dominance epistasis is observed, usually whenever single-locus dominance effects are not significant. Given the definitions of table 1, this occurrence is perhaps not surprising, because these dominance effects are probably colinear, rather than independent, a statistical problem that may be exacerbated by linkage disequilibrium in the F_2 hybrids (see discussion below, and also Blows and Sokolowski 1995; Lynch and Walsh 1998).

A second pattern in the results is that maternal effects are quite common, in particular additive maternal effects (affecting the parentals and F_1 hybrids) and cytoplasmic maternal effects (affecting all generations). With the exception of starvation resistance in the B × ACO cross, for which there is a poor fit in any event, all characters showed maternal effects in at least one of the two crosses. But while additive maternal effects appear to be evenly distributed in the direction of both ancestral and selected populations, cytoplasmic effects are always in the direction of the maternal selected populations. Maternal dominance effects were not common, being clearly detected only for developmental time in the B × ACO cross. Paternal effects, as revealed by the expression of genes in the Y chromosome, seem to be fairly unimportant: even when they were found to improve the fit of two models they were not significant in subsequent *t* tests.

Finally, as already mentioned, digenic composite dominance epistasis effects were significant in a few models. Additive epistasis on the other hand is found only for male starvation resistance for both types of crosses. For B × SO male starvation resistance there is a positive effect within parental populations, but for B × ACO male starvation resistance additive epistasis is negative within parentals. However, the model in the latter case is a generally poor fit (table 2).

Once the final genetic models were obtained, ANCOVAs were used to determine if replicate crosses were significantly heterogeneous (see Materials and methods). This was the case for most characters, with the exceptions of male dry weight ($F_{4,21} = 2.74$, $P = 0.06$) and male starvation resistance ($F_{4,21} = 2.56$, $P = 0.07$) for the B × ACO crosses, and fecundity ($F_{4,21} = 2.10$, $P = 0.12$) as well as female starvation resistance ($F_{4,21} = 2.23$, $P = 0.10$) for the B × SO crosses. Despite heterogeneity among replicate crosses, the estimated parameter values in these models including a replicate cross factor were similar in magnitude and sign to models without it (results of analyses not shown).

The *C* and *D* scalars estimated for each character and each type of cross are shown in table 3, with associated significance testing. Only for B × SO male starvation resistance is there statistical evidence that the *C* value is negative, although only marginally so. For developmental time in the same cross, there is also an indication of a positive *C* value. Significant differences from zero were

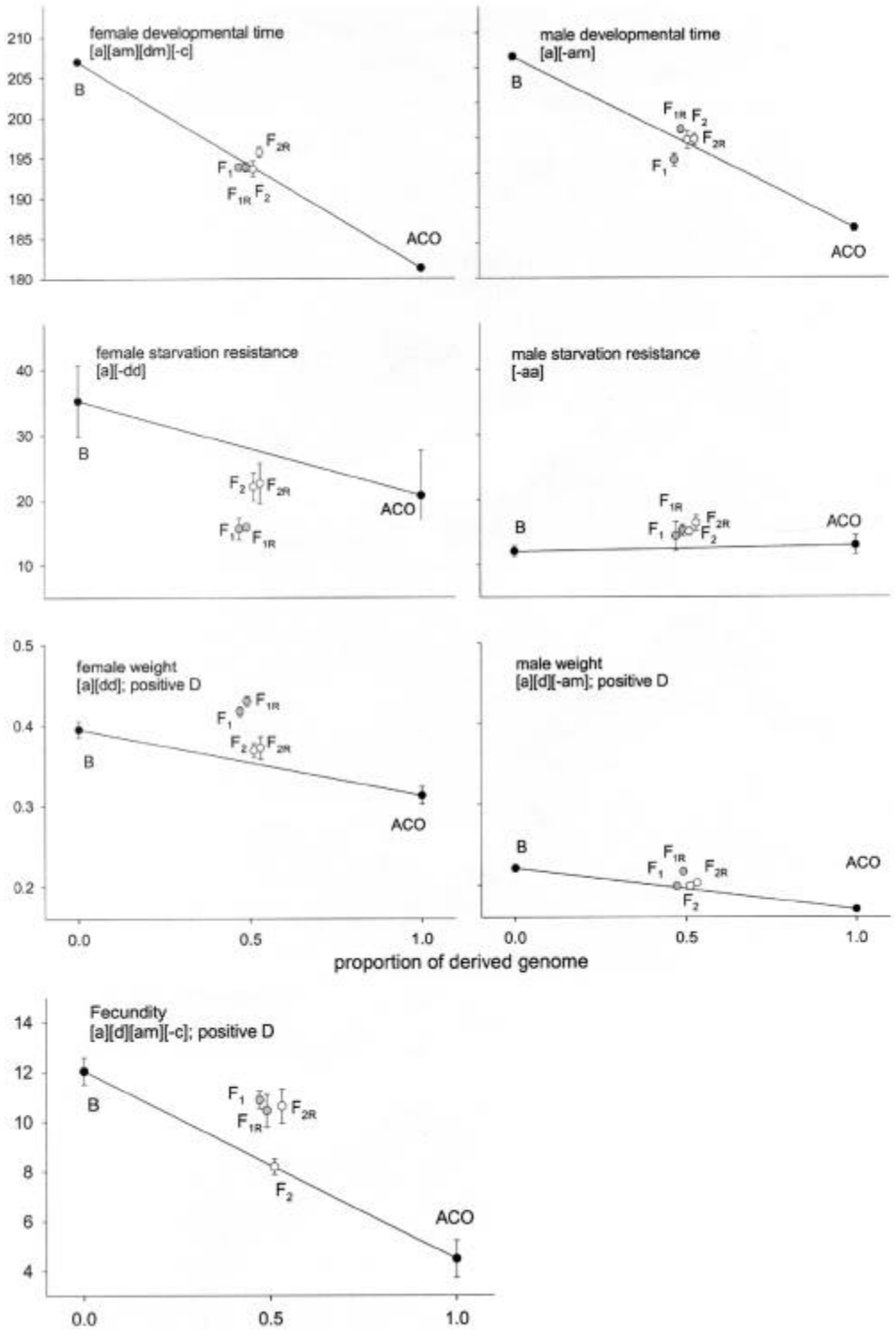

Figure 2. Line-cross generation means for the crosses between the two-week-life-cycle B populations and the ACO populations selected for faster egg-to-egg life cycles. Female and male developmental time is shown as hours from egg to adult, female and male starvation resistance as hours until death from starvation, female and male weight as individual dry body weight in mg, and fecundity as the number of eggs per female per hour (see Materials and methods). For each plot, the mean of the five replicate lines is shown with standard error bars. Parental populations are in black, F_1 hybrids in gray, and F_2 hybrids in white. Hybrid symbols are slightly offset from the 0.5 mark on the x-axis for ease of visualization. The line connecting parentals indicates the expected mean values for an additive model. Insets list significant genetic parameters estimated by stepwise regression and their sign (table 2), as well as the scaling parameters found to be significant (table 3).

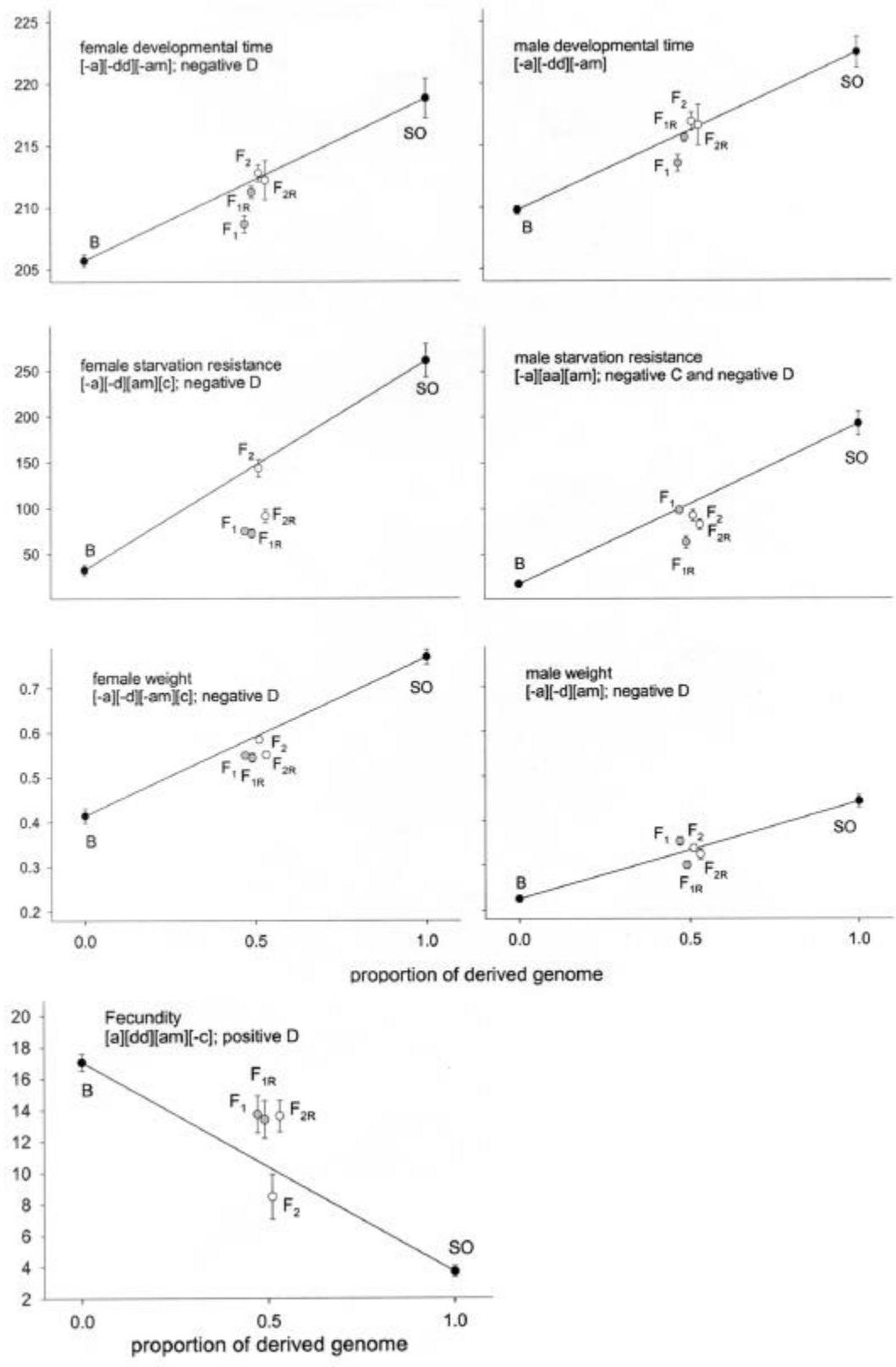

Figure 3. Line-cross generation means for the crosses between the B populations and the SO populations selected for starvation resistance. Labelling, symbols and character value units are as in figure 2. Note however that the scaling of the y-axes differs from that in figure 2.

more common for the *D* parameter. These are found in eight out of 14 characters, being both positive and negative. In nine of the measured characters, *D* estimates are significantly different from *C* estimates.

Discussion

Common effects

As expected, single-locus additive effects are predominant for the traits studied in the two types of crosses. This occurs for two reasons. The first one is biological in that population differentiation is more likely to occur by frequency changes in genes of additive effects within each of the parental populations since it is with these genes that natural selection in large populations is expected to be most effective, in particular for characters with a polygenic basis (Fisher 1930; Turelli and Barton 1994; Weber 1996; Coyne *et al*. 1997). The second reason is methodological, since the parametrization of the models maximizes the possibility of introducing single-locus additive parameters more often than any other effect, so long as the parental populations are fairly differentiated (see figures 2 and 3 and the regression coefficients in table 1). When such differentiation has not arisen, linear regression analyses are likely to have low power, as is readily observed for the results with B × ACO starvation resistance (see table 2).

Other statistical shortcomings are also evident in the estimates of the population mean intercept *m*. Since standardization of data was performed by subtracting the average within-replicate cross-character value from line-generation means, the expectation for this parameter is zero. Remember that *m* estimates the mean character value of the reference line-generation F_∞, which is zero by definition (Mather and Jinks 1982; Kearsey and Pooni 1996). But *m* is different from zero in most cases in our study. Our interpretation is that this pattern is a consequence of the maintenance of linkage disequilibrium in the F_2 generations, leading to nonlinearities that are difficult to control with least-squares linear regression. Since *D. melanogaster* is known to have lower than average recombination rates, this distortion from linearity could be substantial (Lynch and Walsh 1998). Even when genetic heterogeneity among replicate crosses is controlled in the accepted models with ANCOVAs, *m* continues to be significantly different from zero, which indicates that real genetic effects are being incorporated into this term. In addition, any genetic effect that potentially augments linkage disequilibrium within parentals and first-generation hybrids, such as additive epistasis not explicitly modelled (e.g. trigenic epistasis), would also bias *m* towards a value different from zero. Only models using information from more recombinant generations could test these interpretations (Fenster and Galloway 2000).

Table 2. Genetic models obtained with weighted stepwise regression (mean estimate ± SE).

Cross		Developmental time		Starvation resistance		Dry body weight		Fecundity
B × ACO	Parameter	Female	Male	Female	Male	Female	Male	
	m	– 0.43 ± 0.22 (0.3)	– 0.35 ± 0.23 (0.2)	1.24 ± 1.47 (0.1)	2.7 ± 1.33 (0.4)	– 0.28 ± 0.05	– 0.06 ± 0.01	– 28.98 ± 5.81
	[*a*]	12.76 ± 0.47	14.73 ± 0.5	7.87 ± 2.37		0.46 ± 0.08	0.29 ± 0.04	76.04 ± 10.5
	[*d*]				– 2.74 ± 1.84 (0.2)		0.12 ± 0.02	53.91 ± 10.42
	[*aa*]				– 4.82 ± 1.61			
	[*dd*]			– 7.51 ± 1.97		0.67 ± 0.04		
	[*am*]	1.22 ± 0.38	– 2.24 ± 0.27				– 0.06 ± 0.02 (0.6)	27.34 ± 9.74 (0.7)
	[*dm*]	0.79 ± 0.36 (0.4)	0.57 ± 0.36 (0.2)					
	[*c*]	– 1.15 ± 0.28				– 0.08 ± 0.04 (0.3)		– 22.02 ± 5.12
	[*Y*]				0.61 ± 0.4 (0.2)		0.03 ± 0.02 (0.3)	
	ANOVA	< 0.001	< 0.001	0.001	0.014	< 0.001	< 0.001	< 0.001
	Adjusted R^2	0.97	0.97	0.38	0.25	0.85	0.95	0.89
		Female	Male	Female	Male	Female	Male	
B × SO	*m*	1.04 ± 0.31	0.83 ± 0.31 (0.6)	40.39 ± 9.45	– 8.62 ± 2.72	0.26 ± 0.05	0.07 ± 0.04 (0.2)	– 31.97 ± 9.42
	[*a*]	– 5.41 ± 0.61	– 5.62 ± 0.61	– 124.36 ± 10.68	– 101.24 ± 6.24	– 1.78 ± 0.05	– 1.32 ± 0.05	124.6 ± 19.86
	[*d*]			– 79.45 ± 11.09		– 0.48 ± 0.05	– 0.12 ± 0.05 (0.5)	
	[*aa*]				23.97 ± 5.94			
	[*dd*]	– 2.83 ± 0.52	– 2.26 ± 0.53					69.49 ± 19.42
	[*am*]	– 1.65 ± 0.45	– 1.22 ± 0.46 (0.6)	24.16 ± 9.61 (0.6)	17.27 ± 3.33	– 0.16 ± 0.06 (0.6)	0.24 ± 0.03	66.67 ± 18.11
	[*dm*]							
	[*c*]	0.41 ± 0.28 (0.2)	0.39 ± 0.28 (0.1)	26.03 ± 8.35		0.18 ± 0.06		– 62.34 ± 9.67
	[*Y*]							
	ANOVA	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Adjusted R^2	0.87	0.86	0.89	0.95	0.98	0.97	0.85

Grey cells indicate that parameter is not different from zero by a two-tailed *t* test; power for each parameter is shown in parentheses when smaller than 0.8.

After single-locus additive effects, significant maternal effects are estimated for almost all characters. It is not surprising that life-history characters are influenced to a large extent by maternal genotypes, given the physiology of somatic maintenance and reproduction. Nonrecombinant maternal genotypes affect progeny character values in no particular parental population direction, as revealed by the [*am*] parameter. For example, female developmental time in the B × ACO cross F_1 progeny is influenced by positive effects in the direction of B character values, whereas male developmental time appears to be influenced by the overall sum of positive effects of the ACO mothers and negative effects of B mothers. On the other hand, the same negative additive maternal effect of B mothers appears to occur in both female and male B × SO developmental time (compare the differences between F_1 and F_{1R} in figures 1 and 2). Contrary to some other studies with insects (Gilchrist and Partridge 1999; Bieri and Kawecki 2003), effects due to F_1 hybrid mothers are usually not found in our crosses. Finally, cytoplasmic effects increasing progeny character values are biased in the direction of the selected populations. For example, in both figures 1 and 2, it is apparent that the F_2 generation fecundity mean fits midway between the parentals whereas the reciprocal F_{2R} does not. In other words, there is a tendency for cytoplasmic genetic factors to bring character values closer to the average within-cross character values. The reasons for these patterns of maternal genetic effects are not clear at this time. Overall, and while keeping statistical shortcomings in mind, laboratory differentiation in our populations as a consequence of adaptation to novel demography and stress seems to involve single-locus additive effects and maternal additive and cytoplasmic effects to a larger extent than any other genetic effects.

Nonadditive effects

Several methods are typically used to estimate the contribution of nonadditive gene effects to phenotypic differences (Fenster *et al*. 1997). These include analysis of two-way interactions between induced mutations (Clark and Wang 1997; Peters and Keightley 1999), analyses of linkage disequilibrium and interactions among physically mapped chromosomal regions (Long *et al*. 1995; Cheverud 2000), among-population comparisons of additive and nonadditive genetic variance components (Carriére and Roff 1995; Wade 2000), studies of deviations from linearity during response to laboratory selection (Weber 1996; Teotónio and Rose 2000), or the simultaneous use of some of these methods (Agrawal *et al*. 2001). Despite technological advances, however, one of the most powerful methods available is still the analysis of the phenotypic means of hybrids produced by crossing diverged populations of known evolutionary history (Coyne *et al*. 1997; Fenster *et al*. 1997). This is so because only measuring the phenotypes of crosses can directly address questions about the genetic basis of changing phenotypes under inbreeding and crossbreeding. In the context of adaptive landscapes, where the genetic basis of fitness is sought, this is the only method that directly allows the inference of selective optima and fitness depressions generated by nonadditive gene action (Wright 1931, 1982; Lynch 1991; Whitlock *et al*. 1995; Weber 1996; Arm-

Table 3. Mean scaling parameter estimates ± SE.

		Female developmental time		Male developmental time		Female starvation resistance		Male starvation resistance	
Cross	Parameter		*P* value*		*P* value		*P* value		*P* value
B × ACO	*C*	2.8 ± 2.43	–	2.1 ± 2.67	–	1.97 ± 5.27	–	8.08 ± 2.79	0.04
	D	– 0.61 ± 0.81	–	– 0.73 ± 1.09	–	– 24.5 ± 10.2	0.07	4.52 ± 2.12	0.1
	*C = D?***		–		–		0.02		–
B × SO	*C*	5.57 ± 2.13	0.06	5.66 ± 2.73	–	29.2 ± 28.7	–	– 21.7 ± 9.14	0.08
	D	– 4.6 ± 1.3	0.02	– 3.13 ± 1.21	0.06	– 145.97 ± 17.5	0.001	– 48.42 ± 5.33	< 0.001
	C = D?		0.01		0.04		0.02		0.03

**P* values for two-tailed *t* tests with '–' indicating $P > 0.1$; **indicates difference between *C* and *D* parameter with a two-tailed paired *t* test.

		Female weight		Male weight		Fecundity	
Cross	Parameter		*P* value		*P* value		*P* value
B × ACO	*C*	– 0.73 ± 0.37	–	– 0.03 ± 0.09	–	– 3.9 ± 32.9	–
	D	1.4 ± 0.08	< 0.01	0.25 ± 0.01	< 0.01	96.3 ± 22	0.01
	C = D?		0.06		0.04		–
B × SO	*C*	– 0.08 ± 0.37	–	0.03 ± 0.13	–	– 73.5 ± 57.5	–
	D	– 0.91 ± 0.18	0.007	– 0.14 ± 0.06	0.08	127.17 ± 28.8	0.01
	C = D?		0.01		–		0.05

bruster *et al*. 1997; Coyne *et al*. 1997; Fenster and Galloway 2000; Wolf *et al*. 2000). It is also important to note that this approach measures whole-genome departures from additivity that do not depend on allele frequency and that are statistically less demanding in terms of sample size requirements (Mather and Jinks 1982; Kearsey and Pooni 1996; Lynch and Walsh 1998). Of course this advantage comes with its own weaknesses as well, some of which we have discussed before. The most severe of these are likely to be the difficulty of using linear regression techniques to infer genomewide epistatic effects involving more than two loci, and the cancelling out of within-parental genome effects when the effects are of similar magnitude but of opposite sign. The extent to which these problems will underestimate the degree of epistasis affecting population differentiation and adaptation is unknown (see Jinks and Perkins 1969; Mather and Jinks 1982; Lynch and Walsh 1998, Phillips *et al*. 2000).

Our analysis clearly shows that dominance evolves as a consequence of divergence in the laboratory. Directional selection is believed to generate new dominance patterns for the characters being specifically selected (Whitlock *et al*. 1995; Bourguet 1999), and this is what we find. Fecundity is a case worth mentioning since these effects appear to be quite strong in the direction of the B populations. More generally, we find that alleles from populations that evolved under the ancestral environmental conditions are dominant to alleles from populations subjected to divergent selection pressures. This pattern may be generated because the characters in this study were measured in an environment resembling the ancestral environment; if the genetic value of alleles is hyperbolically related to character value, then ancestral alleles are expected to be close to the maxima of this function, compared to alleles from diverged populations. Whether dominance is due to relationships within or between loci is difficult to resolve given the statistical and biological problems mentioned above. In particular, if linkage disequilibrium is maintained up to the F_2 generations, then their genomes will be more similar to those of the F_1 generations than expected under free recombination. The consequence of this effect is that single-locus dominance and epistatic dominance will be confounded during estimation (see table 1). Indeed, single-locus dominance and epistatic dominance do not appear to vary independently of each other in our data.

In partitioning single-locus and two-locus interactions, the scaling parameters *C* and *D* taken together with the regression analyses are revealing. First, consider the fecundity results: in the B × ACO crosses only single-locus dominance is inferred, while in the B × SO crosses epistatic dominance is inferred. The positive *D* scaling values of both types of crosses are significantly different from zero which means that single-locus dominance, two-locus dominance, or both together, generate this significant result (see table 3; $D = 2[d] + [dd] - 2[aa]$). The *C* value result, on the other hand, suggests that dominance epistasis is not significant in either cross ($C = -2[aa] - [dd] + 4[dm]$). Thus, for fecundity, single-locus dominance may be sufficient to explain the remaining phenotypic differences, without epistasis. A similar argument can be made for B × ACO dry body weight. For this character, however, an overdominant phenotypic effect is relatively larger than the single-locus additive effect (figure 1, table 2). Because of this, dominance effects on body weight are probably different, with respect to number of genes involved and the magnitude of their effects, from those involved in fecundity. A good case for dominance epistasis can be made for B × SO developmental time, at least in females. Here *D* values are significantly negative, which, ignoring additive epistasis, means that dominance genetic effects are negative (see table 2 dominance coefficient). The *C* value is positive and marginally significant, which indicates negative dominance epistasis in hybrid generations, and not single-locus dominance. The scaling parameter estimates for male developmental time do not, however, indicate dominance epistasis. It is somewhat surprising that female developmental time shows epistasis in the B × SO cross, because it is only indirectly selected to increase with selection for starvation resistance in the SO populations (Chippindale *et al*. 1996). Since dominance epistasis is only expressed in hybrid generations, it probably results from the pleiotropic or linked effects, or both, of other genes that were selected during divergent selection within parental populations. These genes might themselves have additive effects on the character being directly selected, starvation resistance, while having nonadditive effects on the correlated character of developmental time.

If the interpretation of dominance epistasis is difficult, no such problems occur for the inference of additive epistasis. We have detected clear evidence for additive epistasis for male starvation resistance in the B × SO cross. This genetic effect is also reflected in a significant negative *D* value and a marginally significant negative *C* value. There is therefore good evidence for the evolution of positive epistasis with divergence, which some authors take to indicate the evolution of coadapted gene complexes (Hedrick *et al*. 1978; Whitlock *et al*. 1995; Fenster *et al*. 1997; Wolf *et al*. 2000). We feel that the finding of additive epistasis in these crosses is notable for several reasons. As already mentioned, it is quite difficult to estimate epistasis even if it is present, in outbred populations, owing to the inherent limitations of regression analysis. This difficulty is intensified by the particular experimental design we devised: because we are interested in the mean effects of natural selection in the laboratory, and not in variable selection pressures, genetic drift or mutational histories particular to each replicate population, we analysed data from all the replicate crosses

together. As a result, the genetic effects idiosyncratic to each individual replicate population, including epistasis, are incorporated into the error terms and not into the mean effects estimated in our analysis, as illustrated by the ANCOVA analyses. Most studies model the genetic structure for each replicate cross individually (Blows and Sokolowski 1995; Gilchrist and Partridge 1999; Bieri and Kawecki 2003). In those studies, however, additive epistasis contributing to replicate differentiation is explicitly sought; in our case it is explicitly left out. Finally, processes leading to recombination among loci will act against selection for positive additive epistasis. In the populations we studied, which have large size and where recombination has ample opportunity to occur, selection in the laboratory has nevertheless generated within-population additive epistasis. As for dominance epistasis discussed above, second-order epistatic effects must be relatively large for them to be picked up by selection, if population sizes are close to 1000 reproducing individuals. If the detected additive epistasis is revealing of very close physical linkage among loci then its dynamics are well described by processes similar to single-locus dynamics and in this case selection will be effective whenever the relationship $Ns > 1$ is met, where N is effective population size and s the strength of selection (Crow and Kimura 1970). On the other hand, if linkage disequilibrium among physically distant loci needs to be generated, then the strength of selection must be at least on the order of recombination rates among the loci generating additive epistasis (Fox and Hastings 1992). Epistasis has been shown to occur in relatively small and inbred populations or otherwise structured populations where genetic drift and inbreeding may be more relevant than natural selection in establishing epistasis (Wright 1977; Burton 1990; Hard *et al*. 1992; Blows 1993; Armbruster *et al*. 1997; Starmer *et al*. 1998; Wade 2000), while in populations of larger sizes epistasis is not readily observed (Cohan *et al*. 1989; Weber 1996; but see Bieri and Kawecki 2003). Interestingly, while additive epistasis is present in males, it is not found in females. This difference reflects hemizygous effects in the interaction of X-chromosome genes with autosomal or Y-chromosome genes. Usually it is the homogametic sex that should show more of this within-parental epistasis, not the hemizygous sex (see Whitlock 1995; Wade 2000), and the reason for the results observed here is not clear at this time.

Genetic differentiation and adaptation

Part of the interest in estimating the genetic structure of life-history-related characters lies in the hope that these estimates will give insights into the nature of adaptation and speciation (Fisher 1930; Wright 1931, 1982; Coyne *et al*. 1997; Wade and Goodnight 1998; Wolf *et al*. 2000). In particular, epistasis is often thought to generate rugged adaptive landscapes that can limit future evolution if populations become stranded at selective optima (Whitlock *et al*. 1995; Coyne *et al*. 1997). The question is: do the genetics of functional characters other than fitness reflect this hypothetical pattern?

The answer is that they do not, at least in our laboratory *D. melanogaster* populations when they adapt to ancestral environmental conditions. Previous work with these laboratory differentiated populations when they were undergoing reverse evolution found no evidence that nonadditive gene action for fitness was a determining factor in their evolutionary dynamics (Teotónio and Rose 2000). Convergence to ancestral levels was not complete or uniform for differentiated populations when they were subjected to selection in their ancestral environment, despite their recent common ancestry. A possible explanation for these patterns is that evolutionary constraints are created by rugged adaptive landscapes owing to nonadditive gene action. Since populations derived from crossing the differentiated populations (hybrid populations) will often shift gene frequencies and linkage disequilibrium levels generated by additive epistasis, they are expected to converge more rapidly and more extensively than nonhybrid populations. However, no clear differences were found between the rates of convergence of hybrids between diverged populations and nonhybrid diverged populations undergoing reverse evolution (Teotónio and Rose 2000).

In particular, the additive epistasis for starvation resistance that we find does not apparently impinge on its response during reverse evolution. Even though starvation resistance does not completely converge to ancestral levels after 50 generations of reverse evolution, hybrid populations do not respond to selection more rapidly or more extensively than nonhybrids (Teotónio and Rose 2000; H. Teotónio, M. Matos and M. R. Rose, unpublished data). We also note that the dominance epistasis for developmental time found for female hybrids resulting from the B × SO cross is in the direction of ancestral character values, which if anything would facilitate reverse evolution. Again, hybrid and nonhybrid populations have similar reverse-evolution trajectories for developmental time. Therefore, there is no evidence that epistasis is a limiting factor for adaptation in these populations (cf. Armbruster *et al*. 1997).

The dynamics of reverse evolution might instead be explained by the nonlinear interaction of genotypes resulting from diversifying selection before reverse evolution with the newly restored ancestral environment, not by constraining genetics (Teotónio and Rose 2000). Characters that were tightly connected to fitness during differentiating selection are no longer so during reverse evolution. It is known that the genetic correlation of fitness with other characters can change with selection regime in the laboratory and in nature, both in magnitude and sign (e.g. Bohren *et al*. 1966; Jernigan *et al*. 1994; Gun-

trip and Sibly 1998; Teotónio and Rose 2000; Chippindale *et al*. 2003). Therefore, the quantitative-genetic features of functional characters may not provide much information concerning the subsequent evolution of these characters during the process of adaptation.

Acknowledgements

We thank Y. Chau, T. Vu, N. Vu, C. Hammerle and E. Gass for technical help. A. D. Long, L. D. Mueller and P. C. Phillips have given advice during the project or suggestions on the manuscript. M. Matos received a travel grant from Fundação Luso-Americana para o Desenvolvimento. H. Teotónio is supported by the Fundação Calouste Gulbenkian, Fundação para a Ciência e a Tecnologia and FEDER.

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Received 11 October 2004; in revised form 2 November 2004