ECOPHYSIOLOGY

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Genotypic variation in tolerance and resistance to fouling in the brown alga *Fucus vesiculosus*

Abstract In this study, we examined genetic variation in resistance and tolerance to fouling organisms in the brown alga *Fucus vesiculosus*. We first grew 30 algal genotypes in the field, where we allowed fouling organisms to colonise the genotypes at natural levels. We then conducted a manipulative experiment, where we grew 20 genotypes of algae in aquaria with or without fouling organisms. We measured host resistance as the load of fouling organisms and tolerance as the slope of the regression of algal performance on fouling level. Fouling organisms decreased host growth and contents of phlorotannins and thus have the potential to act as selective agents on algal defenses. We found significant among-genotype variation in both resistance and tolerance to fouling. We did not find a trade-off between resistance and tolerance. We found a marginally significant cost of resistance, but no cost of tolerance. Our results thus indicate that both the tolerance and resistance of *F. vesiculosus* can evolve as a response to fouling and that the costs of resistance may maintain genetic variation in resistance.

Keywords Cost of resistance · Cost of tolerance · Host-fouling organisms interactions · Phlorotannins · Tolerance trait

Introduction

Fouling organisms, which include micro- and macro-algae, bacteria, fungi, protozoans and multicellular animals, often cover the thalli of perennial seaweeds. The abundance of fouling organisms is determined by

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abiotic factors (e.g. nutrients, light and water flow) and by biotic interactions (host characteristics, herbivory and competition; e.g. Hillebrand et al. 2000, 2001). Epiphytic micro- and macroalgae on the thallus surface of macroalgae compete with their hosts for light and nutrients. The hosts are often slow-growing and long-lived perennials with a low nutrient uptake and surface-to-volume ratio, while epiphytic algae have a delicate structure, high surface-to-volume ratio and high growth rate. It has been shown that fouling organisms can negatively affect growth and reproduction in seagrasses and macroalgae (e.g. Williams and Seed 1992; Neckles et al. 1993; Williams and Ruckelshaus 1993; Jernakoff and Nielsen 1997; Worm and Sommer 2000; Duffy and Harvilicz 2001). Furthermore, associated fouling organisms may attract grazers and thereby increase tissue loss of the host (Bernstein and Jung 1979; Williams and Seed 1992; Littler and Littler 1999).

Since fouling can be detrimental to host fitness, it is reasonable to assume that selection has favoured traits that reduce the probability and/or extent of fouling or traits that reduce fitness effects of fouling. There have been some attempts to identify the mechanisms by which macroalgae may reduce fouling. Chemical resistance against fouling organisms may exist through allelopathic compounds (reviewed by Steinberg and de Nys 2002; Steinberg et al. 2002). Schmitt et al. (1995), for instance, found that diterpene alcohols produced by the brown seaweed *Dictyota menstrualis* prevent fouling organisms from colonising the surface of alga. Wikström and Pavia (2003) have suggested that phlorotannins in *Fucus vesiculosus* inhibit the settlement of *Balanus improvisus* larvae. On the other hand, Jennings and Steinberg (1997) did not find evidence that phlorotannins affected the distribution or abundance of epiphytes on the kelp *Ecklonia radiata*. It has also been suggested that macroalgae may reduce fouling by periodically shedding layers of cells from their surface (e.g. Kiirikki 1996), although the general effectiveness of this process in avoiding overgrowth remains unclear (Keats et al. 1994).

Algae may also increase the abandonment/proliferation of blades as a response to fouling (Littler and Littler 1999).

Resistance and tolerance are two general classes of defences that plants use to cope with their enemies. Resistance has usually been defined as host traits that either prevent enemy attack or reduce enemy performance (Karban and Baldwin 1997). Tolerance, in turn, is the ability of a plant to sustain a certain amount of damage without a corresponding reduction in fitness (e.g. Paige and Whitham 1987; Strauss and Agrawal 1999). In several plant species, genetic variation has been found in resistance to herbivory (e.g. Zangerl and Berenbaum 1997; Mauricio 1998; Tiffin and Rausher 1999; Juenger and Bergelson 2000) and in tolerance (e.g. Juenger et al. 2000; Siemens et al. 2003). Explaining the maintenance of this variation has become a major research problem: if tolerance and resistance are beneficial to plants, plants should gradually lose additive genetic variation and be maximally tolerant and/or resistant. It has been suggested that the disappearance of genetic variation for plant defense may be counteracted by negative genetic correlations between resistance and/or tolerance and other fitness components within populations or by spatially variable selection. Costs of resistance and/or tolerance have been reported in many studies (e.g. see Bergelson and Purrington 1996; Strauss et al. 2003 for reviews), but several studies have failed to document such costs (Mauricio et al. 1997; Agrawal et al. 1999; Fornoni and Núñez-Farfán 2000).

Both tolerance and resistance may be based on several different underlying mechanisms. Resistance may involve different types of secondary metabolites and physical defenses, such as thorns and hairs, while tolerance has been associated with a high relative growth rate, high photosynthetic rates after damage, increased branching after release from apical dominance and large root-mass and carbon stores (Strauss and Agrawal 1999; Stowe et al. 2000; Strauss et al. 2003). In order to understand the evolution of tolerance and resistance against fouling, we need to know what algal characteristics may constrain or facilitate the evolution of tolerance or resistance. Due to the simple morphology and functional organisation of macroalgae, there are few potential traits related to tolerance. Macroalgae do not have a root system for the storage of resources, and there are few or no lateral buds to be activated as a consequence of apical disturbances. However, macroalgae are able to store nutrients and photosynthates in the thallus (Lehvo et al. 2001; Honkanen and Jormalainen 2002). A strong effect of thallus biomass on tolerance would indicate that stored resources and/or photosynthetic biomass are important mechanisms underlying tolerance.

Here we use a novel study system, namely that between fouling organisms and their macroalgal host, *F. vesiculosus* to investigate the evolutionary potential of both resistance and tolerance. We are especially interested in genetic variation in resistance and tolerance to fouling organisms. Our previous study suggests that *F. vesiculosus* shows genetic variation in resistance to fouling (Jormalainen et al. 2003). Given that fouling organisms have negative effects on host growth and reproduction, it is possible that fouling organisms may impose selection on host traits that confer resistance and/or tolerance, and that algal populations have the potential to respond to this selection if algal defenses are genetically variable. We conducted both observational field studies and manipulative experiments in aquaria to investigate the occurrence of genetic variation in host resistance and tolerance. Furthermore, we investigated possible costs of tolerance and resistance and examined responses to fouling of putative resistance and tolerance traits. We also studied phlorotannin-fouling relationship and whether phlorotannins can act as a resistance trait.

Materials and methods

Study objects

Fucus vesiculosus is a widely distributed, perennial dioecious brown alga along the North Atlantic coast, including the Baltic Sea. The thallus of *F. vesiculosus* is attached by a broad discoid holdfast, from which arise bilaterally branched fronds with a distinct midrib. The growth of *F. vesiculosus* occurs mainly by means of apical cells (Van den Hoek et al. 1998). Dichotomous branching, the main rule of spatial organisation, results from the division of an apical cell. Nutrients are absorbed from the water through the thallus, which can store nutrients and photosynthates to be used to maintain growth under low resource levels (Lehvo et al. 2001; Honkanen and Jormalainen 2002). Reproduction occurs via the formation of receptacles, which are the enlarged, swollen distal ends of branches. During reproductions the apical meristems in the reproductive part die (Knight and Parke 1950). The number of remaining vegetative apical parts determines future growth and reproduction, thus representing an important fitness component.

The snail *Theodoxus fluviatilis* occurs commonly on *F. vesiculosus* in densities of up to hundreds of individuals per single alga (Segerstråle 1927; observation by the authors). It feeds on fouling organisms covering the thallus of *F. vesiculosus*, but does not feed on the thallus itself (Jormalainen et al. 2003). We consider utilising snails to control fouling as a "natural" manipulation due to the nearly continuous presence and high abundance of this species on algal thallus. On the other hand, such manipulation is potentially confounding because the snails, in addition to their effects on epibiota, may have direct effects on algae. Such multiple snail effects, however, are likely to coexist also in the field.

Experimental designs

The algae for the two experiments were collected from the Archipelago Sea and Northern Baltic Sea (field

experiment: 22°18, 65′E, 60°06, 50′N; aquaria experiment: 22°07, 40′E, 60°07, 28′N), randomly along a *Fucus* belt from a depth of one meter. Genetic individuals of *F. vesiculosus* were defined as entities growing from a single thallus on a holdfast. Each genotype was split into apical pieces; each about 5 cm in length and carrying two to seven dichotomously divided apical tips. Each genotype was divided, i.e. cloned, into separate parts to both control and evaluate the extent of genetic variation in growth, phlorotannins and the amount of covering fouling organisms, and the fitness response to fouling. In the following, we use "genotype" to refer collectively to all the algal parts clipped from a single genetic *F. vesiculosus* individual.

Fouling in the field

The experiment consisted of two temporally separate parts. In order to minimize environmentally derived differences among genotypes (e.g., maternal effects), we first reared algae in aquaria for 44 days. The rearing was arranged in six aquaria, with a volume of 60 l each and with a seawater through-flow of 300 l day^{-1}. The aquaria were located outdoors, under natural light and diurnal rhythm; they were protected from rainfall by a thin plastic cover, permeable to UV light. To ensure adequate nutrient availability in the aquaria, the algae received fertiliser in the form of a controlled release fertiliser, 20 g of 37:0:0 and 5 g 10:21:0 (N:P:K; Polyon, Pursell Industries Inc., USA), in diffusion bags that were replaced every 2 weeks. We cut six apical pieces from each of the 30 genotypes. All algal parts were randomly assigned to the aquaria in such a way that each genotype was represented once in each aquarium. Each alga was attached to a clothes peg and anchored to the bottom of the aquarium. To ensure equal light conditions for all algae, the apical tips were placed at the same level, about 10 cm below the surface of the water.

For the second part of the experiment, we chose a shore with a vigorous *Fucus* belt in the close vicinity of the Archipelago Research Institute of the University of Turku. On 01 June 2000, all algal parts were randomly assigned to six plots, with each genotype represented once in each plot. The algae were attached to a clothes peg and anchored to plots made of plastic grid (60×40 cm). The plots were placed at a depth of 1 m, where the natural *Fucus* belt was vigorous and dense. At the beginning and end of the experiment (19–20 September), we weighed the algal parts and measured their length; we also counted the number of apical tips. At the end of the experiment, we also measured the thallus area and measured the total amount of fouling organisms on the surface of algae by carefully washing each algal piece in a small amount of seawater, filtering the fouling biota on pre-weighted filter papers (Schleicher and Schuell GF 50) and weighing the biomass after 48 h at 60°C. Fouling consisted mainly of periphyton and epiphytic algae; barnacles and bryozoans were absent at this shallow

depth, although they are common on algal thallus in deeper environments. We took samples from the uppermost ∼2 cm of the thallus that had grown during the experiment for the analyses of phlorotannins. To obtain enough material for the analyses, we had to pool three samples within each genotype. The samples were freeze-dried, finely ground and stored at −20°C until the analysis (see below).

Fouling in aquaria: removal of periphyton by the snail *T. fluviatilis*

The experiment in aquaria was conducted at the Archipelago Sea Research Institute of Turku. Aquaria, with a volume of 60 l each and with a seawater through-flow of 202 l day^{-1}, were located outdoors, under natural light and diurnal rhythm; they were protected from rainfall by a thin plastic cover, permeable to UV light. To ensure water movement, one water filtration pump was placed in each aquarium. The effects of fouling organisms on the growth and phlorotannin content of *F. vesiculosus* were investigated from 11 July to 10 August 2000. In the experiment, we manipulated the abundance of fouling organisms (control and removal of fouling). Fouling organisms were either present at natural densities (we did not manipulate densities), or were absent due to removal by the herbivorous snail *T. fluviatilis*; 370 snails were placed in each fouling removal aquarium. Six aquaria were randomly assigned to each treatment. Twelve apical pieces were cut off from 20 genotypes. The algal pieces were randomised into two treatments [fouling and non-fouling (snail) treatment] and each genotype was replicated six times in both treatments. Pieces of each genotype were randomised into aquaria, so that one piece of each genotype was placed in each aquarium. Each piece was attached to a clothes peg and anchored to the bottom of the aquarium. To ensure equal light conditions for all pieces, the apical tips were placed at the same level, about 15 cm below the surface of the water.

At the beginning and end of the experiment, as in the previous experiment, we weighed the algal parts, measured their length and counted the number of apical tips. Fouling organisms on the surface of the thallus were determined as described earlier. We took samples for the analyses of phlorotannins from the uppermost ∼2 cm of the thallus. We had to pool samples within each genotype and treatment level to obtain a sufficient amount of thallus for the analyses. All samples were freeze-dried, finely ground and stored at −20°C until the analysis (see below).

Quantification of phlorotannins, a potential resistance trait

We used the modified Folin-Ciocalteu method and phloroglucinol as a standard agent to quantify the amount of phlorotannins in each genotype. The Folin-

Ciocalteu method is an improvement on the Folin-Denis reagent, making the method more sensitive to reduction by phenolics and less prone to precipitate (Waterman and Mole 1994). The Folin-Ciocalteu assay also quantifies some non-phenolic hydroxylated aromatic compounds; according to Van Alstyne (1995), however, non-phenolic compounds make up less than 5% of the total Folin-Ciocalteu reactive compounds in marine brown algae.

Statistical analyses

Our level of genetic analysis is a cloned genotype. Clonal material is commonly used to evaluate genetic effects (Schwaegerle et al. 2000). However, conclusions from clonal material concern total genetic variation, not just additive genetic variance, and hold under the assumption of minimal maternal or 'carry-over' effects from the clonal parent.

Field experiment: genetic variation for resistance

In the field experiment, we tested differences in fouling among genotypes with analysis of variance, using the genotype and experimental plot as random factors and biomass of fouling organisms per thallus area as the dependent variable. Significant differences in fouling among genotypes indicate among-genotype variation in host resistance.

To determine the relationship between the amount of fouling with the phlorotannin content of the thallus, we calculated Pearson's correlation coefficients using genotypic means.

Field experiment: genetic variation for tolerance

Tolerance is often measured as the difference between the average fitness of individuals attacked by herbivores (or by other biotic factors, such as parasites) and that of individuals not attacked. This measure of tolerance assumes that all plants attacked experience the same level of damage. In the study conducted in the field, however, we did not manipulate the amount of fouling and the algae had different amounts of fouling organisms on their surface. In those cases where the level of damage varies among host individuals, tolerance can be considered a norm of reaction: described by the regression of fitness (final algal size) on damage (fouling biomass; Simms and Triplett 1994; Mauricio et al. 1997). Using the reaction norm approach, we tested differences among genotypes in tolerance using a mixed-model analysis of variance with PROC MIXED (SAS ver. 8.2, SAS Institute, Cary, NC, USA), with restricted maximum likelihood estimation (Littell et al. 1996). In the analyses, the biomass of fouling organisms was a covariate, and the algal genotype and its interaction with the covariate were random factors. A significant interaction term between genotype and biomass of fouling organisms indicates differences in tolerance among genotypes. Since the initial size of the algal part significantly affects the growth of the part, in the analyses we used residuals of final size from the regression of final size on initial size as our dependent variable.

Field experiment: correlation between tolerance and resistance

To study a trade-off between tolerance and resistance, we calculated Pearson's correlation coefficient between the amount of fouling organisms and tolerance (slope from regression of fitness on fouling) using genotypic means.

Field experiment: cost of tolerance

To estimate the cost of tolerance we used the method proposed by Mauricio et al. (1997). This method uses only plants that have been damaged (or, in our case, have undergone fouling); in other words, there is no treatment without damage. In this method, fitness in the absence of damage is estimated by the intercept of the regression of fitness on damage. The cost of tolerance is then assayed by a genotype-mean regression of intercept on slope. The problem with this method is that even in the absence of a cost, the slope and the intercept are statistically dependent on each other because errors are correlated. To remove this bias and to arrive at the true cost of tolerance, we used the correction proposed by Mauricio et al. (1997).

Aquaria experiment: genetic variation for tolerance and fitness effects of fouling

In the experiment conducted in the aquaria, we had two environments for the algae: one in which the amount of fouling organisms was not restricted, and one in which fouling organisms were removed from the surface of the algal thallus by snails (*T. fluviatilis*). We therefore had two fixed treatment groups. We tested the effects of genotype and fouling on algal performance with a mixed-model ANOVA (PROC MIXED, SAS ver. 8.2, SAS Institute). Fouling treatment was considered a fixed effect, while the aquarium, the genotype and their interactions with fouling were treated as random effects. Under this model, a significant genotype × fouling interaction indicates either that the genetic correlation between traits in the control and fouled treatment is significantly less than one (norms of reaction are not parallel), indicating significant genetic variation for tolerance, or that there is significant heteroscedasticity of variances between the treatments. Because thallus biomass variances were unequal for the different fouling

treatments, we employed an unequal variance model, in which genotype effects were estimated separately for the two fouling treatments (Littell et al. 1996, p. 162).

In order to remove the effect of initial size of the algal part on final size, we used initial size as a covariate in all analyses. When the slopes from the regression of initial size on final size were significantly different between fouling treatments, we used a model that estimated separate slopes for treatments. We compared the treatments at the 25th percentile, median and 75th percentile values of the covariate with the t test (see details in Littell et al. 1996). We did these analyses to determine the fitness effects of fouling.

Aquaria experiment: cost of tolerance and resistance

To detect the cost of tolerance, we used the reaction norm approach described above: we investigated the presence of a genetic trade-off between the level of tolerance (the slope of the regression of thallus biomass on fouling) and biomass in the absence of fouling organisms. Because the biomass measures in the absence of fouling now came from a different treatment, no correction was needed. To study the cost of resistance, we calculated Pearson's correlation coefficients between the amount of fouling organisms and host fitness (measured as length increment, number of apical tips and increase of biomass) of the non-fouled hosts ($n = 20$, genotypic means used). In the correlations, we used residuals of final size from the regression of final size on initial size.

Results

Field experiment: genetic variation for resistance and tolerance and correlation between tolerance and resistance

We found significant variation among genotypes in the amount of fouling organisms (Fig. 1; log likelihood ratio test for random effects, $\chi^2 = 6.5$, $P < 0.025$), indicating that in this population there was significant genetic variation in resistance to fouling. Algal growth generally decreased with increasing fouling (Fig. 2; Table 1). We measured tolerance as the slope of the regression of growth on the amount of fouling (Fig. 2). We found a significant interaction between biomass of fouling organisms and genotype (Table 1, Fig. 2), indicating that tolerance is also genetically variable.

We did not find a significant correlation between tolerance and resistance ($n = 30$, $r = -0.03$, $P = $ NS), indicating that there was no trade-off between these two traits.

Field experiment: cost of tolerance

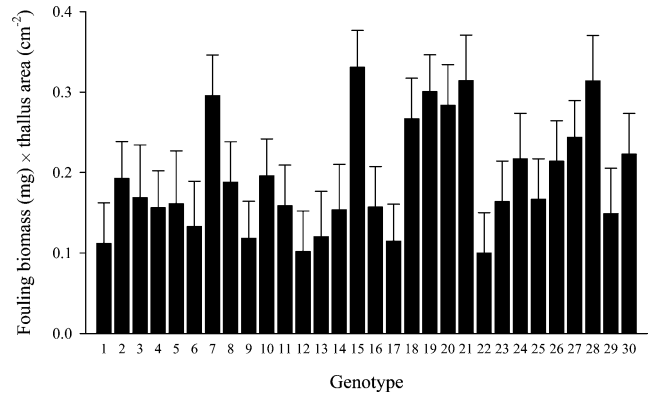In order to test the costs of tolerance, we determined the correlation between the slope and intercept of the

Fig. 1 Fouling biomass (mean $\pm$ SE) in different genotypes of the host alga, *Fucus vesiculosus*. Note that a lower biomass of fouling organisms indicates higher resistance of the host
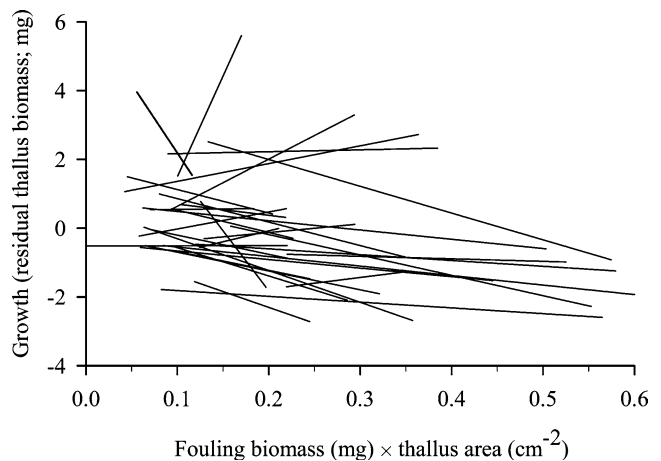

Fig. 2 Relationship between fouling load and increase of thallus biomass of different genotypes of the host alga, *F. vesiculosus*, in the field. The biomass increase is given as residuals from the regression of final weight on initial weight, and thus represents the difference from the average growth of the population. Each line represents the reaction norm of one host genotype

regression of biomass on tolerance. This was negative ($r = -0.85$, $P < 0.001$), and the covariance between tolerance and biomass was -32.2. However, after applying the correction by Mauricio et al. (1997) the unbiased estimation of covariance was -2.61 ± 13.4 (jack-knifed 95% confidence intervals). Since this interval overlaps zero, there is no indication of a significant negative covariance of tolerance with biomass in the absence of fouling, and thus no evidence of fitness costs of tolerance.

Aquaria experiment: genetic variation for resistance and tolerance

Unlike in the field experiment, in this experiment we did not find significant genetic variation among genotypes in the biomass of fouling organisms (Table 2), indicating that

Table 1 Variance components (VarComp ± SE) due to genotype and its interactions with the covariate (biomass of fouling organisms on the surface of algal thallus) with significance tests

Random factors	Thallus biomass		Length		Apical tips	
	VarComp	χ^2	VarComp	χ^2	VarComp	χ^2
Genotype	1.15 ± 0.42	21.9****	88.69 ± 39.07	12.8****	54.58 ± 18.64	18.3****
Genotype × biomass of fouling organisms	8.99 ± 7.61	2.7*	367.31 ± 517.95	0.6	0	
Plot	0.25 ± 0.17	29.3****	0		7.52 ± 6.68	5.7**
Residual	0.57		148.17		70.41	
Fixed effects	ndf, ddf	F	ndf, ddf	F	ndf, ddf	F
Biomass of fouling organisms	1, 13.8	4.86*	1, 19	0	1, 133	1.45

A significant genotype × biomass of fouling organisms interaction indicates genetic variation for tolerance. Tests of variance components are one-tailed (Littell et al. 1996, p. 44); the chi-squared values shown are the differences in two times the log likelihood of that factor included versus excluded from the model
*$P < 0.05$; **$P < 0.01$; ***$P < 0.005$; ****$P < 0.001$

that in this population (algal genotypes for aquaria and field experiments came from different populations) there was little genetic variation in resistance to fouling. There were significantly less biomass of fouling organisms on the thallus of algal hosts with snails than on algae without snails (mean ± SE = 0.0072 ± 0.0098 biomass of fouling organisms mg/algal biomass mg, and 0.10 ± 0.0098 biomass of fouling organisms mg/algal biomass mg respectively; Table 2). The interaction between snail treatment and host genotype on the biomass of fouling organisms was not significant (Table 2), indicating that the snails did not choose among the host genotypes. Thus, the snail treatment effectively manipulated fouling across all genotypes.

Table 2 Variance components (VarComp ± SE) with significance tests for fouling treatment, genotype and their interaction

Random factors	Biomass of fouling organisms		Length		Apical tips		Phlorotannin	
	VarComp × 10^{-4}	χ^2	VarComp	χ^2	VarComp	χ^2	VarComp	χ^2
Genotype	0.27 ± 0.32	1	2.94 ± 1.17	13.9**	0.60 ± 0.26	8.9***	0.50 ± 0.2	13.5***
Genotype × fouling	0	0	0.15 ± 0.37	0.2	0.07 ± 0.12	0.5	0.48 ± 0.09	2.7*
Aquarium (fouling)	3.9 ± 1.92	56.5***	3.12 ± 1.52	63.1****	0.06 ± 0.07	2.6	Not applicable	
Residual	7.32		5.84		1.55		0.2	
Fixed effects	ndf, ddf	F	ndf, ddf	F	ndf, ddf	F	ndf, ddf	F
Fouling	1, 9.94	54.91***	1, 57.6	1.17	1, 104	0.03	1, 19.3	213.52***
Initial size			1, 206	945.24***	1, 214	229.90***		
Initial size × fouling			1, 52.4	9.99**	1, 141	16.61***		

Random factors	Thallus biomass	
	VarComp	χ^2
Genotype fouled	7.61 ± 239.7	0
Genotype non-fouled	6.339 ± 3.217	35.4****
Aquarium (fouling)	2.141 ± 1.049	60.3****
Residual	3.604	
Fixed effects	ndf, ddf	F
Fouling	1, 56.4	9.13**
Initial size	1, 202	2388.70***
Initial size × fouling	1, 202	63.71***

Fouling treatment is a fixed effect, the rest are random effects. Initial size of algal part is a covariate. Tests of variance components are one-tailed (Littell et al. 1996, p. 44); the chi-squared values shown are the differences in two times the log likelihood of that factor included versus excluded from the model. Since we found significantly unequal variances among genotypes between fouled (snails present) and non-fouled (no snails) treatment groups for the thallus biomass, violating the assumption of equal variance, we employed an unequal variance model in which genotype effects were estimated separately for the two treatment groups (Littell et al. 1996, p. 162)
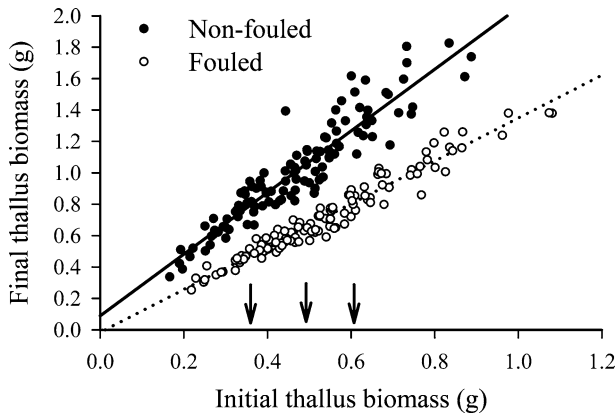*$P < 0.05$; **$P < 0.01$; ***$P < 0.005$; ****$P < 0.001$

Fig. 3 Scatterplot of initial size of alga by final size of alga with separate regression lines for thallus biomass. *Arrows* indicate the positions of 25th percentile, median and 75th percentile. *t* test results at the corresponding positions ($t = 10.3$, $P < 0.0001$, $t = 12.36$, $P < 0.0001$, $t = 14.01$, $P < 0.0001$). Similar patterns were found in the number of apical tips and length (data not shown)
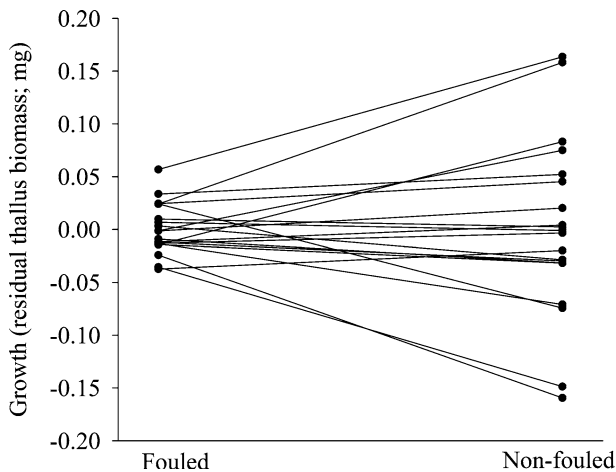

Fig. 4 Relationship between fouling load and increase of thallus biomass of different genotypes of the host alga, *F. vesiculosus*, in the aquarium experiment. The biomass increase is given as residuals from the regression of final weight on initial weight, and thus represents the difference from the average growth of the population within each fouling treatment. *Each line* represents the reaction norm of one host genotype

The regression slopes between initial and final length, between initial and final number of apical tips and between initial and final thallus biomass were significantly different between the fouling treatments, and separate tests at the values of the 25th percentile, median and 75th percentile of the covariate indicated that the algae grew less in the fouling than in the non-fouling treatment throughout the initial size range of the alga (Fig. 3 for thallus biomass—figures for final length and number of apical tips are not shown—Table 2). We found a significant fouling treatment-by-genotype component of variance in thallus biomass (log likelihood ratio test for random effects, $\chi^2 = 15.6$,

$P < 0.0001$, Fig. 4), indicating the existence of genetic variance for tolerance to fouling in this experimental population. However, since we found significantly unequal variances among genotypes between fouled (no snails) and non-fouled (snails present) treatment groups for thallus biomass, violating the assumption of equal variance, we employed an unequal variance model in which genotype effects were estimated separately for the two treatment groups (Table 2; Littell et al. 1996, p. 162). Thus, there is an increase in the variance of biomass production in the non-fouled treatment compared to the fouled treatment, and the genotype × fouling treatment interaction is thus probably due both to changes in the ranks of genotypes between the environments, i.e. genetic variation for tolerance and to heteroscedasticity (Fig. 4).

Aquaria experiment: cost of resistance and tolerance

We did not find a significant cost of tolerance, since the correlation between biomass in the non-fouled treatment and tolerance (measured as the slope of regression of thallus biomass on fouling) was not significant ($n = 20$, $r = -0.33$, $P = 0.15$). We found a marginally significant cost of resistance in terms of length and thallus biomass (measured as correlations between the opposite of the amount of fouling organisms on fouled hosts and length increment and thallus biomass of the non-fouled hosts; $n = 20$, $r = -0.43$, $P = 0.06$, $n = 20$, $r = -0.51$, $P = 0.08$, respectively). There was no cost of resistance in terms of apical tips ($n = 20$, $r = 0.04$, $P = 0.87$).

Phlorotannins as a resistance trait, genotypic and phenotypic variation of phlorotannins

In the field experiment, phlorotannin content and the biomass of fouling organisms were not correlated ($n = 30$, $r = 0.11$, $P = NS$), indicating that fouling did not affect the allocation of resources to phlorotannins or that phlorotannins are not a resistance trait in this system.

In the aquaria experiment, the phlorotannin content in the snail treatment group was 21% higher than in the fouling treatment group (Table 2; mean ± SE = 9.82 ± 0.3 and $7.76 \pm 0.3\%$ d wt., respectively). Hence, either snails induced phlorotannin production or fouling decreased allocation to phlorotannin production. Unfortunately, on the basis of our data we cannot separate these two possibilities. There was significant among-genotype variation in the concentration of phlorotannins, and a significant genotype-by-fouling treatment interaction (Table 2). Although fouling decreased the phlorotannin content of all genotypes, a significant interaction indicates that genotypes had different reaction norms in relation to fouling. The variation due to genotype and its interactions with fouling comprised over 80% of the phenotypic variation.

Discussion

Fouling organisms decrease the fitness of hosts in several ways. Growth and reproduction may be impaired (Williams and Seed 1992; Neckles et al. 1993; Williams and Ruckelshaus 1993; Duffy and Harvilicz 2001). Increased drag causes loss of host seaweeds during storms (Williams and Seed 1992). Associated fouling organisms may attract grazers, which cause tissue loss in the host alga as well (Bernstein and Jung 1979; Karez et al. 2000). In this study, both in aquaria and in the field, fouling organisms on the thallus surface had a strong negative effect on the growth of *F. vesiculosus*. Obviously fouling has the potential to select for algal characteristics that minimize its negative effects.

In the aquarium experiment, we used *T. fluviatilis* snails to remove fouling organisms from the thallus. Snails were extremely efficient cleaners as they decreased the amount of epibiota to less than one tenth of that in the treatment without snails. However, although *T. fluviatilis* does not feed on the thallus of *F. vesiculosus*, it can cut and remove hyaline hairs that the alga uses for nutrient intake (Hurd et al. 1993; Jormalainen et al. 2003). This may affect resource allocation of *F. vesiculosus*, especially; it can under some resource conditions lead to increased phlorotannin production (Jormalainen et al. 2003). We, however, consider the fouling removal effect as the most important one in the present experiment. The presence of snails increased both the growth and production of secondary metabolites of the alga, suggesting that the possible direct manipulation of nutrient intake efficiency did not lead to trade-offs in resource allocation (see Jormalainen et al. 2003 for discussion).

Genetic variation in resistance and tolerance

Another requirement for the evolution of resistance and tolerance against natural enemies is the occurrence of heritable variation for these traits and for fitness. In the field study, we found significant among-genotype variation in both resistance and tolerance. In the experiment conducted in aquaria, we did not find among-genotype variation in resistance to fouling, but found significant among-genotype variation in tolerance. In our previous study conducted on *F. vesiculosus*, we found significant among-genotype variation for resistance to fouling over a range of different growing environments (Jormalainen et al. 2003). Thus, there is genetic variation in both resistance and tolerance to fouling, the extent of which may vary among *F. vesiculosus* populations.

The variable outcomes of our field and aquaria experiments may be due to differences in experimental designs and environmental conditions. For instance, the much higher level of fouling in the aquaria compared to the field may have masked genetic differences in resistance in the experiment conducted in aquaria. The same may explain the difference between the current and earlier (Jormalainen et al. 2003) experiments in aquaria. In the fouling treatment of the current aquarium experiment, the level of fouling was over tenfold compared to the earlier experiment, where we found genotypic variation in resistance.

Both in aquaria and in the field, the genotypes were plastic in their responses to fouling. In most genotypes, fouling had a negative effect on growth, but in the field, some genotypes actually responded positively to fouling. Thus, the positions of the genotypes in the growth hierarchy may change in relation to fouling. In the aquarium experiment, the variance in biomass increase among genotypes in the fouling-free situation was significantly greater than that in the situation with fouling. This suggests that a high level of fouling may largely disguise variation in growth, thus weakening selection.

Mechanisms of resistance and tolerance

The resistance of *F. vesiculosus* was measured as the reduced load of all fouling organisms as a whole. Thus, we did not distinguish different species of fouling organisms; resistance in this study represents general resistance against colonisation of the thallus surface. Since macroalgae are covered most of the time with several kinds of fouling organisms, we assume that our approach is appropriate.

The detection of genetic variation in responses and reaction norms to fouling is the first step in understanding the evolution of resistance and tolerance to fouling. The next step would be to identify the algal traits that provide the mechanisms for resistance and tolerance. Resistance mechanisms that lead to the unsuccessful establishment of fouling organisms may include various defense chemicals (reviewed by Steinberg and de Nys 2002; Steinberg et al. 2002). In this study, we measured the phlorotannin content of algal tissue as a possible resistance trait. The phlorotannin content of tissue may, however, not be a good indicator of resistance against fouling, since it may not affect fouling (Jennings and Steinberg 1997); instead, the amount of phlorotannins that is exuded through the cell wall (see Koivikko et al. 2005) could be better related to fouling. Furthermore, if phlorotannins in tissue act as anti-fouling compounds, we could expect a negative correlation between phlorotannins and fouling in the field as well as in the fouled treatment of the aquarium experiment. Instead, there was no such correlation. If phlorotannins act as inducible anti-fouling compounds, we could expect them to decrease when fouling is removed. On the contrary, phlorotannins increased by 21% in the fouling removal treatment. However, here a possibility remains that the direct effects of snails may have contributed to the increase in phlorotannins (see Jormalainen et al. 2003). There was also a significant genotype-by-fouling-treatment interaction for the concentration of phlorotannins. This indicates that even

though fouling decreased the phlorotannin content, genotypes had variable reaction norms in relation to fouling. If phlorotannins have adaptive functions, such as protection against ultraviolet radiation or defence against herbivory (e.g. Peckol et al. 1996; Pavia et al. 1997), the susceptibility of genotypes in relation to these threats may change as a consequence of fouling.

Plant tolerance mechanisms have been divided into two sets of traits: resource reallocation and plant architecture (Stowe et al. 2000). Replacing tissue lost to consumption involves the reallocation of mobile resources and available meristems. Furthermore, patterns of vascular architecture can restrict the flow of resources among plant parts, limiting the use of existing resources to tolerate tissue loss. The morphology of seaweeds is much simpler than that of vascular plants. For example, *F. vesiculosus* does not have a root system for the storage of resources or lateral buds to be activated as a consequence of apical disturbances. Furthermore, the different parts of most seaweeds show a high level of independence; they absorb most of their nutrients, produce their own photosynthates and lack vascular connections for efficient resource translocation. In *F. vesiculosus*, however, a small-scale translocation of resources occurs from the older thallus to the growing apical tips (Honkanen and Jormalainen 2002). This may explain why thallus biomass can be an important tolerance trait. Fouled genotypes with more stored resources in thallus could allocate more stored resources to the dividing apical tips than fouled genotypes with less stored resources in thallus.

Cost of tolerance and resistance

The maintenance of genetic variation for tolerance and/or resistance is a dilemma: if tolerance and resistance are beneficial in terms of algal fitness, algal populations should evolve towards a high level of tolerance and/or resistance and lose genetic variation in these traits. One possible factor that constrains the evolution of high tolerance or resistance to fouling would be negative genetic correlations between tolerance or resistance traits and other algal functions, i.e. the costs of tolerance or resistance. We found a marginally significant cost of resistance in the aquarium experiment, although we did not find genetic variation in resistance in this particular experiment possibly because the great amount of fouling in the aquaria masked genotypic differences in resistance in that environment. Although we found significant variation in tolerance, we did not find any costs for it. This may indicate that tolerance to fouling is achieved by a trait that is not costly in terms of growth in our experimental conditions, such as efficient photosynthetic machinery or amount of stored resources. It has been found that the cost of tolerance is environmentally dependent (e.g. Stinchcombe 2002; Siemens et al. 2003). In order to find the cost of tolerance, it may be necessary to examine tolerance in multiple environments.

In general, we found significant among-genotype variation in both resistance and tolerance to fouling. We found a marginally significant cost of resistance, but no cost of tolerance. Our results thus indicate that both the tolerance and resistance of *F. vesiculosus* can evolve as a response to fouling and that the costs of resistance may maintain genetic variation in resistance.

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