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Local genetic adaptation to grazing pressure of the green alga *Desmodesmus armatus* in a strongly connected pond system

Pieter Vanormelingen,[1] *Wim Vyverman, Dries De Bock, and Katleen Van der Gucht*
Gent University, Laboratory Protistology & Aquatic Ecology, Krijgslaan 281—S8, 9000 Gent, Belgium

Luc De Meester
Catholic University of Leuven, Laboratory of Aquatic Ecology, Ch. Debériotstraat 32, 3000 Leuven, Belgium

Abstract

Dispersal potentially homogenizes genetic variation among populations and thus may prevent local genetic adaptation. If selection gradients are strong and the selection response efficient, however, local genetic adaptation may persist in the face of high dispersal rates. We compared grazing-resistance traits among populations of the green microalga *Desmodesmus armatus*, which inhabit ponds that are ecologically different but part of a strongly interconnected pond system. *Desmodesmus* clones were isolated from a clear-water and a turbid pond. For 16 clones from an internal transcribed spacer 2 clade with low sequence variation (1.3%) corresponding morphologically to *D. armatus*, coenobial dimensions and the average number of cells per coenobium, in both the absence and the presence of water conditioned by their main grazer, the waterflea *Daphnia*, were determined. Clones from the clear-water pond had four-celled coenobia with a higher greatest axial linear dimension and an increased average number of cells per coenobium in response to *Daphnia* kairomone, contrary to clones from the turbid pond. Unexpectedly, they were also characterized by a lower average number of cells per coenobium. No differences among populations were detected for cell length. Genetic variation was present in both populations for all traits, except for the response to kairomone. Continuous dispersal through overflows and rivulets in this pond system is thus incapable of preventing strong among-population genetic differentiation for ecological relevant traits, testifying both to the capacity of phytoplankton populations to adapt to local conditions and to the importance of grazing as a structuring factor in natural phytoplankton populations.

There is increasing evidence for rapid genetic tracking of natural populations to changes in local environmental conditions (Cousyn et al. 2001; Grant and Grant 2002). In a natural setting, an important question is the relative importance of local environmental factors (local selection pressures) and exchange with the regional gene pool (dispersal and gene flow) in determining the genetic composition of local populations. Although dispersal has the potential to homogenize genetic variation among populations, it is the relative strength of dispersal and differential natural selection that will determine to what extent local adaptation can occur (Slatkin 1987). Indeed, in the case of very strong selection pressure, dispersal may not result in genetic homogenization because of high mortality rates in the sink habitat.

Predation is a key driver of community and population structure in freshwater ecosystems (Kerfoot and Sih 1987; Tollrian and Harvell 1999). In zooplankton, for instance, it is well recognized that positive size-selective predation by visually hunting fish shifts communities to a dominance of small-sized taxa (Brooks and Dodson 1965). In parallel, the presence of fish has been shown to lead to genetic shifts toward more effective avoidance behavior (Cousyn et al. 2001) or smaller size at maturity (Leibold and Tessier 1991) in natural *Daphnia* populations. Similarly, the invertebrate predator *Chaoborus* modifies populations of *Daphnia pulex* by favoring clones forming neck-teeth in its presence (Parejko and Dodson 1991). Grazing by zooplankton daily removes substantial amounts of the phytoplankton biomass (Haney 1973) and has been shown to affect phytoplankton community structure (Cottingham 1999). Especially large-bodied *Daphnia* spp. are efficient phytoplankton grazers because of their high clearance rates and wide food size spectrum (Brooks and Dodson 1965).

However, planktonic primary producers are not defenseless food particles that are easily harvested by their consumers. Instead, a large number of phytoplankton taxa have evolved a wide variety of either constitutive or inducible defense mechanisms against zooplankton grazing. These include large cell or colony size (constitutive: large colonial forms such as those of *Pediastrum*; inducible: Hessen and Van Donk 1993), spine formation (Luo et al. 2006), toxin production (constitutive: Huisman et al. 2005;

[1] Corresponding author (Pieter.Vanormelingen@UGent.be).

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inducible: Jang et al. 2003), adjustment of recruitment from the sediment (Hansson 1996), excretion of substances reducing zooplankton grazing rate (Wagner and Kamjunke 2001), and resistance to digestion by the presence of thick cell walls (Van Donk and Hessen 1993) or gelatinous sheets (Porter 1975). Whereas these defenses have been described in many taxa and their importance is generally acknowledged, there are very few studies that have investigated whether natural populations show genetic variation for these traits, except for some scant reports on genetic differences among lineages (toxicity in *Microcystis aeruginosa*: Carillo et al. 2003). We wanted here to investigate whether local genetic adaptation for grazing resistance traits can be detected in natural populations that are exposed to contrasting grazing pressures by zooplankton.

One of the most intriguing properties of members of the phenotypically highly plastic green alga *Scenedesmus sensu lato* (*Scenedesmus s. l.*, recently split into *Scenedesmus* and *Desmodesmus*; An et al. 1999) is the ability to form colonies (coenobia) in response to the presence of zooplankton (Hessen and Van Donk 1993). This is commonly considered an inducible defense against zooplankton grazing. Indeed, colony formation fulfills all basic requirements for the evolution of such a defense (Tollrian and Harvell 1999): (1) the selective pressure, in this case zooplankton grazing, is variable; (2) there is a reliable cue (kairomones associated with the zooplankton; Yasumoto et al. 2005); (3) the defense is effective (colony formation increases particle size, which reduces the grazing speed of *Daphnia*, especially in the spined *Desmodesmus* and in smaller *Daphnia*; Hessen and Van Donk 1993); and (4) there is a cost to forming colonies (increased sedimentation losses; Lürling and Van Donk 2000). Lürling (1999) has shown that there is intraspecific genetic variation for the colony-formation response, so that this trait may respond to selection.

In shallow eutrophic freshwater ponds and lakes, *Scenedesmus s. l.* is one of the most common constituents of the phytoplankton. Basically, these water bodies can show two contrasting ecological states: a clear-water state characterized by an abundant submerged vegetation and a high proportion of large-bodied zooplankton (especially of the cladoceran genus *Daphnia*) that exert a high grazing pressure on the phytoplankton and a turbid state characterized by the lack of underwater vegetation and by a dominance of small zooplankton that exert a relatively low grazing pressure on the phytoplankton (Scheffer 1998).

Because of the different selection pressures exerted by zooplankton grazing in both pond types, we hypothesized that this would lead to local genetic adaptation, with clear-water ponds showing a shift in *Scenedesmus s. l.* population structure toward better-defended genotypes having larger coenobia and displaying a stronger response to *Daphnia* kairomone. Moreover, we wanted to test this hypothesis in a highly interconnected pond system. In systems characterized by high potential dispersal rates, the observation of local genetic adaptation provides strong evidence for rapid responses to local selection regimes, leading to efficient lineage sorting. We therefore selected a clear-water and a turbid pond in ''De Maten,'' a highly interconnected system of 34 shallow ponds that share the same water source and nutrient levels but that are characterized by contrasting food webs (Cottenie and De Meester 2003). We obtained multiple clonal isolates of *Scenedesmus s. l.* from these two ponds and identified them to species level using sequences of the internal transcribed spacer 2 (ITS2) ribosomal deoxyribonucleic acid (rDNA) region and checked for congruence with light microscopy morphology. We chose the ITS2 region, as it is generally used for species delimitation and identification in *Scenedesmus s. l.* (Vanormelingen et al. 2007) and ITS2 sequence similarity corresponds well with reproductive isolation barriers in several microalgal model taxa (Coleman 2001; Denboh et al. 2003). Next, we used strains of the most common species, *Desmodesmus armatus*, in laboratory experiments to test the hypotheses that (1) genotypic variation for grazing resistance traits is present within the populations, (2) genotypic differences in grazing avoidance traits between the two populations exist despite the high connectivity between the ponds, and (3) these differences correspond to the expectation for adaptive microevolution (local adaptation).

Methods

Sampling was done during the spring zooplankton bloom on 24 May 2002 in a clear-water (pond no. 15) and turbid pond (no. 18) in the De Maten pond system (Cottenie and De Meester 2003; Vanormelingen et al. 2008). The geographic distance between both ponds is at about 300 m, and they are indirectly connected to each other, with water flowing from pond 18 to pond 15. At the time of sampling, water clarity differed considerably between the two ponds, with a Secchi disc depth of 17 cm in the turbid pond and 50 cm in the clear-water pond. Moreover, zooplankton communities differed strongly, as was seen by a qualitative examination of 30- and 65-μm filtered micro- and macrozooplankton samples. The turbid pond contained high densities of small zooplankton, mainly rotifers, *Bosmina*, small *Daphnia* (median carapace length 0.61 mm, range 0.40–0.99 mm, $n = 20$), and cyclopoid copepods. In the clear-water pond, zooplankton was less abundant and consisted mainly of large *Daphnia* (1.1 mm, 0.7–1.7 mm, $n = 20$), *Alona*, and *Ceriodaphnia*. Phytoplankton samples were taken from four sites in the pelagic of each pond and pooled to exclude possible small-scale spatial variation. The phytoplankton samples were kept at 4°C in the dark until further processing. The same day, after concentration of the clear-water sample by gentle vacuum filtration over a glass-fiber filter (Whatman), aliquots of the two live samples were transferred to 50-mm Petri dishes. After the phytoplankton had settled down, single *Scenedesmus s. l.* coenobia were isolated by micropipette and cultured as described in Vanormelingen et al. (2007). A total of 126 *Scenedesmus s. l.* monoclonal cultures was established this way. Mutational change in the clones was prevented by keeping the stock cultures of the clones at low cell numbers and growth rates (low light, 5 μmol photons m^{-2} s^{-1}, and temperature, 4–5°C).

For species identification of our clones, the ITS2 sequences of a total of 87 randomly selected clones were determined, plus those of four additional strains from the

University of Texas (UTEX) culture collection. Methods of deoxyribonucleic acid extraction, polymerase chain reaction conditions, and sequencing are as described in Vanormelingen et al. (2007). Alignment of the ITS2 sequences was performed manually in the program DCSE (De Rijk and De Wachter 1993) based on their secondary structure (folded in RNAstructure ver. 4.2.; Mathews et al. 2004). As the microscopical examination and a preliminary phylogeny showed that all clones belonged to *Desmodesmus*, a phylogeny was constructed with the ITS2 sequences of our clones together with all *Desmodesmus* ITS2 sequences available in GenBank using the Bayesian inference method (MrBayes ver. 3.1.1; Ronquist and Huelsenbeck 2003), as in Vanormelingen et al. (2007). A more restricted Bayesian inference phylogeny consisting of the clade containing the *D. armatus* clones selected for this study, the most similar other *Desmodesmus* ITS2 sequences, and a selection of other sequences of the genus was constructed in the same way. *Scenedesmus obliquus* UTEX72 was selected as outgroup. Sequence divergence (uncorrected *P*-distance), and the number of point mutations between sequences were calculated in PAUP version 4.0b.10 (Swofford 2001).

Sixteen randomly chosen *D. armatus* clones were used for determination of grazing resistance traits, of which seven were isolated from the turbid and nine from the clear-water pond. The following possible grazing resistance traits were determined for these clones: average number of cells per coenobium in the absence and presence of *Daphnia* kairomone and greatest axial linear dimension (GaLD) and cell length of four-celled spined coenobia. The GaLD is the longest linear dimension that can be measured in a coenobium, which is the distance between the tips of diagonally opposite spines. Only the last two traits were determined for one of the clones from the clear-water pond, EH65. Both for growing the clonal cultures to sufficient density as well as for the experiment, cultures were kept at 19°C, a 12 : 12-h light : dark cycle, a light intensity of 120 μmol photons $m^{-2}\,s^{-1}$, and a shaking speed of 120 rpm. Clones were grown to sufficient number in 250-mL Erlenmeyer flasks, whereas 50-mL bottles were used for the experiment. A TD-700 fluorometer (Turner Design) was used for cell density estimations. The measurements on the clones were made in two separate experiments, each involving clones isolated from the two ponds. The results of the two experiments were combined afterward since no significant differences (*t*-tests) could be found for a common clone (EH43). Three replicas were used for each combination of clone and treatment (control or kairomone). *Daphnia* kairomone was produced by letting a multiclonal assemblage of adult *D. magna* at a density of 300 individuals L^{-1} graze for 24 h on a mixture of the *D. armatus* clones in fresh medium with sufficient *Scenedesmus* as food (Lürling and Beekman 1999). After filtration over a glass-fiber filter (Whatman), the culture water with kairomone was added in a concentration of 10% to the appropriate cultures; the kairomone thus corresponded to a situation of 30 *Daphnia* L^{-1}. The production of *Daphnia* kairomone was verified in advance by testing the response of a *Scenedesmus* strain known to exhibit colony formation in the presence of *Daphnia* kairomone, *S. obliquus* UTEX1450 (Lürling 1999). For the control treatment, filtered sterile medium was used. Maternal effects were excluded (or at least minimized) by reinoculating all clones at the same low density 3 d before the start of the experiment, which, according to the growth rate of EH43, corresponds to 2.4 generations. At the start of the experiment, all bottles were inoculated at a final density of 100,000 cells mL^{-1} and placed in a randomized position on the shaker. After 2 d, the experiment was ended, and the average number of cells in a coenobium and the response to the kairomone were estimated by counting the number of cells of 200 coenobia for each bottle under an inverted microscope (Zeiss Axiovert 135, Zeiss). GaLD and cell length (of one of the inner cells) were measured on 10 four-celled coenobia from one of the control bottles. Measurements were made on pictures taken with a Zeiss Axioplan 2 Universal microscope equipped with a monochrome digital camera, AxioCam MRm (Zeiss Gruppe) using the program Axiovision, release 4.4 (Zeiss Gruppe).

All statistical analyses were performed with Statistica version 5.0 for Windows (StatSoft). A critical significance level of $p = 0.05$ was used for all tests. Nested ANOVA was used to test for effects of pond of origin, clone (random factor nested within pond) and kairomone treatment (only for the first trait) and their interactions on the average number of cells per coenobium, GaLD, and cell length, respectively. One or two clones from the clear-water pond were omitted from the analysis to obtain a fully balanced design. These were EH43 for the average number of cells per coenobium and EH9 and EH63 for GaLD and cell length. Before performing the nested ANOVAs, the assumption of normality was checked using the Shapiro–Wilks *W*-test and histograms for each clone in the case of cell length and GaLD. Because of the more limited number of replicates for the number of cells per coenobium, we used the residuals of a one-way ANOVA with the clones as grouping variable to check normality for this trait. No deviations from normality were detected, except for clone ET31b, which was significantly left-skewed for both cell length and GaLD. A Least Significance Difference (LSD) post hoc test after a two-way ANOVA with clone and kairomone treatment as fixed factors was used to test the effect of the kairomone on the average number of cells per coenobium for each separate clone. We used the LSD test instead of the more conservative Tukey test, as only a small subset of the matrix with pairwise comparisons was used. Pairwise comparisons between clones were performed using a one-way ANOVA with a post hoc Tukey test with clone as fixed factor and the respective trait as dependent variable.

The broad-sense heritability of a trait represents the portion of the total phenotypic variance comprised by the total genetic variance and was estimated for the measured traits through a clonal repeatability analysis (Falconer and Mackay 1996). Clonal repeatabilities (a measure of broad-sense heritability) were calculated using a one-way ANOVA for each trait with clone as random factor. The proportion of the sum of all variance components that is genetic (among clone) is the clonal repeatability value or broad-sense heritability. Since GaLD and cell length were measured for only one bottle, possible bottle and genetic

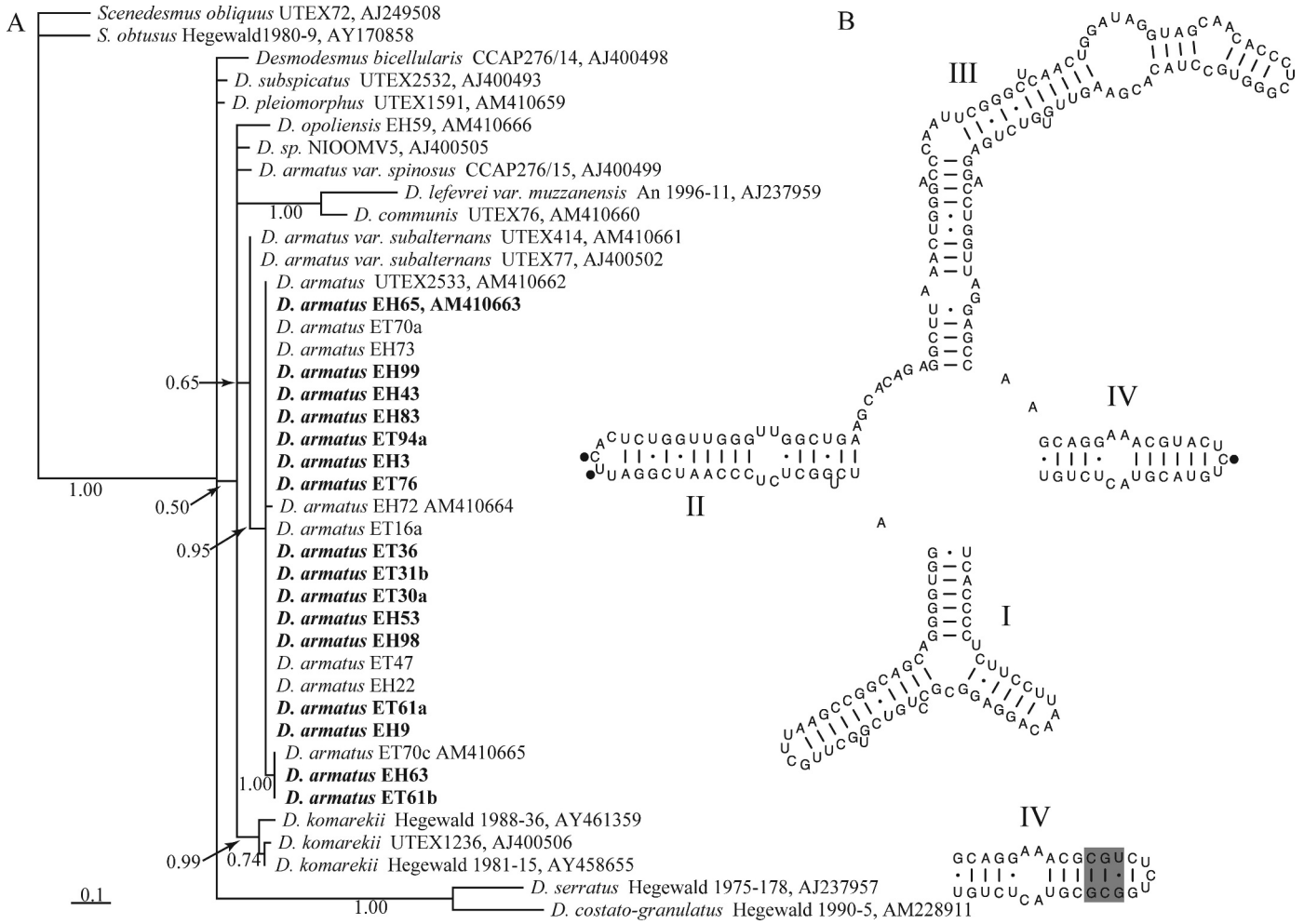

Fig. 1. *Desmodesmus* phylogeny based on ITS2 rDNA sequences and the ITS2 secondary structure of *D. armatus*. (A) 50% majority-rule consensus tree of a Bayesian inference analysis. Posterior probabilities >0.5 are shown at the nodes. Clones used for studying local genetic adaptation are in bold. For each sequence, species name, strain number, and GenBank accession number are indicated (accession numbers starting with AM are new to this study). (B) Secondary structure of the internal transcribed spacer 2 sequence of *D. armatus* clone UTEX2533. Dots indicate the variable positions within the *D. armatus* clade. The helices are given roman numbers. For comparison, the fourth helix of the ITS2 region of the most closely related taxon, *D. armatus* var. *subalternans* (strain UTEX414), is shown as well. The six bases (including two compensatory base changes) differing from the *D. armatus* sequences are shaded in gray.

effects could not be separated, resulting in a potential artificial inflation of differences among clones and thus also of the heritability estimates. Because of the highly standardized experimental setup, we expect the bottle effect to be very small however. Genetic differentiation between populations was calculated as Q_{ST} values; Q_{ST} is a measure of the degree of population differentiation, as it quantifies the fraction of the total variation for a particular trait that is due to differences among populations and is thus the quantitative functional analogue of F_{ST} as calculated for allele frequencies (Spitze 1993; calculation: Storz 2002).

Results

Species identification of the *Desmodesmus* clones isolated from the clear-water and turbid pond in De Maten was done using a Bayesian inference phylogeny that included the ITS2 sequences of 87 of our *Desmodesmus* clones and all published *Desmodesmus* sequences, on the basis of which we outlined different clades corresponding to the species level (P. Vanormelingen unpubl.). The light microscope morphology of the clones was also investigated and was overall in good agreement with their position in the ITS2 phylogeny. The lineage with the highest number of clones was subsequently selected for the present study and presented in Fig. 1. This lineage had a posterior probability of 0.95. Based on light microscope morphology of its members, the clade was identified as *D. armatus*, as described by Hindák (1990). The morphological identification was confirmed by the ITS2 sequence of *D. armatus* UTEX2533, which also fell in this lineage. Intraspecific variation in ITS2 comprised no more than three point mutations, corresponding to 1.3% divergence, and was located in the loop region of helices II and IV. The sister clade consisted of two sequences of *D. armatus* var. *subalternans*, which differed by two deletions and five to eight point mutations (2.1–3.0% divergence) in the stem region of helix IV, resulting in two compensatory base

Table 1. Results of three- and two-way nested ANOVAs testing for the effect of kairomone presence (only for number of cells per coenobium), population (pond of origin), and clone (random factor nested in population) and their interactions (indicated by ×) on, respectively, the average number of cells per coenobium, the greatest axial linear dimension (GaLD), and cell length of *Desmodesmus armatus* clones isolated from a clear-water and turbid shallow pond in "De Maten."

Factor	df effect	MS effect	df error	MS error	*F*	*p*
Average number of cells per coenobium						
Kairomone (K)	1	0.213	12	0.009	24.95	0.00031
Pond (P)	1	2.780	12	0.289	9.62	0.00916
Clone (C)	12	0.289	56	0.006	44.69	<0.0001
P × K	1	0.143	12	0.009	16.69	0.00151
C × K	12	0.009	56	0.006	1.32	0.23331
GaLD						
Pond (P)	1	1738.319	12	361.086	4.81	0.04865
Clone (C)	12	361.086	126	12.698	28.44	<0.0001
Cell length						
Pond	1	11.169	12	3.825	2.92	0.11320
Clone (C)	12	3.825	126	0.577	6.63	<0.0001

changes (Coleman 2000; Fig. 1). The ITS2 sequences of other closely related *Desmodesmus* taxa differed from *D. armatus* by 5.5–6.4% (*D. armatus* var. *spinosus*), 5.5–6.3% (*Desmodesmus* sp.), and 4.6–6.7% (*D. komarekii*).

Daphnia kairomone production was verified in advance by the test strain *S. obliquus* UTEX1450, which showed a highly significant increase from 1.8 ± 0.06 to 2.6 ± 0.10 in response to the kairomone treatment (*t*-test, df = 4, $p = 0.0003$). The results of a three-way nested ANOVA for the average number of cells per coenobium of the *D. armatus* clones are presented in Table 1, and pairwise comparisons of the clones are given in Table 2. A graphic representation is shown in Fig. 2. The coenobia of all clones counted one, two, three, or four cells. Coenobia with three cells were only occasionally observed, however. All cultures contained at least 50% four-celled coenobia. As a consequence, all clones had a rather high number of cells per coenobium, being not lower than 2.9. The character differed significantly between clones within a pond (significant clone effect) as well as between the ponds (significant pond effect). Moreover, while clones from the clear-water pond showed a small but consistent increase in the percentage of four-celled coenobia under influence of the *Daphnia* kairomone, clones from the turbid pond were not responsive, resulting in a significant interaction effect between pond and kairomone treatment and an insignificant clone × kairomone treatment interaction (Table 1). Indeed, as shown by an LSD test, seven out of eight clones from the clear-water pond showed a significant increase in the average number of cells per coenobium versus none of the seven clones from the turbid pond (Fig. 2).

The results of two-way nested ANOVAs for the GaLD and cell lengths of four-celled coenobia of the clones are presented in Table 1, and the results of pairwise comparisons between the clones in Table 2. A graphic representation is shown in Fig. 3. The average GaLD of the four-celled coenobia, the distance between the tip of diagonally opposite spines, differed almost twofold between the isolates, ranging from 31 to 57 μm. Moreover, the six clones with the largest GaLD were all isolated from the clear-water pond. Clones from the turbid pond had a maximal average GaLD of only 37 μm. This resulted in a significant pond effect with four-celled coenobia of clones from the clear-water pond having on average a higher GaLD than clones from the turbid pond, while genotypic variation within each pond was also present (significant clone effect). An additional morphological screening of these clones revealed that the largest three strains were the only ones for which low percentages of eight-celled coenobia (with a GaLD of 58–86 μm) could be observed. The difference in GaLD was not reflected by the cell lengths, for which there was no significant differentiation between the ponds (insignificant pond effect), even though there was genetic variation for cell length between the different clones (significant clone effect). The high GaLD of some of the clones from the clear-water pond appears to be due mainly to the formation of extremely long spines, reaching more than two times the cell length, whereas in small clones, spines tend to have approximately the same length as the respective cells (Fig. 4).

Broad-sense heritability estimates and Q_{ST} values are given in Table 3. Average heritabilities range from 0.34 to 0.86. Heritabilities in both populations were highly significantly different from zero for all traits examined except for the response to kairomone, for which heritabilities were marginally significant or insignificant. The Q_{ST} values are high for all traits examined, ranging from 0.18 to 0.51, except for cell length, having a Q_{ST} value of 0.034.

Discussion

Given the very low intraclade variability in ITS2 sequence of 3 base pairs (bp), or 1.3% located in the extremely variable loops of the helices, and the observation that the sequences cluster in a well-supported clade, there is little doubt that all *D. armatus* clones used in our experiments belong to the same biological species. Indeed, obvious intrinsic barriers to gene flow are absent at this level of ITS divergence in the few green microalgae examined (Volvocaceae: e.g., Coleman 2001; desmids: Denboh et al. 2003). Intraspecific variability in ITS2 for other *Desmodesmus* and *Scenedesmus* species is generally in

Table 2. Significance of pairwise differences between the *Desmodesmus armatus* clones for the measured antipredation traits as revealed by Tukey tests. These are the average number of cells per coenobium in absence and presence of kairomone, the response to kairomone, cell length, and greatest axial linear dimension, respectively. nd, not determined; ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

	ET61b	ET61a	ET94a	ET76	ET31b	ET30a	ET36
ET61b							
ET61a	ns/ns/ns/ns/ns						
ET94a	**/ns/ns/ns/*	ns/ns/ns/ns/ns					
ET76	***/***/ns/***/ns	ns/ns/ns/ns/ns	ns/**/ns/ns/ns				
ET31b	***/**/ns/*/ns	ns/ns/ns/ns/ns	ns/ns/ns/ns/ns	ns/ns/ns/ns/ns			
ET30a	***/***/ns/*/ns	ns/ns/ns/ns/ns	ns/*/ns/ns/ns	ns/ns/ns/ns/ns	ns/ns/ns/ns/ns		
ET36	***/***/ns/**/ns	ns/ns/ns/ns/ns	ns/*/ns/ns/ns	ns/ns/ns/ns/ns	ns/ns/ns/ns/ns	ns/ns/ns/ns/ns	
EH98	***/ns/*/ns/***	***/***/*/ns/***	***/**/***/ns/***	***/***/*/ns/***	***/***/***/ns/***	***/***/**/ns/***	***/***/**/ns/***
EH99	**/***/ns/***/ns	***/***/ns/ns/ns	***/***/*/ns/ns	***/***/ns/ns/ns	***/***/ns/ns/ns	***/***/ns/ns/ns	***/***/ns/ns/ns
EH63	**/*/ns/ns/ns	***/***/ns/*/ns	***/***/*/ns/ns	***/***/ns/***/ns	***/***/ns/**/ns	***/***/ns/**/ns	***/***/ns/**/ns
EH53	ns/ns/ns/***/***	***/***/ns/**/ns	***/*/**/**/ns	***/***/ns/ns/ns	***/***/ns/*/ns	***/***/ns/*/ns	***/***/ns/ns/ns
EH43	ns/ns/ns/ns/***	ns/ns/ns/ns/***	*/ns/ns/ns/***	**/***/ns/ns/***	**/*/ns/ns/***	**/**/ns/ns/***	**/**/ns/ns/***
EH83	ns/ns/ns/*/***	ns/ns/ns/ns/ns	ns/ns/**/ns/ns	*/ns/ns/ns/ns	*/ns/ns/ns/ns	**/ns/ns/ns/ns	**/ns/ns/ns/ns
EH9	ns/*/ns/ns/***	ns/ns/ns/ns/***	ns/ns/*/ns/***	ns/ns/ns/ns/***	ns/ns/ns/ns/***	ns/ns/ns/ns/***	ns/ns/ns/ns/***
EH3	ns/**/ns/*/ns	ns/ns/ns/ns/ns	ns/ns/ns/ns/ns	ns/ns/ns/ns/ns	ns/ns/ns/ns/ns	ns/ns/ns/ns/ns	ns/ns/ns/ns/ns
EH65	nd/nd/nd/***/***	nd/nd/nd/**/*	nd/nd/nd/**/ns	nd/nd/nd/ns/**	nd/nd/nd/ns/**	nd/nd/nd/*/**	nd/nd/nd/ns/**

the same order of magnitude or somewhat higher (3–13 bp; Hegewald et al. 2005) but can also be a lot more extensive, up to 7.4% (Vanormelingen et al. 2007). In this last case, additional cryptic diversity might be present, however. Our conclusion that the clones used for this study belong to a single species is further corroborated by morphological observations, with all clones having "*D. armatus*–like" coenobia. Spine length and shape do differ between the clones and have been used as the main distinguishing

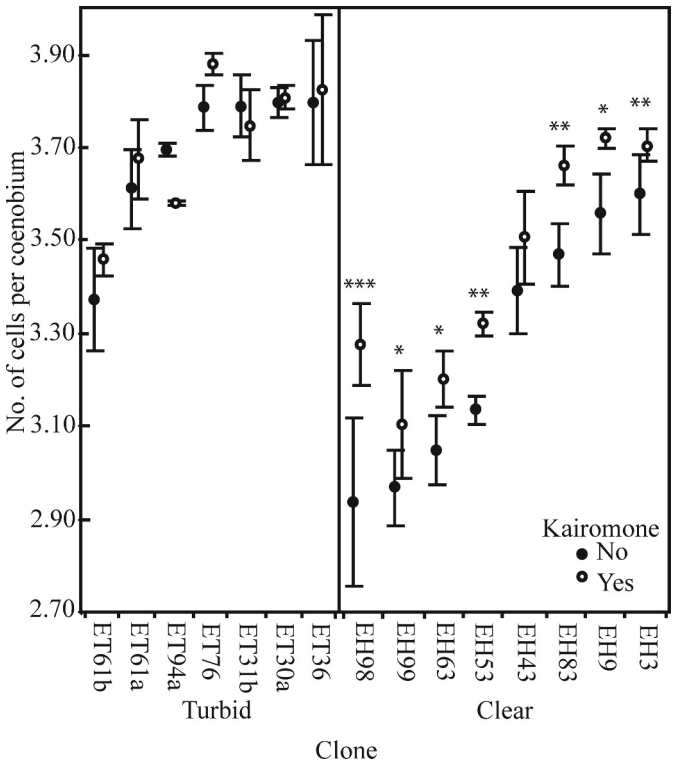

Fig. 2. Number of cells per coenobium (average ± SD) of the *D. armatus* clones from the turbid and clear-water pond, respectively. Within each population, the clones are ordered according to increasing values of the number of cells per coenobium in the absence of *Daphnia* kairomone. The significance of the response to *Daphnia* kairomone was evaluated for each clone by an LSD test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

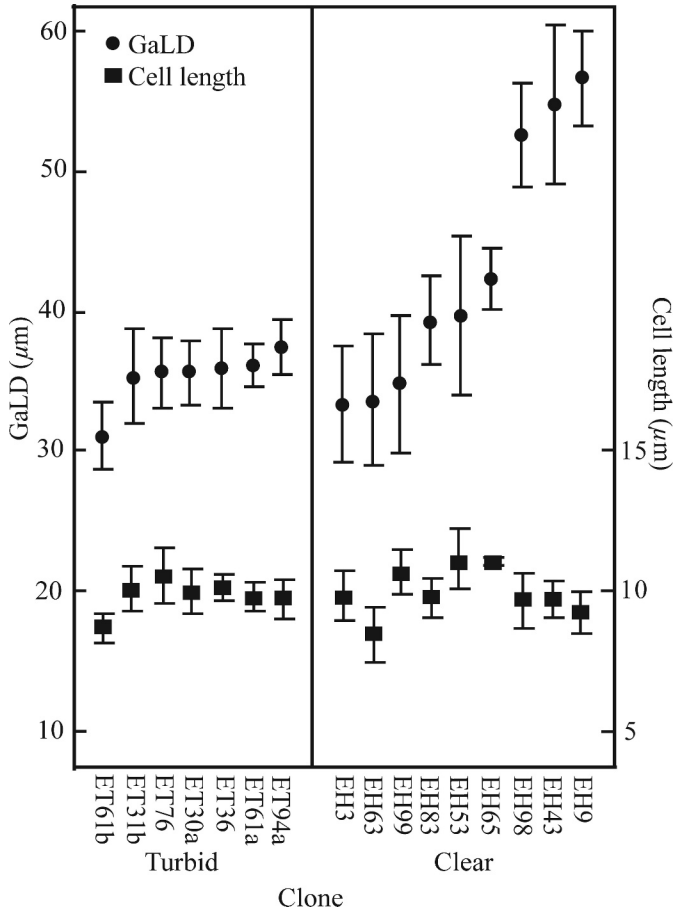

Fig. 3. Greatest axial linear dimension and cell length (average ± SD) of the clones from the turbid and the clear-water pond in the absence of *Daphnia* kairomone.

Table 2. Extended.

EH98	EH99	EH63	EH53	EH43	EH83	EH9	EH3	EH65
ns/ns/ns/ns/***								
ns/ns/ns/*/***	ns/ns/ns/***/ns							
ns/ns/ns/**/***	ns/ns/ns/ns/ns	ns/ns/ns/***/*						
***/*/ns/ns/ns	***/***/ns/ns/***	**/**/ns/*/***	ns/ns/ns/**/***					
//ns/ns/***	***/***/ns/ns/ns	***/***/ns/*/*	**/***/ns/**/ns	ns/ns/ns/ns/***				
//ns/ns/ns	***/***/ns/**/***	***/***/ns/ns/***	***/***/ns/***/***	ns/ns/ns/ns/ns	ns/ns/ns/ns/***			
//ns/ns/***	***/***/ns/ns/ns	***/***/ns/**/ns	***/***/ns/*/*	ns/ns/ns/ns/***	ns/ns/ns/ns/*	ns/ns/ns/ns/***		
nd/nd/nd/**/***	nd/nd/nd/ns/***	nd/nd/nd/***/***	nd/nd/nd/ns/ns	nd/nd/nd/**/***	nd/nd/nd/*/ns	nd/nd/nd/***/***	nd/nd/nd/*/***	

characters between *D. armatus* and two other species with *D. armatus*–like coenobia, *D. setifera* and *D. longispina* (Hindák 1990). Whereas the spines of the former are approximately as long as the cell length, the spines of the latter are 1–1.5 and 2 (up to 3) times as long, respectively. Our observation that clones with identical ITS2 sequences show large divergence in spine shape and length (Fig. 4) suggests that the variation in spine length and shape used to characterize *D. setifera* and *D. longispina* may rather concern intraspecific morphological variation.

Whereas we observed only three different ITS2 types, the pairwise comparisons between the clones for the measured traits suggest the presence of at least nine genotypes (each having a significant pairwise difference with the other genotypes for at least one trait; see Table 2) among the 16 clones tested, including seven genotypes out of nine clones for the clear-water pond and three out of seven from the turbid pond. Only one or two of these genotypes were found in both pond populations. This suggests that the *D. armatus* populations consist of a relatively large number of genotypes that may differ in their ecological characteristics. This contrasts with the classically held view that microalgal species show a strong clonal population structure due to their predominant asexual mode of reproduction. Theoretical work has shown, though, that a small number of sexual individuals per generation is sufficient to make an apparently asexual population highly genotypically variable (Bengtsson 2003). This is confirmed by the high genotypic diversity recently found in diatom (Rynearson et al. 2006) and dinophyte populations (Hayhome et al. 1987), although at least some ciliates indeed seem to have a clonal population structure (Kusch 1998). Unfortunately, the frequency of sexual reproduction in *D. armatus* is unknown, although the mating system is probably heterothallic (Trainor 1998), and gamete formation in the genus has been suggested to be a seasonal phenomenon (Lürling 2003). Species showing an alternation of sexual and asexual reproduction have a high potential for local adaptation since novel gene combinations are made while at the same time beneficial gene combinations are not broken up each generation (De Meester 1996).

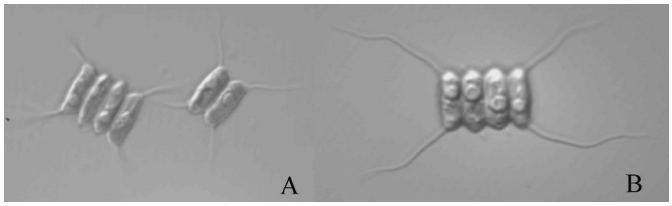

Fig. 4. Coenobia of clones ET31b (A) and EH98 (B), showing typical spines and the long spines found in several clones from the clear-water pond.

The capacity of a local population to show a genetic response for any trait is critically dependent on the heritability of the trait. Populations having a high genotypic diversity and low pure phenotypic (not genetically based) variation potentially have a faster and more efficient selection response. Genetically based variation within the *D. armatus* populations studied here with respect to grazing avoidance traits was substantial and highly significant, with broad-sense heritabilities of 0.34–0.92, except for the response to *Daphnia* kairomone (heritabilities 0.30 and 0.39). These values are similar to or higher than the average heritability values of 0.3–0.5 found for life history, morphological, and behavioral traits (often related to predator avoidance) in populations of the cyclical parthenogen *Daphnia*, although values from single populations can vary widely (De Meester 1993; Spitze 1995; Cousyn et al. 2001).

Extensive and significant genetically based population differentiation was observed between the *D. armatus* populations for all investigated traits (Table 1) with Q_{ST} values ranging from 0.18 to 0.51, except for cell length, which was not correlated with the ponds (Q_{ST} value of 0.034). These Q_{ST} values are in the same range as found between *Daphnia* populations strongly differentiated for fish avoidance behavior (Q_{ST} values of 0.19–0.20; Cousyn et al. 2001) or body size (average $Q_{ST} = 0.40$; Spitze 1993), a trait strongly related to predation resistance. The genetic differ-

Table 3. Broad-sense heritability estimates as determined by a clonal repeatability analysis and Q_{ST} values for antipredation traits of *Desmodesmus armatus* from a clear-water and a turbid pond; *p*-values are given between brackets and were obtained from the ANOVAs used to estimate the heritabilities. GaLD, greatest axial linear dimension.

	Broad-sense heritability			
	Clear-water	Turbid	Average	Q_{ST}
Number of cells − kairomone	0.88 ($p<0.001$)	0.78 ($p<0.001$)	0.83	0.45
Number of cells + kairomone	0.92 ($p<0.001$)	0.80 ($p<0.001$)	0.86	0.27
Response to kairomone	0.30 ($p=0.079$)	0.39 ($p=0.045$)	0.35	0.51
Cell length	0.51 ($p<0.001$)	0.34 ($p<0.001$)	0.43	0.034
GaLD	0.82 ($p<0.001$)	0.34 ($p<0.001$)	0.58	0.18

entiation found between the *D. armatus* populations is largely in accordance with the expectations under different zooplankton grazing regimes. Clones from the clear-water pond were sensitive to the *Daphnia* kairomone in contrast to clones from the turbid pond, and their four-celled coenobia, the dominant morphotype, had on average a higher GaLD. The long spines on the coenobia of some of the clones should not only protect them from being eaten but also reduce their sinking velocity. The higher number of cells per coenobium for clones of the turbid pond in the experimental conditions was not predicted and suggests that other factors besides grazing might also be important. For instance, the risk of sinking out of the euphotic zone (the main cost to being colonial; Lürling and Van Donk 2000) is less of a problem in turbid ponds because of the higher turbulence, as the effect of wind on the water column is strongly reduced by vegetation (Van den Berg et al. 1998). Overall, the congruence of the observed genetic differences with the expected differences based on the selection pressures in both habitats and the presence of sufficient intrapopulation genetic variation to allow rapid microevolution strongly suggest that local genetic adaptation to the conditions in the respective habitats is involved (De Meester 1996). The alternative explanations of drift or persistent founder effects are very unlikely in this strongly connected system, characterized by continuous dispersal through overflows and rivulets.

Gene flow can counteract divergent selection and prevent local adaptation (Slatkin 1987). Despite the high connectivity between the ponds in our study system, which are all connected through overflows and rivulets, extensive genetic differentiation was found for grazing avoidance traits among the *D. armatus* populations inhabiting ponds that differ in ecological characteristics. This indicates that divergent selective pressures are very strong. The pattern observed by us parallels the results found for zooplankton and phytoplankton communities in the same pond system that have been shown to be structured primarily by local environmental factors with almost no signature of dispersal limitation or of mass effects (Cottenie et al. 2003; Vanormelingen et al. 2008).

In conclusion, in the present study a green microalga was shown to exhibit among-population genetic differences in mean phenotype for quantitative traits that are related to differential grazing pressure by zooplankton. Moreover, considerable within-population genetic variation was present for each of the traits studied, except for the response to kairomone. The fact that not all traits showed the expected among-population differences suggests that other factors than grazing might also be important, as genetic drift is highly unlikely in this pond system. Additional studies on local adaptation to different selection pressures in other ponds and with other *Scenedesmus s. l.* species or other phytoplankton should therefore be conducted to broaden our understanding of the spatiotemporal scale of local adaptation of phytoplankton populations and the traits and selection pressures involved. In any case, our results suggest striking local genetic adaptation in a system in which potential gene flow is very high. We conclude that grazing by zooplankton, itself a function of the trophic structure of the system (Scheffer 1998), exerts a sufficiently strong selection pressure on the local phytoplankton populations so as to lead to local genetic adaptation in the face of massive dispersal potential. Our results therefore also emphasize the importance of divergent natural selection in structuring local phytoplankton populations.

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