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Evolution, Vol. 45, No. 3. (May, 1991), pp. 668-679.

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THE ECOLOGY AND GENETICS OF FITNESS IN *CHLAMYDOMONAS* III. GENOTYPE-BY-ENVIRONMENT INTERACTION WITHIN STRAINS

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Abstract.—The fitness of genotypes created by crossing strains of *Chlamydomonas reinhardtii* was measured in axenic pure culture in a set of chemically defined environments. There was substantial and highly significant genotype-by-environment interaction, with genetic correlations between environments averaging only about +0.1 for both r and K . Higher-order interactions with combinations of environmental factors appeared to be no less important than simple interactions with single factors. The importance of genotype-by-environment interaction increased with the number of environmental factors manipulated. The linear reaction norms of genotypic score on environmental mean score varied substantially among genotypes and often intersected. There was also some evidence that nonallelic genetic interactions were present, and varied among environments. The genetic correlation of r with K also varied among environments, being significantly negative in some but not in others. These results are similar in all important respects to those previously obtained with different species, and suggest that genotype-by-environment interaction is important at all genetic scales. It is argued that they provide empirical support for a general theory of diversity, the “Tangled Bank,” based on the different response of genotypes to the range of conditions found in heterogeneous natural environments.

Key words.—Environmental heterogeneity, genetic homeostasis, genetic variance, multiple-niche hypothesis, norm of reaction, phenotypic plasticity, phenotypic stability.

Received February 26, 1990. Accepted July 28, 1990.

The major theme of this series of papers is the maintenance of variation in heterogeneous environments. It reports a sequence of experiments designed to investigate the most important empirical issues involved, using laboratory cultures of the unicellular green alga *Chlamydomonas* as a model system. The present paper deals with variation within a single species of *Chlamydomonas*, and complements an earlier paper (Bell, 1990a) dealing with variation among species. A general introduction to the series is given in this earlier paper.

The fitness of a genotype growing in a uniform environment can be expressed in terms of demographic parameters that represent the time-course of population growth. The logistic equation provides a simple and widely used model of the growth of a density-regulated population in terms of two parameters, r (the limiting rate of increase as population density approaches zero) and K (the equilibrium or asymptotic population density, or “carrying capacity”). Either r or K may be more important in determining fitness, depending on how often the environment is disturbed, but the two together supply a complete description of fitness in asexual unicells: given that geno-

types do not interact, the values of r and K measured in pure culture will govern changes in the frequencies of genotypes through time in a mixed population. In a heterogeneous environment the situation is more complicated, because fitness will depend on how genotypes are distributed among environments, as well as on demography within each environment. In these circumstances, fitness can hardly ever be measured. What can be measured is the performance of each genotype in isolation in each environment, or in each of a random sample of environments. By “performance” I mean demographic parameters such as r and K , which would be sufficient to define fitness if the environment were uniform. If the relative performance of different genotypes does not vary much over environments, then the environment is essentially uniform; a single genotype will be superior in all environments, and this genotype will eventually eliminate all its competitors. On the other hand, if relative performance varies widely over environments, then directional selection is less effective, and genetic variance in performance will be eliminated more slowly, or conserved indefinitely. This amounts to a theory of variation, referred to by Bell

(1982) as the "Tangled Bank," in which biotic diversity is underlain and supported by environmental heterogeneity. The theoretical issues involved are discussed in part below and in part in a later publication (see also Bell, 1990a, 1990b; the topic is reviewed by Hedrick, 1986). The central empirical issue is the extent to which the ranking of genotypes with respect to performance varies among environments. This is most succinctly expressed as the magnitude of genotype-by-environment variance in performance, relative to the main-effect genetic variance; it can also be thought of in terms of the lack of correlation among genotypes cultured in different environments. The purpose of this paper is to estimate these quantities and thereby to arrive at statistical generalizations relevant to theory.

The laboratory culture of *Chlamydomonas* has both strengths and weaknesses as a system for investigating genotype-by-environment interaction. Its principal strength is that it enables demographic parameters to be estimated quickly and cheaply by measuring the optical transmittance of liquid cultures. Other laboratory organisms, such as *Tribolium*, *Drosophila*, and *Mus* have such long and complex life histories that the routine measurement of fitness is impracticably laborious and expensive; thus, investigations that use such organisms have concentrated on characters whose relationship with fitness is usually rather remote (see Hedrick and Murray, 1983; for a summary of work on fitness in *Drosophila*). *Chlamydomonas* has the further advantages of being haplontic and clonable, while it can be crossed at will, so that estimating and partitioning genetic variance is unusually simple; and unlike yeasts and bacteria, it can be grown on simple defined media of inorganic salts. Finally, its genetics are well known and a large variety of mutants are available (Harris, 1989), though this study makes no use of them. Its principal drawback, which it shares with many other laboratory organisms, is that very little is known about the ecology or genetics of natural populations. Subsequent papers in this series will address this problem more directly. However, the present investigation is necessarily conducted over a fixed range of contrived environments, and uses a very nar-

row genetic base. The interactions between genotype and environment that it describes are therefore arbitrary rather than evolved interactions.

Experimental Design

I used five wild-type isolates of *Chlamydomonas reinhardtii*, one being mt+ and the other four mt-. The strain references are: CC-1010 (mt+); CC-1009 (mt-); CC-410 (mt-); CC-1418 (mt-); CC-S1D2 (mt-). All the strains were obtained through Dr. E. Harris of the Chlamydomonas Genetics Center at Duke University, except S1D2, which was kindly provided by Dr. P. Lefebvre of Minnesota. Strains 1010 and 1009 are the original *C. reinhardtii* isolates obtained by Smith, and almost certainly came from the same zygote; the other strains are probably only very distantly related. The reason for using so few strains is that very few others are available; with the exception of two Japanese isolates, these five strains are probably the only wild-type *C. reinhardtii* of indisputably independent provenance. A description of the strains and a history of *C. reinhardtii* isolates is given by Harris (1989), who should also be consulted for most of the laboratory protocols used here.

The culture media used as environments were modifications of Bold's medium, a mixture of inorganic salts lacking a carbon source. Eight environments were created by factorial manipulation of the standard concentrations of nitrate (100% or 25%), phosphate (100% or 25%) and bicarbonate (zero or 250 mg/liter). All media were sterile and the cultures remained axenic throughout the experiment. These conditions are identical with those used in the previous study of different species (Bell, 1990a).

About 40 random spores were isolated from each of the four possible crosses, using standard techniques, and 12 chosen at random from each cross as the experimental genotypes. It will be recalled that the F₁ is a segregating generation in haplonts with a zygotic meiosis. The experiment therefore included 4 sets of 12 spores, spores in the same set being full sibs and spores in different sets being half-sibs, plus the five parental strains.

Experimental procedure was as follows.

Each of the 53 genotypes were inoculated from agar slants into Bold's medium and allowed to grow for five days under continuous light. The optical density of these log-phase cultures was adjusted to the same value by dilution with sterile medium, and one loopful then used to inoculate 40 ml of growth medium in a culture tube of 1 cm internal diameter. The inoculated culture tubes were placed in racks in a growth cabinet under constant illumination from two fluorescent lamps, renewed weekly, situated 20 cm from the tops of the tubes. The temperature was uncontrolled and varied between 25°C and 30°C during the experiment. These conditions are dimmer and warmer than those usually used to culture *Chlamydomonas*. The $53 \times 8 = 424$ genotype-environment combinations were arranged as a single unreplicated randomized block on a shelf of the growth cabinet in 12 racks with a total capacity of 480 tubes, the gaps being filled with blanks, contamination checks, and reference genotypes. A second shelf was then laid out in precisely the same way. The two replicates of each genotype-environment combination thus represent replication over shelves.

The experiment, referred to as the Random Spore Pure Culture Experiment or RSPCE, opened on 20th June 1989 and closed on 4th August 1989, with each culture being scored 16 times during this interval. The score used to represent population density was $1,000 \cdot 10T$, where T is percent transmittance at 665 nm, near the peak absorbance of chlorophyll *a*. At the conclusion of the experiment, 80 cultures were chosen randomly and each rescored twice, the readings being about one hr apart, at 450 nm, 665 nm, and 750 nm. The mean scores were correlated as follows: 450/665 nm, $r = 0.982$; 450/750 nm, $r = 0.965$; 665/750 nm, $r = 0.977$. Transmittances at different wavelengths are thus highly correlated, doubtless because they are influenced primarily by the scattering of light from the cells. A small sample was then taken from each tube and cell density measured by haemocytometer; the remainder was filtered, dried (40°C for 24 hr) and weighed. Surprisingly, cell density and culture dry weight are only weakly correlated ($r = +0.209$, $P = 0.07$), perhaps because of a negative cor-

relation of cell size with cell number. Both are correlated with transmittance at 665 nm (cell density, $r = -0.559$; dry weight, $r = -0.656$). Finally, 10 other cultures were chosen so as to span a wide range of transmittances, and serially diluted with equal volumes of sterile medium in six increments, to a final concentration of 1/64. If transmittance declines proportionately with cell density, the regression of log transmittance on log dilution should have a slope of $-\log 2 = -0.301$. The common slope of all 10 samples was -0.235 , SE 0.005, suggesting the presence of some interference at high densities. These trials indicate that optical transmittance, which is the only practicable way of routinely monitoring population density, gives results that are consistent with more direct measures in this system, and that the wavelength chosen is appropriate.

The 16 scores, V_i , obtained for each culture were fitted to the logistic equation.

$$V_i = K/[1 + (K/V_0 - 1)\exp(-rt)]$$

by nonlinear least-squares, using the Gauss-Newton option on PROC NLIN of SAS release 6.3 (SAS Institute, 1988). Units of time are days. From the 848 cultures, there were 3 missing values for both r and K owing to tube breakage; in addition 9 values of r and 17 values of K were missing because of failure to fit the model (r but not K can be estimated for cultures that show very slow growth throughout the experiment). Both genotype-environment means and deviations of replicates from these means were approximately normally distributed for log r (but not for r) and for K (but not for log K). Consequently, log r and K are the parameters analyzed below.

There are three sources of error in this design. The first is the departure of the observations from the logistic model used to summarize them, which can be expressed by the asymptotic standard errors of the estimates. For r , standard error was related to the estimated value:

$$\log \text{SE}(\hat{r}) = -2.163 + 1.388(\text{SE } 0.032)\log \hat{r},$$

with $r^2 = 0.71$, $P < 0.0001$. Low values of r are thus well estimated, with standard errors about 10% of the estimate, but large values are poorly estimated, with standard errors about 50% as large as the estimate.

Nevertheless, in all cases predicted values were checked by eye and found to conform with the observations. The corresponding relationship for K was:

$$\log \text{SE}(\hat{K}) = 1.414 + 0.247(\text{SE } 0.086)\log \hat{K},$$

which is formally significant ($P = 0.004$), but with $r^2 = 0.011$ shows that standard errors are almost independent of estimated values; for the overall mean value of $K = 5,894$, the predicted standard error of 221.5 represents only 4% of the estimated value. The second source of error is between cultures at the same position on different shelves. This was found to be significant, with one shelf yielding greater values of r and lower values of K than the other. This shelf effect was removed in advance of further analysis by subtracting the shelf effect from each culture, leaving the shelf-by-culture interaction as the residual variance. The third source of error is among cultures at different positions on the same shelf. There are no replicates available to estimate this error: a minimal value is given by the residual variance, and a maximal value by the highest-order interaction (spore \times nitrate \times phosphate \times bicarbonate). The residual variance is similar to that observed among replicate cultures on the same shelf in comparable experiments (e.g., Bell, 1990a), and has been used here as the fundamental error variance in hypothesis-testing.

RESULTS

The Overall Magnitude of Genotype-by-Environment Interaction.—The set of 12 random spores obtained from each of the four mt – parents was cultured in each of 8 macroenvironments, yielding 32 estimates of the variance of full sibs within environments for both measures of performance. The estimate of this variance was positive in 29 cases for r and in 30 cases for K ; it was significantly different from zero at $P < 0.05$ in 17 cases for r and in 13 cases for K , and was significant at $P < 0.01$ in 13 cases for r when in 10 cases for K . There is often, then, substantial genetic variance for fitness expressed in the F_1 when F_1 spores are grown as pure cultures in a uniform environment.

The basic analyses of variance are shown in Table 1. Their most conspicuous feature is the substantial contribution made by ge-

notype-by-environment interaction. This is most simply expressed by the intraclass correlation coefficient

$$t_G = \sigma^2_G / (\sigma^2_G + \sigma^2_{GE}),$$

which will be close to unity if the performance of genotypes is highly consistent across environments, but close to zero if relative performance is nearly independent in different environments. Values of t_G obtained for random spores in RSPCE can be compared with those for random species obtained under very similar conditions in a previous trial (PCE; Bell, 1990a).

	t_G :	
	$\log r$	K
Random species	0.354	0.496
Random mt – strains	0.305	0.280
Random spores, 1010 \times 1418	0.166	0.056
Random spores, 1010 \times 410	0.212	0.261
Random spores, 1010 \times S1D2	0.335	0.080
Random spores, 1010 \times 1009	0.123	0.300

It is clear that genotype-by-environment interaction, relative to the main-effect genetic variance, is at least as great among spores from the same cross as it is for different strains within a species, or for different species. Figure 1 is a scatter-plot of σ^2_{GE} on σ^2_G . Genetic variance, roughly speaking, generally decreases in the sequence species-strains-spores for both r and K , as expected. The interaction variance, however, shows little trend, and thus, if anything, tends to increase in importance as relatedness decreases. Estimates of the interaction variance at these various levels of genetic distinctness range from being about equal to the genetic variance to being about 10 times as great. Genotype-by-environment interaction therefore makes a major contribution, or the predominant contribution, to the overall genotypic variance of fitness regardless of the range of genotypes surveyed.

Genotype-by-Environment Interaction Expressed as a Genetic Correlation.—If the performances of a genotype in two different macroenvironments are interpreted as two different characters, their genetic correla-

TABLE 1. Variance of fitness in crosses of *Chlamydomonas reinhardtii*. The columns define the type of material (see below), the source of variance (G, genotype; E, culture macroenvironment; G × E, genotype-by-environment interaction), the number of degrees of freedom for the estimates (*df*), and Mean Squares (MS) and variance components (VC) for log *r* and *K*. The first four blocks refer to 12 random spores from each of four crosses of strain 1010 mt+ with different mt- strains (1418, 410, S1D2 and 1009). The fifth is the combined analysis for the 48 spores. The sixth refers to the four mt- parents themselves. MS and VC have been multiplied by 10⁶ for log *r* and by 10⁻² for *K*. Variance components for environmental effects have been calculated for purposes of comparison with genotypic effects as though they were random effects, although they are in reality fixed. All MS cited are significant at *P* < 0.01 (most at *P* < 0.0001) when tested against the residual MS (G and G × E) or the interaction MS (E) except the MS for G × E of log *r* in cross 1010 × S1D2, for which *P* = 0.08. Residual *df* are 97 (less a few missing values) for the crosses, 372 for the combined analysis and 32 for the mt- parents.

Material	Source	<i>df</i>	Log <i>r</i>		<i>K</i>	
			MS	VC	MS	VC
×1418	G	11	88,302	2,738	25,595	428
	E	7	218,799	7,231	65,344	1,991
	G × E	77	44,510	13,765	18,730	7,277
×410	G	11	161,644	6,214	54,960	2,205
	E	7	572,200	21,616	70,788	20,520
	G × E	77	63,181	23,148	20,631	6,256
×S1D2	G	11	83,955	2,760	26,742	501
	E	7	212,164	7,412	78,095	2,629
	G × E	77	40,768	5,485	18,888	5,794
×1009	G	11	86,275	2,510	52,198	2,336
	E	7	266,112	9,303	17,457	104
	G × E	77	46,332	17,859	15,173	5,453
Combined	parent	3	308,898	1,001	207,935	841
	spore (parent)	44	104,906	3,527	39,917	1,371
	env	7	1,070,556	10,733	140,933	1,243
	parent × env	21	65,478	622	30,813	460
	spore (parent) × env	305	48,991	13,861	18,372	5,648
mt- parents	G	3	149,801	6,816	48,944	2,009
	E	7	146,801	1,326	45,603	3,600
	G × E	21	40,741	15,503	16,802	5,167

tion can be calculated (Falconer, 1952). Each of the four crosses yields 28 pairwise comparisons among environments, and thus 28 estimates of the genetic correlation, each with 10 *df*. The frequency distributions of the 112 estimates for *r* and *K* are shown in Figure 2, where they are compared with the corresponding distributions for species. These frequency distributions have two main features. First, the average of genetic correlations is about +0.1 for both measures of fitness. Fitness in one of the eight macroenvironments is thus a poor predictor of fitness in another random macroenvironment. Many of the estimates (40/112 for *r* and 44/112 for *K*) are negative. Second, these correlations do not appear to be greater on average than those displayed by different species; if anything, indeed, they may be substantially smaller.

Properties of the Genotype-by-Environment Interaction. — Trials with different

species suggested two major generalizations about the pattern of genotype-by-environment interaction: that higher-order interactions were as great as lower-order interactions, and that the genetic correlation was smaller when the environments compared were more different (Bell, 1990a).

The magnitudes of variance components are shown in Figure 3. By far the most important environmental determinant of *r* is the level of bicarbonate, but second-order interactions of environmental factors are also substantial in about half the cases. By contrast, no main effects contribute to variance in *K*, which is affected only by second-order interactions. Thus, fitness is strongly influenced by the relative levels of environmental factors. The figure makes it clear that the variance attributable to genotype-by-environment interactions is as great for higher-order as for lower-order interactions. Thus, relative as well as absolute perfor-

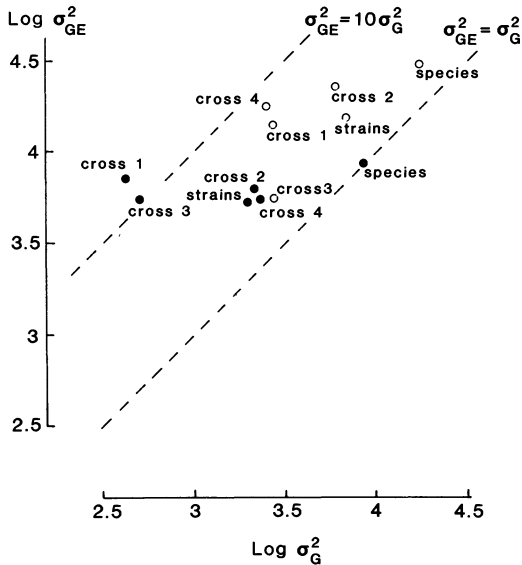


FIG. 1. The magnitude of genotype-by-environment interaction for $\log r$ and K . Values for the four crosses refer to 12 random spores cultured in each of eight macroenvironments. Values for strains refer to the four mt-parents involved in these crosses, scored in the same experiment (RSPCE). Values for species refer to 24 species of *Chlamydomonas* cultured by Bell (1990a) in the same eight macroenvironments in a separate experiment, and are included in the figure for comparison. The two lines drawn on the figure are not regressions: they define $\sigma^2_{GE} = \sigma^2_G$ and $\sigma^2_{GE} = 10 \sigma^2_G$ respectively. Open circles refer to $\log r$ and solid circles to K . Cf. Table 1.

mance is sensitive to particular combinations of environmental factors.

Table 2 shows the average values of genetic correlation when the environments being compared differ with respect to one, two or all three environmental factors. The factors differ among themselves, with bicarbonate eliciting much lower correlations than either nitrate or phosphate. Nevertheless, the average genetic correlation for both r and K is greatest when only one factor has been manipulated, and least when all three have been manipulated. I conclude that the genetic correlation becomes smaller as environments become more different. The data suggest that it may approach zero for environments that are sufficiently different. Bell (1990a) expressed the environment as the mean value of the character over species, and found that the regression of genetic correlation on the absolute difference between environments was negative for both

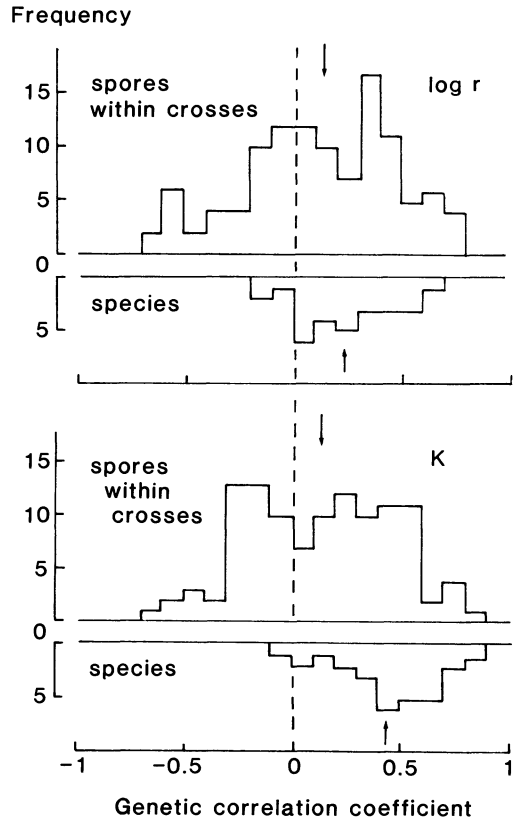


FIG. 2. The frequency distribution of the genetic correlation coefficient for $\log r$ and K . For each of the four crosses there are 28 pairwise combinations of the eight macroenvironments, each yielding an estimate of the correlation coefficient from 12 spores. The frequency distributions above the line refer to these $4 \times 28 = 112$ coefficients. The inverted frequency distributions below the line refer to the 24 species of *Chlamydomonas* cultured by Bell (1990a) in the same eight macroenvironments, and are included here for comparison with the observations of spores. Mean values for the crosses separately are as follows.

mt-parent	log r:		K:	
	mean	SE	mean	SE
S1D2	+0.162	0.070	+0.034	0.063
410	+0.108	0.065	+0.188	0.065
1009	+0.150	0.049	+0.213	0.064
1418	+0.094	0.075	+0.039	0.063
overall	+0.129	0.032	+0.118	0.052
species	+0.232	0.040	+0.448	0.042

Overall means are indicated by arrows in the figure.

r and K . Estimates of the corresponding regressions for spores within crosses were likewise negative, but were only marginally significant for r ($P = 0.04$) and were not significant for K ($P = 0.19$).

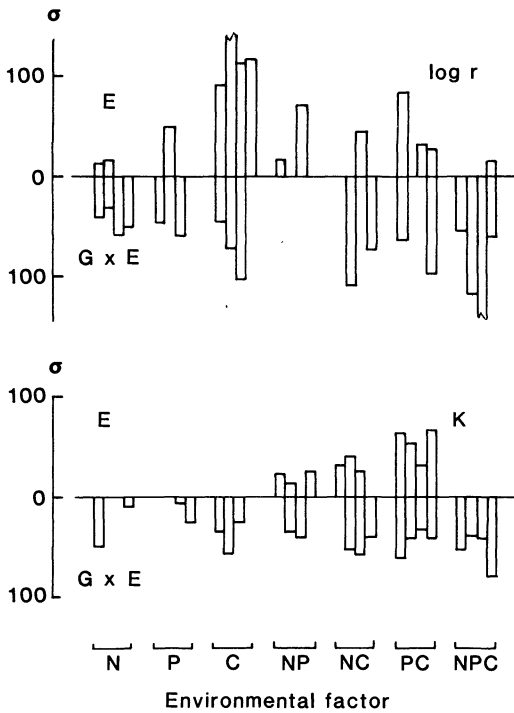


FIG. 3. Structure of the environmental and interaction variances. Each group of four columns represents one of the seven environmental factors or groups of factors (nitrate N, phosphate P, bicarbonate C). The four columns stand for the four crosses using the mt-parents S1D2, 410, 1009, and 1418 respectively. The height of each column above or below the line represents the square root of the estimate of the appropriate variance component, that for environment shown above the line and that for genotype-by-environment interaction below the line. The upper figure refers to $\log r$ and lower figure to K .

The Environmental Variance of Sexual and Asexual Progeny.—The extensive genotype-by-environment variance among spores from a single sexual cross suggests that the mean fitness of sexual progeny may be less sensitive to environmental variation than are their parental strains, or equivalently the mean of the asexual progeny of these parents. This argument is supported by Figure 4, which shows that the mean fitness (r or K) of sexual sibships varies less over macroenvironments than does the fitness of asexual clones developed from their mt-parents.

Environmental Sensitivity.—The mean over spores provides a measure of environment for a given sibship for any given parameter. The genotype-by-environment

TABLE 2. Genetic correlation in relation to environmental difference. Environments may differ with respect to one (N, P, C), two (NP, NC, PC) or all three (NPC) environmental factors. In each case, four comparisons are available for the 12 spores from each cross. Each mean is therefore based on 4 comparisons \times 4 crosses = 16 estimates of genetic correlation. In addition, means over all comparisons involving manipulation of a given number (one, two, or three) of factors are given. These are compared with the corresponding values obtained using different species as genotypes (Bell, 1990a).

	Log r		K	
	Mean	SE	Mean	SE
N	+0.246	0.106	+0.227	0.102
P	+0.264	0.100	+0.276	0.083
C	+0.037	0.082	+0.003	0.079
All singles	+0.182	0.057	+0.169	0.053
(Species:	+0.312	0.070	+0.575	0.066)
NP	+0.063	0.084	+0.144	0.074
NC	+0.065	0.072	+0.087	0.078
PC	+0.103	0.083	+0.048	0.082
All doubles	+0.077	0.045	+0.093	0.044
(Species:	+0.184	0.051	+0.399	0.061)
NPC	+0.062	0.069	+0.044	0.094
(Species:	+0.132	0.065	+0.236	0.187)

variance can then be partitioned into two components, one representing a linear response to environment and the other representing deviations from linearity (Finlay and Wilkinson, 1963). The regression of an individual genotype on the environmental mean can be thought of as a norm of reaction, and the heterogeneity of slopes among spores then represents the genetic variance of norms of reaction. This analysis is illustrated by Figure 5, which shows the regression of r for the spores from one cross, and gives summary analyses of r and K for all four crosses. The figure shows how the norms of reaction cross within the range of environments, and so provides a graphic illustration of genotype-by-environment interaction; indeed, in this case the genotype with the greatest value of r in the worst environment has the lowest value in the best environment, while the genotype with the lowest value of r in the worst environment has the greatest value in the best environment. The former genotype is the most stable, in the sense of having the least environmental variance, and the latter the least stable, or most plastic, of the 12 genotypes from this cross, with respect to r . The anal-

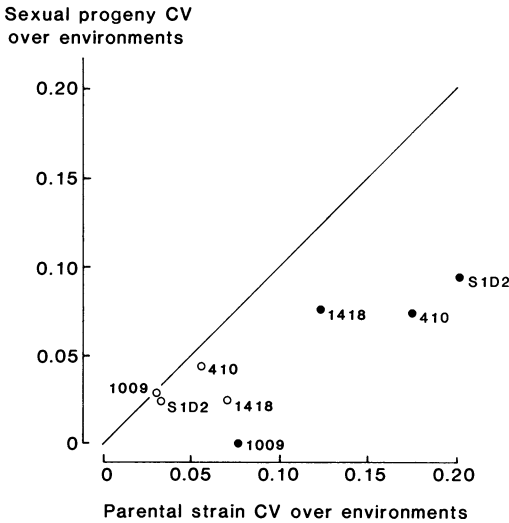


FIG. 4. Environmental variance of fitness of asexual and sexual progeny. The variance of spores over macroenvironments was calculated for asexual progeny (replicates of the parental strain) by single-classification ANOVA, and for sexual progeny (means of spores produced by crossing with mt+ strain 1010) from a model in which the effects were environments, spores within environment, and replication. The variable plotted in the figure is $CV = (\text{square root of variance})/\text{mean}$. The four mt- parents used are named in the figure; solid circles are for *K* and open circles for *log r*.

yses of variance suggest that regression slopes vary among random spores with respect to *r* in 3/4 cases, and with respect to *K* in 2/4 cases; deviations from linearity are large and highly significant in 7/8 cases.

Genetic Analysis. — Because these algae are

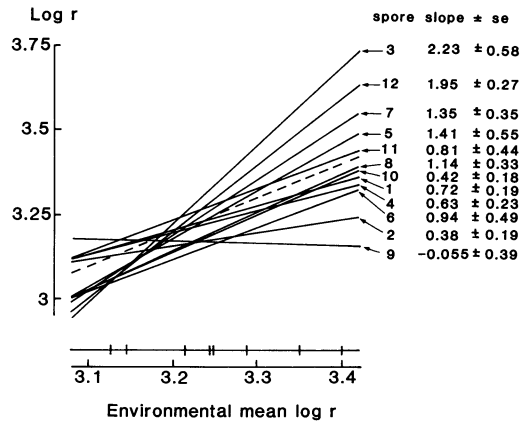


FIG. 5. Linear stability analysis. The figure shows the regressions of $\log r$ on the environmental mean of $\log r$ for all 12 spores from the cross 1010 × 410, with the estimates and standard errors of the regression coefficients. The broken line is the common regression, with unit slope and zero intercept. Ticks on the bar above the X-axis indicate the eight environmental means on which the data for each spore were regressed; each regression is thus based on eight points. The analyses of variance for $\log r$ and *K* for each of the four mt- parents in RSPCE are as listed below.

haplontic, the analysis of the crosses is straightforward (see Mather and Jinks, 1971; Caten, 1979). The expected value of the F_1 is the midparental value, and deviations from the mean over spores from the midparent represent nonallelic interaction. Since the parental strains were randomized together with their sexual progeny, the values of these deviations can be used to assess the importance of nonallelic interactions. Such

(a) Linear stability analysis for $\log r$

Source	df	mt- parent:			
		S1D2	410	1009	1418
Spore	11	0.0439**	0.0971***	0.0458*	0.0455**
Env. and G × E:					
Env. (linear)	1	0.7293	2.1170	0.9623	0.7530
Spore × Env. (linear)	11	0.0446**	0.0799**	0.0321	0.0389*
Deviations	72	0.0168	0.0247***	0.0208**	0.0181***
Pooled error	96	0.0150	0.0088	0.0055	0.0088

(b) Linear stability analysis for *K*

Source	df	mt- parent:			
		S1D2	410	1009	1418
Spore	11	1338081	2634772**	2580512***	1348662*
Env. and G × E:					
Env. (linear)	1	27217842	23647509	6068447	24651526
Spore × Env. (linear)	11	1517040*	1047158	711715	2151376**
Deviations	72	834873***	1014232***	711300***	703558***
Pooled error	96	375298	433688	218578	219352

TABLE 3. Analysis of nonallelic interaction. The variable analyzed is the deviation of the fitness measure from the midparental value. Significance of F is evaluated on the basis that Parents are random and Environments are fixed factors.

	df	Log r			K		
		MS	F	P	MS	F	P
Parent	3	0.18383	6.17	0.0004	14,247,452	13.14	<0.0001
Environment	7	0.19295	2.26	0.071	10,038,526	2.77	0.033
Par \times Env	21	0.08555	2.87	<0.0001	3,621,329	3.34	<0.0001
Residual	352	0.02980			1,084,534		

interactions are difficult to study in diplonts, and our knowledge of epistatic effects on fitness is therefore very slight; the only comparable results from haplonts concern the growth-rate of fungal mycelia (see Caten, 1979), and the present results are thus of some interest despite the restricted parentage of the spores. Among 32 mt-parent-environment combinations, each represented by a set of 12 spore means, 9 were significantly different from the midparent at $P < 0.1$ for log r and 17 for K , 5 at $P < 0.01$ for log r and 7 for K , and 2 at $P < 0.001$ for log r and 3 for K . Nonallelic interaction is therefore detectable in a substantial minority of cases. Analysis of variance (Table 3) shows that the magnitude of deviations varies among parents, and perhaps also among environments; in addition, there is a highly significant tendency for deviations to vary among genotype-environment combinations. This can be expressed by saying that there are extensive genotype-

by-genotype-by-environment interactions: different combinations of genes vary in fitness among different combinations of environmental factors.

Correlation of r with K .—The correlation between the two fitness measures r and K is shown in Table 4. Over all spores in all environments, r and K are uncorrelated. However, this overall result conceals a great deal of variation among environments, the correlation being significantly negative (at $P < 0.01$) in three environments, and significantly positive (at $P = 0.015$) in a fourth. The factorial design of the experiment allows the environmental factor responsible to be identified: correlations tend to be negative in environments with added bicarbonate, but zero or positive in environments with no bicarbonate. Very similar results were obtained when species rather than spores were the units of comparison (Table 4). Thus, the significance of genotype-by-environment interaction extends

TABLE 4. The relationship between r and K . Regression and correlation coefficients were calculated on the 48 spore means available from the four parents in each environment. Analysis of covariance showed that regression slopes were significantly ($P < 0.01$) heterogeneous among parents for only one environment. Nested analysis of covariance confirmed that the intrinsic correlation coefficients (variance component correlations) for spores within parents were very similar to the observed correlations reported here. Each environment has two levels (symbolized - and +) of each of the three environmental factors nitrate N, phosphate P and bicarbonate C. The correlations among species reported in the final column are from an independent experiment with a similar design (PCE) reported by Bell (1990a).

Environment	Regression		Genetic correlation		Correlation among species	
	$b (\times 10^4)$	SE (b)	r	P	r	P
N-P-C-	+0.089	0.137	+0.095	0.52	-0.050	0.80
N+P-C-	+0.200	0.155	+0.188	0.20	-0.330	0.10
N-P+C-	+0.243	0.097	+0.348	0.015	+0.038	0.90
N+P+C-	+0.110	0.070	+0.231	0.12	+0.041	0.90
N-P-C+	-0.937	0.310	-0.408	0.004	-0.505	0.015
N+P-C+	-1.83	0.361	-0.598	0.0001	-0.393	0.05
N-P+C+	-1.28	0.341	-0.485	0.0005	-0.239	0.20
N+P+C+	-0.561	0.362	-0.223	0.13	-0.615	0.001
Overall	-0.089	0.090	-0.051	0.32	-0.149	0.50

beyond simple characters to the relationship between characters, with the relationship between fitness measures varying among environments.

DISCUSSION

The leading feature of these results is the extent to which relative fitness changes over environments. In describing them, I have emphasized their similarity to previous work in which different species were cultured in the same set of environments. The interaction of genotype with environment is thus a major component, or the predominant component, of overall genotypic variance in fitness, whether the genotypes concerned are random species from the same genus, or random spores from the same sexual cross. Moreover, it can be shown in both cases that relative fitness is highly sensitive to particular combinations of environmental factors, and that genetic correlation decreases as environments become more different. These are, therefore, important generalizations about the variance of fitness which appear to be independent of genetic scale.

It may be argued that these generalizations cannot be extended to natural populations because the conditions of culture in the laboratory are entirely unrepresentative of natural environmental variation. It is true that the levels of bicarbonate represented in the experiment span the entire range normally encountered in natural waters, and that the levels of nitrate and phosphate exceed those present even in highly eutrophic ponds. The fact that the culture conditions represent novel and extreme environments, however, strengthens rather than weakens the conclusions. Genotype-by-environment interaction may arise in two ways. In the first place, there may exist a set of physiological constraints such that (say) success when the ratio of nitrate to phosphate is high necessarily implies that performance will be inferior when this ratio is low. Such constraints would imply that relative fitness will vary over environments even for novel genotypes generated by mutation or inbreeding, or for random genotypes exposed to novel conditions. Second, selection will tend to remove genetic variance contributed by genes that are uniformly successful or unsuccessful over a wide range of environ-

ments, but will be less effective in eliminating variance due to genes that enhance fitness in some environments but reduce it in others. Genotype-by-environment interaction arising in this way, as the result of disruptive selection among environments, will be a property of evolved populations. The experiment described here tests only the first of these two possibilities. The results described here are therefore conservative, in that they are likely to understate the magnitude of interactions occurring in natural populations. In the same way, the correlation between fitness components such as r and K might be expected to be positive when random genotypes are cultured in novel environments (Service and Rose, 1985; Bell and Koufopanou, 1986), and the predominantly negative correlations that were found are the more surprising.

It may still be urged, however, that natural environments are not sufficiently heterogeneous, on spatial scales comparable to the scale on which dispersal occurs, for large amounts of genotype-by-environment interaction to be expressed. This is an empirical issue outside the scope of the present study, which will be investigated in a separate series of papers (Bell and Lechowicz, 1990).

Genotype-by-environment interaction combined with local population regulation creates negative frequency-dependent selection and therefore acts to maintain diversity (Levene, 1953; Maynard Smith, 1966; Bell, 1985). This represents a particularly powerful general theory of diversity because it can be applied with the same force in a very wide range of circumstances—sexual and asexual populations, haploids and diploids, genes and species. It does not require that the environment consists of discrete patches, but can be restated in terms of the different resource requirements of individuals in a physically homogeneous environment (Clarke, 1972; Maynard Smith and Hoekstra, 1980). In highly heterogeneous environments, genetic variation will be maintained under selection if on average genetic correlations are slightly negative or, in the limit, zero (Via and Lande, 1985). It is however debatable whether the precise conditions for maintaining stable equilibria in unchanging environments are crucial to the

interpretation of levels of diversity in natural populations or communities; extensive genotype-by-environment interaction will, at the least, retard the loss of genetic variance under selection and thereby lead to elevated levels of diversity when rates of mutation, immigration or speciation are given.

Much of the theory of population genetics is based on assigning fixed values of fitness to genotypes and calculating the consequences of variation in fitness in uniform environments. A conspicuous example of this procedure is the stochastic theory of nearly neutral alleles (Kimura, 1983). Many of the genotypes used in this investigation had indistinguishable effects on fitness in some given environment. It did not in the least hold, however, that they were indistinguishable in any other environment. It seems entirely possible, given these results, that most genotypes will prove to be nearly neutral in any particular set of conditions, but will express very different fitnesses if these conditions are changed. The same point has been made by de Jong and Scharloo (1976). Diversity would then be maintained, not by near-neutrality, but by very powerful but roughly balanced forces of selection.

Not only single fitness measures in isolation but also the covariances of fitness measures, such as r and K , vary with circumstances. Gebhardt and Stearns (1988) obtained a similar result for developmental variables in *Drosophila*. To this extent, arguments about the stability of genetic variance-covariance matrices through time are beside the point; covariances are not fixed at any particular time in heterogeneous environments, and may change radically through time as the result of environmental changes extrinsic to the selection scheme.

The main conclusion of this paper, then, is that selection is likely to be almost indefinitely contingent. The relative performance of different genes or combinations of genes and the relationship between different aspects of performance are all sensitive to changes in environmental factors and in combinations of environmental factors. The organic diversity that we see is underlain by the richness and complexity of these interactions.

ACKNOWLEDGMENTS

This work was supported by an Operating Grant from the National Science and Engineering Research Council of Canada. I was assisted in setting up and scoring the experiment by L. Pilkonis. The parental strains of *C. reinhardtii* were supplied by the Chlamydomonas Genetics Center at Duke University through the kindness of Dr. E. Harris. Final copies of the figures were prepared by R. Kara and of the typescript by S. Bocti.

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