The meaning of protist diversity: ecology meets taxonomy*

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Abstract: Taxonomists and ecologists working with protists use different methods and different concepts to assess interspecific and intraspecific variability. The former usually study relatively few individuals in much detail and infer species-specific characteristics inductively, based upon the range of variability observed among those selected specimens. Microbial ecologists, in contrast, study populations and variations between them; species-specific and individual characteristics are inferred mainly from the variability observed at the population level. The different approaches used by taxonomists and ecologists have important implications for the assessment of biological diversity at the different levels.

Key words: Clones, ecophysiology, morphology, species.

Introduction

Morphological research has the longest tradition of all subdisciplines of protistology, dating back to the pioneering work of O.F. Müller (1730–1784), G.A. Goldfuss (1782–1848) and C.G. Ehrenberg (1795–1876) (for brief overviews see Foissner 1996a and Hausmann et al. 2003). Morphological features that were discernible with the relatively simple early light microscopes were used to characterize protist species and to classify them based upon their morphological similarity. More and more characters became visible with improved optical techniques and the development of sophisticated methods for fixing and staining of individual cells. In the mid 20th century, the morphology-based taxonomy gained new momentum when electron microscopy was applied to detect ultrastructural details that were invisible with conventional light microscopy. Electron microscopy also enabled protistologists to study the ontogenetic development of internal structures (e.g., stomatogenesis in ciliates, Foissner 1996b).

More recently, this classical, morphology-based taxonomic approach has been complemented by the application of molecular, mainly PCR-based techniques for the identification and phylogenetic classification of protist species. At the beginning of the genetic era of classification, when the methods of DNA extraction, purification and amplification were not that much refined as they are today, single-cell analysis was impossible with most protist species. Single-cell analysis is still problematic with many nanosized (<20 µm) species; in particular, if more than one gene shall be investigated. Amplification following molecular cloning of DNA fragments or clonal cultures can be used to obtain enough DNA of a given genotype. The latter approach is conceptually similar to experimental ecophysiological research with clonal protist cultures. In both cases the target signal, i.e. a gene or a physiological trait, that is too weak to be measured with the current methodology at the individual cell level, is enhanced by the use of many genetically identical copies. If clonal cultures are used, the population mean may be comparable to measuring individual traits of single cells or organisms. More often than using clonal cultures, microbial ecologists used non-clonal or undefined cultures or natural populations to measure ecophysiological key parameters such as abundance, biomass, ingestion, growth and production rates (e.g., Müller & Weisse 1994; Hansen 1995; Weisse 2004). This population-based approach is conceptually different from the individual-based approach used by taxonomists and many ecologists dealing with larger metazoans. Probably because there was too little exchange of results and ideas between the various biological disciplines, the implications of this conceptual difference for assessing biodiversity have been ignored. It is, therefore, the major goal of this article to explore the pros and cons of the different approaches with respect to the ongoing debate about diversity of natural, free-living microbes.

* This article is dedicated to Wilhelm Foissner on the occasion of his 60th birthday.

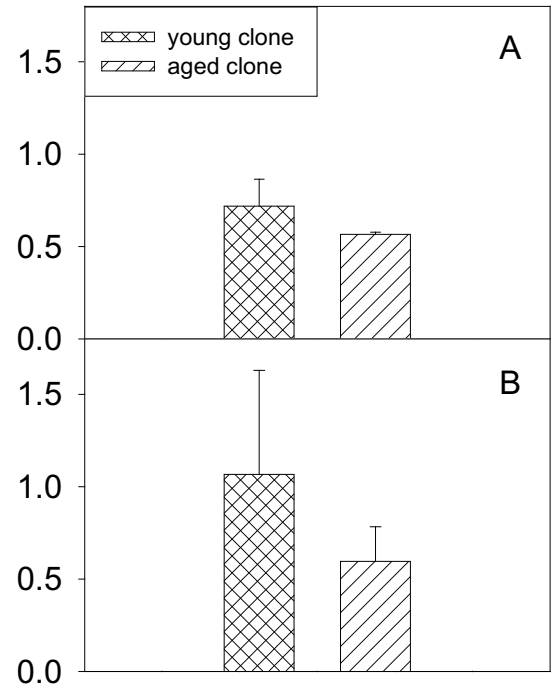

Fig. 1A, B: Population growth rates of two clonal cultures (AU3, A and AU2, B) of the freshwater ciliate *Meseres corlissi* (Ciliophora, Spirotrichea). Both clones were isolated from Salzburg, Austria. The young clones were investigated approximately two months after their isolation, the old cultures six months later under otherwise identical culture conditions. Results show the mean values, the error bars denote one standard deviation (modified from GÄCHTER & WEISSE 2008).

Methods of protist ecology and some typical results

Microbial ecology uses a suit of methods to measure ecophysiological parameters and processes (KEMP et al. 1993) that can not be reviewed here in any detail. I will use some representative examples from aquatic microbial ecology, primarily from own previous research, to illustrate the general approach commonly applied in ecological field studies and laboratory experiments.

Natural populations and field studies – drawbacks from limited resolution

Accurate species identification and an inventory of the species diversity is difficult if not impossible with most natural protist populations, because a compromise has to be made between the research goal and the sampling effort. Most aquatic ecological investigations focused on the dominant players that contributed most to the pools and fluxes within the pelagic food web. Accordingly, the rare protist species which occur in cell numbers of <100 per liter in the ocean and in fresh waters have been largely ignored. The crude resolution of sampling in combination with commonly applied microscopic techniques is problematic, considering that, e.g., a medium-sized lake (e.g. 20 km in length, 12.5 km in width, thermocline in 20 m) with an epilimnetic volume of 5 km^3 may harbour 5×10^{12} cells of a 'rare' ciliate species (assuming a mean abundance of 1 cell/l). Even if the samples are concentrated by filtration or by using settling chambers, the detection limit is approximately 10 cells/l, which would yield one cell in a 100-ml sample. The detection limit cannot be improved by using plankton nets, because most of the nanosized (<20 μm) species will squeeze through the mesh gauze or may be damaged to an extent that species identification is prevented. The quantitative protargol staining (QPS; MONTAGNES & LYNN 1987; SKIBBE 1994) has been successfully applied for the identification and quantification of small ciliate and flagellate species in their natural habitats. However, the sampling volume cannot be increased ad libitum by filtering large volumes and investigating the retentate, because many tiny cells will disintegrate upon filtration, if the filtered volume is large, the filter starts clogging, and the filtering pressure becomes increasingly high.

Considering the limited resolution of the conventional methods used for identification and quantification of protists, it is not surprising that the recent application of culture-independent, DNA/RNA-based molecular techniques revealed a much larger molecular protist diversity, in particular in oligotrophic or little studied extreme biotopes (LOPÉZ-GARCÁ et al. 2001; STOECK et al. 2003a, 2007). Many novel protist clades and lineages were recently detected in the ocean and in freshwater (AMARAL ZETTLER et al. 2002; DAWSON & PACE 2002; STOECK et al. 2005). The current question is how this molecular diversity compares with the diversity assessed by conventional taxonomic techniques. An important step in this direction is that novel gene sequences could already be linked to organisms identified by electron microscopy (STOECK et al. 2003a, b; KOLODZIEJ & STOECK 2007).

Estimates at the population level

Ecologists investigate the abiotic and biotic factors and processes that determine the occurrence and abundance of organisms. Qualitative aspects are studied by evolutionary ecology, aiming to identify the proximate (= immediate) and ultimate (= selective) factors responsible for the development of a certain physiological or morphological character. For instance, the actual light level may trigger the daily vertical migration of phytoflagellates in a lake (proximate factor), while differential light requirements and/or access to nutrient reserves in deeper waters provide an adaptive advantage (ultimate factor), relative to non-migrating species (SOMMER 1985). Most ecological field studies conducted since the early 1980s, i.e. after the detection of the significance of the microbial food web in the ocean and freshwater (POMEROY 1974; AZAM et al. 1983; PORTER et al. 1988), were focused on quantification of ecological key processes. Since quantification is notoriously difficult at the single cell level, the primary target level of the microbial ecologist is not the individual, but the population. Ingestion rates, for instance, are derived from changes in cell numbers of prey (e.g., small algae)

and predator (e.g., an herbivorous ciliate) during the course of the experiment. The difference between gross growth rates (in controls, without predators) and net growth rates (in treatments with predators) of the algae yields the grazing coefficient (g, unit per time). Multiplication of the grazing coefficient with the geometric mean algal cell numbers during the experimental period (P_m) yields population loss rate (= total ingestion rate of the predator). Per capita ingestion is then calculated as the total ingestion rate of the predator population divided by the mean cell number of the predators (R_m):

$$I = \frac{P_m \times g}{R_m}$$

The result (I, prey cells ingested per ciliate and time) is considered representative for the population. If clonal cultures are used, the mean ingestion rate calculated for the population may be comparable to measuring ingestion rates of individual larger metazoan freshwater predators such as *Daphnia* or copepods. For practical reasons, i.e. to obtain a larger signal with improved statistical significance, experiments with the latter are usually conducted with more than one individual. Note that ingestion and growth rate are not constant for an individual *Daphnia* or a clonal protist culture, but depend, for the latter, on the cell cycle (Boenigk 2002), nutritional status (Jürgens & DeMott 1995; Boenigk et al. 2002), and age of the organisms investigated (Gächter & Weisse 2008). This phenotypic flexibility is clearly visible with clonal cultures that are kept under identical laboratory conditions (Fig. 1). An estimate of the variance within and between populations is obtained if more than one population are measured with several replicates each. In the example shown in Figure 1 three replicates were used for each *Meseres corlissi* clone and each treatment. All treatments had been kept under identical laboratory conditions, but for different time (young vs. aged cultures). In the young, fast growing clone AU2 (Fig. 1B) variation between the replicates was unusually large; the standard error in such laboratory growth rate experiments with small ciliates normally ranges from 10–15 % (own unpubl. res.). According to the high variance, the decline of growth rates in the aged culture of clone AU2 was statistically insignificant. Growth rates and other fitness related parameters of ciliates tend to deteriorate if sexual reproduction is prevented in laboratory cultures ('clonal decay'; Bell 1988; Montagnes 1996; Weisse et al. 2001). A significant decline of growth rates with time, similar to the pattern observed with clone AU3 (Fig. 1A), might have been obtained if more than three replicates had been investigated. This need to characterize population parameters with statistical reliability is laborious and adds another facet to the limited resolution of many ecological studies.

Some parameters of natural protist populations such as abundance (in cells/ml) and the percentage of dividing cells can be estimated directly from microscopic observations of living or, more frequently, fixed samples. Although individual cells are being counted, only the estimate for the population is of interest. This may be similar or different in compound parameters such as population biomass. In the case of autotrophic protists, chlorophyll *a* concentration (in mg/m^3 on a volumetric or mg/m^2 on an areal basis, if the biomass is averaged over the euphotic zone or the whole water column) is often used as a proxy for their biomass. Similarly, the biomass of a given size class (e.g., nanoplankton, 2–20 µm) can be expressed in terms of wet weight, dry weight or carbon units (all in mg/m^3 or mg/m^2), without differentiating between individual populations. More often, estimates of the population biomass are calculated by multiplying the total abundance with the average cell volume in a population. To obtain the latter, a statistically representative number of individual cells (>30 per sample) have to be measured by optical (image analysis) or electronic (coulter counter, flow cytometry) techniques. Flow cytometry allows to measure several thousands of individual cells in a sample within one minute, and this technique has been applied to characterize protist cells by their cell size, autofluorescence and relative DNA content (Lindström et. al. 2002, 2003). Flow cytometry is superior to electronic particle counting and image analysis because it measures several characteristics of each cell simultaneously. It is therefore possible to discriminate between populations that overlap, for instance, in their size and/or fluorescence characteristics (Fig. 2). Flow cytometry can also be used to measure grazing rates of ciliates and dinoflagellates feeding on small algae directly (Kenter et al. 1996; Weisse & Kirchhoff 1997).

The individual size variability, which is obvious from Figure 2A and B, is neglected if only the population mean biomass is being reported. Since sizing of individual cells is labour intensive, analysis of several subsamples from the same population to obtain an estimate of the stochastic variation of the mean biomass remains understudied with natural populations. Rather, the biomass of a given protist population has been calculated from the actual abundance determined in the sample and assuming an average cell volume reported in the literature. Considering that the individual cell volume of most small protist species varies by a factor of up to 4 (Weisse et al. 2002, Weisse 2004, Weisse & Stadler 2006) the potential error inherent in such calculations is large.

Measurements of ingestion rates are conceptually similar to biomass estimates if the per capita uptake of

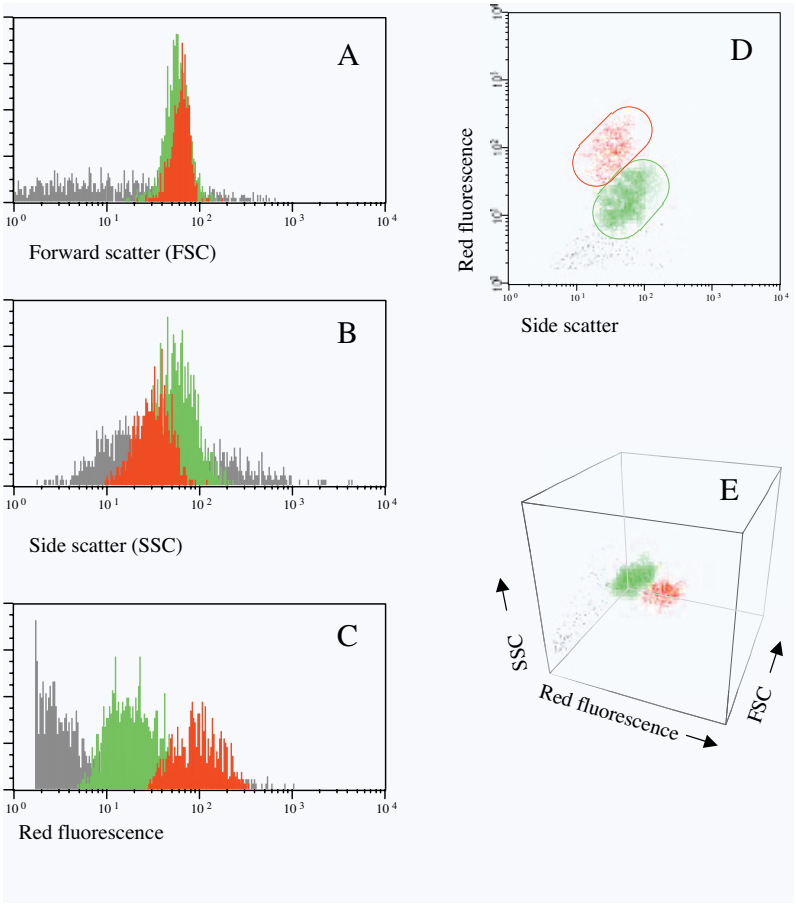

Fig. 2A–E: Flow cytometric analysis of individual cells of a natural sample taken from an acid mining lake (pH = 2.9) at Langau, Lower Austria, at 3 m depth on August 29, 2007. Forward scatter (FSC, A) is a measure of cell size, side scatter (SSC, B) is related to size and surface properties of the cells, red fluorescence indicates the cellular chlorophyll *a* content. All parameters were measured after logarithmic amplification in relative units. The red population represents the green alga *Chlamydomonas acidophila*, the green population the mixotrophic chrysomonad *Ochromonas* sp. The histograms show that both populations largely overlap in their cell size (A, B) and also, to some extent, in their autofluorescence (C). The populations can be discriminated unequivocally in the red fluorescence vs. side scatter dot plot (D) and in the three-dimensional plot (E). Each dot in D and E denotes one individual cell. The flow cytometer used was a FacsCalibur (Becton Dickinson) equipped with an Argon laser.

individual cells is assessed and the mean is then extrapolated for the population. Examples of ingestion rate measurements are the uptake of fluorescently labelled algae (FLA, SHERR et al. 1991) or bacteria (FLB, SHERR et al. 1987). Grazing (loss) rates can also be derived from bulk parameter estimates such as radioactive labelling; in this approach, the prey species is being labelled by incorporation of ^{3}H, ^{14}C or ^{32}P and offered to the predator in experimental enclosures, and the uptake rates are then calculated from measuring collectively the radioactivity in the predator fraction. For example, the small cryptophyte *Rhodomonas minuta* was labelled with ^{14}C by offering radioactive $NaHCO_3$; the labelled prey suspension was then added to a grazing chamber filled with the natural grazer populations from Lake Constance and incubated in situ for 20 min (WEISSE et al. 1990). Community grazing rates were calculated separately for two grazer size fractions (microzooplankton, 50–170 µm, and macrozooplankton, >170 µm) by comparing the radioactivity in the respective grazer fraction to the radioactivity in the suspension. Accordingly, this approach yielded a species-specific loss rate of the protist prey, but did not give a species-specific grazing rate of the predators.

Species-specific ciliate growth rates were also estimated in Lake Constance from in situ growth rate experiments using 4-l diffusion chambers (MÜLLER & WEISSE 1994, WEISSE & MÜLLER 1998). For some ciliates and most of the smaller heterotrophic nanoflagellates, which cannot be identified unequivocally with conventional light or epifluorescence microscopy, this approach yielded genus-specific growth and production rates (WEISSE 1997, 2006a).

It is difficult to estimate the overall error inherent in measurements with populations or even communities. Random sampling and counting errors, artifacts originating from manipulation of the natural populations, inadequate fixation and identification all contribute to the overall bias. A simple example for the measurement of cellular abundance of a cultured phytoflagellate species, comparing four different counting and two different fixation methods, is shown in Table 1. The standard error of the mean was higher for the first two, manual-counting methods than for the last two, automatic methods (Tab. 1). Note that the variance associated with the mean values for these methods represents the overall variance resulting from subsampling, fixation, and 'operator error'. For replicate natural samples from oligotrophic lakes, the 95 % confidence level may be >30 % of the mean abundance for small phytoflagellates (ROTT 1981). Precision (but not necessarily accuracy) increases when samples are analysed by a single researcher: Subsampling and counting of easily identifiable aquatic microbes by inexperienced observers results in a typical counting error of 5 %. The precision of the counting method may be increased, that is, the counting error my be reduced to <2 %, if an experienced observer uses an optimal technique under ideal laboratory conditions. The precision of the method is positively related to the sample size and abundance of the target microbe. This is one of the reasons why the variance of automatic counting techniques such as flow cytometry (WEISSE & KIRCHHOFF 1997; LINDSTRÖM et. al. 2002) or image analysis (PSENNER 1993) is normally lower than that of manual counting.

While the accuracy of many ecological field measurements may be relatively low, the precision appears to be distinctly better. This is because, if the level of reso-

lution and the overall bias remain unchanged, a comparison of similar measurements made with the same population at different times or with different populations may yield consistent results. There is no doubt that microbial ecology has progressed dramatically over the past three decades by analysing natural populations in space and time. Analyses of single cells that are increasingly applied in the laboratory will soon be available for field studies if the technological improvement advances at a similar pace as it did over the past decades.

Laboratory experiments

Laboratory experiments offer many logistic advantages, relative to field experiments. For instance, studying clonal cultures requires (i) manipulation of a natural population and (ii) selective rearing of a (clonal) subpopulation which is usually impossible under natural conditions. However, ecologists working with laboratory cultures face a trade-off between increased accuracy and limited ecological meaning. In vitro, it is much easier than in situ to provide 'controlled' experimental conditions, i.e. to study the effect of one or the interaction of a few selected factors on the fitness of a population and to keep other, potentially interacting factors constant during the experiment. Similarly, the precision can be improved by using more replicates than in the field, because experimental manipulation is easier in the laboratory. However, the increased accuracy and precision of the results are often obtained at the expense of their ecological meaning. A typical example is the temperature response of protist species, which is usually studied in the laboratory over a range of constant temperatures, reflecting the temperature range that will be likely experienced by the species in its natural environment. The temperature effect is measured in terms of population growth rates, which can be used as a proxy for fitness in asexually reproducing species (WEISSE 2006a). To rule out that food limitation affects the temperature response, abundant prey is provided during the experiments. This combination of constant temperatures and unlimited food is, however, rarely met in the field, both in terrestrial and aquatic habitats. Recent evidence suggests that daily fluctuating temperatures of a few centigrade, as they occur in most natural environments, may stimulate the growth rates of free-living ciliates (MONTAGNES & WEISSE 2000). Similarly, there is a significant interaction of temperature and food on the growth and survival rates of ciliates and flagellates (WEISSE et al. 2002; KIMMANCE et al. 2006. The latter findings may have far reaching consequences for the carbon flow in aquatic food webs in the course of global warming (MONTAGNES et al., submitted). Ignoring the interactive effects of temperature and food by assuming laboratory derived maximum temperature dependent growth rates grossly overestimated the significance of freshwater ciliates in carbon flow models (GAEDKE & STRAILE 1994).

Table 1: Cell counts of *Cryptomonas* sp. (cells/ml) obtained with four different methods. Cell counts by the Sedgewick rafter cell used Lugol's fixed material and light microscopy; epifluorescence microscopy and flow cytometry used formalin fixed subsamples. Electronic counting was conducted with live cells (SD = standard deviation of the mean, SEM = standard error of the mean; the former quantifies the variability in the population, the latter quantifies uncertainty in the estimate of the mean and depends on the size of the sample).

Method	N	Mean	SD	SEM
Sedgewick rafter cell	3	14666.7	2248.0	1297.9
Epifluorescence microscopy	3	9669.0	2053.7	1185.7
Flow cytometry	3	13895.7	1115.0	643.7
Electronic counting	3	15510.0	1342.3	775.0

Table 2: Major differences and similarities between the taxonomic and the ecological approach to assess protist diversity. Accuracy is the degree of veracity, i.e. degree of conformity of a measured value to its actual (= true) value, while precision is the degree of reproducibility of a given measurement.

Parameter	Taxonomic study	Ecological study
Primary target level	individual cell	population
Number of cells studied	few	many
Resolution at the individual level	high	low
Resolution at the population level	low	high
Accuracy of results	high	mostly unknown
Precision of results	high, but rarely studied	moderate
Typical range of intraspecific variation	5–15 %	10–100 %
Criteria used for species circumscription	qualitative and quantitative, but arbitrary	primarily quantitative, objective
Phylogeny oriented	yes	no
Fitness oriented	no	yes
Complemented by molecular genetics	yes	yes

Discussion

The individual oriented concept of the taxonomist

The individual oriented, morphospecies concept favoured by most taxonomists has some distinct advantages. By comparing several to many individuals from the same sample and from different populations, the taxonomist immediately may identify the individual variability. In many cases, this is not a trivial task, but requires sophisticated methods of isolation, fixation and preparation, before advanced techniques such as scanning and transmission electron microscopy may be applied to study morphological details. Artifacts may arise at each step necessary to characterize a given species, but, relative to most ecological studies, the taxonomist does not have to make many assumptions to obtain realistic results. Another question is how representative the results are. Species circumscriptions are arbitrary and largely rely upon the expertise and general approach of the taxonomist. In fact, the present situation is not much different from DARWIN's view: "I look at the term species, as one arbitrarily given for the sake of convenience to a set of individuals closely resembling each other... Hence, in determining whether a form should be ranked as a species or a variety, the opinion of naturalists having sound judgement and wide experience seems the only guide to follow"[1] (DARWIN 1859). Taxonomists are often called "splitters" or "lumpers" by their colleagues, depending upon their personal broad or narrow approach to recognizing species (MAYR 1975). The interspecific degree of variation a taxonomist allows depends, however, not only on his view and expertise, but also on the total number of individuals of a given species studied. According to the International Code of Zoological Nomenclature (ICZN 1999; also available online at http://www.iczn.org/iczn/index.jsp) investigations and microscopic preparations ("type slide", which need to be deposited in museums and made available upon request) of "one or more" individuals is required if a new protistan species is to be described. Accordingly, the number of specimens studied for the description of a new species varies widely from one to many, and this quantitative aspect affects the quality of the inductive conclusion about the species identity. It appears that many species have been described without any idea of their intraspecific morphological variation. This approach is problematic, as far as diagnostic characters are concerned. DARWIN was already aware of the significance of individual differences: "Hence I look at individual differences, though of small interest to the systematist, as of high importance for us, as being the first step towards such slight varieties as are barely thought worth recording in works on natural history. And I look at varieties which are in any degree more distinct and permanent, as steps leading to more strongly marked and more permanent varieties; and at these latter, as leading to sub-species, and to species" (DARWIN 1859). Estimates of the intraspecific morphological variation can only be obtained reliably from a few alpha-taxonomists who studied many specimens of each newly described species. For numeric characters, such as the number of ciliary rows and adoral membranelles, an intraspecific variation of 5 % is typical for most ciliates (FOISSNER 1984, 1993). Size-related distance measures such as body length or macronucleus width vary typically by approximately 15 % (FOISSNER 1984, 1993) Other problems inherent in the current rules of ciliate nomenclature have been discussed by FOISSNER (2002).

In addition to getting relatively direct access to the individual and to intraspecific variation, the taxonomic approach offers some other advantages. By comparing the development of morphological characters in evolutionary lineages, this approach promotes phylogeny and cladistic analyses. A major difference to evolutionary ecology is that the taxonomic approach is oriented towards past selection. Although the taxonomic literature contains many speculations about the adaptive value of certain characters, the evidence is obtained with hindsight. Since the more sophisticated detailed investigations are performed with fixed material, taxonomists have no means to test the effect of a given character on the fitness of the individuals observed. Another traditional disadvantage of the morphological taxonomic approach is that, as a typological concept, it is closely related to phenotypic diversity and neglects the genotype. The powerful combination of morphological with molecular genetic identification (e.g., FOISSNER et al. 2001, 2004; MODEO et al. 2003; AGATHA et al. 2004; SHAO et al. 2007) enabled taxonomists to overcome this disadvantage. Moreover, studying phenotypic diversity is conceptually important because selection acts on the phenotype.

The population based concept of microbial ecology

Ecology studies the abiotic and biotic interactions that determine the distribution and abundance of organisms. Traditionally, those interactions have been investigated at levels ranging from individuals, population, communities, and ecosystem to the biosphere (LAMPERT & SOMMER 2007). Similar to taxonomic research, ecological investigations have recently included the molecular level by investigating genes and proteins. The adaptations of the organisms to their specific habi-

[1] This and the following quotation are taken from Chapter 2, Variation Under Nature, of "The Origin of Species...". The full text is available online at http://www.talkorigins.org/faqs/origin.html.

tat are investigated with respect to their fitness, i.e. the adaptive value of a given trait is high if the individuals carrying the respective genotype will reproduce successfully. In other words, ecology investigates the forces driving natural selection (LAMPERT & SOMMER 2007). Even without applying genetic methods, the link between phenotype and genotype is, therefore, close in ecological investigations.

Methodological constraints have hampered the study of the individual variability of microorganisms such as bacteria and most protist species in the past. Although sophisticated technologies such as high speed video microscopy and flow cytometry that allow studying the behaviour of individual cells less than 50 µm in size have recently become available (WEISSE & KIRCHHOFF 1997; BOENIGK & ARNDT 2000), little is known on the individual phenotypic and genotypic variability within protist species (WEISSE 2002, 2003, 2006b). Recent investigations with clonal cultures revealed an unexpectedly large intraspecific variation for aquatic ciliates and flagellates (WEISSE & LETTNER 2002; KIM et al. 2004; LOWE et al. 2005; WEISSE & RAMMER 2006; BOENIGK et al. 2007). Accordingly, the deductive reasoning inherent as tacit assumption in many ecophysiological studies that investigating a particular population is representative for the species has been criticized (WEISSE 2002; LOWE et al. 2005; WEISSE & RAMMER 2006).

The classical unit of study in microbial ecology is the population. A drawback of the 'population approach' is that usually only the mean value and, if at all, the range of a parameter studied is reported, and the individual variation is neglected. From a conceptual point of view, this 'population approach' is unsatisfactory, because natural selection does not select for the mean (WEISSE 2002, 2006a). However, although natural selection acts on the individual, the resulting evolutionary change (i.e., a shift in the gene pool) can only be measured at the level of population. The difficulties of analysing individual cells are also the main reason why population genetics of natural protists is still in its infancy, compared to metazoans such as *Daphnia* and rotifers (WEISSE 2006a).

Studying populations, communities and ecosystems is, however, a necessity for ecological investigations, because new properties emerge at each higher level that cannot be explained by the lower levels. Similarly, it is important to consider the cascading effects of the higher levels on the fitness of individuals. The current global warming, for example, already led to an increase in the water temperature of central European lakes (LIVINGSTONE & DOKULIL 2001, DOKULIL et al. 2006) that will eventually alter the species composition. Ecological research, therefore, addresses the higher levels of diversity that are usually not considered in taxonomical research.

The major differences and similarities between the taxonomic and the ecological approach discussed in the foregoing text have been summarized in Table 2.

Ecology meets taxonomy and population genetics – needs for future research

Diversity needs to be assessed at the various levels ranging from genes to the biosphere. The taxonomic approach traditionally favours the level of the individual and increasingly includes the genetic level. The higher levels of diversity received comparatively little attention in taxonomic studies with protists. Driven by the technological advancement, microbial ecology progressed in the other direction; while organismic interactions affecting pools and fluxes in ecosystems have been classified in broad categories such as functional guilds (e.g., bacterivorous heterotrophic nanoflagellates, HNF; e.g., WEISSE 1991; GAEDKE & STRAILE 1994) in the 1980s and early 1990s, numerous species-specific interactions have been published since the turn of the century (e.g., BOENIGK & ARNDT 2000; ŠIMEK et al. 2000; WEISSE & FRAHM 2001, 2002; BOENIGK et al. 2004). Based upon more reliable estimates at the lower levels, the ecological role protists play at the higher levels of ecosystem and the biosphere gained momentum in the course of the global change scenario (MONTAGNES et al., submitted).

It is crucial for an improved understanding of protist diversity to integrate the variability observed at the genetic, morphological and ecological level. A comprehensive recent study with several clones of the cosmopolitan oligotrich freshwater ciliate *Meseres corlissi* concluded "the intraspecific variation was low at the genetic level (0–4 %), moderate at the morphological level (5–15 %), and high at the ecophysiological level (10–100 %) (WEISSE et al. 2008). The genetic level referred to the SSrDNA and ITS genes, the morphological investigations comprised 14 characters of trophic cells, and the ecophysiological variability was primarily derived from the experimental response to temperature and pH. Since the results were obtained with clonal cultures, the different cell numbers studied by the various methods applied should have been of minor importance. In future work on protist diversity, morphological investigations at the single cell level should be more closely coupled with corresponding genetic studies, and both should be linked to ecophysiological measurements at the population level. The latter should be complemented by an assessment of the genetic structure of the populations, yielding an estimate of the genetic similarity and, accordingly, the gene flow between the populations under study. The increased cooperation between experts of the subdisciplines leads to an augmented awareness of the pros and cons of the concepts and methods applied

in each field. I hope that this article will stimulate future cooperative research between taxonomists and ecologists – we complement and need each other, more than most of us have thought until recently.

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