Local adaptation among geographically distant clones of the cosmopolitan freshwater ciliate *Meseres corlissi.* II. Response to pH

Thomas Weisse[1],*, Ulrike Scheffel[1], Peter Stadler[1], Wilhelm Foissner[2]

[1]Institute for Limnology of the Austrian Academy of Sciences, Mondseestrasse 9, 5310 Mondsee, Austria
[2]University of Salzburg, Department of Organismic Biology, Hellbrunnerstrasse 34, 5020 Salzburg, Austria

ABSTRACT: We investigated the pH response of 5 clones of the oligotrichine ciliate *Meseres corlissi* originating from 2 temperate localities in Austria, from a subtropical habitat in China, and from a warm-temperate habitat in Australia. The pH reaction norm was investigated under standard laboratory conditions, with the small cryptophyte *Cryptomonas* sp. provided as food at saturating food levels over the pH range 4.0 to 9.5. Experiments were conducted at 22.5°C and lasted for 24 h. We measured growth rate, cell length and volume, and cellular production at each pH. We found significant clone-specific differences in each measured parameter, which increased with geographical distance of the isolates. Overall, the pH reaction norms of the Austrian isolates were significantly different from those of the Australian and Chinese clones. The tolerance to low pH differed by up to 1.5 pH units between the clones, i.e. intraspecific differences were comparable with interspecific differences measured earlier under similar experimental conditions. Our results suggest that the pH reaction norm is not homogenous for the species, but that genetically and phenotypically different ecotypes may exist among free-living, cosmopolitan ciliates that are adapted to a particular habitat.

KEY WORDS: Local adaptation · Oligotrich ciliates · pH response · Intraspecific differences · *Meseres corlissi*

INTRODUCTION

Free-living ciliates may exhibit extensive intraspecific differences within a morphologically defined species (Nanney 1999, 2005), and the extent of intraspecific ecophysiological differences recorded for aquatic ciliates may reach or exceed interspecific or even intergeneric differences (Pérez-Uz 1995, Weisse 2002, Weisse & Rammer 2006). Previous work on intraspecific differences among widely distributed aquatic heterotrophic protists has focused on temperature and salinity adaptation (Weisse & Montagnes 1998, Weisse et al. 2001, Lowe et al. 2005a,b, Weisse & Rammer 2006). Recently, clonal differences in the temperature response and adaptation to the habitat temperature were demonstrated for the oligotrich ciliate *Meseres corlissi* Petz & Foissner 1992 (Gächter &

Weisse 2006). This ciliate has become a model organism for investigating local ecophysiological adaptation among cosmopolitan but rare freshwater ciliates (Weisse 2004, 2006, Müller et al. 2006).

Other obvious intraspecific differences in the life cycle of this species have been identified with respect to the factors that trigger its encystment and excystment (Müller et al. 2006). Müller et al's (2006) study showed that the presence of soil or soil extract may be essential for sustained optimal growth and completion of the life cycle of at least some Austrian *Meseres corlissi* clones. The freely swimming, trophic cells of *M. corlissi* reach growth and feeding rates that are among the highest hitherto recorded for aquatic ciliates (Weisse 2004, 2006). However, *Meseres* is not a typical planktonic species, but thrives in ephemeral aquatic habitats such as river flood plains (Gächter & Weisse

2006) and the reservoirs of tree bromeliids (Weisse 2004, Foissner et al. 2005). The benthic phase, with its characteristic resting cyst (Foissner 2005, Foissner et al. 2005, 2006, Foissner & Pichler 2006), is an important part of the life cycle of *M. corlissi*. We extend previous research by investigating the response of *M. corlissi* to changing pH, another environmental factor that may limit the distribution of ciliates in freshwater (Weisse & Stadler 2006) and soil (Foissner et al. 2002).

The effect of pH on the growth and survival of freshwater ciliates has received little attention until recently (Weisse & Stadler 2006). Weisse & Stadler's (2006) investigation revealed pH niche separation among 3 species of the common prostome freshwater genus *Urotricha*. The primary goal of the present study was to assess, for the first time, the extent of intraspecific differences in the pH tolerance of a freshwater ciliate, using *Meseres corlissi*. Our null hypothesis was that there would be no significant differences in the pH response of *M. corlissi* clones under standard experimental conditions. Because we found significant differences in the pH response of geographically distant clones, we subsequently provided further evidence for the significance of genotypic and phenotypic differences (ecotypes, Turesson 1922) among cosmopolitan, free-living ciliates.

MATERIALS AND METHODS

Origin of the cultures and clones. The geographic origin of the 5 *Meseres corlissi* clones used in this study and the procedure of their isolation were described in detail by Gächter & Weisse (2006). We used the simplified codes suggested by these authors for the clone designation in the following text; the original clone designations are reported in parentheses. Briefly, the clones AU3 (M10) and AU5 (E4; isolated by H. Müller) were sampled at the type locality in Salzburg, Austria, at different times. Clone AU6 (KMP1) originated from a meadow pond near the town of Kefermarkt, Upper Austria, located approximately 200 km NE of the type locality. Clone AUS (AUA4) was isolated from a warmtemperate environment in SE Australia, and clone CHI (CH4W4) from subtropical SE China. The ciliates were isolated from air-dried soil samples that were swamped with distilled water in the laboratory (Foissner et al. 2002), i.e. our cultures were obtained from excysted cells. All clones used in this study exhibited minor divergence in their small ribosomal subunit RNA sequences (SSU rRNA) and their internal transcribed spacer regions (ITS, M. Strüder-Kypke et al. unpubl. data), and were morphologically almost identical (Foissner 2005, Foissner et al. 2005), suggesting that they were the same species.

Stock clonal cultures of *Meseres corlissi* were fed with the small alga *Cryptomonas* sp. Strain 26.80 (Culture Collection of Algae [SAG], Göttingen) or Strain 979/44 (UK National Culture Collection of Algae and Protozoa [CCAP], Windermere) and kept in modified Woods Hole Medium (MWC; UKNCC 2001) as described previously (Weisse 2004, Weisse & Stadler 2006). In contrast to previous ecophysiological investigations with *M. corlissi*, all strains used in this study were maintained at a temperature of 22.5°C.

pH measurements. pH was measured using a microprocessor pH-mV meter (WTW, model pH 526) to the nearest 0.01 unit. The pH sensor was 2-point calibrated with standard buffer solutions of pH = 6.87 and pH = 9.18 before each series of measurement. The pH-mV meter was also used to measure pH at the type locality in Salzburg and at the site close to Kefermarkt, Upper Austria. pH in the soil samples was measured colourmetrically in water according to ÖNORM L 1083 (2006).

Experimental design. *Meseres corlissi* was taken from exponentially growing cultures containing *Cryptomonas* sp. in MWC medium and Volvic table water. Ciliates and their prey were acclimated to the experimental conditions, which ranged from pH 4.0 to 9.5, in steps of 0.5 pH unit change d^{-1} for 2 to 5 d. With 1 exception, pH levels of <4.0 were not tested because *Cryptomonas* spp. do not tolerate such highly acidic conditions for more than a few hours (Weisse & Stadler 2006).

After the acclimation period, 4 wells of 12-well tissue plates were filled with 4 ml each of the food suspension containing *Cryptomonas* sp. at saturating concentrations in a cocktail composed of MWC medium, Eau de Volvic, and soil extract (SE, 5 to 10% by vol.). The SE was prepared from a commercial garden soil (Qualitäts-Pflanzenerde, SagaFlor) as described by Müller et al. (2006). The pH of the suspension was adjusted by addition of small amounts of 0.1 mol l^{-1} NaOH or HCl. Ten ciliates each were pipetted into the first 3 wells of each tissue plate under a dissecting microscope at the beginning of the experiments, while the 4th well served as control without ciliates in order to monitor the pH response of the food algae in the absence of ciliates. Experiments were conducted at 22.5°C and lasted for 24 h.

Ciliate cell numbers were counted under a dissecting microscope and pH was measured in each well 6, 12, and 24 h after the beginning of the experiment. Additionally, pH was measured in the controls 0 and 3 h after the beginning of the experiment. Since pH shifted with time, the ciliates were transferred into new wells containing fresh food suspension that was adjusted to the target pH 6 and 12 h after the beginning of the experiment. This procedure allowed us to

maintain the pH within ±0.2 units of the target value in the course of the experiments. A preliminary test that compared ciliate growth rates in wells with and without this transfer confirmed that the transfer does not affect the ciliate growth rate during the experiments (data not shown). The transfer was also necessary to measure the pH in each well without disturbing the ciliates, i.e. pH was measured in each well immediately after pipetting the ciliates into the new wells. Finally, the transfer enabled precise counting of the ciliates, which was otherwise difficult in rapidly growing treatments in which ciliate cell numbers more than doubled within the first 12 h. At the end of the experiments, all ciliates were pipetted out of each well and fixed with acid Lugol's solution (2% vol/vol final concentration).

The acclimation and experiments were conducted at a light intensity of ~50 to 70 µmol photons m^{-2} under a 14:10 h light:dark cycle. Ciliate growth rate (µ, d^{-1}) was calculated from initial (N_0) and final (N_t) cell concentrations measured at the beginning and end of the experiments according to: $\mu = (\ln N_t - \ln N_0)t^{-1}$, where t is the experimental duration (1 d). We did not estimate µ from a linear regression of lnN vs. time because if µ was low (either positive or negative), then the regression lnN vs. time was insignificant in several cases. The average pH in each experimental treatment was estimated from the logarithms of the reciprocals of the concentration of free hydrogen ions measured 0, 3, 6, 12, and 24 h after the beginning of the experiment, i.e. from log pH, and subsequent power transformation.

Additional growth experiments were conducted at pH <6 in the presence of 5 to 10% sterilized peat extract (PE) under otherwise identical experimental conditions. The higher percentage of PE was applied at the lowest pH tested for each clone. The PE was prepared according to the recommendations of the SAG at the University of Göttingen (see www.epsag.uni-goettingen.de/html/culturemedia.html#BM7).

Cell size. Cell length (*L*) and volume (*V*) of ciliate cells were determined from Lugol's fixed material using an inverted microscope and a semi-automatic image analysis system (LUCIA version 4.51, Laboratory Imaging). Subsamples from the 3 wells with the same target pH were pooled, and the length and width of 50 ciliates were measured per treatment. Ciliate cell volume was calculated assuming a prolate spheroid shape with circular cross-section (Weisse 2004). Cell volume was multiplied by µ to calculate cellular production rate.

Statistical analyses. Our attempts to analyze the µ vs. pH curves by predictive models failed because the data shown in Fig. 1 deviated from a normal distribution and any of the other common probability distributions (e.g. Poisson, binomial or negative binomial)

according to chi-square and Kolmogorov-Smirnov (K-S) goodness-of-fit tests. Further, none of the common transform functions yielded data that were normally distributed. Accordingly, we used a Kruskal-Wallis test on ranks and Dunn's post-hoc test to test for overall differences in the µ vs. pH response of the 5 clones. The data from the 3 experiments that yielded the highest growth rate (μ_{max}) for each clone (see Table 1), and the respective pH at μ_{max}, did not deviate from a normal distribution and were compared by 1-way ANOVA and Tukey's post-hoc test. This analysis was also used to identify significant differences in cell size (*L* and *V*) of the clones over the entire pH range (see Table 2). Results were considered significant if p < 0.05, unless stated otherwise.

Predictive models were fitted to the *L* vs. pH data to examine responses to pH for each *Meseres corlissi* clone and to compare the clones. The only equation that yielded significant fits for all *L* (=*y*) vs. pH (=*x*) curves was a 3-parameter Gaussian peak equation of the form

$$ y = ae^{[-0.5(x - x_0/b)^2]} \tag{1} $$

where *a* and *b* are constants. The resulting non-linear regression curves were pair-wise subtracted from each other and the residuals fitted by least-squares linear regressions. The slope of these regressions were then tested for significant differences from zero (Zar 1984, Glantz 1997). If significance from zero was observed, then the *L* vs. pH responses of the respective 2 clones were significantly different. We chose p < 0.001 as α-level for these analyses because we made a Bonferroni correction to compensate for performing 5 tests with each data point. The same statistical procedure was applied to test for significant differences between the production rate and pH response of the clones.

The statistical analyses were performed with Sigma-Stat for Windows (version 2.03, SPSS) and Statgraphics Plus (version 4.0, Manugistics).

RESULTS

Growth rates vs. pH

Population growth rates (µ) vs. pH of the 5 isolates of *Meseres corlissi* differed on a clone-specific basis (Fig. 1). While there was no obvious difference at the highest pH tolerated, the Australian clone AUS (Fig. 1d) was more tolerant to low pH (<5) than was any other clone. At pH 4.4, the lower pH tolerance limit of the algae provided as food (Weisse & Stadler 2006), cells of this clone divided more than once per day. The ciliates were actively swimming even at pH ~4.0, but the cell morphology changed when pH was <5. The

cells appeared more ellipsoidal than at higher pH, and developed a distinct tail (cf. Foissner et al. 2005, their Fig. 4). At pH 3.5, the ciliates formed cysts immediately after the beginning of the experiment; 21 of the initial 30 ciliates were encysted after 6 h. The cysts were clearly visible at the bottom of the wells of the culture plates. After 24 h, we removed the acid medium,
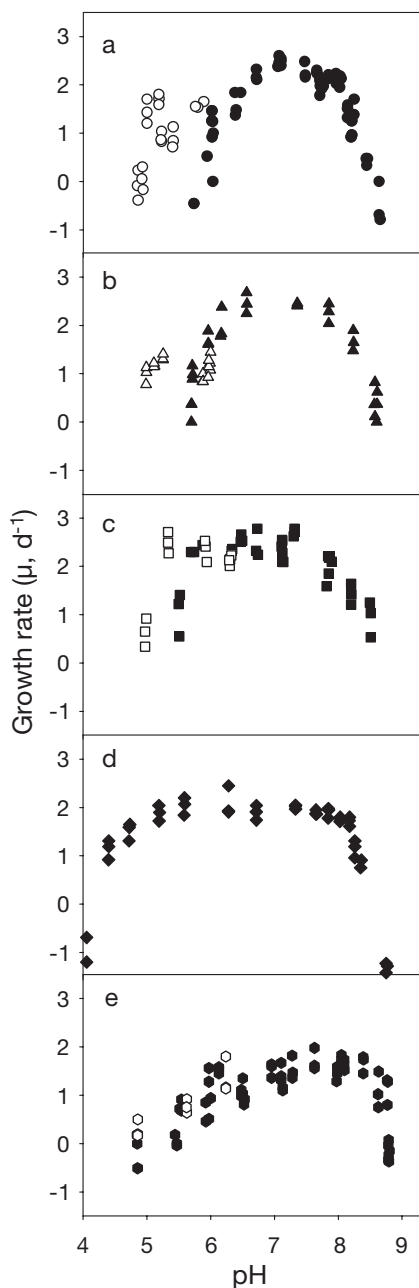
refilled the wells with a fresh *Cryptomonas* spp. suspension of pH 7.9 containing 5% SE, and kept the treatment under the same temperature and light conditions as used in the experiments, in order to test the viability of the cysts. We did not find any trophic cells in these wells over a period of 2 wk. At pH 4.06, only 2 out of the 30 initial cells were encysted after 24 h. Similar observations were made with the Chinese clone CHI, where ~50% of the population formed non-viable cysts at pH 4.5 and 4.0. We did not monitor the formation of cysts at low pH in the Austrian clones.

The lower pH tolerance limit of the other 4 clones ranged from 5.1 to 5.9 under the standard experimental conditions (Table 1). With the addition of 5 to 10% PE to the medium, the tolerance limit of the Austrian clones (Fig. 1a–c) was shifted by 0.6 to 0.9 pH units. The beneficial effect of PE at low pH was less obvious with the Chinese clone (Fig. 1e). We did not try to extend the pH tolerance limit of clone AUS (Fig. 1d) to even more acidic conditions by the addition of PE because the food algae became immobile at pH ~4.0 and rapidly sedimented in the experimental wells. The presence of PE did not affect μ of the Austrian and the Chinese clones in the moderately acidic range.

It was difficult to test for significant differences in the overall growth response of the clones to pH. Mainly owing to the relatively broad plateau close to the maximum growth rates (μ_{max}), where μ was almost constant over ~2 pH units, the data deviated from the normal distribution (K-S test), and none of the common transform functions yielded data that were normally distributed. Our attempts to fit the curves by predictive models failed: even 4th- and 5th-order polynomial equations did not fit the curves adequately. A Kruskal-Wallis test on ranks and Dunn's post-hoc test yielded that clone AU6 (Fig. 1c) was significantly different from all other clones. If the pH was averaged over the 3 experiments that yielded μ_{max}, then clone AUS dif-

Fig. 1. *Meseres corlissi*. Growth rate (μ) of 5 clones vs. experimental pH. (a) AU3 (Salzburg, Austria); (b) AU5 (Salzburg, Austria); (c) AU6 (Kefermarkt, Austria); (d) AUS (Australia); (e) CHI (China). Filled symbols: experiments under standard conditions without peat extract (PE); open symbols: additional experiments with 5 to 10% PE

Table 1. *Meseres corlissi*. Range of pH tolerance (i.e. where $\mu \geq 0$), pH at highest growth rates (μ_{max}), and highest growth rates measured over the pH range under standard experimental conditions, i.e. with addition of soil extract (SE) but without peat extract (PE). Latter values are mean ± 1 SD. Significant differences in **bold**; μ_{max} of AUS was significantly higher than that of CHI and significantly lower than those of AU3, AU5 and AU6

Clone	Range of pH tolerance	pH at μ_{max}	μ_{max} (d^{-1})
AU3	5.9–8.6	7.09 ± 0.02	2.56 ± 0.04
AU5	5.7–8.6	7.26 ± 0.65	2.53 ± 0.13
AU6	5.5–8.6	7.12 ± 0.34	2.76 ± 0.04
AUS	<4.4–8.6	**5.82** ± 0.40	**2.24** ± 0.19
CHI	5.1–8.7	7.65 ± 0.39	**1.88** ± 0.09

fered significantly (1-way ANOVA, Tukey's post hoc test) from all other clones (Table 1). Similarly, μ_{max} of AUS was significantly higher than that of the Chinese clone (CHI) and significantly lower than μ_{max} of the 3 Austrian clones (Table 1). The seeming difference between the Austrian clones AU6 and AU5 was insignificant (p = 0.171).
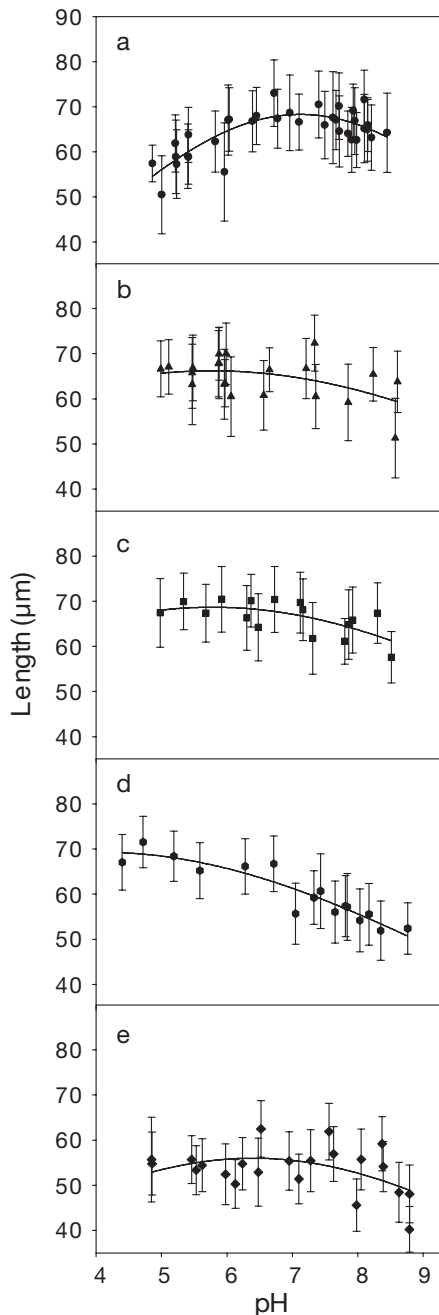

Fig. 2. *Meseres corlissi*. Cell length (*L*, mean ± 1 SD) of 5 clones vs. pH. (a) AU3 (Salzburg, Austria); (b) AU5 (Salzburg, Austria); (c) AU6 (Kefermarkt, Austria); (d) AUS (Australia); (e) CHI (China). Solid lines represent fit of a 3-parameter Gaussian peak equation (see 'Materials and methods')

pH effect on cell size

In contrast to the μ vs. pH response, the data representing the pH effect on cell size did not deviate from a normal distribution and could be fitted by a predictive model, a 3-parameter Gaussian peak distribution (Fig. 2). The non-linear regression yielded a significant result in each case, with R^2 ranging from 0.24 (Fig. 2b) to 0.87 (Fig. 2d). Cell length (*L*) and biovolume (*V*) were highly significantly correlated in each case; accordingly, the *V* vs. pH curves (data not shown) looked similar to the *L* vs. pH curves reported in Fig. 2. The shapes of the curves shown in Fig. 2a & 2d are obviously different, and the Chinese clone (Fig. 2e) was significantly smaller (1-way ANOVA, Tukey's post hoc test) than all other clones (Table 2). The statistical test (see 'Materials and methods' for details) revealed that the length response to pH of the clones AU3 (Fig. 2a) and AUS (Fig. 2d) was significantly different from that of all other clones, and that the Chinese clone was the only one that differed from less than at least 3 other clones (Table 3).

Cell lengths and growth rates were positively and linearly related to each other in 3 out of the 5 clones investigated (Fig. 3). The correlation was highest in the Australian clone, where changes in μ explained 52% of the variance in *L* (Fig. 3d). The *L* and μ were uncorrelated in 2 of the 3 Austrian clones (Fig. 3b,c). If all data were pooled, then the linear regression was highly significant, but the coefficient of determination was relatively low (R^2 = 0.155, n = 103, p < 0.001).

Table 2. *Meseres corlissi*. Mean cell length (L_{avg}) and biovolume (V_{avg}) ± 1 SD of 5 clones. Results were averaged over the pH range tested with the addition of SE but without PE. The Chinese clone (CHI, **bold**) differed significantly from all other clones

Clone	L_{avg} (μm)	V_{avg} (μm^3)
AU3	64.44 ± 4.82	88766 ± 15362
AU5	64.31 ± 4.89	97412 ± 14974
AU6	66.46 ± 362	102543 ± 15063
AUS	62.49 ± 6.12	86110 ± 23585
CHI	**53.57** ± 5.156	**52295** ± 12018

Table 3. *Meseres corlissi*. Differences in cell length (*L*) vs. pH response of 5 clones. Results were obtained by pair-wise tests of the slopes of regression lines of *L* vs. pH curve (see 'Materials and methods' for details); ***significance at p < 0.001

Clone	AU3	AU6	AU5	CHI	AUS
AU3	–	***	***	***	***
AU6	***	–	***	ns	***
AU5	***	***	–	ns	***
CHI	***	ns	ns	–	***
AUS	***	***	***	***	–

Cellular production vs. pH

Similar to the L vs. pH response, cellular production (CP, the product of μ and V) vs. pH curves were fitted by a 3-parameter Gaussian peak distribution (Fig. 4). There was no difference between the maximum production (CP_{max}) and overall shape of the CP vs. pH curves of 2 clones isolated from the type locality in
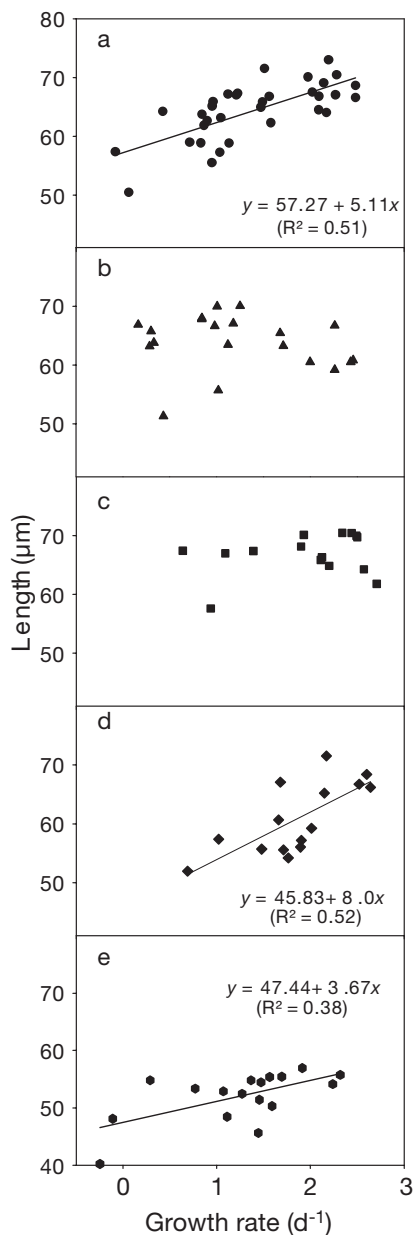

Fig. 3. *Meseres corlissi*. Cell length (L) vs. growth rate (μ) of 5 clones. (a) AU3 (Salzburg, Austria); (b) AU5 (Salzburg, Austria); (c) AU6 (Kefermarkt, Austria); (d) AUS (Australia); (e) CHI (China). Regression line and equation indicated when the correlation yielded a significant least-squares linear regression (a,d,e)
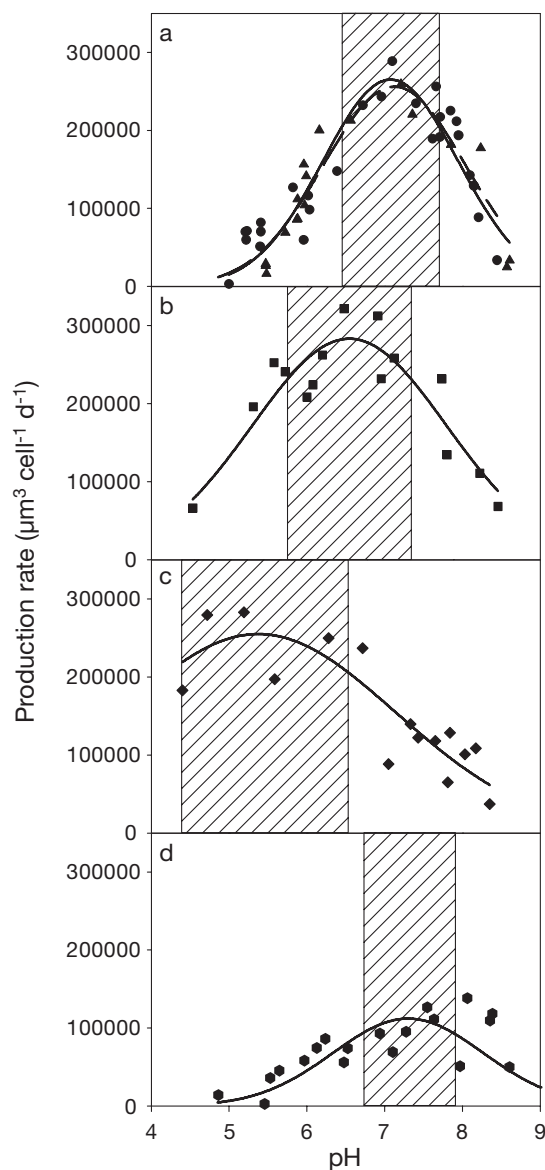

Fig. 4. *Meseres corlissi*. Cellular production (CP) rate of 5 clones vs. pH. (a) AU3 (●) and AU5 (▲); (b) AU6 (■); (c) AUS (♦); (d) CHI (●). Solid lines represent fit of a 3-parameter Gaussian peak equation (see 'Materials and methods'); hatched areas indicate pH optimum at which production reached >80% of the maximum

Salzburg (Fig. 4a). The slopes of the linear regressions of the residuals of the CP vs. pH curves of all other pair-wise clonal combinations were significantly different from zero ($p < 0.001$), i.e. the CP vs. pH response of these clones differed on a clone-specific basis. The CP_{max} of the Chinese clone (Fig. 4d) reached less than 50% of that of all other clones. The hatched areas in Fig. 4 indicate the pH range where CP was >80% of CP_{max}, i.e. the pH optimum of CP. Thus defined, the pH optimum was shifted between the Austrian clones iso-

lated from Salzburg (Fig. 4a) and from Upper Austria (Fig. 4b), and did not overlap between the Australian (Fig. 4c) and the Chinese clone (Fig. 4d).

DISCUSSION

pH response of *Meseres corlissi*

The general growth response of *Meseres corlissi* to pH confirmed results from a recent study of 3 prostome ciliates under comparable experimental conditions (Weisse & Stadler 2006). In both studies, the pH effect was minor in the circumneutral range, but μ declined sharply at pH extremes, both under acidic and alkaline conditions. If all clones were combined, the pH tolerance of *M. corlissi* ranged from <4.4 to 8.7, i.e. *M. corlissi* is the second-most tolerant of the 4 freshwater species investigated thus far. Our results also confirm the earlier conjecture that CP may be best suited to characterize the pH optimum of a clone or species (Weisse & Stadler 2006), because it combines the pH effect on μ and cell size. Furthermore, CP is an important parameter that determines the role of a given species in the aquatic food web with respect to the food that is made available to its predators.

We conducted our experiments under near-to-optimum conditions with respect to food supply and temperature. However, as already noted by Weisse & Stadler (2006), laboratory studies such as the present one cannot adequately describe the fundamental pH niche of a ciliate species because with other food, media, or a combination of several direct and indirect factors, the pH niche of the clones and species may be expanded. This became obvious when we added PE to the medium, which enhanced the growth performance of most clones at low pH (Fig. 1). We assume that the effect of PE on μ of *Meseres corlissi* was rather indirect, mediated by affecting the solubility, bioavailability, and toxicity of ammonium/ammonia, iron, aluminum, and some other heavy metals to the ciliates. The stock solution of our PE had a pH of ~3.7. We conclude that in the presence of PE or other organic acids, the trophic cells of most *M. corlissi* clones may survive in habitats with pH of ≤5.

Clone-specific effects of pH

It was not our goal to analyze the fundamental pH niche of *Meseres corlissi*, which is impossible in principle. Our null hypothesis was that, after careful acclimation, we would not measure any significant differences in the pH responses of the *M. corlissi* clones. However, because we found unequivocal intraspecific differences in the pH responses of this aquatic ciliate,

we can reject our null hypothesis. We reported clone-specific differences for μ, cell size (*L* and *V*), the relationship between μ and *L*, and CP rates. The Australian clone was more tolerant to low pH than was any other clone (Table 1), was largest at low pH (Fig. 2d), and reached its CP_{max} at pH ~5.3 (Fig. 4c), i.e. at a pH that was 1 to 2 units lower than that at the CP_{max} of other clones.

In the Australian and 2 other clones (AU3 and CHI) we observed a significant positive relation between cell size (*L* and *V*) and μ (Fig. 3a,d,e). We assume that this is because high μ is reached at near-maximum ingestion rates in *Meseres corlissi* and other aquatic ciliates (Müller & Schlegel 1999, Weisse et al. 2001, Weisse 2004). Accordingly, since the food vacuoles are more heavily filled and/or more food vacuoles and other cell organelles are formed, *V* also increases asymptotically with increasing food (Jakobsen & Hansen 1997, Montagnes & Lessard 1999). However, 2 of the 5 clones did not follow this rule. The general positive relationship between μ and cell size may be offset if the ciliates divide at smaller cell size under pH stress, similar to cell division triggered by starvation (Fenchel 1987). The comparison between the 2 clones from the type locality suggests that different clones may use a different strategy to optimize their CP, which was identical between these 2 clones: AU3 was smaller but reached higher μ than AU5 at pH <6, thus compensating for its reduced size. To speed up cell division (and thus increase μ) under conditions of environmental stress may be an evolutionary successful strategy that leads to an increased fitness, if there are no other size-related effects. The advantage would be offset if, for instance, smaller cells fall easier victim to predators, or die off earlier owing to starvation. We can only speculate about the possible reasons for the observed differences, but interpret our findings as part of the phenotypic plasticity of *M. corlissi*.

Magnitude of intraspecific variation

The relative differences in the temperature and feeding response of freshwater ciliates may be comparable with interspecific differences (Weisse et al. 2001, Weisse 2002, Weisse & Rammer 2006). The minimum temperature tolerated by 8 clones of *Meseres corlissi* and the temperature of their $μ_{max}$ differed by up to ~5°C. The average cell volume of the clones differed by a factor of 2 (Gächter & Weisse 2006). We found no apparent relationship between the pH in the soil from which the clones were isolated (Table 4) and their pH tolerance. The pH in soil, however, is more important for the survival of the resting cysts than for the trophic cells of *M. corlissi*, and it is obvious from Table 4 that

Table 4. pH in soil samples (pH$_s$) from which *Meseres corlissi* clones were isolated (first 5 rows) and from 3 other localities where the ciliate has been recorded (last 3 rows), and pH in the water (pH$_w$) of meadow ponds in Austrian localities measured on 2 occasions in spring and summer. See text for further explanation

Origin	pH$_s$	pH$_w$	Date	Source
Austria (Salzburg)	6.0	6.69–7.53	March 2005	This study
Austria (Salzburg)	5.3	6.30–6.82	July 2005	This study
Austria (Kefermarkt)	5.3	7.30–9.96	March 2005	This study
Australia	5.8	nd	March 2006	This study
China	5.1	nd	November 2005	This study
Brazil	5.4	nd	October 2005	W. Foissner (unpubl. data)
Venezuela	6.4	nd	February 1996	W. Foissner (unpubl. data)
Namibia	8.0	nd	March 1994	Foissner et al. (2002)

viable cysts were found in soil that differed by 3 pH units. The trophic cells of *M. corlissi* will experience higher pH in the alkaline range, when they excyst under food-replete conditions coupled with high primary production and, accordingly, relatively high pH. For example, in several puddles of a meadow pond at the sampling site (Kefermarkt) of clone AU6, pH ranged from 7.30 to 9.96 in spring (Table 4), with the highest values measured in the presence of dense algal populations. Small-scale variations were lower at the type locality in Salzburg, where we also observed only minor pH differences between spring and summer (Table 4). These sporadic measurements demonstrate that pH may vary seasonally by >3.5 units at a given locality. Daily pH fluctuations measured in a small water body at the type locality in the City of Salzburg in spring amounted to ≥0.3 units (E. Gächter pers. obs.). The variations of the pH in the natural aquatic environments of the *M. corlissi* isolates remain, at present, largely unknown. We speculate that natural pH fluctuations in their suitable habitats may differ on a site-specific basis and persistent, thus providing opportunities for local adaptation of the *M. corlissi* populations.

Similar to previous observations of 3 *Urotricha* species (Weisse & Stadler 2006), the differences among the *Meseres corlissi* clones in response to temperature and pH were similar. It was suggested recently that heat-shock proteins play a major role in tolerance to pH and temperature stress among aquatic protists (Gerloff-Elias et al. 2006), which may explain why eurythermal species are also tolerant to wide ranges of pH. Average μ and *V* reported in the present study were not different from the data obtained in response to temperature by Gächter & Weisse (2006), and the clones ranked similarly with respect to cell size and μ. In both studies, which were performed independently

of each other, the Chinese clone was the smallest and, on average, grew most slowly. There was no or only a minor difference between the 2 clones isolated from the type locality, while we found significant differences between the Austrian clones from Salzburg and Kefermarkt, and highly significant differences between the Austrian clones and exotic strains from China and Australia. The temperature response of *M. corlissi* was related to the habitat temperature of their origin (Gächter & Weisse 2006); however, knowledge of the pH fluctuations in the natural habitats of these strains is at present too rudimentary to allow any firm conclusions regarding the potential adaptation of the pH response to habitat conditions. We conclude that the existence of genetically different thermal ecotypes (Turesson 1922) is likely among free-living, cosmopolitan ciliates such as *M. corlissi*, and that further research on other species is needed to demonstrate the existence of ecotypes that are phenotypically adapted to a particular pH environment.

LITERATURE CITED

Fenchel T (1987) Ecology of protozoa. The biology of free-living phagotrophic protists. Science Tech, Madison, WI/Springer-Verlag, Berlin

Foissner W (2005) The unusual, Lepidosome-coated resting cyst of *Meseres corlissi* (Ciliophora: Oligotrichea): transmission electron microscopy and phylogeny. Acta Protozool 44:217–230

Foissner W, Pichler M (2006) The unusual, Lepidosome-coated resting cyst of *Meseres corlissi* (Ciliophora: Oligotrichea): genesis of four complex types of wall precursors and assemblage of the cyst wall. Acta Protozool 45: 339–366

Foissner W, Agatha S, Berger H (2002) Soil ciliates (Protozoa, Ciliophora) from Namibia (Southwest Africa), with emphasis on two contrasting environments, the Etosha region and the Namib Desert. Denisia 5:1–1459

Foissner W, Müller H, Weisse T (2005) The unusual, Lepidosome-coated resting cyst of *Meseres corlissi* (Ciliophora: Oligotrichea): light and scanning electron microscopy, cytochemistry. Acta Protozool 44:201–215

Foissner W, Pichler M, Al-Rasheid K, Weisse T (2006) The unusual, Lepidosome-coated resting cyst of *Meseres corlissi* (Ciliophora: Oligotrichea): Encystment and genesis and release of the lepidosomes. Acta Protozool 45: 323–338

Gächter E, Weisse T (2006) Local adaptation among geographically distant clones of the cosmopolitan freshwater ciliate *Meseres corlissi*. I. Temperature response. Aquat Microb Ecol 45:291–300

Gerloff-Elias A, Barua D, Mölich A, Spijkerman E (2006) Temperature- and pH-dependent accumulation of heat-shock proteins in the acidophilic green alga *Chlamydomonas acidophila*. FEMS Microbiol Ecol 56:345–354

Glantz SA (1997) Primer of biostatistics. McGraw-Hill, New York

Jakobsen HH, Hansen PJ (1997) Prey size selection, grazing and growth response of the small heterotrophic dinoflagellate *Gymnodinium* sp. and the ciliate *Balanion comatum*: a comparative study. Mar Ecol Prog Ser 158:75–86

Lowe CD, Day A, Kemp SJ, Montagnes DJS (2005a) There are high levels of functional and genetic diversity in *Oxyrrhis marina*. J Eukaryot Microbiol 52:250–257

Lowe CD, Kemp SJ, Montagnes DJS (2005b) An interdisciplinary approach to assess the functional diversity of free-living microscopic eukaryotes. Aquat Microb Ecol 41:67–77

Montagnes DJS, Lessard EJ (1999) Population dynamics of the marine planktonic ciliate *Strombidinopsis multiauris*: its potential to control phytoplankton blooms. Aquat Microb Ecol 20:167–181

Müller H, Schlegel A (1999) Responses of three freshwater planktonic ciliates with different feeding modes to cryptophyte and diatom prey. Aquat Microb Ecol 17:49–60

Müller H, Foissner W, Weisse T (2006) The role of soil in the life cycle of *Meseres corlissi* (Ciliophora: Oligotrichea): experiments with two clonal strains from the type locality, an astatic meadow pond. Aquat Microb Ecol 42:199–208

Nanney DL (1999) When is a rose? The kinds of *Tetrahymena*. In: Wilson RA (ed) Species: new interdisciplinary essays. MIT Press, Cambridge, MA, p 93–118

Nanney DL (2005) The systematics and biogeography of ciliated protozoa. SILnews 44:6–7

ÖNORM L 1083 (1989) Chemische Bodenuntersuchungen. Bestimmung der Acidität. Österreichisches Normungsinstitut, Wien

Pérez-Uz B (1995) Growth rate variability in geographically divers clones of *Uronema* (Ciliophora: Scuticociliatida). FEMS Microbiol Ecol 16:193–204

Turesson G (1922) The genotypic response of the plant species to the habitat. Hereditas 3:211–350

UKNCC (UK National Culture Collection) (2001) Catalogue of the UK National Culture Collection. List of algae and protozoa. UK National Culture Collection, Windermere and Oban

Weisse T (2002) The significance of inter- and intraspecific variation in bacterivorous and herbivorous protists. Antonie Leeuwenhoek 81:327–341

Weisse T (2004) *Meseres corlissi*: a rare oligotrich ciliate adapted to warm water and temporary habitats. Aquat Microb Ecol 37:75–83

Weisse T (2006) Freshwater ciliates as ecophysiological model organisms—lessons from *Daphnia*, major achievements, and future perspectives. Arch Hydrobiol 167:371–402

Weisse T, Montagnes DJS (1998) Effect of temperature on inter- and intraspecific isolates of *Urotricha* (Prostomatida, Ciliophora). Aquat Microb Ecol 15:285–291

Weisse T, Rammer S (2006) Pronounced ecophysiological clonal differences of two common freshwater ciliates, *Coleps spetai* (Prostomatida) and *Rimostrombidium lacustris* (Oligotrichida), challenge the morphospecies concept. J Plankton Res 27:55–63

Weisse T, Stadler P (2006) Effect of pH on growth, cell volume, and production of freshwater ciliates, and implications for their distribution. Limnol Oceanogr 51:1708–1715

Weisse T, Karstens N, Meyer VCM, Janke L, Lettner S, Teichgräber K (2001) Niche separation in common prostome freshwater ciliates: the effect of food and temperature. Aquat Microb Ecol 26:167–179

Zar JH (1984) Biostatistical analysis. Prentice-Hall, Englewood Cliffs, NJ