Coadapted gene complexes for morphological traits in *Drosophila mercatorum*. Two-loci interactions

SERGIO RUSSO MATIOLI*† & ALAN R. TEMPLETON‡

†Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, travessa 14, 321, 05508 São Paulo, SP, Brazil and ‡Department of Biology, Washington University, St. Louis, MO 63130, U.S.A.

The availability of molecular and morphological markers in a parthenogenetically reproducing strain of *Drosophila mercatorum* allowed us to design an experiment in which we could obtain a sample of completely homozygous recombinant females from two different parental homozygous strains. The phenotypic values of body-size-related measures and bristle numbers (sternopleural and abdominal) were measured in females sampled from the parental, F₁ and F₂ generations. The DNA extracted from the F₂ flies was scored for five Mendelian segregating loci through double stringency PCR. In addition, the flies were scored for three morphological recessive loci. We estimated all single-locus and all two-loci associations between the marker loci with the principal components of the morphological data, which allowed us also to estimate the epistatic parameters. The results suggest that the underlying genetic architecture of the morphological phenotypes cannot be regarded as a result of additivity only, but instead, involves many different kinds of interactions that are distributed around an additive mean.

Keywords: body size, epistasis, *Drosophila*, heterosis, morphology, QTL.

Introduction

From the theoretical point of view, the statistical assumptions of quantitative genetics have changed little since its beginnings, until recently (Barton & Turelli, 1989). There are many questions that can be addressed regarding the quantitative genetical variation in evolution, ranging from genetic architecture (Moreno, 1994) to rates of phenotypic modification in evolutionary time (Spicer, 1993) or even to the origin of reproductive isolation (Cabot *et al.*, 1994; Davis *et al.*, 1994). Depending upon how the variation is structured genetically in the species of interest, very different evolutionary processes can act on continuous phenotypic variation. If the phenotypic variation is caused by additive contributions from many individual loci, the selection pressures can act in virtually any direction of the phenotypic space. If epistatic interactions are present, evolution in certain directions becomes likely only in circumstances that involve genetic reorganizations (Templeton, 1980; Wright, 1980; Wade & Goodnight,

1991; Moreno, 1994). Until recently, when an extensive number of genetic markers became available through DNA technology, most of the work concerning quantitative genetic variation was restricted to organisms where genetic markers were available, as in *Zea mays* or *Drosophila melanogaster* (Mitchell-Olds, 1995). We present here a study that focuses on the detection of single-locus and two-loci effects on morphological traits of *Drosophila mercatorum*. This species is specially suitable for this kind of study because it is able to produce completely homozygous, diploid flies by automictic parthenogenesis in a single generation (Carson *et al.*, 1969). Because parthenogenesis is automictic (retains meiosis), the parthenogenetic offspring can carry various combinations of the genomes from the parental strains. By using this system, we are able to discard the interallelic effects (dominance deviations) from the analysis without using special strains. This experimental system has been used successfully in the past in marker-association experiments to document extensive two- and three-locus epistasis for the phenotype of egg-to-adult viability (Templeton *et al.*, 1976a). Our goal in this paper is to assess the genetic architecture of morphological

*Correspondence. E-mail: srmatiol@ib.usp.br

traits that have been analysed in the classical literature of *Drosophila* quantitative genetics.

Materials and methods

A highly inbred triple mutant strain of *D. mercatorum* (*v pm vl*, hereafter named *v*) with visible markers on all three of the major autosomes, and a parthenogenetic strain (K23-0-Im *v. 6*) were used in all experiments. The *v* strain was obtained by Templeton *et al.* (1976a) from spontaneous mutants that appeared in a strain originally collected in El Salvador by Hampton Carson. The K23-0-Im *v. 6* strain, hereafter called Im, was derived from a single wild-caught female from the Island of Hawaii, and was allowed to reproduce parthenogenetically, thereby resulting in a completely homozygous strain (Templeton *et al.*, 1976a).

Males from the *v* strain were crossed with females from the Im strain in vials containing 10 pairs. The F_1 females were separated while virgins, and left in vials (10 females per vial) to reproduce parthenogenetically. F_1 males were backcrossed with females from the *v* strain to determine the chromosome on which the molecular markers were located relative to the known locations of the morphological markers. Daily, the newly emerged F_2 females were put into a microfuge tube with a water supply for at least 24 h to insure morphological maturity. After this 24 h period, the flies were kept at 4°C in 70% ethanol until the measurements could be performed.

Measurements

A subsample consisting of 240 flies (30 from each of the eight possible genotypes with respect to the *v*, *pm* and *vl* loci) was selected. The 30 flies from each visible genotype were also chosen to be uniformly distributed along the interval classes of developmental time from egg to adult of 12, 13 and 14 days, with 10 flies in each. The ethanol-preserved flies (ranging from a few days to three months in the ethanol) were scored for sternopleural bristle numbers (left and right) and abdominal bristles (5th and 6th segments). Measurements were made for the head width (he wdt), eye length (eye lgt), between-eye length (bt eye), thorax width (trx wdt), thorax length (trx lgt) and scutellum width (sc wdt), using bristles as landmarks, as indicated in the scheme presented by Spicer (1993). The measurements were made using a stereomicroscope equipped with a scaled ocular.

DNA extractions and PCR

After the flies were measured morphologically, DNA was extracted from them using a modified Chelex

protocol from Walsh *et al.* (1991). The flies were removed from ethanol and dried for 1–2 h at 65°C. They were ground in 50 µL of Tris-HCl buffer, pH 8.0, 10 mM. One µL of a proteinase K solution (20 mg/mL) was added to each tube, then 200 µL of 5% Chelex in water were added, and the tubes were incubated at 55–60°C overnight. The tubes were boiled in a water bath for 5 min and centrifuged in a microfuge at top speed for 5 min. The supernatant was then transferred to a new tube and stored at –70°C. Further dilutions (usually 1 : 10) were made before using the samples for the DS (double stringency) PCR. To obtain the genetic markers, the procedures of Mاتيoli & Brito (1995) were followed. The reaction mix contained typically Tris-HCl 10 mM, pH 8.4, dNTPs 200 µM each, KCl 50 mM, 2.4 pmol of specific primer, 9.6 pmol of 10mer primer, and 0.25 units of AmpliTaq (Perkin-Elmer). For the first 15 cycles, the temperatures were set at 94°C for 20 s, the microsatellite annealing temperature for 60 s, and 72°C for 20 s. For the last 25 cycles, the temperatures were set at 94°C for 30 s, 35°C for 30 s, and 72°C for 10 s, with the ramp time from 35°C to 72°C set to 60 s. After the cycles, the tubes were kept at 72°C for 10 min, and then cooled to 4°C, until analysed by agarose gel electrophoresis (1.4% agarose in TBE 1×). All the reactions were carried out in a Perkin-Elmer thermocycler model 9600.

The primer combinations yielding useful markers were 5'-GACAGACAGACAGACA and 5'-CTCACCGTCC (markers *g9-1* and *g9-2*), 5'-GACAGACAGACAGACA and 5'-GAAACGGGTG (marker *g7*), 5'-GACAGACAGACAGACA only (marker *GACA*) and 5'-CTCCACC-RCCRAGT and 5' TGCCGAGCTG (marker *m8*). For the first three combinations, we used 50°C as annealing temperature for the first 15 cycles. For the last one, we used 45°C.

Analyses

The principal components were calculated over the correlation matrix among the variables. Each principal component (PC) was normalized, and thus the compared parameters are expressed in standard-deviation units. All these calculations were performed with the JMP 3.02 statistical package (SAS Institute, 1994).

The individual locus effects over each PC were estimated using a modification of Cheverud & Routman's (1995) procedures with the model of Templeton *et al.* (1976a), where the heterozygotes are not available. The significance of the individual locus effects was assessed by using a randomization test (Crowley, 1992), in which the genotypes of a single locus were randomly assigned to each phenotype (keeping the same sample sizes as for the original data), and the absolute mean

difference of the phenotypic values between the genotypes was calculated and compared with the observed value. This procedure was repeated 1000 times. The probability of a deviation as extreme or more than the observed data under the null hypothesis of no genotypic effects is the ratio between the number of times in which the random absolute difference was greater than the observed difference, and the number of times the procedure was carried out. This procedure was carried out by using a simple randomization algorithm written in ANSI C (available upon request from S.R.M., srmatiol@ib.usp.br)

The two-loci effects were assessed in the same way. For each two-loci combination, each mean phenotypic value (the genotypic value, G_{ij}) was calculated. The nonadditive parameter (NA_{ij}) was estimated as:

$$NA_{ij} = G_{ij} - G_{i.} - G_{.j},$$

where $G_{i.}$ and $G_{.j}$ are the additive effects of alleles in the first and second loci, respectively. These additive and nonadditive parameters can be related to the model of Templeton *et al.* (1976a) by

$$\begin{pmatrix} G_{11} & G_{12} \\ G_{21} & G_{22} \end{pmatrix} = \begin{pmatrix} \mu + \alpha + \beta + \varepsilon & \mu + \alpha & \beta & \varepsilon \\ \mu & \alpha + \beta & \varepsilon & \mu & \alpha & \beta + \varepsilon \end{pmatrix},$$

where α is the additive effect of the '1' allele at the first locus, $-\alpha$ is the additive effect of the '2' allele at the first locus, β is the additive effect of the '1' allele at the second locus, $-\beta$ is the additive effect of the '2' allele at the second locus, and ε is the epistatic effect of the interaction between the first and second loci.

In principal components analysis the grand mean (μ) is rescaled to 0, and the epistatic parameter (ε) was estimated using the following equation:

$$\varepsilon = (NA_{11} + NA_{22} - NA_{12} - NA_{21})/4.$$

The significance of this estimate was calculated by random assignment of the genotypes to the phenotype values, keeping the sample sizes invariant, and re-estimating ε a thousand times to generate its sampling distribution under the null hypothesis of no epistatic interaction. The significance level of the ε estimated from the original data was the frequency with which the absolute value of the random ε was greater than the absolute value of the observed ε in the 1000 resamplings.

Results

The results from the backcrosses using F_1 males provide a powerful and simple test of synteny of markers as

there is no recombination in males. All the autosomes but the dot are marked by the three unlinked, autosomal markers v , pm and vl , with v marking a metacentric autosome and pm and vl marking two acrocentric autosomes (Templeton *et al.*, 1976b). Hence, any molecular marker on the major autosomes will show absolute cosegregation with one and only one of these markers. By keeping track of the sex of the backcross progeny, the pattern of segregation of a molecular marker not showing absolute cosegregation with one of the visible marker loci can quickly be classified as being either X-linked or autosomal (in which case it must be on the dot). In this fashion, the chromosomal locations of the molecular markers were determined to be as follows: $g9-1$ is on the X chromosome, $g9-2$, $m8$ and $g7$ are on the metacentric autosome marked by v , and $GACA$ is on the acrocentric autosome marked by pm . By examining the genotype frequencies in the parthenogenetic F_2 , the tightest linkage was between v and $g7$ with a recombination frequency of 0.13. The next smallest recombination frequency was 0.40 between pm and $GACA$. The remaining metacentric markers, $g9-2$ and $m8$, are not tightly linked to each other or to the v - $g7$ pair. Table 1 presents the mean values of the measurements and bristle scores of the strain samples (v , Im , F_1 and F_2). The complete data set is available upon request from S.R.M. The values obtained were highly correlated (Table 2), so we performed a principal components analysis (PCA) to obtain uncorrelated variables that were used in all subsequent analyses. Table 3 summarizes the results of the PCA analysis. The first principal component (1st PC) has positive loadings for all measurements, and therefore can be interpreted as a measure of overall body size.

The second PC is primarily measuring the overall bristle number. The third PC can be interpreted as a contrast of the number of sternopleural bristles vs. the number of abdominal bristles. The PC of order 4 has a less obvious biological interpretation, but appears to be roughly measuring a shape factor in the thorax and head. These first four principal components, responsible for more than 70% of the observed phenotypic variation, were compared across the strains as presented in Fig. 1. The first and the fourth PC display overdominance and underdominance, respectively (the F_1 values are significantly different from the mid-parent value). The second PC shows a dominance effect regarding the Im strain. The third PC shows codominance. The results of the analysis of single-locus effects over the first four principal components are summarized in Table 4.

The results of the two-loci interaction analyses are presented in Table 5. Significant epistatic interactions were found for the first and fourth principal components. To verify whether the different principal

Table 1 Means for the measured traits in females of *Drosophila mercatorum*. The measurements are expressed in units that correspond to 0.02 mm. Numbers in parentheses are the sample standard deviation

Trait	Im (<i>n</i> = 30)	F ₁ (<i>n</i> = 30)	v (<i>n</i> = 30)	F ₂ (<i>n</i> = 240)
st br r	22.80 (1.85)	22.90 (1.22)	20.20 (1.67)	22.52 (2.07)
st br l	22.57 (1.63)	22.57 (1.98)	20.63 (1.35)	22.67 (1.84)
ab br 5	27.17 (2.48)	29.97 (3.01)	26.87 (2.91)	28.41 (3.93)
ab br 6	22.73 (1.96)	24.80 (3.04)	21.27 (2.15)	22.91 (3.64)
he wdt	53.63 (1.19)	55.93 (1.89)	53.20 (1.85)	55.49 (1.73)
bt eye	23.10 (0.80)	23.77 (1.04)	22.60 (1.04)	24.25 (1.11)
eye lgt	29.83 (0.70)	30.93 (1.08)	29.97 (1.07)	30.41 (1.26)
trx lgt	63.60 (1.90)	67.90 (2.51)	64.27 (2.50)	66.55 (2.25)
trx wdt	38.70 (1.21)	39.50 (1.76)	38.87 (1.59)	40.28 (1.98)
sc wdt	19.47 (0.63)	20.53 (0.86)	20.43 (0.97)	20.32 (0.93)

st br r: sternopleural bristles on right; st br l: sternopleural bristles on left; ab br 5: abdominal bristles in the 5th segment, ventral; ab br 6: abdominal bristles in the 6th segment, ventral; he wdt: head width; eye lgt: eye length; bt eye: between-eye length; trx wdt: thorax width; trx lgt: thorax length; sc wdt: scutellum width.

components displayed significantly different patterns of epistasis, a meta-analysis was performed by using a one-way ANOVA to compare the probabilities from the epistatic interactions with the different principal components being regarded as the treatment effects. This analysis shows that there are significantly different epistatic patterns ($F_{3,108} = 2.95$, $P < 0.05$).

Discussion

The use of principal components as phenotypic values can be a powerful tool in detecting genetically meaningful variation in the measured characters (Templeton, 1977). Individual characters are often correlated either because of pleiotropic effects (e.g. a gene that affects overall size), or because of linkage. For example, Long *et al.* (1995) showed correlated responses of sternopleu-

ral bristles, when selecting for abdominal bristles, that could have arisen from pleiotropic effects (e.g. of a gene that deals with overall bristle number) or the effects of linked loci. Our results also indicate strong correlations among the traits, caused by pleiotropic or linked genes that affect overall body size (PC 1), overall bristle number (PC 2), a trade-off between sternopleural vs. abdominal bristles (PC 3), and head and thorax shape (PC 4).

The parthenogenetic production of females in *D. mercatorum* involves a potentially strong selective bottleneck (Annest & Templeton, 1978; Templeton, 1979), and all possible genome rearrangements are not likely to be produced. Thus, the F₂ results are possibly biased in an unknown fashion by selection. This bias can be partially counteracted by uniform sampling of the various phenotypic combinations of the visible markers,

Table 2 Correlations among the traits of the F₂ females of *Drosophila mercatorum*. The numbers above the diagonal are the linear correlation coefficients. The numbers below the diagonal are the probabilities of a random correlation. Bold numbers indicate significant correlations at the 0.05 level

Trait	st br r	st br l	ab br 5	ab br 6	he wdt	bt eye	eye lgt	trx lgt	trx wdt	sc wdt
st br r		0.355	0.190	0.173	0.081	-0.026	0.107	0.158	-0.003	-0.042
st br l	0.000		0.073	0.207	0.001	-0.132	0.050	0.066	-0.059	-0.103
ab br 5	0.003	0.261		0.515	0.117	0.094	0.122	0.014	0.017	0.004
ab br 6	0.007	0.001	0.000		0.032	-0.007	0.102	0.022	0.008	-0.044
he wdt	0.210	0.992	0.070	0.626		0.612	0.604	0.575	0.559	0.486
bt eye	0.693	0.042	0.148	0.911	0.000		0.363	0.415	0.399	0.467
eye lgt	0.097	0.444	0.060	0.115	0.000	0.000		0.383	0.342	0.333
trx lgt	0.014	0.305	0.823	0.740	0.000	0.000	0.000		0.330	0.432
trx wdt	0.967	0.366	0.793	0.896	0.000	0.000	0.000	0.000		0.532
sc wdt	0.519	0.112	0.956	0.502	0.000	0.000	0.000	0.000	0.000	

st br r: sternopleural bristles on right; st br l: sternopleural bristles on left; ab br 5: abdominal bristles in the 5th segment, ventral; ab br 6: abdominal bristles in the 6th segment, ventral; he wdt: head width; eye lgt: eye length; bt eye: between-eye length; trx wdt: thorax width; trx lgt: thorax length; sc wdt: scutellum width.

Table 3 Principal components analysis, showing the parameters of the first four principal components of F₂ females of *Drosophila mercatorum*

Eigenvalue	3.322	1.812	1.159	0.721
Percent:	33.22	18.12	11.60	7.214
CumPercent	33.22	51.34	62.94	70.15
Eigenvectors				
st br r	0.06	0.46	0.45	-0.08
st br l	-0.02	0.43	0.53	0.31
ab br 5	0.08	0.50	-0.52	-0.06
ab br 6	0.04	0.55	-0.40	0.18
he wdt	0.48	0.01	0.03	-0.18
bt eye	0.40	-0.10	-0.14	-0.18
eye lgt	0.37	0.11	0.05	-0.45
trx lgt	0.38	0.04	0.24	-0.19
trx wdt	0.39	-0.10	-0.04	0.59
sc wdt	0.40	-0.16	-0.04	0.46

st br r: sternopleural bristles on right; st br l: sternopleural bristles on left; ab br 5: abdominal bristles in the 5th segment, ventral; ab br 6: abdominal bristles in the 6th segment, ventral; he wdt: head width; eye lgt: eye length; bt eye: between-eye length; trx wdt: thorax width; trx lgt: thorax length; sc wdt: scutellum width.

as all phenotypic classes are equally likely under total homozygosity for this set of three unlinked loci under the assumption of neutrality. However, this uniform sampling might itself produce a bias for the molecular genotypic frequencies, but we have no way of testing for this possibility at present. An additional limitation of the current design is the lack of full coverage of the genome by our markers. The sparseness of the map defined by the current set of genetic markers prevents us from using an interval mapping approach. Instead, we must look for phenotypic associations with genes in the regions surrounding our marker loci. The term ‘locus’ may be regarded operationally as the chromosome region that lies roughly within 20 cM of a marker. Fortunately, because of the small size of the *D. mercatorum* genome, at least 49% of the genome lies within 20 cM of one or more of our markers (this calculation assumes that each major arm is about 70 cM long — as indicated by previous mapping studies — and represents a minimal estimate because terminal or single markers were assumed to be on the ends of chromo-

Fig. 1 Comparisons of the first four principal components among the parental and F₁ strains of *Drosophila mercatorum*. The values on the ordinates are the principal component values. The horizontal lines linked to the mean (dot) are standard errors, and the dashed horizontal lines are standard deviations. Different letters (at the right of the means dots) indicate samples significantly different at the 0.05 level.

Table 4 Effects of the alleles of the Im strain on the principal components of F₂ females of *Drosophila mercatorum* (in standard deviations of the allele effect from the v strain compared to the mean)

Locus	1st PC	2nd PC	3rd PC	4th PC
v	0.064	0.114	0.043	0.043
pm	0.181***	0.291****	-0.123	-0.043
vl	-0.048	-0.206***	0.124*	0.223****
g9-1	0.075	-0.110	0.097	-0.021
g9-2	0.001	-0.182**	0.088	-0.002
GACA	0.041	0.007	0.047	0.031
m8	0.002	-0.076	-0.225****	0.161**
g7	0.143*	0.138*	-0.029	0.041

* $P < 0.05$, ** $0.005 < P < 0.01$, *** $0.001 < P < 0.005$,
**** $P < 0.001$.

somes, thereby marking only 20 cM in this calculation rather than the maximum possible of 40 cM for a centrally located marker), so we can detect the possible genetic effects of a substantial portion of the genome. Nevertheless, some loci that are contributing to the phenotypes could well exist and not be detectable by us.

Despite these limitations of the current sampling and genetic design, many significant genetic effects upon the phenotypic measures were detected. At the coarsest level of strain comparisons, we found evidence for overdominance, dominance, codominance and underdominance for the four principal component phenotypes that we measured. Further genetic effects were detected by marker association analysis, which revealed significant interactions among different regions of the *D. mercatorum* genome for two of the phenotypes under study. The phenotypes that displayed significant over- or underdominance in the F₁ females are the same phenotypes that displayed significant epistatic interactions at the two-loci level. This suggests that perhaps the simulta-

neous over/underdominant and epistatic behaviour are two effects of the same phenomenon, perhaps related to the existence of coadapted gene complexes for the phenotypes of interest. A stronger conclusion that emerges from our studies relates to the genetic architecture of the two phenotypes displaying significant epistasis. For these two phenotypes, the absence of additive or marginal effects of the marker loci did not imply a lack of phenotypic significance through interaction effects. In three out of the four significant epistatic interactions observed, the marker loci involved had no significant effects when analysed at the single-locus level. In the remaining case of the *pm* and *m8* interaction on the first principal component, only the *pm* locus has a significant effect at the single-locus level. These results are similar to those of Templeton *et al.* (1976a) and Templeton (1979) on the phenotype of viability in which one-locus, two- and three-loci analyses were performed. As in the current case, many highly significant two-loci epistatic effects were detected involving markers with no significant single-locus effect. If only the loci that presented an additive effect when analysed alone were further tested for epistasis, as is commonly done (Stuber *et al.*, 1992; Lark *et al.*, 1994; Wu & Li, 1994; Long *et al.*, 1995; Pavan *et al.*, 1995), much of the relevant genetic variation contributing to the phenotypes would be missed and an erroneous conclusion would have been drawn. Our results therefore serve as a strong warning against the use of marginal effects as a prescreening procedure in defining the genetic architecture of a trait.

We must remain extremely conservative in generalizing the conclusions drafted above. First, the sample size was small. Indeed, we can consider our population sample size to be only two haploid genomes, because we started with completely homozygous strains. As *D. mercatorum* is believed to have natural

Table 5 Estimates of the epistatic parameter ε of each two-loci combination of *Drosophila mercatorum*, expressed in standard deviation units, where significant two-loci effects were found. Values above the diagonal are related to the effects on the first PC. Values below the diagonal are related to the fourth PC

	v	pm	vl	g9-1	g9-2	GACA	m8	g7
v		-0.113	-0.027	-0.126	-0.018	-0.010	0.007	0.003
pm	-0.152*		-0.126	0.055	-0.122	-0.135	-0.148*	-0.058
vl	-0.080	0.057		-0.165*	-0.100	-0.049	-0.071	0.071
g9-1	-0.051	-0.068	-0.055		0.003	0.060	-0.003	-0.076
g9-2	-0.059	-0.083	-0.044	-0.213**		-0.094	0.038	0.056
GACA	-0.006	-0.084	0.012	-0.079	0.028		-0.046	0.012
m8	0.006	-0.106	0.017	-0.004	-0.024	0.091		0.037
g7	0.045	-0.083	-0.038	-0.126	-0.081	0.043	-0.059	

* $0.005 < P < 0.01$, ** $P < 0.005$.

Bold numbers indicate significant ε with nonsignificant single-allele effect.

parthenogenesis under low-density ecological conditions (Templeton, unpubl. results), its alleles may have experienced the complete homozygous state on a periodic basis. In this case, the existence of a coadapted gene complex is more likely to occur than in species that have not experienced this dramatic genetic state. This species has been shown to present another kind of adaptive complex involving gene interactions. The abnormal abdomen syndrome genes, although they can promote very strong morphological and life history effects, are present in high frequencies in some natural populations. This syndrome has a genetic architecture characterized by strong interactions between two major X-linked elements, interactions between the X and Y chromosome, and interactions of the major elements with autosomal modifiers (Templeton *et al.*, 1985, 1993; DeSalle & Templeton, 1986; DeSalle *et al.*, 1986; Hollocher *et al.*, 1992). These studies on natural, sexually reproducing populations of *D. mercatorum* also demonstrate the importance of epistasis in determining the genetic architecture of phenotypic variation, and therefore are consistent with the conclusions drawn here using an experimental system with parthenogenetic reproduction. Taken together, these studies on *D. mercatorum* indicate that epistasis is an important contributor to genetic architecture, but also one that is easily overlooked by many current analytical designs.

Acknowledgements

The authors thank Susan Pletcher because of her expertise on dealing with such delicate flies, and Flora M.C. Fernandes-Matioli for helpful comments on the manuscript. This work was supported by NIH Grant R01 GM31571 to A.R.T. and Grant 93/3328-1 from FAPESP to S.R.M.

References

- ANNIST, J. I. AND TEMPLETON, A. R. 1978. Genetic recombination and clonal selection in *Drosophila mercatorum*. *Genetics*, **89**, 193–210.
- BARTON, N. H. AND TURELLI, M. 1989. Evolutionary quantitative genetics: How little do we know? *Ann. Rev. Genet.*, **23**, 337–370.
- CABOT, E. L., DAVIS, A. W., JOHNSON, N. A. AND WU, C.-I. 1994. Genetics of reproductive isolation in the *Drosophila simulans* clade: complex epistasis underlying hybrid male sterility. *Genetics*, **137**, 175–189.
- CARSON, H. L., WEI, I. Y. AND NIEDERKORN, J. A., JR. 1969. Isogenicity in parthenogenetic strains of *Drosophila mercatorum*. *Genetics*, **63**, 619–628.
- CHEVERUD, J. M. AND ROUTMAN, E. J. 1995. Epistasis and its contribution to genetic variance components. *Genetics*, **139**, 1455–1461.
- CROWLEY, P. H. 1992. Resampling methods for computational intensive data analysis in ecology and evolution. *Ann. Rev. Ecol. Syst.*, **23**, 405–447.
- DAVIS, A. W., NOONBURG, E. G. AND WU, C.-I. 1994. Evidence for complex genic interactions between conspecific chromosomes underlying hybrid female sterility in the *Drosophila simulans* clade. *Genetics*, **137**, 191–199.
- DESALLE, R. J. AND TEMPLETON, A. R. 1986. The molecular through ecological genetics of abnormal abdomen. III. Tissue-specific differential replication of ribosomal genes modulates the abnormal abdomen phenotype in *Drosophila mercatorum*. *Genetics*, **112**, 877–886.
- DESALLE, R. J., SLIGHTOM, J. AND ZIMMER, E. 1986. The molecular through ecological genetics of abnormal abdomen. II. Ribosomal DNA polymorphism is associated with the abnormal abdomen syndrome in *Drosophila mercatorum*. *Genetics*, **112**, 861–875.
- HOLLOCHER, H., TEMPLETON, A. R., DESALLE, R. J. AND JOHNSTON, S. 1992. The molecular through ecological genetics of abnormal abdomen. IV. Components of genetic variation in a natural population of *Drosophila mercatorum*. *Genetics*, **130**, 355–366.
- LARK, K. G., ORF, J. AND MANSUR, L. M. 1994. Epistatic expression of quantitative trait loci (QTL) in soybean [*Glycine max* (L.) Merr.] determined by QTL association with RFLP alleles. *Theor. Appl. Genet.*, **88**, 486–489.
- LONG, A. D., MULLANEY, S. L., REID, L. A., FRY, J. D., LANGLEY, C. H. AND MACKAY, T. F. C. 1995. High resolution mapping of genetic factors affecting abdominal bristle number in *Drosophila melanogaster*. *Genetics*, **139**, 1273–1291.
- MATIOLI, S. R. AND BRITO, R. A. 1995. Obtaining genetic markers by using double stringency PCR with microsatellites and arbitrary primers. *BioTechniques*, **19**, 752–755.
- MITCHELL-OLDS, T. 1995. The molecular basis of quantitative genetic variation in natural populations. *Trends Ecol. Evol.*, **10**, 324–328.
- MORENO, G. 1994. Genetic architecture, genetic behavior, and character evolution. *Ann. Rev. Ecol. Syst.*, **25**, 31–44.
- PAVAN, W. J., MAC, S., CHENG, S. AND TIGHMAN, S. M. 1995. Quantitative trait loci that modify the severity of spotting in piebald mice. *Genome Res.*, **5**, 29–41.
- SAS INSTITUTE 1994. JMP — Statistical software for the Macintosh. SAS Institute Inc., Cary, NC.
- SPICER, G. S. 1993. Morphological evolution of the *Drosophila virilis* species group as assessed by rate tests for natural selection on quantitative characters. *Evolution*, **47**, 1240–1254.
- STUBER, C. W., LINCOLN, S. E., WOLFF, D. W., HELENTJARIS, T. AND LANDER, E. S. 1992. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics*, **132**, 823–839.
- TEMPLETON, A. R. 1977. Analysis of head shape differences between two interfertile species of Hawaiian *Drosophila*. *Evolution*, **31**, 630–642.
- TEMPLETON, A. R. 1979. The unit of selection in *Drosophila mercatorum*. II. Genetic revolutions and the origin of coadapted genomes in parthenogenetic strains. *Genetics*, **92**, 1265–1282.

- TEMPLETON, A. R. 1980. The theory of speciation via the founder principle. *Genetics*, **94**, 1011–1038.
- TEMPLETON, A. R., SING, C. F. AND BROKAW, B. 1976a. The unit of selection in *Drosophila mercatorum*. I. The interaction of selection and meiosis in parthenogenetic strains. *Genetics*, **82**, 349–376.
- TEMPLETON, A. R., CARSON, H. L. AND SING, C. F. 1976b. The population genetics of parthenogenetic strains of *Drosophila mercatorum*. The capacity for parthenogenesis in a natural bisexual population. *Genetics*, **82**, 527–542.
- TEMPLETON, A. R., CREASE, T. J. AND SHAH, F. 1985. The molecular through ecological genetics of abnormal abdomen in *Drosophila mercatorum*. I. Basic genetics. *Genetics*, **111**, 805–818.
- TEMPLETON, A. R., HOLLOCHER, H. AND JOHNSTON, J. S. 1993. The molecular through ecological genetics of abnormal abdomen in *Drosophila mercatorum*. V. Female phenotypic expression on natural genetic backgrounds and in natural environments. *Genetics*, **134**, 475–485.
- WADE, M. J. AND GOODNIGHT, C. J. 1991. Wright's shifting balance theory: an experimental study. *Science*, **253**, 1015–1018.
- WALSH, P. S., METZGER, D. A. AND HIGUCHI, R. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques*, **10**, 506–513.
- WRIGHT, S. 1980. Genic and organismic selection. *Evolution*, **34**, 825–843.
- WU, W. R. AND LI, W. M. 1994. A new approach for mapping quantitative trait loci using complete genetic marker linkage maps. *Theor. Appl. Genet.*, **89**, 535–539.