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Reaction norms for developmental time and weight at eclosion in *Drosophila mercatorum*

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Key words: Phenotypic plasticity; reaction norm; genotype-environment interaction; genetic correlation; genetic constraint; larval growth; age and size at maturity.

Abstract

We determined reaction norms for developmental time and weight at eclosion for 2 isozygous and 11 genetically mixed strains of *Drosophila mercatorum* in four culture media differing in yeast concentration. With decreasing yeast concentration, development was delayed, the weight of emerging flies decreased, and the phenotypic variance of both variables increased. Differences among stocks and significant stock × yeast interactions indicated genetic variance for both variables within environment and different phenotypic responses of stocks across environments. The phenotypic correlation between developmental time and weight was negative at low yeast concentrations and disappeared gradually with increasing yeast. The comparison of completely homozygous with genetically heterogenous stocks showed that most of the increase of variability with deteriorating environment was due to the changing expression of genetic variance. The genetic correlation between developmental time and weight turned from negative in poor to positive in rich medium, while the environmental covariance was negative in all media. Plotting the reaction norms in the developmental time-weight plane rather than separately for each trait reveals most of these results at a glance. It also suggests that much of the genetic variance might be additive, because an effect of the half-sib family structure inherent in the design is clearly visible in the plot. We interpret the pattern of changing variances and covariances, pointing out that the special growth physiology of *Drosophila* and the way environmental factors affect it must be taken into account. We briefly discuss the implications of changing genetic correlations among traits for the evolution of phenotypic plasticity in general.

Introduction

Phenotypic plasticity is a pervasive phenomenon that has long been recognized as important for ecology and genetics (Schmalhausen, 1949; Simpson, 1953; Waddington,

1957; Dobzhansky, 1970). One approach to it is the reaction norm, defined by Woltereck (1909) as all phenotypes developed by a single genotype exposed to a gradient of an environmental factor. Phenotypic plasticity was considered by Wright (1931) to be the "chief object of selection", and Dobzhansky (1951) even said that reaction norms are "what changes in evolution". The more directly a trait is related to fitness, the more this view should apply, but only recently have attempts been made to incorporate reaction norms into the theory of life history evolution (Caswell, 1983). Life history theory has traditionally made predictions about optimal phenotypes for a given environment, but plasticity in life history traits should be seen as a trait that itself can be optimized (Via and Lande, 1985; Reznick et al., 1986).

Recent models have addressed several different questions. Caswell (1983) examined the optimal amount of phenotypic plasticity in terms of phenotypic mean and variance as a response to environmental variability. Such predictions do not specify the form of plasticity, i. e. what shape the reaction norms should have. Insight into the shape of reaction norms can be gained from models like those described by Stearns and Crandall (1984) and Stearns and Koella (1986). They predict how an organism should adjust the development of its life history characteristics if it encounters different environmental conditions (e. g. Fig. 1). It is assumed that if a population recurrently experiences a range of environmental conditions in evolutionary time, selection will leave genotypes that are able to maximize fitness within this range.

This assumption obviously hinges on an optimistic view of the genetics and developmental physiology that produce the phenotypes that natural selection sees. However, several mechanisms constraining the evolution of phenotypic plasticity have been recognized (van Noordwijk and Gebhardt, 1987). For example, genetic correlations between character states expressed in different environments (which correspond to different points on the reaction norm) can at least temporarily limit the progress of the evolution of optimal phenotypic plasticity (Via and Lande, 1985).

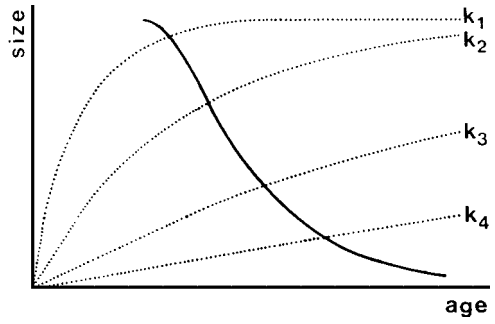

Fig. 1. A reaction norm for age and size at maturity. For each growth rate k_i specified, an optimal age at maturity is calculated by solving the Euler-Lotka relation for the age that results in the maximal intrinsic rate of increase, r. By connecting all the points defining optimal age at maturity and the size of the organism at that age, a norm of reaction for age and size at maturity can be defined in age-size-space. In the figure, one such reaction norm (heavy solid line) and four growth curves (where $k_1 > k_2 > k_3 > k_4$) are shown (after Stearns and Koella, 1986).

To examine the possibility that phenotypic plasticity of life history traits can be optimized by natural selection, we chose *D. mercatorum* as a convenient organism for genetical and ecological experimentation. Here we report on experiments designed to explore the plastic response of developmental time and weight at eclosion to a general environmental stress factor, food shortage. By using several different strains, two of which were completely homozygous due to their parthenogenetic reproduction, we were able to show that there is genetic variability for phenotypic plasticity, and that genetic and environmental variances and correlations differ consistently among environments.

Developmental time and weight at eclosion can be considered as separate traits, and each of them has an environmental variability that can be visualized with separate reaction norms by plotting the trait against the environmental variable. Because we are interested in the simultaneous plastic response of both variables, we have represented the combined reaction norms in the plane defined by developmental time and weight at eclosion (analogous to age and size in Fig. 1) throughout the paper. As discussed by van Noordwijk and Gebhardt (1987) and exemplified in the following, this condensed presentation is helpful because it provides insight into the interactions of the two traits that are obscured in the conventional method. Such insight is particularly valuable when studying genetic and environmental causes of variation at the same time.

Methods

The experiments were carried out on stocks of *D. mercatorum* derived from single inseminated females caught by Dr. A. Templeton at three sites near Kamuela, Hawaii. Table 1 lists the labels by which we refer to them, along with designations of the corresponding strains maintained by Dr. A. Templeton and the date of collection. The a-, b- and c-stocks were maintained as isofemale-lines with discrete generations. D and E are isozygous (completely homozygous) strains derived from isofemale lines

Table 1. Laboratory stocks of *D. mercatorum* used for the experiments. Different characters correspond to three collection sites near Kamuela, Hawaii. The numbers identify the females the stocks were derived from.

Label used in this paper	by Dr. A. Templeton	Date of Collection
a1	IV-74 # 16	June 1985
a2	IV-74 # 15	"
b1	F # 10	"
b2	F # 16	"
c1	B-1 # 03	"
c2	B-1 # 33	"
D	K23-aF-Im-SF2	January 1974
E	K28-O-Im	"

as described in Templeton et al. (1976) and Templeton (1983, p. 367). They are both efficient at parthenogenetic reproduction and are maintained as impaternate (female only) strains. A consideration of the geographical and meteorological situation (changing trade wind direction during the year, humidity) suggests that the three collection sites (corresponding to the a-, b- and c-stocks) are not strongly isolated and that the flies can be considered to derive from one meta-population.

Crosses were made between the isozygous stocks and the isofemale lines. For each cross virgin females and males were mated in a group of about 30 flies for four or five days with about two males per female. Then the females were allowed to oviposit for six to eight hours in petridishes (5 cm diam.) containing a medium that had the same yeast concentration as experienced later by the larvae. This avoided an effect of a different food concentration on the growth of first instar larvae before they were collected (Bakker, 1961). The egg laying medium was dyed black with active charcoal for better visibility of eggs and supplemented with a drop of living yeast suspension which was scraped away before larvae hatched. Larvae were collected 24 hr after the end of egg laying by adding a concentrated sucrose solution which caused them to rise to the surface. They were transferred to small tubes (16 mm diam., 10 larvae per tube) containing 2 ml of the experimental medium and kept under constant experimental conditions as specified below until the last adults had emerged. Adults were collected at 12 hr intervals and time of emergence was recorded. The difference between the midpoints of the egg laying period and the observation interval in which an adult fly had emerged served as our estimate of developmental time. Dry weight of individual flies was determined to the nearest 0.01 mg with a Mettler microbalance after the flies had been dried for 3 hr at 70° C.

Four different environments were created by varying the yeast concentration (in %): 0.1, 0.25, 0.5 and 1.5 (=standard) at otherwise standard conditions (Table 2). The experiment was carried out with the F1 from crosses between males of the isofemale strains a1, a2, b1, b2, c1 and c2 with both isozygous strains D and E, yielding 11 different crosses (b1 × E failed for lack of eggs). Because all the females within an isozygous strain are genetically identical, the experimental design thus comprised essentially two halfsib families, corresponding to the two isozygous strains, barring maternal effects due to different environments experienced by the individual females or due to the maternal genotype. In addition to the crosses, parthenogenetic offspring from D and E females were tested.

Table 2. Standard medium and culture conditions

Medium:	100.0 g cornmeal
(per 1)	60.0 g sucrose
	15.0 g dried yeast
	13.8 g agar
	2.4 g nipagine (non-alcoholic)
Temperature:	25.0+/− 0.1° C
Humidity:	70 +/− 5 % rel.

Because of the large number of different genotype classes, the experiment had to be divided and carried out at several time periods between May and July 1986. We had intended to test all genotypes within a given environment simultaneously with 10 replicates (30 for D and E) and to pool the time factor with the yeast factor. This was not possible due to irregularities in egg laying. The experiment was then carried out in three periods: I. 0.25 % yeast, incomplete set of crosses and replicates. II. 1.5 % yeast, incomplete set of crosses and replicates. III. all yeast concentrations, complement to periods I and II with respect to lines and number of replicates to yield the originally intended full design. Because of a strong effect of the time period and genotype × time interactions, most of the results we report on below derive from the separate analysis of period III which comprised all environments. The number of replicates per genotype × environment combination was thus unequal and varied from 5 to 10, some of the combinations were completely missing, however.

A preliminary genetical analysis was done by comparing the parthenogenetic and the sexual offspring. The presence of isozygous and genetically variable stocks in the same experiment makes it possible to estimate roughly the proportion of phenotypic variability that is due to genetical differences. All variability within the isozygous stocks must have environmental causes. If one assumes that the amount of environmental variance is about the same for all genotypes, the difference between the variability of crossed and parthenogenetic offspring should provide an estimate of the variance due to genetic differences (Robertson, 1957). In the present case, this procedure is likely to underestimate the genetical variance, because complete homozygosity tends to reduce homeostasis and to increase the variability due to environmental factors (Dobzhansky and Wallace, 1953; Lerner, 1954; Sanchez and Blanco, 1984).

We were also interested in the genetic contributions to the phenotypic covariation of developmental time and dry weight. To get estimates for the covariances of dry weight and developmental time within stocks, we calculated analyses of covariance for each combination of yeast concentration and sex. The results from the analyses based on the parthenogenetic flies yielded an estimate of the environmental covariance, the results based on the crosses yielded an estimate of the sum of genetic and environmental covariance. Thus, the genetic contribution to the phenotypic covariance of developmental time and dry weight could be tested as the difference of slopes between crosses and parthenogenetic flies.

The model for the analyses of covariance was a partially nested two factor design, distinguishing for each cross the maternal line (D or E), the collection locality (a, b or c) and the isofemale line within locality (1 or 2). Dry weight was the dependent variable and developmental time the independent covariable.

To compare some of these results with an alternative approach, we also calculated a multivariate analysis of variance with developmental time and dry weight as dependents and the same classification design as in the analysis of covariance. Because each maternal line constitutes a halfsib family, the variance components due to this factor represent half the additive genetic variances of the traits (not one fourth as usual in halfsib designs because the maternal stocks were isozygous; Bulmer, 1985, p. 88).

Results

1. Effects on means

Yeast concentration had a strong and consistent effect on developmental time, dry weight, and their covariance. Table 3 and Fig. 2 show that development was considerably delayed and dry weight smaller when the yeast concentration dropped below 0.5 % to 0.25 %. Table 4 gives the tables of mean squares and crossproducts. It is based on that subset of the data from period III that was almost balanced with respect to yeast level, sex and crosses (no data from b1-crosses, no parthenogenetic offspring). The within replicate means were used as data.

Differences among crosses were statistically significant at all yeast levels, but were more marked at the lower yeast levels. This can be seen in both the standard

Table 3. Summary statistics of developmental time and dry weight for parthenogenetic and sexual offspring (sexes separately). All comparisons of means and variances were made using nonparametric tests (U-test for means, Siegel-Tukey-test for variances, Sachs 1978) and are indicated by the appropriate symbol according to statistical significance.
Symbols are: " > > > " = $P < 0.001$, " > > " = $P < 0.01$, " > " = $P < 0.05$, " = " = not significant, std = standard deviation, CV = coefficient of variation = 100*std/mean, N = number of flies. Developmental time in days, dry weight in micrograms.

variable	class	yeast:	0.1		0.25		0.5		1.5
develop-	females	mean	21.0	> > >	17.0	> > >	13.7	> > >	12.5
mental		std	2.8	> > >	1.7	> > >	1.1	> >	0.7
time		CV	13.3		10.0		8.0		5.6
		N	474		439		587		304
	males	mean	20.4	> > >	17.0	> > >	13.7	> > >	12.6
		std	2.3	> >	1.9	> > >	1.0	=	0.8
		CV	11.3		11.2		7.3		6.3
		N	375		268		361		249
	parth.	mean	20.2	> > >	17.5	> > >	14.0		
		std	1.9	=	1.7	> > >	0.9		
		CV	9.4		9.5		6.3		
		N	314		239		486		
dry weight	females	mean	379	< < <	483	< < <	562	< < <	652
		std	108	> > >	82	> > >	64	>	56
		CV	28.7		17.0		11.4		8.6
		N	352		265		344		221
	males	mean	372	< < <	434	< < <	486	< < <	514
		std	54	=	50	=	45	=	51
		CV	14.5		11.5		9.2		9.9
		N	374		266		361		242
	parth.	mean	345	< < <	415	< < <	522		
		std	48	=	48	=	50		
		CV	13.9		11.6		9.6		
		N	314		238		484		

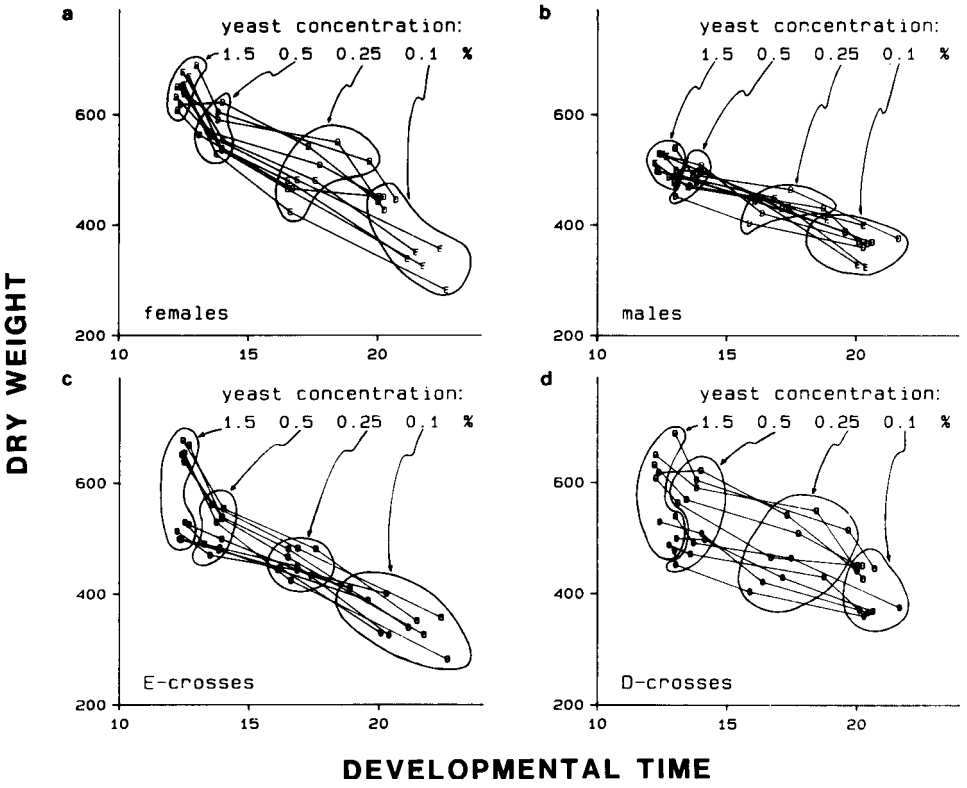

Fig. 2. Reaction norms for developmental time (in days) and dry weight (in micrograms) along a gradient of yeast concentrations (four different values measured), separately for both sexes (a and b, letters indicate the maternal line D or E) and the maternal line in the crosses (c and d, open symbols = females, closed symbols = males).

deviations and the coefficients of variation of the within cross means from the different crosses (Table 5).

Sex differences depended on the yeast concentration: males were faster than females on the poor medium (Table 3, $P < 0.005$), although only slightly (not much more than the measuring accuracy). There were no differences in developmental time between sexes under good food conditions. In contrast, sexual dimorphism for dry weight appeared under good conditions and disappeared at low yeast levels. Fig. 2 (c and d) shows that this is because the females responded more strongly to the yeast reduction: they delayed development more than males and the difference in dry weight among yeast concentrations was larger than in males.

Significant yeast*cross interactions show that crosses responded differently to the yeast gradient (Table 4). Correspondingly, Fig. 2 shows that crosses had differently shaped reaction norms. These differences among crosses were sex-dependent, however. For example, a separation of D- and E-crosses at the lowest yeast level was

Table 4. MANOVA tables for the effects of yeast concentration, cross and sex on developmental time, dry weight and the covariance between these traits. Mean values from replicate tubes were used as data. Only data from sexual offspring from the third experimental period were used. Mean squares and crossproducts are type III. The significance of the mean crossproducts was determined with the exact *F* statistic for Wilks' Lambda.

variable	source	*df*	mean square	*P*
develop-mental time	model	87	82.74	<0.0001
	error	550	1.22	
	yeast	3	1950.00	<0.0001
	cross	10	4.58	<0.0001
	sex	1	11.01	0.0028
	yeast*cross	30	3.71	0.0001
	yeast*sex	3	6.38	0.0015
	cross*sex	10	2.66	0.0178
	yeast*cross*sex	30	2.41	0.0017
dry weight	model	87	0.0626	<0.0001
	error	550	0.0019	
	yeast	3	1.0383	<0.0001
	cross	10	0.0165	<0.0001
	sex	1	0.5881	<0.0001
	yeast*cross	30	0.0058	<0.0001
	yeast*sex	3	0.0883	<0.0001
	cross*sex	10	0.0142	<0.0001
	yeast*cross*sex	30	0.0048	<0.0001
covariance	error	550	−0.0201	
	yeast	3	−44.7657	<0.0001
	cross	10	0.1120	<0.0001
	sex	1	2.5451	<0.0001
	yeast*cross	30	−0.0265	<0.0001
	yeast*sex	3	−0.7385	<0.0001
	cross*sex	10	−0.1660	<0.0001
	yeast*cross*sex	30	−0.0473	<0.0001

much more pronounced in females than in males and qualitatively different (Fig. 2 a and b). There were also differences in sexual dimorphism according to the maternal line: at the lowest yeast level, D-males were lighter than D-females, and there was no difference in developmental time. In the E-crosses, the reverse was true (males faster, but not lighter).

Offspring from parthenogenetic flies were lighter at all yeast levels and developed more slowly at all but the lowest yeast level than offspring from crosses (Table 3). This comparison is confounded with general stock differences. The comparison of the within stock means of the parthenogenetic stocks and the sexual stocks should

Table 5. Variability of developmental time and dry weight among different crosses (third experimental period). Shown are standard deviations and coefficients of variation (CV) from the means over crosses ($N = 11$ different crosses).

variable	sex	yeast:	0.1	0.25	0.5	1.5
develop-	females	std	1.066	1.014	0.290	0.253
mental time		CV	5.1	5.8	2.1	2.0
	males	std	0.671	0.810	0.338	0.294
		CV	3.3	4.8	2.4	2.3
dry weight	females	std	62.3	38.9	30.3	26.1
		CV	15.9	7.8	5.3	4.0
	males	std	25.9	16.5	16.0	19.5
		CV	7.0	3.8	3.3	3.8

indicate differences due to reproductive mode (or the genetical background implied by it), but is significant only for dry weight in this case.

The covariance section of Table 4 shows how the relationship between developmental time and dry weight was affected by the different factors. Although all factors and their interactions had statistically significant effects, by far the strongest correlation is induced by the yeast gradient which produces a negative relationship. This is also visible in the negative slope of the joint reaction norms for developmental time and dry weight (Fig. 2).

2. *Effects on variation*

Phenotypic standard deviations and coefficients of variation were larger in the poor media for both traits (Table 3). Within crosses, females were generally more variable than males, especially in dry weight. This difference was larger at low yeast levels; for developmental time it was significant only at the lowest level. While in general there were no differences among the phenotypic within stock variances, differences between female offspring from parthenogenetic and sexual lines were significant (Table 3). Sexual offspring were much more variable in dry weight than parthenogenetic offspring at all but the highest yeast level. Again, the smaller the yeast concentration, the more pronounced was this difference. Analogous differences existed for developmental time, but were significant only when the variability of individual flies was compared. However, such a comparison confuses general stock differences with differences due to reproductive mode.

3. *Preliminary genetical analysis*

The results of the analyses of covariance are shown in Table 6. There were significant negative correlations between developmental time and dry weight for the

Table 6. Analyses of covariance for dry weight with developmental time as covariate. If the slopes in a complete model with separate regression lines for each replicate were significant, their sum of squares was partitioned by removing one classification factor at a time (maintaining separate intercepts for each replicate). (***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$).

female sexual offspring	yeast = 0.1			yeast = 0.25		
Source	*SQ*	*df*	*F*	*SQ*	*df*	*F*
model	3.635	179	7.62 ***	1.195	114	2.65 ***
different slopes	1.297	81	6.01 ***	0.554	50	2.80 ***
– replicates within cross	0.264	70	1.41 *	0.219	39	1.42 n.s.
– line within locality	0.040	5	2.71 *	0.052	5	2.42 *
– locality	0.014	4	1.11 n.s.	0.005	4	0.27 n.s.
– maternal stock	0.013	1	4.17 *	0.009	1	2.03 n.s.
– mean slope	0.966	1	308.3 ***	0.269	1	61.03 ***
female sexual offspring	yeast = 0.5			yeast = 1.5		
Source	*SQ*	*df*	*F*	*SQ*	*df*	*F*
model	0.825	128	2.32 ***	0.423	104	1.81 ***
different slopes	0.283	55	1.85 ***	0.102	43	1.06 n.s.
– replicates within cross	0.181	44	1.48 *			
– line within locality	0.026	5	1.72 n.s.			
– locality	0.013	4	1.03 n.s.			
– maternal stock	0.004	1	1.39 n.s.			
– mean slope	0.060	1	19.50 ***			
male sexual offspring	yeast = 0.1			yeast = 0.25		
Source	*SQ*	*df*	*F*	*SQ*	*df*	*F*
model	0.775	188	2.50 ***	0.476	147	1.97 ***
different slopes	0.319	82	2.36 ***	0.200	68	1.79 **
– replicates within cross	0.099	71	0.85 n.s.	0.138	57	1.48 *
– line within locality	0.015	5	1.92 n.s.	0.028	5	2.91 *
– locality	0.015	4	2.33 n.s.	0.005	4	1.53 n.s.

Table 6. (continued)

male sexual offspring	yeast = 0.1			yeast = 0.25		
Source	*SQ*	*df*	*F*	*SQ*	*df*	*F*
– maternal stock	0.009	1	5.72 *	0.001	1	0.38 n.s.
– mean slope	0.180	1	108.0 ***	0.028	1	14.04 ***
male sexual offspring	yeast = 0.5			yeast = 1.5		
Source	*SQ*	*df*	*F*	*SQ*	*df*	*F*
model	0.369	176	1.09 n.s.	0.319	114	1.18 n.s.
different slopes	0.105	77	0.71 n.s.	0.135	47	1.21 n.s.
parthenogenetic offspring	ycast = 0.1			yeast = 0.25		
Source	*SQ*	*df*	*F*	*SQ*	*df*	*F*
model	0.451	118	2.55 ***	0.303	59	3.88 ***
different slopes	0.246	59	2.78 ***	0.176	30	4.43 ***
– replicates within line	0.108	57	1.26 n.s.	0.064	28	1.72 *
– line	0.002	1	1.24 n.s.	0.0001	1	0.08 n.s.
– mean slope	0.136	1	85.73 ***	0.112	1	77.43 ***
parthenogenetic offspring	yeast = 0.5			yeast = 1.5		
Source	*SQ*	*df*	*F*	*SQ*	*df*	*F*
model	0.571	119	2.53 ***	1.923	115	1.91 ***
different slopes	0.259	60	2.23 ***	1.058	56	2.16 ***
– replicates within line	0.157	58	1.42 *	0.656	54	1.39 *
– line	0.005	1	2.47 n.s.	0.023	1	2.53 n.s.
– mean slope	0.093	1	46.21 ***	0.379	1	41.09 ***

parthenogenetics at all yeast levels. For both female and male sexual offspring there were negative regressions that were decreasingly steep with increasing yeast concentration and had about a zero slope in the richest medium.

However, where the regressions were significant, there were also significant inhomogeneities of slopes among replicate tubes, although the differences were small.

We determined the factors responsible for the differences among slopes by partitioning the sums of squares for the regression model with separate slopes for each replicate tube into sums of squares attributable to a mean slope and to the design factors. Significance of a design factor was tested using the error mean square of the model with a separate slope for each level of that factor. Table 6 shows that most of the inhomogeneities are due to microenvironmental differences among the replicates within a specific cross. At the lowest yeast level, part of the inhomogeneities are also due to differences between the isofemale lines and between the maternal lines. No differences could be detected between the parthenogenetic lines.

Because of these inhomogeneities of slopes, we estimated mean variances and covariances from the within replicate data. For example, our estimate for the environmental (co)variance was the weighted mean of all within replicate (co)variances from the parthenogenetic flies (within a particular yeast concentration). We calculated an estimate for the genetic (co)variances by substraction of the environmental estimates from the (co)variances calculated for the sexual flies. Table 7a gives the results.

(1) Both genetic and environmental variances in developmental time are highest at the lowest yeast level. The proportions of genetic variance show no consistent trend.

Table 7. a) Estimates of genetic and environmental parts of variances and covariance of developmental time and dry weight, based on averaged within replicate (co)variances from crosses and isozygous flies. the genetic parameters are estimated as the difference between the parameters for crossed and isozygous flies. The calculations for the highest yeast level are based on data from the second experimental period, all others from the third (**: $P < 0.01$, *: $P < 0.05$).
b) Estimates for the additive genetic variances for females (third experimental period), based on the variance component due to the maternal stock.

a) Difference between isozygous and crossed flies

yeast	var(dev. time)			var(dry weight)			covariances		genetic correl.
	gen.	env.	%gen.	gen.	env.	%gen.	gen.	env.	
0.1	2.51	3.38	43	4862	2078	70	−108.1	−41.9	−0.98**
0.25	0.05	2.60	2	3759	1981	65	−22.3	−37.4	−1.62*
0.5	0.38	0.72	35	1110	2139	34	−4.8	−12.1	−0.23
1.5	0.39	0.27	59	4402	10084	30	28.0	−15.3	0.68**

b) Additive genetic variances for developmental time and dry weight

	developmental time			dry weight		
yeast	V_A	$P(V_A > 0)$	% of V_p	V_A	$P(V_A > 0)$	% of V_p
0.1	0.753	0.044	9	7370	0.018	45
0.25	0.0001	>0.1	2.5	2153	0.085	24
0.5	0.0	>0.1	0	1305	0.020	18
1.5	0.04	>0.1	8	0	>0.1	0

(2) Dry weight showed a different pattern. Because only the genetic part of the phenotypic variance decreased with improvement of the substrate (three lowest levels), its proportion declined between the poor and the rich substrate. (The fact that the environmental variance was almost five times as large with 1.5 % yeast as at the other three concentrations may not be attributable to the difference in the yeast concentrations. For the standard food medium, we had to use the data from a different experimental period because the parthenogenetic flies were not represented in period III in this medium.)

(3) There was a marked change in the correlations between the two traits across the different environments. While the genetic correlation was negative in the poor medium, it was positive at the standard yeast concentration, with intermediate values in between. The genetic part of the covariance between the two traits changed sign, but the part caused by environmental noise had negative sign and roughly the same magnitude at all yeast concentrations. This explains why the negative phenotypic correlation that exists at low yeast concentrations disappears with standard medium.

Since there were only two families, our experiment is not particularly powerful to measure additive variances. Nevertheless, a multivariate analysis of variance revealed at least for females significant variance components whose estimates are given in Table 7b. The pattern agrees well with the conclusions that can be drawn from the first analysis: the poorer the environment, the larger the genetic variances.

A graphical representation of these results is given in Fig. 3, where the trajectories for female offspring from the different crosses are shown by their 95 % error

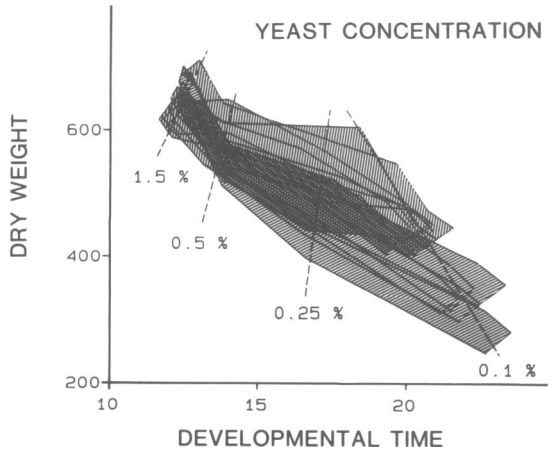

Fig. 3. Reaction norms for developmental time and dry weight along a gradient of yeast concentrations (only for females). Overlapping 95 % confidence envelopes of the reaction norms are plotted for all crosses separately. The broken lines show where the measured points for each yeast concentration cluster (compare Fig. 2) and indicate the correlation between developmental time and dry weight within environment which is due to the maternal lines. The two kinds of hatching indicate the two maternal lines employed (steep: D, shallow: E) for the crosses. Note that the envelopes cluster according to the maternal line, reflecting closer similarity among halfsibs.

Table 8. Survivorship (in % emerged flies) to the adult stage for crossed and parthenogenetic offspring at different yeast concentrations in the third period of the experiment. The chisquares correspond to the comparisons of the number of flies emerging from all crosses and both parthenogenetic lines (D and E). The differences between yeast concentrations are significant at the 0.001 level for all rows shown.

yeast:	0.1	0.25	0.5	1.5
all crosses	77.2	83.2	83.2	77.9
D	42.0	85.0	82.0	—
E	63.0	74.0	80.0	—
D and E	52.3	79.7	81.0	—
chisquare	110.9	1.9	1.3	
P	< 0.001	n.s.	n.s.	

envelopes. The variability within and between crosses is larger in the poor environment (right end of the trajectories) than in the rich environment. The genetic correlation of developmental time and dry weight which is due to the difference of the two maternal lines is negative in the poor environment, changing to positive in the rich one (dotted lines). Furthermore, the halfsib design of the experiment is mirrored in this figure. The crosses made with the same maternal line (D and E, indicated by separte hatchings) are more similar to each other than crosses made with the other maternal stock.

Note that the negative sign of the environmental correlation within yeast concentrations corresponds to the negative slope of the reaction norms along the yeast gradient in the developmental time-dry weight-space (cf. Fig. 2). This negative slope represents an environmental correlation, too, caused by the yeast gradient.

4. Viability

Different yeast concentrations affected the probability that a larva reached the adult stage ($P < 0.001$, Chisquare test), but inconsistently for different lines. The overall survival was highest at intermediate yeast levels and somewhat lower at both ends of the gradient (Table 8). However, this pattern did not show up within different crosses. The most notable difference was that between offspring from crosses and the parthenogenetic lines at 0.1 % yeast: the parthenogenetic larvae survived much less frequently. This is true for both parthenogenetic lines separately. At other yeast levels, there was no difference between parthenogenetic and sexual offspring.

Discussion

The general response we got in our experiments agrees well with the results found by other authors. Some of the most relevant studies were done by Robertson and

Sang, who obtained comparable results by raising larvae on axenic food media deficient for a number of different components, such as casein or RNA (Robertson and Sang, 1944a, b; Robertson, 1960a, b, c; 1963; 1964). As in our experiments, they found that deficiencies can reduce developmental speed as well as the growth rate (and the subsequent weight), but the details depended on which specific component was lacking. Most relevant here is that different nutrients affect many different processes of development which can be expected to depend on genetic differences (Robertson, 1960a; Church and Robertson, 1966). This explains the strong effects on both developmental time and size that a reduction of the yeast level had in our experiment as well as the large differences among genotypes, for we reduced many important food components simultaneously.

It is also commonly observed that variability of developmental time and dry weight increase when the environment is deteriorating (Imai, 1933, 1934; Robertson, 1960a; Parsons, 1961; Barker and Podger, 1970). We could show that a substantial part of this increased variability had genetic causes.

In the following discussion we focus on two points. First we suggest an explanation for the different patterns found for genetic and environmental variances and covariances. We apply knowledge on the physiology of growth in *Drosophila* that has been established by other authors.

Secondly, we elaborate on the role of genetic correlations in evolution. We found genetic variability for the plastic response that may be additive to a large extent (most easily seen by comparing the D- and E-crosses, Fig. 3). Why should this variability be maintained if we assume that there exists an optimal plastic trajectory?

1. Patterns of variability

We found increased variability in poorer environments. By comparing genetically variable with isozygous stocks we concluded that the effect is due to larger differences between the genetic values expressed in poorer environments. This interpretation agrees with Robertson's (1960a) interpretation of his own data which showed a similar pattern.

Similarly we can argue that there is a dramatic change of the genetic relationship between developmental time and weight across environments. Developmental time and weight were phenotypically uncorrelated in the rich medium, but showed a negative correlation in the poor medium. The genetic correlations, however, changed from strongly positive to negative (Table 7). Similar results have been found recently either by analysis of halfsib families in different environments (Giesel, 1986), or by the measurement of correlated responses to selection in different environments (Robertson, 1964; Gilbert, 1986).

Some details on the physiology of growth help in the interpretaiton of our results. Bakker (1959) and Robertson (loc. cit.) established that the larval period of *Drosophila* can be subdivided into two periods (Fig. 4a). During the first two instars, the larvae feed and grow until they reach a minimum weight which enables them to pupate with a fair probability. This period is variable in time and can be extended if

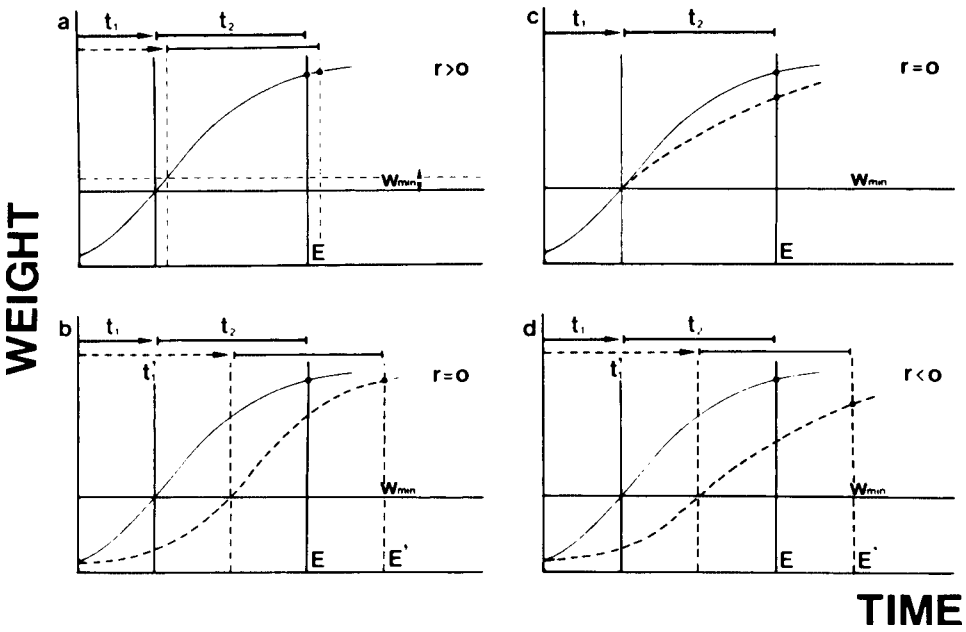

Fig. 4. A growth model for *Drosophila*, showing how different correlations r between developmental time and weight at eclosion (E) can arise. There are two main growth phases: t_1 covers the first two larval instars and ends when a particular minimum weight (w_{min}) is reached. t_2 includes the third larval instar and the pupal stage and is not very variable. (a): small variations in w_{min} cause variations in t_1. Without additional variation in growth rates, the correlation r is positive. (b): Variation in growth rates only during t_1 results in variable developmental time and $r = 0$. (c): Variation in growth rates only during t_2 results in variable weight and $r = 0$. (d): Variation in growth rates during the whole larval period causes developmental time and weight to covary negatively.

growth conditions are bad. The second period corresponds to the third instar and is quite constant in time. The weight at pupation (and at eclosion; Bakker, 1969) will largely be dependent on the growth conditions during this period. So, developmental time and hatching weight will depend on two distinct periods that can be influenced by genetic or environmental factors either separately or simultaneously, depending on the circumstances.

We found a positive genetic correlation between developmental time and dry weight in the rich medium. This suggests that there was little genetic variability for growth rate in that environment, as expected if growth is not limited by resources but by processes of development that cannot be easily accelerated (for example cell division rate or DNA-production). In support of this view, Church and Robertson, (1966) found that when there is a positive correlation between developmental time and weight, the DNA to protein ratio is quite invariable, indicating that changes in size come about by changes in cell number. What we see under such conditions is essentially genetic variation for the minimal weight after the second instar. This variation is probably not large (Bakker, 1961) (Fig. 4a).

On the other hand, the genetic correlation between developmental time and weight was negative in the poor medium. Bakker (1969) showed that growth rates vary under limiting conditions. A situation where the growth rates of both developmental periods are varying is shown in Fig. 4d, where the negative correlation between developmental time and weight is evident.

We want to emphasize that it was necessary to consider a special growth model and the details of growth physiology that apply for *Drosophila*. With different assumptions about growth, completely different patterns might be expected, such as lack of correlations in certain environments (Fig. 4b and 4c). While we observed more genetic variability in the poor environment, the reverse can be true if a different growth model applies. An example for the latter is provided by van Noordwijk (1987), who discusses effects of sibling competition in Great Tits.

In contrast to the pattern found for the genetic correlations between developmental time and weight, the environmental correlations were more or less the same in all environments and had negative signs. This is what we expect if we assume that the environmental noise which produced this correlation was composed of factors acting collectively in a similar manner as the yeast concentration in the experiment. Bell and Koufopanou (1986) sketched a method for deriving expectations about the signs of environmental correlations if we know the dependence of each trait on the environmental variable (that is, their reaction norms). This analysis is straightforward for the variation in yeast concentration. Because the slopes of the reaction norms are negative, this factor apparently caused a negative environmental correlation between dry weight and developmental time.

2. The evolution of reaction norms

What can we infer from these results for the potential evolution of reaction norms? First we must keep in mind that our results describe the total genetic variances, not their additive parts. The implications for responses to selection depend strongly on the relative proportions of additive and non-additive genetic variance. These proportions not only may differ greatly for different traits, but also according to the environment.

Inspection of Fig. 3 shows that the reaction norms of the crosses cluster according to the maternal lines. Because the similarity among halfsibs is due to additive effects, this suggests that the additive proportion of the genetic variance might be substantial. However, because we have only two "families" (maternal lines), it is hard to substantiate this statistically. At least for female dry weight, we found significant variance components and heritabilities between 0.2 and 0.4 (Table 7b).

If there is additive genetic variance for both traits within each environment and also for different plastic responses across environments, we may ask how this variability is maintained. Models predicting optimal plastic trajectories have been successful when applied to life history data of very different organisms (Stearns and Koella, 1986). They suggest that selection may be strong enough to deplete the genetic variability for these strongly fitness related traits.

A possible explanation may be seen in the changing sign of the genetic correlations between the two traits within environments. For example, a response to selection for a simple shift of the reaction norms parallel to the weight axis may be hindered or even prevented if it is associated with selection for developmental time that is different for different environments. The outcome of such selection depends not only in the within-environmental correlation between the traits, but also on the across-environment correlation of each trait (Via and Lande, 1985). Unfortunately, it is not possible to calculate these reliably for our data, but they may be negative for dry weight and positive for developmental time (Fig. 3), implying that the reaction norms cross in intermediate environments. We can imagine, therefore, that there may be little genetic variance for moulding reaction norms as a whole although there is much genetic variance for each trait within environments.

It has been recognized for several years that genetic correlations among different traits can constrain evolution if selection on one of the traits is opposite to the correlated response due to selection on the other (see Bell and Koufopanou, 1986 for a review). As an extension of this principle, Via and Lande (1985) pointed out that correlations between character states in different environments can constrain evolution as well, in this case the evolution of phenotypic plasticity for one trait. Our results provide an example of the more general picture that correlations between character states in different environments interact with correlations between traits within each environment in a complex way that can either enhance or diminish constraining effects on the evolution of phenotypic plasticity. In our example, the crucial question is whether the reaction norms cross.

One can work out which combinations of positive or negative correlations between or within environments among character states or different traits will constrain evolution. However, this becomes a rather complicated undertaking as the number of possibilites increases rapidly. Constraints on the evolution of phenotypic plasticity can be viewed as "narrow regions" of genetic variability (e. g. where two bundles of reaction norms cross each other). Such regions can be detected without difficulty if the reaction norms of all genotypes in a population can be plotted in the trait space.

Any predictions concerning the evolution of phenotypic plasticity will depend on a rather complete description of all the above mentioned components. It will not be possible in general to conclude from the observation of only one kind of correlations how selection would affect phenotypic plasticity. We feel that our graphic represention of reaction norms may help to decide whether there are genetic constraints or not. However, only further theoretical work and careful experimentation will show whether more quantitative predictions can be made.

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