Heredity **78** (1997) 498–506 *Received 10 June 1996*

Experimental evolution in *Chlamydomonas* II. Genetic variation in strongly contrasted environments

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Experimental populations of *Chlamydomonas* were selected in Light (photoautotrophic) or Dark (heterotrophic) environments. Each population was a clone, founded by a single spore and propagated vegetatively thereafter. A heterogeneous environment was simulated by mixing Light and Dark lines in each growth cycle and redistributing them between the two environments in the next cycle. Some lines maintained permanently in the Dark evolved greatly increased growth within fewer than 300 generations, at the expense of reduced growth in the Light. Lines maintained in both Light and Dark environments evolved a negative genetic correlation between Light and Dark growth, and displayed more genetic variance of fitness than lines maintained in either environment exclusively. It is possible that genetic variance near mutation–selection balance is greater in heterogeneous environments because selection is weaker. However, the evolution of distinctly specialized lineages in these experiments suggests that in the conditions of batch culture a cost of adaptation creates negative frequency-dependent selection that maintains genetic variance. Genetic variance was greater in the more permissive environment (Light) than in the more restrictive environment (Dark).

Keywords: autotroph vs. heterotroph, *Chlamydomonas*, correlated response, cost of adaptation, fitness, genetic variance, selection experiment.

Introduction

It is widely accepted that environmental heterogeneity may support genetic diversity, either transiently through obstructing selection and retarding the loss of genetic variance, or permanently through the maintenance of a stable genetic equilibrium under disruptive selection (Levene, 1953; Maynard Smith & Hoekstra, 1980; Via & Lande, 1985; Hedrick, 1986). In a previous paper, using the unicellular chlorophyte *Chlamydomonas* as a model system, Bell (1997) has shown how selection in a diverse environment, consisting of a range of culture media with different concentrations of macronutrients, was associated with higher levels of genetic variance in fitness than selection in a comparable uniform environment. This result is consistent with surveys of genotypic variance, which have shown that the genetic correlation between environments differing in the dilution of macronutrients declined towards zero as environmental variance increased, but did not become consistently negative (Bell, 1992). It might be argued that the effect will be much greater when environments differ qualitatively, rather than merely quantitatively. We might then anticipate that genetic correlations will become negative through antagonistic adaptations to different environments — a general 'cost of adaptation' — and that in consequence populations that experience both, or all, environments will be much more variable than those that experience only one. More specifically, we can define six propositions describing how genetic variance in fitness is expected to be maintained in populations that are exposed simultaneously to qualitatively different environments.

1 Allopatric lines (maintained in isolation) will become adapted to a novel or stressful environment.
2 Adaptation will be specific: the direct response to selection will be greater than any indirect

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response in other environments, creating a negative genetic correlation across environments.

3 This negative correlation is caused in part by a cost of adaptation, advance over the founding genotype in the environment of selection being associated with regress in other environments.

4 Sympatric populations that are regularly distributed among environments will show less specific adaptation to a given environment than allopatric populations that are maintained in that environment only, but they will also show less regress in other environments.

5 Selection in sympatric lines that experience a variety of conditions of growth is less effective because genes that improve performance in one environment but reduce it in others will be fixed more slowly, if at all; this will create a negative genetic correlation within sympatric populations.

6 Consequently, the genetic variance of fitness will be greater in sympatric than in allopatric treatments.

This paper is an attempt to investigate these propositions in an experimental system using *Chlamydomonas*, and thus to evaluate the argument linking environmental heterogeneity to genetic diversity. Our experimental populations are clones that are propagated vegetatively; consequently, the genetic variances and covariances that we estimate are the consequence of novel mutations that have arisen during the course of the experiment. Our results are not therefore influenced by any pre-existing genetic variances or covariances in the base populations.

Materials and methods

Base populations

The base population for each selection line was a single spore. Two mt^+ (CC-1010 and CC-2343) and two mt^- (CC-1952 and CC-2342) strains of *Chlamydomonas reinhardtii* were crossed in all combinations, and from each cross one mt^+ and one mt^- spore were isolated, a total of eight selection lines. Routine laboratory procedures are described by Harris (1989).

Environmental treatments

The two physical environments used were:

- Bold's minimal liquid medium, under continuo s illumination (Light treatment);
- the same medium supplemented with $1.2\,g\,L^{-1}$ sodium acetate, kept dark (Dark treatment).

In both cases, the cultures comprised 300 mL of medium in 500 mL Erlenmeyer flasks bubbled with sterile air; Light and Dark flasks were maintained on the same shelf, the Dark flasks being wrapped in aluminium foil. The over-riding difference between the two environments was thus the wholly photoautotrophic growth in one and wholly heterotrophic growth in the other.

Environmental heterogeneity was created by mixing Light and Dark cultures. In the allopatric treatment, Light and Dark cultures were maintained separately. In the sympatric treatment, Light and Dark cultures were mixed after each cycle of growth, the mixture being used to inoculate both Light and Dark flasks at the beginning of the next cycle. The allopatric treatment thus represents a uniform environment, and the sympatric treatment a diverse or heterogeneous environment.

Each of the eight founding spores gave rise to three selection lines: an allopatric line maintained in the Light, an allopatric line maintained in the Dark, and a sympatric line. These were unreplicated. There were thus 24 selection lines in all.

Selection

The experiment was propagated by serial transfer of asexual cultures for one year, at the end of which the Light lines had completed 66 cycles of growth and the Dark lines 24 cycles. Each transfer involved inoculating 100 μL of culture ($\approx 6 \times 10^4$ cells) into 300 mL medium, permitting 11–12 doublings (to a final population size of roughly 2×10^8 cells) per cycle; Light lines thus completed about 750 generations and Dark lines about 275 generations.

Assay

After selection, spores were isolated from each line and from its founder, the eight founding genotypes having been stored meanwhile on solid medium in dim light. Four spores were isolated from each flask; the sympatric lines were thus each represented by eight spores, four from the Dark flask and four from the Light flask of the final cycle. Each spore was then grown in Dark and Light conditions, except that the assay was carried out (for reasons of practicality) in culture tubes rather than in flasks. Single colonies from plates were grown in 10 mL of liquid medium in culture tubes for 5 days, at which point they were in vigorous growth. These preinoculation

cultures were then diluted to a standard optical density, and used to inoculate 20 mL of fresh medium with 100 μL of culture. Two replicate cultures of each genotype–environment combination were used. Growth was measured on a spectrophotometer at intervals of about two days, providing complete growth curves from which the logistic parameters r and K can be estimated, although growth was markedly nonlogistic in some Dark cultures. The measure of growth analysed here is the optical density of the culture after 10 days, P_{10}. This is a simple and model-free statistic that reflects both r and K, and is closer to fitness in the circumstances of the experiment than either. The results reported here, particularly the response to selection in allopatry and the greater genetic variance of the sympatric lines, apply quantitatively to r and K as well as to P_{10}. The relationship between r and K in the evolved populations will be described in a later paper. The assay thus comprised 5 flasks (the three experimental treatments, the sympatric treatment being represented by two flasks, plus the founder) $\times$ 8 lines $\times$ 4 spores $\times$ 2 environments $\times$ 2 replicates = 640 cultures.

Because the conditions of growth in the selection environment (flasks) and in the assay environment (tubes) were somewhat different, the assay procedure was itself tested, after the completion of the experiment. Forty independent isolates of *C. reinhardtii* were grown in replicated flask and tube cultures, in Light and Dark conditions, in order to estimate the genetic correlation between flask and tube growth.

Results

Assay procedure

Discrepancies between flask and tube scores may arise from two sources: error variance (which reduces the correlation between scores of replicate cultures in either flasks or tubes) and systematic differences between flask and tube environments. The correlation between flask and tube scores, independently of error, can be estimated as the intraclass genetic correlation coefficient:

$$t_G = \sigma^2_G/[\sigma^2_G + \sigma^2_{GE}],$$

where G refers to genetic main effects and GE to genotype–environment interaction, the environment being flask vs. tube. Estimates from the flask–tube assay were:

Light growth: $\sigma^2_G = 1887$; $\sigma^2_{GE} = 535$; $t_G = 0.78$.

Dark growth: $\sigma^2_G = 14419$; $\sigma^2_{GE} = 14979$; $t_G = 0.49$.

Estimates of the genetic variance were highly significant ($P<0.001$) in both cases; genotype–environment interaction was significant for Dark growth ($P<0.01$) but not for Light growth ($P>0.1$).

Light and Dark growth

The genetic variance of growth is similar among the 40 isolates scored for the flask–tube assay and among the eight founding spores of the main experiment. In both cases the variance of Dark growth is about an order of magnitude greater than the variance of Light growth. All isolates are capable of growing well in the light, but there is a wide range of behaviour in the dark, some isolates growing as well as they do in the light, whereas others can scarcely grow at all. The Dark environment is thus the more stressful, in the sense that the algae are initially less well-adapted to it.

Response to selection in allopatry

The population statistics before and after selection are given in Table 1. The response to selection can be evaluated in two ways. In the first place, all pairs of Light and Dark lines were tested both in the environment of selection and in the other environment. The interaction of selection environment with assay environment (Light lines growing better than Dark lines in the Light environment but worse in the Dark environment, and vice versa) shows that selection has caused sister lines exposed to different environments to diverge. This effect was significant (at a level per test of 0.05/8 = 0.00625) in six of eight cases. Secondly, a comparison of each line with its founder, tested in the environment of selection, shows whether selection has substantially increased adaptedness to that environment. Two lines (B– and D–) evolved a markedly enhanced ability (relative to their founders) to grow in the Dark, but otherwise the degree of adaptation, in Light or Dark, was modest. The overall increase of adaptedness in the Dark was not formally significant, primarily because the variance among lines was inflated by the highly exaggerated response of the B– and D– lines. In both environments, the genetic variance within lines was low at the beginning of the experiment, and increased markedly over time. In the Light environment, the variance among lines increased during the course of the experiment; in the Dark environment, the variance among lines remained the same or decreased.

Table 1 Response to selection in allopatry. Scores are optical density at 10 days. The table shows the mean growth in environment B of spores selected in environment A, Y_{AB}, where L = Light, D = Dark and F = Founder. Means are based on two replicate cultures of each of four spores from each line

Line	Y_{LL}	Y_{LD}	Y_{DL}	Y_{DD}	Y_{FL}	Y_{FD}
A+	658	174	544	316	585	362
A−	673	55	484	419	609	380
B+	561	88	480	237	635	242
B−	612	39	340	293	669	40
C+	654	149	563	462	633	446
C−	371	466	517	416	524	400
D+	617	160	523	436	640	354
D−	517	367	349	590	617	220
Mean	583	187	475	396	614	305

Divergence caused by selection can be evaluated for each line, because spores should grow better in the environment in which they were selected. Spores isolated from Light and Dark selection lines were all tested in both Light and Dark conditions, with two replicates of each genotype–environment combination.
The model was thus:

Source	df
Selection environment	1
Assay environment	1
Selection × Assay	1
Spores within lines	12
Replication	16

The occurrence of selection is detected by the Selection × Assay interaction; this was tested by the Spores mean square, unless it were smaller than the Replication MS, in which case the Replication MS was used instead. The results of this line-by-line analysis were:

Line	F	P
A+	9.6	0.021
A−	56.1	<0.001
B+	5.6	0.056
B−	90.0	<0.001
C+	26.5	0.002
C−	9.9	0.005
D+	21.2	0.004
D−	23.1	0.003

Correlation of Light and Dark growth

The growth of Light and Dark allopatric lines in both environments is shown in Fig. 1. It is clear that the Light and Dark lines have diverged: in seven of the eight lines, the Dark lines grow better in the Dark and worse in the Light, whereas the Light lines grow better in the Light but worse in the Dark. This effect is highly significant in most cases (Table 1, Selection × Assay interaction). The probability that the direction of the divergence (lines more highly adapted in the environment of selection, less highly adapted in the other, in seven of eight cases) resulted from chance is 9/256 = 0.035. Selection has thus created negative genetic correlation between isolated populations within a few hundred generations.

Table 1 *Continued*
Overall means and variances are as follows

Treatment	Mean	SE (mean)	Variance components: Among lines	Within lines	Error
Light					
Before selection	614	14	1490	131	4655
After selection	583	33	8647	3330	5102
Dark					
Before selection	305	46	16 811	1605	1988
After selection	396	36	10 469	7059	1241

The specificity of the response to selection can be evaluated by comparing lines tested in the environment with the founders, tested in the same environment. This is highly significant ($P<0.001$) in only two cases, B− and D− selected in the Dark. The mean difference between lines selected in the Light and their founders was −31 (SE 28, $t_7 = -1.1$, $P>0.25$). The mean difference between lines selected in the Dark and their founders was +91 (SE 51, $t_7 = 1.8$, $P \approx 0.1$). The error variance does not change during the experiment. The variance within lines (genetic variance) is greater after selection (Light treatment, $F_{24,24} = 25.4$, $P<0.001$; Dark treatment, $F_{24,24} = 4.4$, $P<0.001$). The variance among lines is greater after selection in the Light treatment ($F_{7,7} = 5.8$, $0.01<P<0.025$) but did not change appreciably in the Dark treatment ($F_{7,7} = 1.6$, $P>0.1$).

Cost of adaptation

The divergence of Light and Dark lines need not reflect a cost of adaptation: it is conceivable that each line exceeds its founder in either environment, as the result of adaptation to general features of laboratory culture. There is a cost of adaptation only if increased growth in the Dark is accompanied by

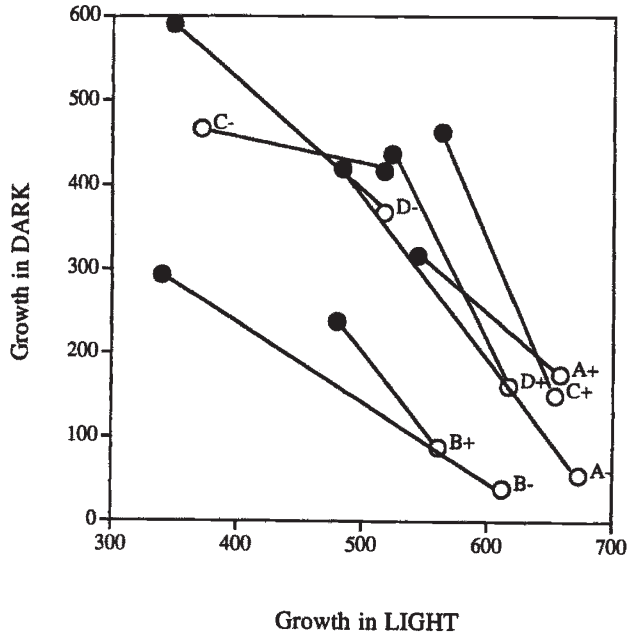

Fig. 1 Genetic divergence of allopatric lines. The eight selection lines obtained from the four original crosses are labelled A+, A−, etc. The open circles are mean values for the Light selection lines, solid circles for the Dark selection lines. Light and Dark sister lines are connected to show the negative correlation between environments.

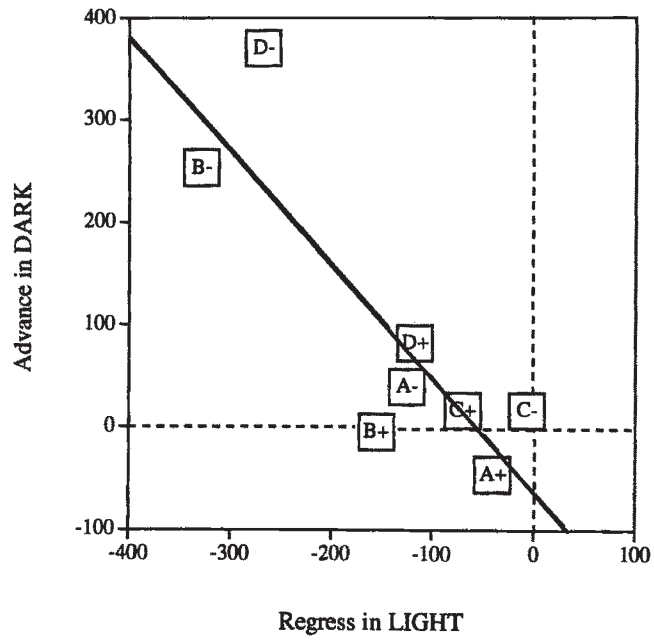

Fig. 2 Cost of adaptation to the Dark environment. The values plotted are the deviations of the means of selection lines from the parental spore; thus, positive values indicate an advance, and negative values a regress. The solid line is the least squares regression: $y = -1.11x - 63$, $r^2 = 0.72$. This is of course strongly levered by B− and D−.

reduced growth in the Light. Fig. 2 shows that all the Dark selection lines regressed in the Light, and the most marked regress in the Light was shown by the lines that achieved the greatest advance in the Dark.

Genetic correlation in sympatric lines

The growth of spores extracted from the sympatric lines in Light and Dark environments is shown in Fig. 3. There is a broad range of behaviour within the populations, but a tendency towards specialization: spores that grow well in the Dark tend to grow poorly in the Light, and vice versa. The cost of adaptation observed in the allopatric lines thus gives rise to a negative genetic correlation within sympatric populations.

Response to selection in sympatry

The growth of sympatric populations in a given environment is less than that of allopatric populations that have been selected in that environment (Fig. 4(a)). However, their growth in either environment is greater than that of allopatric populations that have *not* been selected in that environment (Fig. 4(b)). In either case, the effect is greater in the Dark than in the Light.

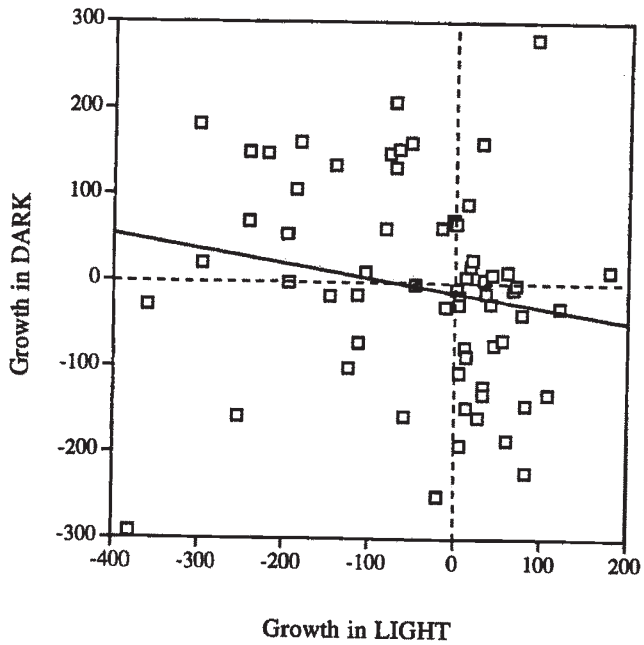

Fig. 3 Light and Dark growth in sympatric lines. Each point is a spore ($N = 64$). The genetic correlation is −0.40 ($P < 0.01$).

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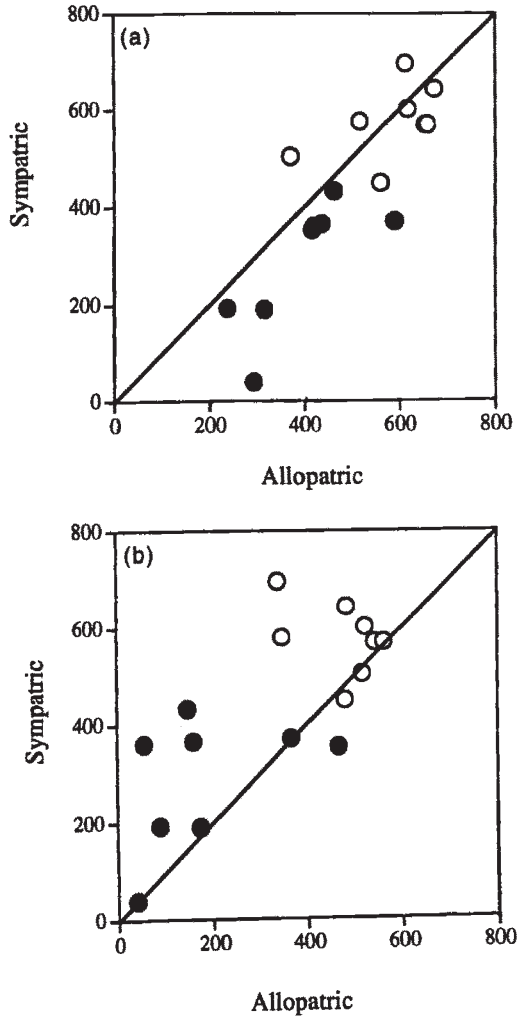

Fig. 4 Response to selection in allopatric and sympatric lines. Open circles indicate spores tested in the Light and solid circles in the Dark. (a) In the upper diagram, sympatric lines are compared with allopatric lines, in the environment in which the allopatric lines had been selected. The allopatric lines exceed the sympatric lines in 13/16 cases ($P = 0.021$, binomial test). (b) In the lower diagram, sympatric lines are compared with allopatric lines, in the environment in which the allopatric lines had not been selected. The sympatric lines exceed the allopatric lines in 13/15 cases (equal values in one case) ($P = 0.007$, binomial test).

Genetic variance of fitness

Estimates of genetic variance within lines are given in Table 2. In the Light environment, seven of eight sympatric lines yield a greater estimate than the corresponding allopatric Light selection lines (one-tailed $P = 0.035$), and the means ± 1 SE among lines do not overlap. Genetic variance was on average nearly three times as great in sympatry as in allopatry. There was a trend in the same direction in the Dark environment, but it was not nearly as marked, and cannot be shown to be significant.

Table 2 Genetic variance of fitness in selection lines. Estimates were obtained by equating observed with expected mean squares in single-classification analysis of variance, and may be negative. Estimates for the allopatric lines are equivalent to the 'within lines' variance component in Table 1. The sympatric population compared with the allopatric Light line is that extracted from the Light flask of the two used to propagate the sympatric line after the last cycle of growth; similarly for the Dark treatment

	Light		Dark	
Line	Allopatric	Sympatric	Allopatric	Sympatric
A+	5197	25 767	29 883	7696
A−	−2119	12 036	7264	5831
B+	−1545	14 840	6676	13 322
B−	−3601	−567	9959	132
C+	2195	3429	−359	3384
C−	21 581	7791	1034	20 433
D+	−150	8170	1847	20 787
D−	5080	6481	167	2776
Mean	3330	9743	7059	9295
SE	2849	2838	3524	2829

Discussion

Broadly speaking, these results provide experimental documentation of the conventional account of how genetic variation for site-specific fitness is maintained in heterogeneous environments. Specific adaptation to heterotrophic conditions is accompanied by a loss of fitness in photoautotrophic conditions, and in a heterogeneous environment this cost of adaptation is reflected in a negative genetic correlation between Light and Dark growth that retards or prevents the loss of genetic variance.

Response to selection in uniform environments

Selection was effective despite the genetic uniformity of the founding populations. The input of new variation by mutation was thus adequate to fuel adaptation in these large populations of 10^7-10^8 individuals. The rate of input can be calculated from Table 1 as:

$$(3330-131)/750 = 4.265/4655 \approx 1 \times 10^{-3}\ \sigma_e^2$$

per generation in the Light lines, and

$$(7059-1605)/275 = 19.833/1988 \approx 1 \times 10^{-2}\ \sigma_e^2$$

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per generation in the Dark lines. The value of about 10^{-3} of the environmental variance per generation is comparable with other guesses and estimates (Maynard Smith, 1989), although it is not clear why microenvironmental effects causing the variance of replicate cultures should provide a standard for comparing different systems. These estimates are minimal, because some of the new variation that appears will be harvested by selection.

The variance among the Dark lines decreased somewhat during the experiment because lines that at first grew poorly became adapted to heterotrophic conditions, an example of phenotypic convergence. The divergence of the Light lines, where selection was ineffective, is probably spurious. It is caused solely by the low score of the C– selection line, (a prominent outlier in Fig. 1), where three of four spores at first grew slowly, although they later achieved a normal asymptotic density.

Adaptive divergence and the cost of adaptation

Adaptation in experimental populations may be specific or general: specific, in that it refers to a particular environment among all those tested, and general, in that it applies broadly to the laboratory conditions of growth common to all treatments. If adaptation is to any degree specific, as in practice it almost always will be, then it will cause populations to diverge so that the genetic correlation among them is negative, as in Fig. 1. This need not imply that adaptation involves a cost of adaptation, as usually understood. Adaptation is costly only if advance in the environments of selection is achieved at the expense of regress in other environments. To make this point more clearly, we have defined three new terms in Fig. 5. These refer to selection in two environments such as Light and Dark. There is a direct response, the increase of growth in the environment in which the population has been selected, and an indirect, or correlated, response in the other environment. If both direct and indirect responses are positive, but the direct exceeds the indirect response, selection may be said to be *synclinal*: the response to selection is in the same direction in both environments. If the direct response is positive and the indirect response zero, selection is *aclinic*: adaptation to one environment has no effect on growth in the other. Finally, the direct response may be positive and the indirect response negative: this is *anticlinal* selection, advance in the environment of selection causing regress in the other environment. It is only anticlinal selection that implies a cost of adaptation. Thus, the cost of adaptation can be evaluated only by reference to an ancestor, and not solely by the divergence of lines selected in different environments.

The main response to selection in this experiment was the adaptation to Dark growth by lines B– and D–, and to a lesser extent by D+. These responses were anticlinal, as shown in Fig. 2 by the reduced growth of these lines in Light conditions, relative to their founders. Figure 6 shows in more detail the

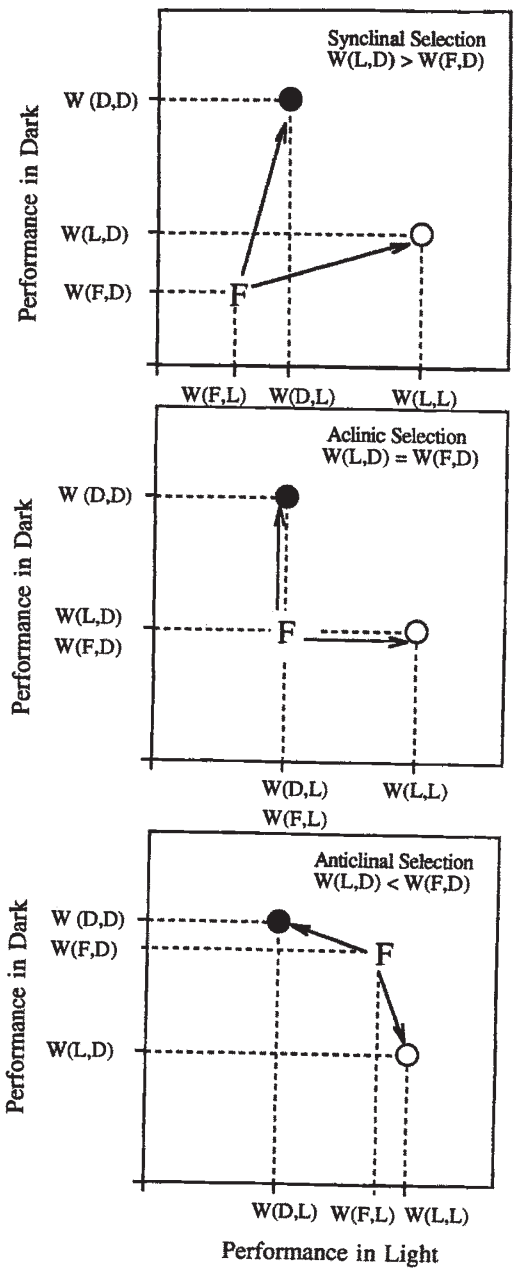

Fig. 5 The concepts of synclinal, aclinic and anticlinal selection. The open and solid circles represent Light and Dark selection lines, respectively; F indicates the founder.

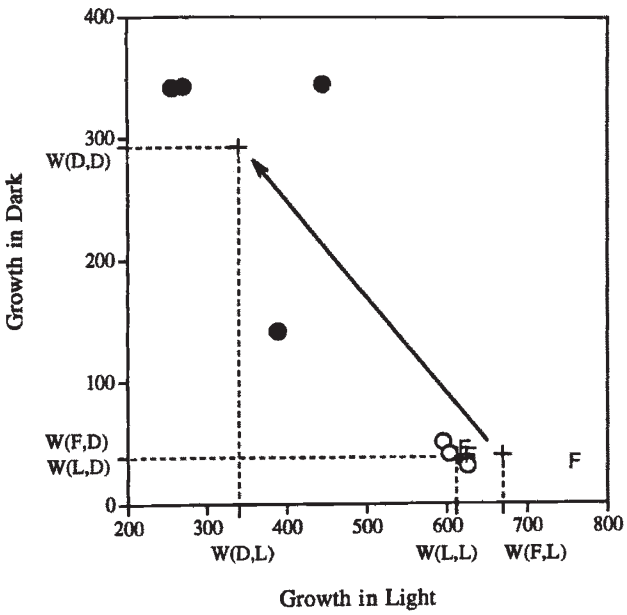

Fig. 6 History of B–lines. Each plotted point is a spore.

behaviour of the B– line. There is no discernible direct or indirect effect of selection in the Light; in the Dark, the line evolves greatly improved growth, but no longer grows as well in the Light. (In parenthesis, we note that after about 150 generations this line grew yellow in the dark. Genes causing this character are well known; they arise as a lesion in the final stage of the synthesis of chlorophyll in the dark, leading to an accumulation of protochlorophyllide. Whether or not this character in itself causes greater fitness in the Dark has not been investigated.)

The only directly comparable experiment involving large asexual populations cultured for hundreds or thousands of generations (Bennett *et al.*, 1992; Bennett & Lenski, 1993) showed that adaptation to different temperatures by allopatric lines of *E. coli* over 2000 generations was not always, or even usually, accompanied by decreased performance at other temperatures, relative to the ancestral strain. There is some evidence for a cost of adaptation from reciprocal transplant experiments, both in natural environments (Antonovics & Primack, 1982; van Tienderen, 1992) and in environments severely disturbed by human activity (Davies & Snaydon, 1976).

Response to selection in heterogeneous environments

In heterogeneous environments, selection may vary in direction at different sites, or in different conditions of growth. Local (within-site) regulation of density, as in the original model by Levene (1953), creates negative frequency-dependent selection that may retain genetic variance for site-specific fitness permanently in the population, although the conditions for stable genetic equilibrium are quite severe (Maynard Smith & Hoekstra, 1980; Via & Lande, 1985; Gillespie & Turelli, 1989). A less onerous hypothesis is that directional selection is less intense in heterogeneous environments, so that genetic variance, although eventually eliminated, declines more slowly than in comparable environments with uniform conditions of growth. We presume that this implies a higher level of genetic variance in heterogeneous environments at mutation–selection equilibrium, although we have not found a formal treatment of this situation.

In a previous experiment (Bell, 1997), lines descending from a genetically diverse base population retained higher levels of genetic variance in fitness when cultured in a heterogeneous environment than when cultured in a uniform environment. In this case, conditions of growth in the heterogeneous environment differed with respect to the concentrations of macronutrients in minimal media. The effect was attributed to the slower elimination of variance under less intense directional selection, primarily because there was no difference between treatments with and without deliberate site-specific density-regulation. In the present experiment, there was no deliberate attempt to impose local density-regulation (within the Light and Dark flasks), but the effect cannot be explained merely from the slower elimination of variance in heterogeneous environments, because there was no genetic variance, or very little, present in the founding populations. We suggest that local density-regulation was inadvertently imposed by our experimental design: in batch culture, growth is inevitably limited to some extent by the density of cultures within flasks. It remains conceivable that the quantity of variance in an initially clonal population eventually tends to an equilibrium under mutation–selection balance, and that this equilibrium is higher in a heterogeneous environment because directional selection is weaker. However, this seems much less plausible when, as in the present case, genotypes that are distinctly specialized for light or dark growth arise during the course of the experiment, rather than being merely retained from an initially diverse stock. The most economical interpretation of our results seems to be that a cost of adaptation, demonstrated by the anticlinal response of lines initially unable to grow well in Dark conditions, generates negative frequency-

dependent selection through the limitation of population growth within flasks, leading to the evolution of higher levels of genetic variance in the sympatric lines.

The effect is quite modest. The variance of the sympatric populations exceeds, on average, that of either allopatric population; but it does not equal that of the *combined* allopatric populations. This is because the massive immigration implied by mixing and redistributing the cultures in each generation counteracts the effect of selection. More pronounced specialization might be displayed if migration were restricted.

Genetic variance is markedly greater in the Light, but not in the Dark. This would follow if selection in the Light against spores selected in the Dark is less intense than selection in the Dark against spores selected in the Light; as is probably the case. This suggests the general rule that in a heterogeneous environment genetic variance will be greater in more permissive and less in more restrictive habitats.

Acknowledgements

This work was funded by a Research Grant from the Natural Sciences and Engineering Research Council of Canada. X.R. was supported by a Postdoctoral Fellowship from the Ministère de la Recherche et de la Technologie, France. We are grateful to Lori Pilkonis for technical assistance, and to Arnold Bennett for critical comments on an earlier draft.

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