

# Variation in natural selection for growth and phlorotannins in the brown alga *Fucus vesiculosus*

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## Abstract

Directional selection for plant traits associated with resistance to herbivory tends to eliminate genetic variation in such traits. On the other hand, balancing selection arising from trade-offs between resistance and growth or spatially variable selection acts against the elimination of genetic variation. We explore both the amount of genetic variation and variability of natural selection for growth and concentration of phenolic secondary compounds, phlorotannins, in the brown alga *Fucus vesiculosus*. We measured variation in selection at two growing depths and two levels of nutrient availability in algae that had faced two kinds of past growing environments. Genetic variation was low for growth but high for phlorotannins. The form and strength of selection for both focal traits depended on the past growing environment of the algae. We found strong directional selection for growth rate in algae previously subjected to higher ultraviolet radiation, but not in algae previously subjected to higher nutrient availability. Stabilizing selection for growth occurred especially in the deep growing environment. Selection for phlorotannins was generally weak, but in some past-environment–current-environment combinations we detected either directional selection against phlorotannins or stabilizing selection. Thus, phlorotannins are not selectively neutral but affect the fitness of *F. vesiculosus*. In particular, there may be a fitness cost of producing phlorotannins, but the realization of such a cost varies from one environment to another. Genetic correlations between selective environments were high for growth but nonexistent for phlorotannins, emphasizing the high phenotypic plasticity of phlorotannin production. The highly heterogeneous selection, including directional, stabilizing, and spatially variable selection as well as temporal change in selection due to responses to past environmental conditions, probably maintains a high amount of genetic variation in phlorotannins. Such variation provides the potential for rapid evolutionary response of phlorotannins under directional selection.

## Introduction

Herbivory often generates directional selection for plant traits mediating resistance to herbivory (Berenbaum *et al.*, 1986; Rausher & Simms, 1989; Pilson, 2000; Shonle & Bergelson, 2000). Directional selection, in a large panmictic population, is expected to eliminate genetic variation of the selected traits. Contrary to this

expectation, however, plant resistance to herbivory has often been found to show high genetic variation (Berenbaum & Zangerl, 1992; Tiffin & Rausher, 1999; Juenger & Bergelson, 2000). Explaining the maintenance of variation in plant resistance has, therefore, become one of the major challenges in research on plant–herbivore interactions; it is usually attributed either to stabilizing selection (Rausher & Simms, 1989; Simms, 1990) or to temporally or spatially variable selection (Stratton, 1994; Dudley, 1996; Tiffin & Rausher, 1999).

Stabilizing selection arises when the trait is associated with costs that offset its benefits. For example, a high

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level of resistance may be costly in terms of growth or reproduction, whereas a high growth rate may be costly in terms of higher palatability for herbivores (Herms & Mattson, 1992; Koricheva, 2002). Thus, trade-offs mediate the evolution of intermediate trait levels, and variation in selective pressures may allow the occurrence of relatively high genetic variation (Berenbaum *et al.*, 1986; Mauricio & Rausher, 1997). In some cases, stabilizing selection has been documented both for resistance to herbivory (Rausher & Simms, 1989; Nunez & Dirzo, 1994) and for the concentrations of secondary metabolites (Mauricio & Rausher, 1997; Shonle & Bergelson, 2000) that form the basis for chemical resistance.

The role of variable selection for the maintenance of genetic variation is less clear. Temporally variable selection alone is supposed to be unable to maintain genetic variation; the effect of temporal variations in selection should not differ from the average selection over time (Hedrick, 1986). On the other hand, spatial heterogeneity in selection, especially when individuals face the same environment throughout their life, may maintain genetic variation (Maynard Smith, 1998). When different phenotypes are favoured in distinct environments, selection may either preserve genetic variation, assuming an insignificant gene flow, or may lead to the evolution of phenotypic plasticity (Via & Lande, 1985, 1987; de Jong, 1995).

Variable selection among microhabitats is often generated by heterogeneity with respect to resource availability or grazing pressure. The availability of resources also sets the stage for variation in trade-offs (Tuomi *et al.*, 1983; Van Noordwijk & de Jong, 1986). For example, the carbon-nutrient balance and growth-differentiation balance hypotheses predict that the availability of mineral nutrients will affect the costs of producing carbon-based defensive metabolites: when nutrients are in short supply, the production of such compounds may be less costly than when nutrients are available for growth (Tuomi *et al.*, 1988; Herms & Mattson, 1992). Thus, among-microhabitat variability in resource availability may modify selection for resistance by influencing the costs of resistance.

Here, we explore the variability of natural selection for growth and the amount of secondary metabolites in a littoral brown alga *Fucus vesiculosus* (L.). Brown algae produce phenolic secondary metabolites, phlorotannins, the production of which often constitutes a considerable sink of photosynthetic carbon (Ragan & Glombitza, 1986; Van Alstyne *et al.*, 1999; Schoenwaelder, 2002). Phlorotannin production shows plasticity with respect to a variety of environmental factors, such as nutrients and light (e.g. Yates & Peckol, 1993; Pavia & Toth, 2000a; Jormalainen & Honkanen, 2001). The adaptive role of phlorotannins has been attributed mainly to defence against herbivory; phlorotannins have been found, in some cases, to be induced by herbivory and to act as feeding deterrents or to reduce the assimilation efficiency

of herbivores (reviewed by Targett & Arnold, 1998). In addition, phlorotannins may play a role in tolerating ultraviolet radiation. UV radiation has been shown to induce increases in brown algal phlorotannins (Pavia *et al.*, 1997, unpublished data by the authors), which has been interpreted as adaptive plasticity (Pavia *et al.*, 1997; Cooper-Driver & Bhattacharya, 1998). Although such plastic responses of phlorotannins may well be adaptive, selection analyses are needed in order to show that phlorotannin variation has fitness consequences.

Sedentary plants living in steep environmental gradients are expected to face spatially variable selection. Aquatic littoral habitats provide such gradients on a relatively small spatial scale. The depth gradient generates heterogeneity in the selective environment in many ways. The light quality changes: for example, UV radiation disappears, and the amount of light decreases quickly with depth (Lobban & Harrison 1994). Depth also correlates with sea surge and water temperature, both of which may influence grazing pressure (Peckol *et al.*, 1996). Ambient nutrient availability may affect selection by modifying the trade-off between growth and the production of secondary metabolites. The allocation of carbon to phlorotannins is expected to be costly in terms of growth, especially when nutrients are ample (e.g. Yates & Peckol, 1993; reviewed by Herms & Mattson, 1992). Nutrients and depth may further modify the selective environment by influencing the amount of epibiota on the thallus or by changing the palatability of the algae for herbivores. In a littoral environment, fluctuations in sea level and water movements generate temporal variability in the availability and quality of light and nutrient resources for littoral algae. If algal growth and secondary metabolism respond plastically to such variability, this may affect the strength and form of later selection.

In this study, we explore variation in selection for growth and phlorotannins between the shallow and deep ends of the depth distribution of *F. vesiculosus*, and under low and high nutrient availability. We further evaluate the effect of temporal variations in selective environments, i.e. the effects of past environmental conditions, on current selection. Since evolutionary response to selection depends on the amount of genetic variation, we also measured the broad-sense heritability of growth and phlorotannins.

## Materials and methods

*Fucus vesiculosus* is a perennial, dioecious brown alga that forms dense belts in rocky littoral habitats. In our nontidal study area in the Finnish Archipelago Sea in the northern Baltic Sea, these belts occur from the edge of the water to a depth of 2–4 m. There is also a clear gradient in the area in the amount of ambient nutrients, from high nutrient areas close to the mainland to low nutrient areas in the outer Archipelago (Hänninen *et al.*,

2000). Thus, the species faces variable growing conditions on a small spatial scale due to depth-related factors and on a larger spatial scale due to variation in nutrient availability.

The most important herbivore inhabiting *F. vesiculosus* belts in our study area is the isopod *Idotea baltica* (Pallas) (Jormalainen *et al.*, 2001), judging from the abundance of herbivores and the amount of grazing marks on the algae. The epibiota, especially epiphytic macroalgae, by modifying the availability of resources for the host algae, forms another potential selective agent. The amount of epibiota usually increases with nutrient availability (Lotze *et al.*, 2000; Honkanen & Jormalainen, 2002).

### Environments prior to the measurement of selection and the broad-sense heritability of growth and phlorotannins

We collected algae from the Archipelago Sea and reared them in through-flow aquaria prior to the selection period in the sea. The rearing was performed to study the effect of past environment on selection and to measure the amount of genetic variation in growth rate and phlorotannin concentration in conditions where the direct effects of herbivory on these traits are excluded. To ensure a representative amount of genetic variation, we collected a total of 30 genotypes originating from three separate *Fucus* belts, all within a distance of 15 km, from a depth of 1 m. The genotypes from different belts did not differ significantly in phlorotannins but did in their growth rate. However, ranges were overlapping. Therefore, we pooled the data for further analyses. Genotypes of *F. vesiculosus* were defined as entities growing from a single branch of thallus on a holdfast.

We arranged the algae in 12 aquaria, with a volume of 60 L each and with seawater through-flow of 300 L day<sup>-1</sup> and a water pump to provide turbulence. The aquaria were located outdoors, with natural light and a diurnal rhythm; they were protected from rainfall by a thin plastic cover. Each genotype was divided into 32 apical parts; each part was about 4–6 cm in length and consisted of one or two dichotomous branching points. All the algal parts were randomized among the aquaria, in such a way that each genotype was replicated two to three times in each aquarium. Each alga was attached to a clothespin and anchored to the aquarium.

To explore the possible consequences of past environment on natural selection, we reared the algae in two different environments (hereafter 'past-environment'). The rearing began on 17 April 2000 by assigning six aquaria randomly to each environment: (1) addition of UV irradiation by fluorescent tubes (hereafter 'UV+') and (2) nutrient enrichment (hereafter 'Nutrient+'). These environments were chosen because natural variation in sea level predisposes algae periodically to high UV radiation and there is considerable spatial and temporal variability in the availability of nutrients. Furthermore,

these treatments are known either to increase (UV+) or decrease (Nutrient+) phlorotannin production in *F. vesiculosus* (Jormalainen & Honkanen, 2001; Jormalainen *et al.*, 2003). We suspected that such plasticity, by revealing more phenotypic variation in phlorotannin concentrations, might affect selection for phlorotannins.

UV was added by placing fluorescent tubes (Philips TL40W/12 RS F40 T12/UVB, Philips Lighting, Eindhoven, The Netherlands) emitting both UVB (4.5 W) and UVA (2.7 W) radiation 25 cm above the aquaria. The UV lamps were on for 6 h daily, from 10:00 to 16:00. When on, they increased the total irradiance (280–400 nm) by 9.16 W m<sup>-2</sup> at the surface of the aquaria. The aquaria in the nutrient enrichment treatment received extra nutrients in the form of a controlled release fertilizer, 20 g of 37 : 0 : 0 and 5 g 10 : 21 : 0 (N : P : K; Polyon, Pursell Technologies Inc., Sylacauga, AL, USA) in diffusion bags that were replaced every 2 weeks. In the beginning of the period this nutrient addition increased the ambient NH<sub>4</sub> concentration in the aquaria from 5.3 (SE = 0.31, *n* = 2) to 12.6 µg/L (*n* = 1) and the PO<sub>4</sub> concentration from 6.0 (SE = 1.1, *n* = 2) to 67.5 µg/L (SE = 26.3, *n* = 2). Since the water temperature rose steadily during the course of the experiment and the solubility of the fertilizer increases linearly with temperature, these estimates represent minimum effects.

We weighed the algae and measured their maximum length at the beginning and end of the 44-day period in the two environments. At the end, we sampled eight algae per genotype, each alga coming from a different aquarium, four algae per treatment for the quantification of phlorotannins. For a single determination, two algae originating from two different aquaria within a treatment were combined; we thus had two replicate measures from each genotype and treatment. Samples were freeze-dried, finely ground and stored at -20 °C until the analysis. We measured the total content of phenolic compounds by a modification of the Folin-Ciocalteu method (Nurmi *et al.*, 1996), using phloroglucinol as the reference compound. In the following, we use the term 'phlorotannins' for these total phenolics, since brown algae are not known to contain other polyphenolics (Ragan & Glombitza, 1986).

### Natural selection in the sea

For the measurement of natural selection gradients, we chose a sheltered shore with dense *F. vesiculosus* vegetation (60°14'N, 21°40'E). We transferred the algae from the past-environments in aquaria into the sea for a period of about 4 months. We anchored the algae to the bottom, in plots made of a plastic grid (60 × 40 cm). We assigned two algae of each genotype to each experimental plot: one had undergone the UV+ and the other the Nutrient+ past-environment.

We assigned the algae to four different selective environments in a two-by-two factorial manner, three plots in each: (1) shallow site, no nutrient enrichment;

(2) shallow site + nutrient enrichment; (3) deep site, no nutrient enrichment; and (4) deep site + nutrient enrichment. The shallow and deep environments were at depths of 1 and 3 m, respectively. Each nutrient enrichment plot received controlled release fertilizer, a total of 1665 g of 37 : 0 : 0 and 185 g of 10 : 21 : 0 (N : P : K; Polyon, Pursell Industries, Inc.). The fertilizer was applied using four elongated diffuser bags (5 × 30 cm; see Worm *et al.*, 2000 for the method) per plot, and the bags were replaced four times during the course of the experiment. The nutrient manipulation was effective; the fertilizer addition significantly increased algal growth in the shallow environment (T. Honkanen & V. Jormalainen, unpublished data).

At the end of the experiment, on 20 September, we determined the success of each alga by counting the number of live apical meristems. The number of apical meristems was used as an estimate of fitness. It is a function of growth rate and of losses due to herbivory and/or disintegration of the thallus. Growth of *F. vesiculosus* occurs by means of apical meristems (Van den Hoeck *et al.*, 1995). Dichotomous branching, the main rule of spatial organization, results from the division of an apical cell. When reproducing, some of the apical meristems differentiate into gamete-producing receptacles. After forming the receptacle and releasing gametes, the apical meristem dies (Knight & Parke, 1950), and future growth and reproduction occur by means of the remaining vegetative apices. At the end of our experiment, it was possible to identify those apices that had initiated the process of differentiation into a receptacle for the forthcoming summer. The genotypic average proportion of such reproductive apices was 77% ( $n = 30$ ,  $SD = 12.6$ ). The number of apical meristems clearly limits reproductive output and is intimately associated with both future growth and reproduction, thus representing a crucial fitness component. As the measure of growth rate we used length increment during the sea period, measured as the average length of branches with ungrazed apical meristems. The phlorotannin concentration for each genotype was measured from a pooled sample of all phenotypic individuals within a treatment combination, as described above.

### Data analysis

We calculated heritability estimates for growth rate and phlorotannin production separately within both past-environments. The proportion of the between-genotype variance component out of the total variance was used to estimate the broad-sense heritability (Lynch & Walsh, 1998). The variance estimation was based on the restricted maximum likelihood method (SAS Institute, 1990). Because some of the variance could be due to the use of several aquaria, aquarium was used as a blocking factor in the initial determination of variance components. Variance between aquaria, however, did not differ

significantly from zero in any of the analyses; we, therefore, ignored it in the further analysis. Standard errors for the heritability estimates were obtained by the Jackknife resampling procedure (Dixon, 1993).

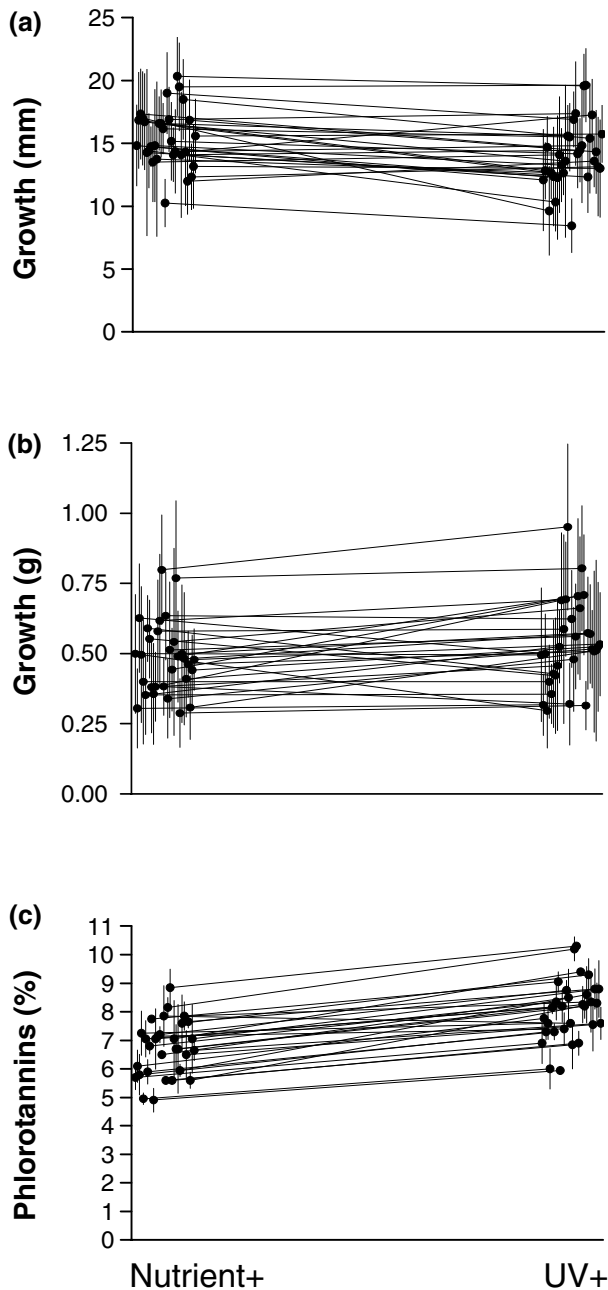
Selection in each environment was measured according to the regression method (Lande & Arnold, 1983; Arnold & Wade, 1984). Our analyses are based on the genotypic means of the focal traits and phenotypic fitness after the selection period in the sea. To control for the effect of the initial size of the alga on the fitness measure, we adjusted the number of apical meristems at the end of the selection period for the initial number of apical meristems in the beginning of the selection period. This was accomplished by using the residual fitness from the regression of initial to final number of apical meristems in the selection gradient analysis. We first calculated selection differentials for growth and phlorotannins, including both direct and indirect selection for a trait, as the covariance of the standardized trait with the relative fitness (Lande & Arnold, 1983). We did this separately for algae from both past-environments and from all selective environments. We judged the significance of the selection differentials by the nonzero Pearson correlation coefficient between trait and fitness. Differences in selection differentials between past-environments and between depths and nutrient environments were tested by pairwise comparisons of the correlation coefficients (Sokal & Rohlf, 1981; Dudley, 1996). Second, to study direct selection for growth and phlorotannins, we calculated selection gradients from a multiple regression (Lande & Arnold, 1983), again separately for past-environments and selective environments. Directional selection was estimated by fitting a model including only linear terms, and stabilizing or disruptive selection by fitting a model including linear, quadratic and interaction terms. In all the analyses, we used relative fitness with an average of one and standardized trait values. The differences in linear selection gradients between past-environments, and between selective environments within a past-environment, were tested by means of a test of heterogeneity of slopes in an ANCOVA of relative fitness, using the traits as covariates and past-environments or selective environments as categorical variables (following Dudley, 1996).

All analyses were performed with the SAS 6.12 software (procedures CORR, GLM, MIXED; SAS Institute, 1990). In the regression analyses, the distribution of residual variation did not deviate significantly from the normal distribution, as judged by the Kolmogorov test, and significance levels from the parametric analyses are therefore used.

## Results

### Broad-sense heritability of growth and phlorotannins

Growth showed both considerable genetic variation and phenotypic plasticity with respect to environmental



**Fig. 1** Genotypic mean growth, in terms of length (a) and weight gain (b), and phlorotannin concentration (c) in Nutrient+ and UV+ past-environments. Horizontal lines connect genotypic means in two environments, vertical bars indicate  $\pm 1$  SD.

conditions (Fig. 1a,b, Table 1). In terms of length, the algae grew slightly better in the Nutrient+ environment (mean  $\pm$  SE:  $15.5 \pm 0.44$  mm,  $n = 30$ ) compared to the UV+ environment ( $14.1 \pm 0.44$  mm,  $n = 30$ ; Table 2). Growth in terms of weight, on the other hand, was slightly higher in the UV+ ( $0.53 \pm 0.02$  g,  $n = 30$ ) than

in the Nutrient+ environment ( $0.48 \pm 0.03$  g,  $n = 30$ ; Table 2). Despite some crossing of the reaction norms (Fig. 1a,b, Table 1), genetic correlations across the two past-environments were positive (length:  $r = 0.63$ ,  $n = 30$ ,  $P < 0.001$ ; weight:  $r = 0.73$ ,  $n = 30$ ,  $P < 0.001$ ).

Similarly, the phlorotannin concentration showed both high genetic variation and environmental plasticity (Fig. 1c, Table 1); algae grown in the UV+ environment had significantly higher phlorotannins ( $8.07 \pm 0.18$ ,  $n = 30$ ) than those grown in the Nutrient+ environment ( $6.71 \pm 0.18$ ,  $n = 30$ ; Table 1). The genetic correlation of phlorotannin production between the past-environments was positive (Fig. 1c;  $r = 0.80$ ,  $n = 30$ ,  $P < 0.001$ ).

The broad-sense heritability estimates for growth varied between 0.25 and 0.33 (Fig. 2). Compared to these relatively low heritabilities for growth (although significantly higher than zero as judged by jack-knifed SEs), the heritability for phlorotannin concentration was clearly higher, around 0.65 (Fig. 2). The heritability estimates did not differ between environments, as shown by the overlapping standard errors (Fig. 2).

#### Effects of past and current environments on selection

Genetic correlations among the past-environments in the aquaria and sea environments were close to zero for phlorotannins (Table 2), suggesting that the reaction norms were highly variable among the genotypes. The corresponding correlations for growth, in contrast, were positive in all comparisons (Table 2). Accordingly, genetic correlations between the environments in the sea were significantly positive for growth, but nonexistent or weakly positive for phlorotannins (Table 3).

We found significant differences in directional univariate selection differentials between algae coming from different past-environments: selection for growth differed in the shallow control plots, and selection for phlorotannins differed in the deep control plots (Table 4). We therefore analysed selection separately for the two past-environments. Positive selection differentials for growth, including both direct and indirect selection, were found in the shallow environment in all groups (Table 4). In the deep growing site, selection for growth was found in algae from the UV+ past-environment, in both nutrient environments. Selection differentials for phlorotannins were nonsignificant in all selective environments in the algae originating from the Nutrient+ past-environment. However, in the algae from UV+ past-environment there was selection against phlorotannins in the shallow, nutrient-enriched environment and in the deep, control environment (Table 4).

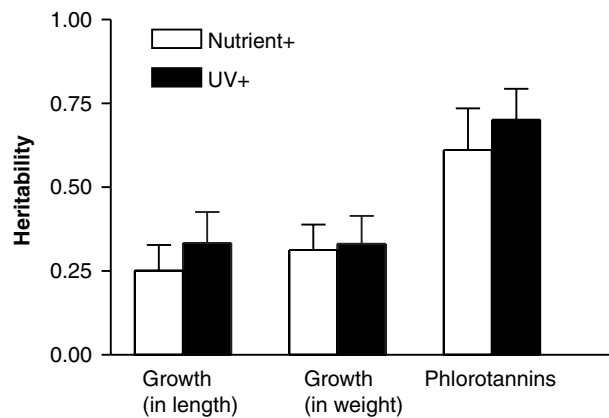
The linear selection gradients from the multiple regression model (Table 5), measuring direct selection for growth or for phlorotannins, showed an overall pattern similar to the univariate selection differentials. Because the selection gradients also showed certain differences between algae from different past-environments

**Table 1** Results of mixed model ANOVA on the effects of environment, genotype and their interaction on growth and phlorotannin concentration of algae during the past-environment period. Initial size of algae, either length or weight, was used as a covariate in the analyses of growth. For the random effects, variance estimates  $\pm$  SE calculated by the restricted maximum likelihood method are given.

Source of variation	Growth, in length		Growth, in weight		Phlorotannin concentration	
	$F_{\text{ndf, ddf}}$	$P$	$F_{\text{ndf, ddf}}$	$P$	$F_{\text{ndf, ddf}}$	$P$
<i>Fixed effect</i>						
Environment	14.18 <sub>1, 28.9</sub>	<0.001	5.30 <sub>1, 17.9</sub>	<0.05	133 <sub>1, 29</sub>	<0.0001
Initial size	15.97 <sub>1, 685</sub>	<0.0001	573 <sub>1, 680</sub>	<0.0001		
	Variance estimate	$P$	Variance estimate ( $\times 10^3$ )	$P$	Variance estimate ( $\times 10$ )	$P$
<i>Random effects</i>						
Genotype	3.4 $\pm$ 1.2	<0.01	11.1 $\pm$ 3.5	<0.01	8.0 $\pm$ 2.4	<0.001
Genotype $\times$ environment	1.1 $\pm$ 0.5	0.05	2.3 $\pm$ 1.1	<0.05	0.0	ns
Aquarium (environment)	0.0	ns	0.0 $\pm$ 0.2	Ns	–	
Residual	11.8 $\pm$ 0.7		38.9 $\pm$ 2.2		2.1 $\pm$ 0.5	

**Table 2** Genetic correlations of growth rate and phlorotannin concentration between past-environments and the four selective environments in the sea. Precise probabilities are given in parentheses for statistically significant correlation coefficients; nonsignificant ones are denoted as ns. Coefficients remaining significant at 0.05 risk level after the Bonferroni correction are printed in boldface. Ns vary from 29 to 30.

Sea environment	UV+ past-environment		Nutrient+ past-environment	
	Growth rate	Phlorotannin concentration	Growth rate	Phlorotannin concentration
Shallow (1 m), Control	<b>0.74</b> (<0.0001)	0.13 <sup>ns</sup>	<b>0.50</b> (0.0046)	0.17 <sup>ns</sup>
Shallow (1 m), Nutrient enriched	<b>0.70</b> (<0.0001)	0.12 <sup>ns</sup>	0.40 (0.031)	0.10 <sup>ns</sup>
Deep (3 m), Control	<b>0.63</b> (0.0002)	–0.06 <sup>ns</sup>	<b>0.48</b> (0.0090)	0.28 <sup>ns</sup>
Deep (3 m), Nutrient enriched	<b>0.60</b> (0.0005)	–0.05 <sup>ns</sup>	0.46 (0.0127)	–0.10 <sup>ns</sup>



**Fig. 2** Broad-sense heritability estimates ( $h^2$  and jack-knifed SE) for growth and phlorotannins in Nutrient+ and UV+ past-environments.

(Table 6), we analysed the two past-environments separately. We tested the differences in directional and quadratic selection gradients between the selective environments, within the two past-environments, by means of the heterogeneity of slopes tests in ANCOVA on relative fitness (Table 7). Within both past-environments there was significant overall directional and stabilizing selection

for growth (Table 7); otherwise, the selection patterns differed depending on past-environment.

In the algae originating from the UV+ past-environment, we found significant directional selection for a high growth rate in all selective environments (Fig. 3, Table 5). However, directional selection for growth differed significantly between the nutrient environments, being stronger in the control than in the nutrient-enriched environment (Fig. 3, Table 7). In addition, there was stabilizing selection for growth in the shallow nutrient-enriched and the deep control environments, as implied by the significant, negative quadratic selection gradients (Fig. 3b,c, Table 5). We found significant overall directional selection against phlorotannins (Table 7); this was especially clear in the deep control environment (Fig. 3c, Table 5). Both the directional and quadratic selection gradients for phlorotannins also tended to differ between the selective environments (Fig. 3, Table 7).

In algae originating from the Nutrient+ past-environment, directional selection for growth was weaker, and was statistically significant only in the shallow, nutrient enriched environment (Fig. 4, Table 5). Stabilizing selection for growth differed between the depth zones (Table 7): stabilizing selection was strong in the deep environment (Fig. 4c,d, Table 5) and was absent in the shallow environment (Fig. 4a,b, Table 5). We found

**Table 3** Genetic correlations between the four selective environments in the sea for growth rate (at the lower left side of the matrix) and phlorotannin concentration (at the upper right side of the matrix), calculated for algae originating from the UV+ past-environment. Exact risk probabilities (in parentheses) are given when  $P < 0.10$ , otherwise significance is denoted as 'ns'. Coefficients remaining significant at 0.05 risk level after the Bonferroni correction are printed in boldface. Ns vary from 28 to 30.

	Shallow (1 m), Control	Shallow (1 m), Nutrient enh.	Deep (3 m), Control	Deep (3 m), Nutrient enh.
Shallow (1 m), Control		<b>0.47</b> (0.0092)	0.27 (ns)	<b>0.47</b> (0.0095)
Shallow (1 m), Nutrient enh.	<b>0.77</b> (<0.0001)		0.15 (ns)	0.25 (ns)
Deep (3 m), Control	<b>0.60</b> (0.0004)	<b>0.64</b> (0.0002)		<b>0.51</b> (0.0039)
Deep (3 m), Nutrient enh.	<b>0.82</b> (<0.0001)	<b>0.72</b> (<0.0001)	<b>0.72</b> (<0.0001)	

**Table 4** Estimates of standardized, univariate, directional selection differentials, calculated as the covariance of the standardized trait with the relative fitness, for growth and phlorotannins and their statistical significance, separately for algae from the UV+ and Nutrient+ past-environments. *t*-Test statistics tests for differences between the past-environments. Marginally significant ( $P < 0.10$ ) coefficients are indicated by °,  $P < 0.05$  by \*,  $P < 0.01$  by \*\* and  $P < 0.001$  by \*\*\*.

Trait	Shallow (<1 m)						Deep (3 m)					
	Control			Nutrient enriched			Control			Nutrient enriched		
	UV+ past-env.	Nutrient+ past-env.	<i>t</i>	UV+ past-env.	Nutrient+ past-env.	<i>t</i>	UV+ past-env.	Nutrient+ past-env.	<i>t</i>	UV+ past-env.	Nutrient+ past-env.	<i>t</i>
Growth	0.25***	0.08*	2.02*	0.16**	0.07*	0.56	0.18***	0.08	1.27	0.18**	0.08°	0.98
Phlorotannins	-0.08	-0.06	0.07	-0.14*	-0.03	1.11	-0.12*	0.06	2.33*	-0.08	-0.04	0.34

stabilizing selection for phlorotannins in the shallow, nutrient-enriched environment (Fig. 4b, Table 5).

In general, we found little evidence of genetic trade-offs between phlorotannins and growth. The correlations between them were nonsignificant in most past-environment-selective-environment combinations. There were two exceptions to this trend: we found a significant negative relationship in the deep, nutrient-enriched environment in algae from the Nutrient+ past-environment ( $r = -0.44$ ,  $P < 0.05$ ,  $n = 29$ ), and in the shallow, control environment in algae from the UV+ past-environment ( $r = -0.40$ ,  $P < 0.05$ ,  $n = 30$ ).

## Discussion

### Maintenance of genetic variation in phlorotannin contents

The co-evolution of plant defences with grazing pressure presumes that plant resistance to herbivory will show heritable variation (Futuyma & Keese, 1992). Plant secondary compounds typically form the basis for chemical resistance. Estimates of the heritability of plant secondary compounds vary from zero to complete genetic control (Berenbaum *et al.*, 1986), but they usually indicate considerable genetic influence on secondary chemistry (e.g. Berenbaum & Zangerl, 1992; Nichols-Orians *et al.*, 1993; Han & Lincoln, 1994; Orians *et al.*, 1996; Marak *et al.*, 2000). Despite the putative role of brown algal phlorotannins as defences against

herbivory, there are no prior estimates of the amount of genetic variation or selection gradients for them. Instead, the emphasis in research on phlorotannins has been on the environmental plasticity and inducible responses to herbivory (e.g. Van Alstyne, 1988; Ilvessalo & Tuomi, 1989; Arnold *et al.*, 1995; Steinberg, 1995; Cronin & Hay, 1996; Peckol *et al.*, 1996; Hammerström *et al.*, 1998; Pavia *et al.*, 1999; Pavia & Toth, 2000b), which have typically been found.

We found a relatively high (average 0.63) broad-sense heritability of phlorotannin contents in *F. vesiculosus* in two different environments. Since heritability is always context-dependent and the estimates were obtained in controlled environments, the higher phenotypic variance in the field may decrease the actual heritability. When calculated from the field data at the end of the experiment, genotype alone contributed 30.6% and genotype together with genotype-by-environment interactions 46.5% of the phenotypic variation in phlorotannins (T. Honkanen & V. Jormalainen, unpublished data), implying that a high heritability of phlorotannins persists under more variable natural conditions. In addition, our estimate may be biased upwards by a high level of dominance and/or epistatic genetic variance and potential transgenerational effects (Schwaegerle *et al.*, 2000) inherited from the clonal 'mother'. However, the broad-sense heritability estimates of another trait, growth, obtained from the same material and the same environments, provide a comparison: the amount of genetic variation in phlorotannins was clearly higher than that in growth.

**Table 5** Estimates of multiple linear ( $\beta$ ) and quadratic ( $\gamma$ ) selection gradients and correlational selection gradient with their standard errors from multiple regression analyses of phlorotannins and growth on relative fitness under two growing depths and two nutrient regimes, separately for algae from the UV+ and Nutrient+ past-environments. For visualization of the simplest well fitting models, see Figs 3 and 4. Marginally significant  $P$ s are indicated in parentheses,  $P < 0.05$  by \*,  $P < 0.01$  by \*\* and  $P < 0.001$  by \*\*\*.

Trait	UV+ past-environment				Nutrient+ past-environment				Pooled over past-environments and selective environments
	Shallow (<1 m)		Deep (3 m)		Shallow (<1 m)		Deep (3 m)		
	Control	Nutrient enriched	Control	Nutrient enriched	Control	Nutrient enriched	Control	Nutrient enriched	
	$\beta$	$\beta$	$\beta$	$\beta$	$\beta$	$\beta$	$\beta$	$\beta$	$\beta$
Growth	0.26*** ± 0.058	0.13* ± 0.062	0.20*** ± 0.057	0.17** ± 0.060	0.08 ± 0.045	0.07* ± 0.034	0.07 ± 0.055	0.08 ± 0.051	0.10*** ± 0.024
Phlorotannins	-0.03 ± 0.055	-0.10 ± 0.063	-0.12* ± 0.053	-0.03 ± 0.058	-0.04 ± 0.047	-0.01 ± 0.033	0.05 ± 0.055	-0.015 ± 0.054	-0.04 <sup>(0.09)</sup> ± 0.024
	$\gamma$	$\gamma$	$\gamma$	$\gamma$	$\gamma$	$\gamma$	$\gamma$	$\gamma$	$\gamma$
Growth <sup>2</sup>	-0.08 ± 0.059	-0.19** ± 0.059	-0.14* ± 0.053	-0.10 ± 0.070	-0.07 ± 0.055	0.02 ± 0.028	-0.13* ± 0.048	-0.12** ± 0.041	-0.07** ± 0.025
Phlorotannins <sup>2</sup>	-0.06 ± 0.049	-0.07 ± 0.046	0.04 ± 0.054	0.01 ± 0.038	0.02 ± 0.056	-0.09** ± 0.034	-0.03 ± 0.048	-0.02 ± 0.047	-0.005 ± 0.018
Growth × phlorotannins	0.003 ± 0.066	-0.06 ± 0.060	0.07 ± 0.057	-0.004 ± 0.088	0.005 ± 0.058	-0.03 ± 0.040	0.05 ± 0.065	-0.07 ± 0.074	0.02 ± 0.026

Maintenance of genetic variation in a putative defence trait calls for explanation, since selection for the trait tends to reduce genetic variation. One possibility is that genetic trade-offs between defence investment and other fitness components contribute to the maintenance of genetic variation. We found such a trade-off between growth and phlorotannin concentration, but in only two of the eight past-environment–current-environment combinations, suggesting that trade-offs alone cannot explain the maintenance of genetic variation.

Depending on the environment, we found either no selection, stabilizing selection or selection against phlorotannins. The effect of varying selection on genetic variation depends on the genotype-by-environment interaction, i.e. the genetic variation of the reaction norms. Via & Lande (1987) suggested that the genetic architecture, namely genetic correlations between environments, constrains the evolution of genetic variation in heterogeneous habitats. According to their model, disruptive selection between environments may increase genetic variation when the genetic correlation of trait levels between environments is close to one and the mean phenotype thus cannot evolve to a habitat specific optimum. On the other hand, if the inter-environment genetic correlation of the trait is low, the mean phenotype expressed in each environment can evolve to an optimum for that environment. As a result, adaptive phenotypic plasticity will evolve and selection alone cannot maintain genetic variation (Via & Lande 1987; but see Pigliucci 1996 for criticism of simple predictions on evolution of plasticity). Whereas the plasticity of phlorotannins seems to be true, so does their high genetic variation; in other words, the low inter-environment correlations do not lead to a reduction in the amount of genetic variation. We suggest that the small-scale heterogeneity of the selective environment with respect to nutrient availability and possibly depth gradient, together with gene flow between the habitats, contribute to the maintenance of genetic variance in the phlorotannin concentration.

### Selection depends on past environment

Past environment affected the strength and form of selective gradients for growth and phlorotannins. This implies that the responses of algae to the environment may change the future fitness ranks of the genotypes. In the analyses of the selection gradients, we adjusted fitness for the initial number of apical meristems at the beginning of the selective period. Thus, differences in the initiation of new meristems in the past cannot account for the persisting influence of the past environment.

The persistence of environmental effects in clonal organisms has been attributed to somatic mutation, to persistent changes in gene expression or to differences in the quality or quantity of stored resources (Schwaegerle *et al.*, 2000). We cannot exclude the possibility of the first

**Table 6** Test statistics of ANCOVA testing the difference in linear and quadratic standardized selection gradients on growth and phlorotannins between UV+ and Nutrient+ past-environments, separately in the four selective environments. Tests of interaction of the past-environment with the linear or quadratic term are shown. Separate ANCOVAs were run for testing linear and quadratic terms. The selection gradients are given in the Table 5.

Trait	Shallow (<1 m)				Deep (3 m)			
	Control		Nutrient enriched		Control		Nutrient enriched	
	$F_{df}$	$P$	$F_{df}$	$P$	$F_{df}$	$P$	$F_{df}$	$P$
Linear selection gradients								
Growth	6.57 <sub>1,152</sub>	<0.05	0.91 <sub>1,152</sub>	ns	2.91 <sub>1,151</sub>	0.09	1.35 <sub>1,144</sub>	ns
Phlorotannins	0.02 <sub>1,152</sub>	ns	1.65 <sub>1,152</sub>	ns	4.66 <sub>1,151</sub>	<0.05	0.02 <sub>1,144</sub>	ns
Quadratic selection gradients								
Growth <sup>2</sup>	0.00 <sub>1,146</sub>	ns	11.84 <sub>1,146</sub>	<0.001	0.03 <sub>1,145</sub>	ns	0.07 <sub>1,138</sub>	ns
Phlorotannins <sup>2</sup>	0.27 <sub>1,146</sub>	ns	0.16 <sub>1,146</sub>	ns	0.98 <sub>1,145</sub>	ns	0.23 <sub>1,138</sub>	ns
Growth × phlorotannins	0.01 <sub>1,146</sub>	ns	0.14 <sub>1,146</sub>	ns	0.03 <sub>1,145</sub>	ns	0.33 <sub>1,138</sub>	ns

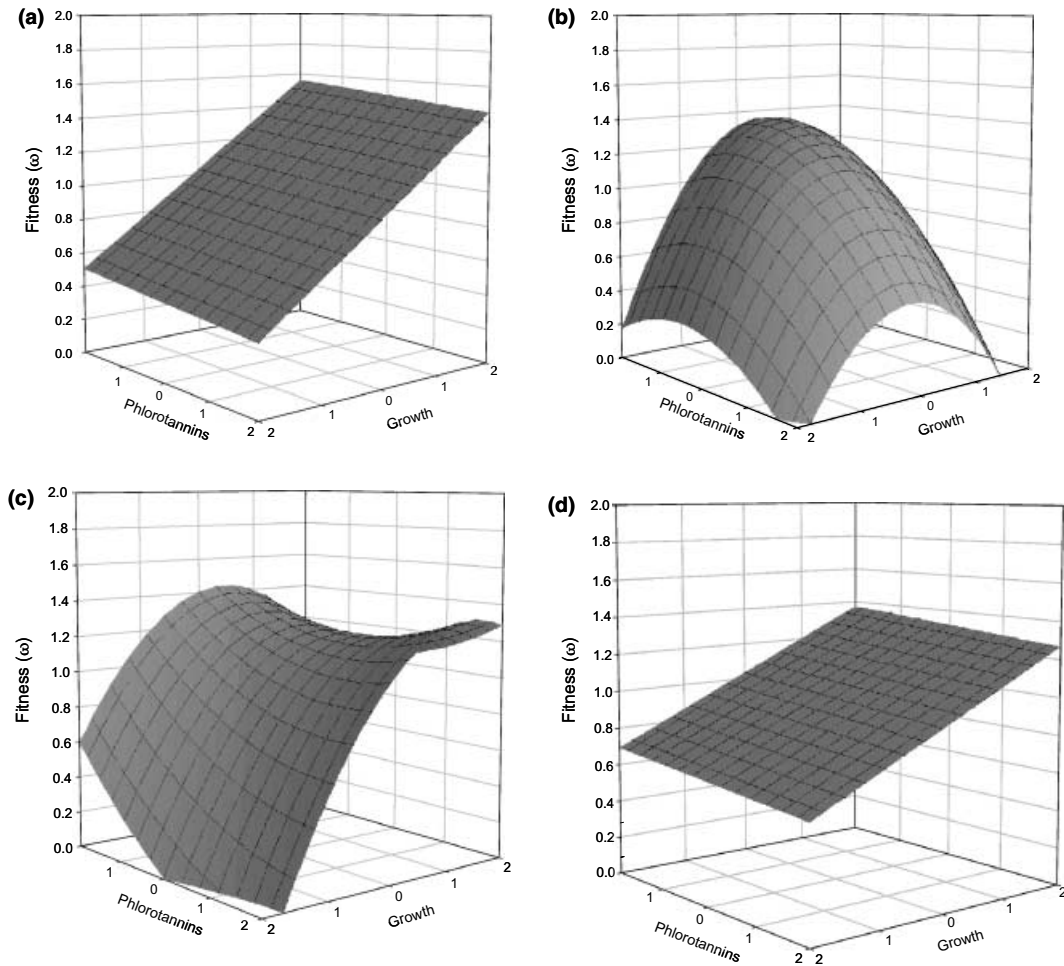
**Table 7** Analysis of differences in the multiple linear and quadratic selection gradients between depths and nutrient environments by means of ANCOVA on relative fitness. Relative fitness is calculated within each environment; thus the statistics tests differences in the shape of selection surfaces (Figs 3 and 4), not absolute fitness. For the quadratic terms, only interactions significant at the level of  $P < 0.10$  are shown. Separate analyses for both past-environments are shown.

Source	UV+ past-environment			Nutrient+ past-environment		
	d.f.	$F$	$P$	d.f.	$F$	$P$
Growth	1	38.52	<0.0001	1	9.86	<0.01
Growth <sup>2</sup>	1	18.79	<0.0001	1	11.98	<0.001
Phlorotannins	1	5.65	<0.05	1	1.96	ns
Phlorotannins <sup>2</sup>	1	0.15	ns	1	1.94	ns
Growth × phlorotannins	1	0.04	ns	1	0.17	ns
Growth × depth	1	0.93	ns	1	0.00	ns
Growth × nutrients	1	4.22	<0.05	1	0.24	ns
Growth × depth × nutrients	1	1.12	ns	1	0.62	ns
Phlorotannins × depth	1	0.57	ns	1	0.22	ns
Phlorotannins × nutrients	1	0.69	ns	1	0.01	ns
Phlorotannins × depth × nutrients	1	3.20	0.07	1	0.85	ns
Growth <sup>2</sup> × depth				1	4.73	<0.05
Phlorotannins <sup>2</sup> × nutrients	1	3.10	0.08			
Error	283			294		

two mechanisms, but we suggest that nutrients stored in the thallus during the past-environment period may explain the observed differences in selection for growth. Perennial brown algae such as *F. vesiculosus* are able to store nitrate, ammonium, free amino acids, phosphorus and polysaccharides in their thallus (Lundberg *et al.*, 1989; Carlson, 1991; Lobban & Harrison, 1994). The basal thallus is important in this species as a source of resources for apical growth (Honkanen & Jormalainen, 2002). We found no or only weak directional selection for growth in algae that experienced the nutrient-enriched past-environment, but strong selection for growth in those from the UV+ past-environment. If the nutrient-intake efficiency of the genotypes were the crucial trait determining growth rate, we would expect to see selection for growth precisely in those algae that have little or no internal nutrient resources to utilize for growth.

Selection for phlorotannins in the deep control environment depended on the past-environment of the algae.

There, directional selection against phlorotannins was found in algae from the UV+ past-environment but not the Nutrient+ past-environment. We suggest that this may be due to the different initial levels of phlorotannins in the beginning of the selective period in the sea: the phlorotannin concentration was over 20% higher in algae from the UV+ past-environment than in those from the Nutrient+ past-environment. However, selection against phlorotannins was restricted to the deep control environment. In all other cases there was either no selection, or – in algae that experienced nutrient enrichment in the past – stabilizing selection for phlorotannins in the shallow nutrient-enriched environment. Our interpretation of these differences in selection is that the prior phenotypic manipulation helped to detect selection that would otherwise been masked by the lack of variation in the low and high extremes of trait values (see Wade & Kalisz, 1990; Dudley & Schmitt, 1996 for further discussion of phenotypic manipulations in detecting selection). That the history of a phenotype may affect



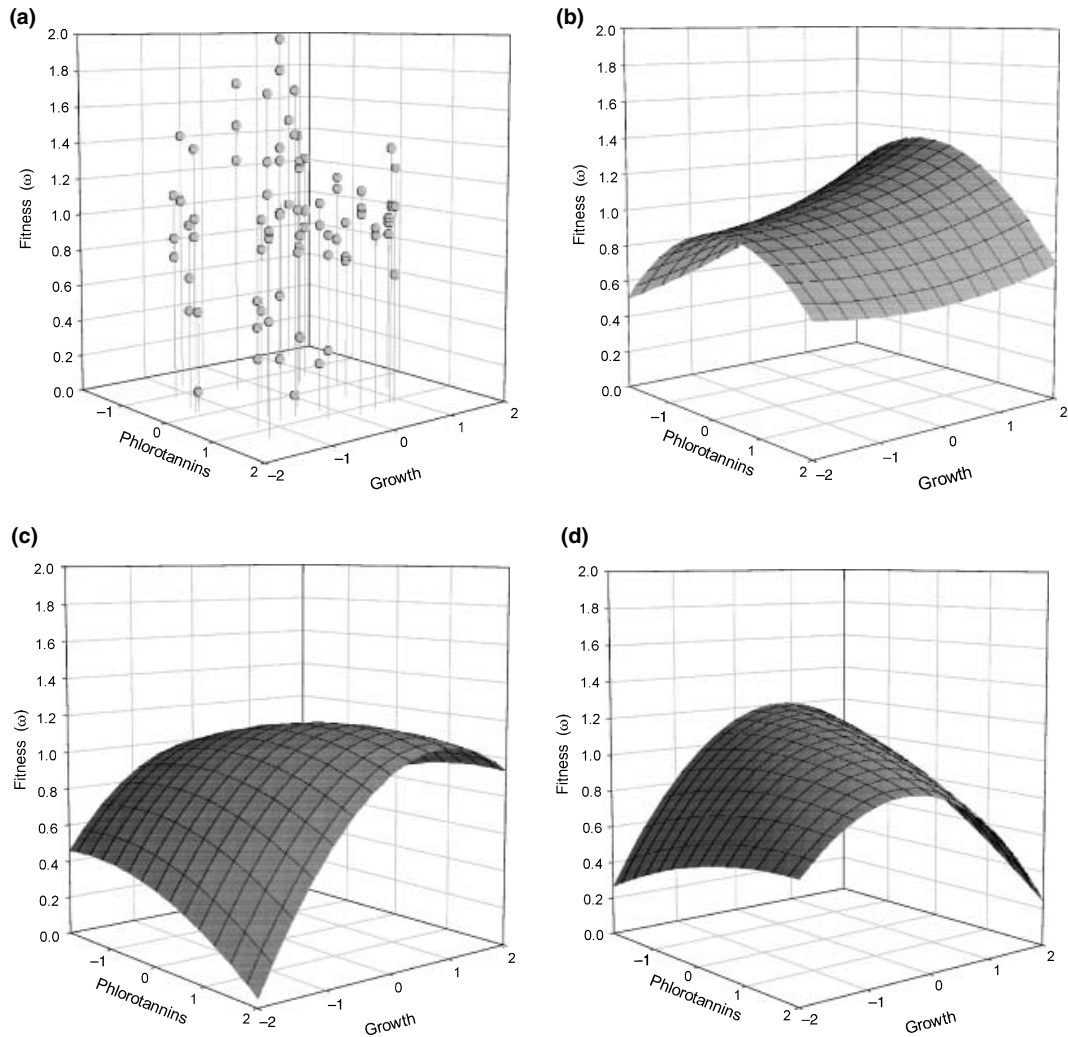
**Fig. 3** Relative fitness surfaces representing selection for growth and phlorotannins for algae that experienced the UV+ past-environment, separately for the four selective environments. The model surface is drawn only if the model significantly explains the variation in relative fitness. (a) Shallow, no nutrient increment; multiple, linear regression:  $r^2 = 24\%$ , model  $P = 0.0001$ . (b) Shallow, nutrient increment; multiple, quadratic regression:  $r^2 = 31\%$ , model  $P = 0.0001$ . (c) Deep, no nutrient increment; multiple, quadratic regression:  $r^2 = 28\%$ , model  $P = 0.0001$ . (d) Deep, nutrient increment; multiple, linear regression:  $r^2 = 13\%$ , model  $P = 0.009$ .

the form and strength of selection also implies that past-environmental influences may either hide a trait from selection or reveal it. This has methodological implications for detecting selection (Schwaegerle *et al.*, 2000), but, in addition, it may have evolutionary consequences for the maintenance of genetic variation especially in long-lived organisms.

### Spatial variation in selection

Nutrient availability affected selection for growth in algae from the UV+ past-environment: directional selection for growth was stronger in the control than in the nutrient-enriched environment. We suggest that a high level of ambient nutrients may mask genetic differences in nutrient intake ability and consequent growth, thereby lessening selection for growth. Another difference in

selection between environments was the stabilizing selection for growth, found in algae from the Nutrient+ past-environment in the deep but not the shallow environment. The growth rate measured in shallow and deep environments is likely to represent a different underlying trait. In the shallow, light-saturated habitat, nutrient intake capability may largely determine the ability to grow. In the deep, light-limited environment, on the other hand, the effectiveness of the photosynthetic apparatus in fixing carbon probably contributes much more to the growth rate. Thus, variable selection for growth along the depth gradient may reflect selection for different ultimate traits. In the deep habitat, however, the genotypes with the highest growth rates did worse than those with average growth rates. Herbivory was more intense in the deep than in the shallow habitat, as judged by the number of grazing marks on algae



**Fig. 4** Relative fitness surfaces representing selection for growth and phlorotannins for algae that experienced the Nutrient+ past-environment, separately for the four selective environments. The model surface is drawn only if the model significantly explains the variation in relative fitness. (a) Shallow, no nutrient increment; multiple, linear regression:  $r^2 = 5\%$ , model  $P = 0.10$ . (b) Shallow, nutrient increment; multiple, quadratic regression:  $r^2 = 16\%$ , model  $P = 0.02$ . (c) Deep, no nutrient increment; multiple, quadratic regression:  $r^2 = 14\%$ , model  $P = 0.05$ . (d) Deep, nutrient increment; multiple, quadratic regression:  $r^2 = 15\%$ , model  $P = 0.03$ .

(T. Honkanen & V. Jormalainen, unpublished data), and may well have contributed to stabilizing selection for growth found there.

The patterns of selection for phlorotannins were interesting with respect to the putative adaptive roles of brown algal phlorotannins in defence against herbivory or in tolerating ultraviolet radiation. If herbivory were the main selective agent for phlorotannins, we would expect directional selection for them, especially in the deep environment where herbivory was higher than in the shallow one (T. Honkanen & V. Jormalainen, unpublished data). On the other hand, if the primary adaptive role of phlorotannins were to enhance tolerance against ultraviolet radiation, we would expect directional selec-

tion for them in the shallow but not the deep habitat, since the penetration of UV into the water column in the Baltic Sea usually extends no deeper than a maximum of 2 m (Piazana & Häder, 1994). We did not find selection for high phlorotannins in either environment. Instead, in algae from the UV+ past-environment we found selection against phlorotannins in the deep environment, but this depended on nutrient availability; there was no selection in the nutrient-enriched environment. In algae that had experienced nutrient enrichment in the past we found stabilizing selection for phlorotannins in the shallow, nutrient-enriched environment. Thus, phlorotannins were not selectively neutral but affected fitness, although their fitness effects depended in a complex way on both

past and current environment. The patterns of selection were not consistent with the predictions from their suggested adaptive roles as defence compounds against herbivory or the harmful influences of UV radiation.

In conclusion, we found highly variable patterns of selection for growth and phlorotannins in *F. vesiculosus*. Past environmental conditions, nutrient availability and the depth gradient all contributed to variation in selection. In most cases we found directional and/or stabilizing selection for growth, but either no selection, stabilizing selection or selection against phlorotannins. Thus, phlorotannins seem to be causally related to fitness variation in *F. vesiculosus*, although their adaptive functions still remain unclear. There may be a fitness cost of producing phlorotannins, at least when herbivory is low or moderate, but the realization of such a cost depends on the environment. Trade-offs between growth and phlorotannins were found in some combinations of past and current environments, but they did not lead to correlated selection on the traits. Genetic variation in phlorotannins is probably maintained by spatially variable selection together with variation in the selection gradients generated by plastic responses to past environments. Due to the high genotypic variation, phlorotannin production may respond rapidly to directional selection.

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