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Distribution and diversity of aquatic protists: an evolutionary and ecological perspective

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Abstract Assessment of the distribution and diversity of free-living protists is currently hampered by a limited taxonomic resolution of major phyla and by neglecting the significance of spatial and temporal scaling for speciation. There is a tremendous physiological and ecological diversity that is hidden at the morphological level and not apparent at the level of conserved genes. A conceptual framework linking the various levels of diversity is lacking. Neutral genetic markers are useful indicators of population structure and gene flow between populations, but do not explain adaptation to local habitat conditions. The correspondence between protein-coding genes, ecophysiological performance, and fitness needs to be explored under natural conditions. The area and the associated typical temporal dimension of active cells (their 'home range') are much smaller, respectively shorter, than the area and time period potentially covered during passive dispersal of protist resting stages. The assumptions that dispersal rates are generally high in free-living protists and that extinction of local populations is, therefore, infinitesimally small wait rigorous testing. Gene flow may be uncoupled largely from dispersal, because local adaptation and numerical effects of residents may strongly reduce or even prevent successful invasion (immigration). The significance of clonal selection depends on the as yet unknown frequency and timing of sexual reproduction, and on the stability of the environment. The extent of local adaptation and the fitness-related ecophysiological divergence are critical for the speciation process and, hence, for defining protist species.

Keywords Clones · Dispersal · Distribution · Diversity · Free-living protists · Home range concept · Sexual reproduction · Speciation

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Introduction

Protist diversity and their geographic distribution, which are ultimately linked to each other, were controversially debated in recent years (Finlay and Fenchel 1999; Foissner 1999; Lachance 2004) despite, or perhaps because of, a limited understanding of the determining processes. The current debate centres on the old hypothesis by the Delft school of microbiology that free-living microorganisms are globally distributed and ubiquitous, i.e., that they can be found wherever a suitable habitat exists (see Quispel 1998 for a short review of the historic background, and Whitfield 2005 and Foissner 2004, 2006 for brief summaries of the "everything is everywhere" view). The controversial discussion and the advent of novel, mainly molecular tools stimulated research on microbial diversity and their potential biogeography over the past decade (reviewed by Foissner 2006, 2007; Hahn 2006; Weisse 2006a). In spite of the increased efforts, the range and extent of protist dispersal, the genotypic and phenotypic diversity, the frequency of sexual reproduction and, accordingly, the extent of reproductive isolation are at present unknown for the vast majority of extant, free-living protist species.

The special issue on "Biogeography of Freshwater Algae" (Kristiansen 1996a), the theme section on "Biogeography of aquatic microbes" (Dolan 2005), the recent reviews by Foissner (2006, 2007) and several articles in this issue of BIODIVERSITY and CONSERVATION present numerous examples on limited dispersal and biogeographic differences among major phyla of terrestrial and aquatic protists. The purpose of the present paper is to discuss the distribution and diversity of protists from an ecological and evolutionary perspective. In line with Horner-Devine et al. (2004) and Hughes Martiny et al. (2006), I follow the hypothesis that protist biogeography and diversity is, in principal, similar to biogeography and diversity of macroorganisms. In particular, I will highlight the population genetic literature on parthenogenetic zooplankton and suggest that many of the processes shaping populations in protists will be common to these organisms. This conceptual article will focus on heterotrophic aquatic protists but also include some findings from autotrophs and from terrestrial environments where appropriate.

Distribution of free-living protists

Significance of the taxonomic resolution

Any census of biological diversity across phylogenetically diverse taxa relies on a meaningful unit that renders the diversity of the different taxa comparable. Traditionally, most estimates of biodiversity have used the species as the 'common currency', although it is well known that species-based approaches ignore other levels of biodiversity (e.g., Maddock and Du Plessis 1999; Giller et al. 2004). As outlined by Kristiansen (1996b) and Schlegel and Meisterfeld (2003), the species problem is still unresolved for major protist phyla. It is important, in this context of protist diversity, to differentiate between the meaning of species as an evolutionary unit ('real' species, see Claridge et al. 1997; Bachmann 1998; Hey 2001) and the meaning of species as a category. Recent work with bacteria demonstrated that the level of taxonomic resolution is crucial for detecting biogeographic differences among microbes (Horner-Devine et al. 2004; Schauer et al. 2006; Ramette and Tiedje 2007). Accordingly, a pragmatic definition of the species as a category has to be commonly accepted before any firm conclusion can be drawn on the species-specific distribution of protists and their biogeography (Mitchell and Meisterfeld 2005).

The rapidly growing molecular data on diatoms (Amato et al. 2007), dinoflagellates (Kim et al. 2004), cryptomonads (Hoef-Emden and Melkonian 2003), bodonid (von der Heyden et al. 2004; von der Heyden and Cavalier-Smith 2005) and chrysomonad flagellates (Boenigk et al. 2005, 2006), amoebae (Wylezich et al. 2002; Smith and Wilkinson 2007), foraminifera (Darling et al. 1999, 2000), and ciliates (Katz et al. 2005; Barth et al. 2006; Finlay et al. 2006; Gächter and Weisse 2006; Weisse 2006b; Weisse and Rammer 2006) implies that the morphospecies concept is, in many cases, too conservative when comparing protist diversity to that of most macroorganisms. Application of the morphospecies concept is also problematic in highly polymorphic species such as *Pfiesteria*-like dinoflagellates (Marshall et al. 2006).

Similarly to the morphological approach, evolutionary conserved genes such as the small subunit ribosomal DNA gene (SSU rDNA), may underestimate protist diversity. In several major protist phyla, a 1–2% sequence divergence of the SSU rDNA, which has been equated with morphospecies designations of protists (Jerome et al. 1996; Walochnik et al. 1999; Snoeyenbos-West et al. 2002; Katz et al. 2005), may not reflect a species resolution comparable to that of higher taxa (von der Heyden and Cavalier-Smith 2005). For instance, significant differences in the growth rate response to salinity and a huge genetic variation in the SSU rRNA gene were found for *Bodo designis* from seawater, freshwater and soil (Koch and Ekelund 2005). Strains of *B. designis* clustered in several groups, which were not obvious from morphological characters. Another comprehensive genetic and ecological study with the chrysophyte morphospecies *Spumella* from many different environments arrived at the same conclusion (Boenigk et al. 2007). Similarly, distinct genetic differences, up to 66% divergence of their 5.8 S rDNA and ITS sequences, and highly significant differences in growth rate response to salinity, with maximum growth rates ranging from 0.3 to 1.2 d^{-1}, have been reported for the marine flagellate *Oxyrrhis marina* (Lowe et al. 2005). Some more examples of similar recent investigations with ciliates are listed in Table 1. It appears that the variability of the ribosomal genes is different in the various protist phyla (Table 1). Alternatives to the taxonomy based upon SSU rDNA analyses and the pros and cons of the molecular approach to protist phylogeny have recently been reviewed (Schlegel 2003; Schlegel and Meisterfeld 2003).

In conclusion, there is currently no molecular 'gold standard' for protist taxonomy to solve the species problem, and the units of interest, i.e., species, cannot be recognized with standard tools across the protist realm (Amato et al. 2007). The current challenge is to decipher the correspondence between DNA sequence clusters, ecotypes (Turesson 1922) and (morphologically defined) biological species (Dini and Nyberg 1999; Foissner et al. 2001; Finlay 2004; Boenigk et al. 2005). In other words, the priority is to understand the interplay of molecular mechanisms with organismic and ecosystem biology (Jackson et al. 2002).

Terminology and the significance of scaling

Similar to the unresolved species problem, the use of ambiguous terminology contributed to the often emotionally led debate on protist diversity (Finlay et al. 2004). Terms such as 'ubiquitous', 'ubiquitous dispersal' and 'cosmopolitan' are virtually meaningless without clear spatial and temporal dimensions. The *potential* for regular, world-wide distribution (= unlimited distribution) may be high in frequent, widespread taxa that are dispersed passively; the *actual* dispersal rates, i.e., dispersal on relatively short time scales (i.e., related to the organisms' generation time), will be much lower in most taxa (Bohonak and Jenkins 2003).

Table 1 Examples for sequence divergence and ecophysiological differences in morphologically indistinguishable (at the light microscopical level) free-living protist taxa

Taxon investigated	Origin of strains	Genes investigated	Sequence divergence (%)	Physiological variation in	Reference
Karenia brevis (Dinophyceae), 5 strains	M, from Texas and Flv	18S rRNA; ITS-1, 5.8S, and ITS-2	0	Max. growth rate, toxin content, salinity tolerance	Loret et al. (2002)
Oxyrrhis marina (incertae sedis), 11 strains	M, TP; from 3 cont	18S rRNA; ITS-1, 5.8S, and ITS-2	0.6–10.5 0–65.7	Growth rate, salinity tolerance	Lowe et al. (2005)
Bodo designis (Kinetoplastida), 9 strains	FW, P, M, S; from 3 cont	18S rRNA	Huge, seq. fall into 4 different phylogentic clusters	Growth rate, salinity tolerance	Koch and Ekelund (2005)
Paramecium spp., 3 species, 19 clones	P, from 3 cont	ITS-1, 5.8S, and ITS-2; COI*	0–2.4 6.0–9.5	n.d.	Barth et al. (2006)
Meseres corlissi, (Ciliophora, Oligotrichea), 5–10 clones	FW, from 4 cont	18S rRNA, ITS-1, ITS-2	<1 0–2	Max. growth rate, temperature and pH tolerance, cell volume	Gächter and Weisse (2006), Weisse et al. (2007)
'Spumella-like' flagellates, 3 strains	FW, from 3 cont	18S rRNA	0	Bacterial grazing rate, growth rate	Boenigk et al. (2004)
'Spumella-like' flagellates from FW and soil, >50 strains	FW, P, M, S; from 5 cont	18S rRNA	0–10	Growth rate, temperature tolerance	Boenigk et al. (2005, 2006, 2007)

FW, Freshwater; M, marine; P, pond; S, soil; TP, tide pool; cont, continents; FL, Florida; seq., gene sequences

*For *Paramecium* spp., sequence variation denotes to variation within one species

Note that even actual dispersal rate is not identical with distribution, because actual dispersal becomes *effective* dispersal only after successful establishment of immigrants. Although the meaning of effective dispersal and distribution of a species may be largely identical, there is a conceptual difference, because only the former refers to a process. Similarly, there are important conceptual differences between colonization, invasion and immigration (Table 2).

It is the interplay of passive dispersal, which may be a primarily physically mediated (i.e., dispersal by air, water currents) or primarily biologically mediated process (e.g., dispersal by water fowl), with various physico-chemical and biological processes in the new habitat that determine the distribution of species with their temporal (frequency and persistence of occurrence) and spatial (extention of occurrence) dimension. Temperature, salinity and pH are major physico-chemical variables controlling the occurrence of protist species (reviewed by Weisse 2006b). Biological processes such as food limitation, predation pressure and infection by parasites contribute to temporarily or permanently low abundances in some otherwise suitable habitats.

Species-to-area curves plot the (log) number of species of a given taxon versus the (log) area sampled; typically, there is an increase in species numbers when progressing from local to global spatial dimensions. With some exceptions (Hillebrand et al. 2001), species-to-area curves of protists are virtually unknown. An increase of species number at continent-wide and global levels has recently been reported for the autotrophic synurophycean genus *Mallomonas* (Řezáčová and Neustupa 2007). If phylogenetically diverse organisms of widely differing size such as protists and mammals are to be compared (Finlay 2002), species-to-area curves must relate habitat size to the body size of the organisms to yield statistically sound results (Hillebrand et al. 2001; Hillebrand and Blenckner 2002). The area, where the organisms usually range in the course of a day or season, may be termed their home range. The home range concept was coined by Burt (1943) for territorial mammals. The size (area) of the home range is of utmost biological importance, because it sets the upper limits of biological interactions. This concept is similar to Cornell and Lawton's (1992) definition of local scales of species richness, where ecological factors such as competition and grazing determine species richness. Free-living protists have typical home ranges varying between 10^{-3} and 10^2 m^2, corresponding to linear dimensions ranging from a few cm to several m. The typical linear home range dimensions of macroorganisms with known biogeographies range from several m to several km. The home range can be roughly equated with the 'local' dimension typical for active organisms of different sizes. It does not include the potentially much larger area covered by passive dispersal of inactive resting stages. In those taxa that disperse primarily with asexual propagules, the equivalent of the home range for a given genotype (clone or 'genet', i.e., all individuals or 'ramets' belonging to this clone) may be orders of magnitude larger.

The significance of spatial scaling is obvious for the speciation process. A swimming flagellate, with a typical generation time of one to a few days in temperate lakes (Weisse 1991, 1997), has virtually no chance to meet its congener dwelling at the other shore of a small lake of ~100 m length (Weisse 2006a). Accordingly, two individuals of a fish population living in a small lake are likely to be sympatric; however, two individuals of a given flagellate species living in the same lake at the same time may be sympatric, parapatric or even allopatric. It is, therefore, misleading to relate the term 'sympatric speciation' to a well defined geographic area such as, e.g., Lake Victoria, without specifically referring to the organisms under study (Gavrilets 2003).

A corollary of the foregoing considerations is that the 'local' dimensions, at which α-diversity (species richness at a single site) is assessed, may be worlds apart for passively

Table 2 Operational definitions of key terms used in this article

Terms and definition	Source
Potential dispersal: The random, unlimited movement of individuals as active cells or resting stages (cysts) across population boundaries at all spatial scales. This definition is similar to 'ubiquitous dispersal' as defined by Finlay et al. (2004).	This study
Actual dispersal: The movement of individuals as active cells or resting stages (cysts) across population boundaries per unit time (e.g., d^{-1}, $season^{-1}$, y^{-1}, generation $time^{-1}$).	This study
Effective dispersal: The movement and successful establishment of individuals as active cells or resting stages (cysts) across population boundaries per unit time.	This study
Distribution: The area, over which a species has been recorded at any time. The temporal dimension of distribution is referred to as occurrence.	This study
Specific distribution: The variability of habitats, over which a species has been recorded at any time. Species with a narrow specific distribution occur in only a few, peculiar habitats.	This study
Cosmopolitan species: A species that thrives wherever its required habitat is realized.	Finlay et al. (2004)
Gene flow: The exchange of genes between populations, or 'gene pools.'	Bohonak and Jenkins (2003)
Metapopulation: A set of populations that (1) are spatially discrete, (2) may differ in size, demography and carrying capacity, (3) may be subject to extinction and recolonization, and (4) interact via dispersal and gene flow.	Bohonak and Jenkins (2003)
Colonization: The initial step of establishing a new population by one or more dispersers.	Modified after Bohonak and Jenkins (2003)
Invasion: Colonization that impacts other species already inhabiting an area.	Bohonak and Jenkins (2003)
Immigration: Result of successful invasion, i.e., the propagules of an invader must have reproduced and survived over many generations, thus establishing a new (sub)population within the new habitat. In contrast to colonization, invasion will be successful only if, among the flow of invaders, there are some cells better adapted (i.e., with a higher fitness) to the specific local environment than those already present. Different from effective dispersal, immigration is not necessarily a rate, because the temporal dimension of immigration remains, in many cases, unknown.	This study
Local adaptation: The ability of individuals within a population to survive and reproduce better than immigrants from other populations. If local adaptation exists, immigrants can affect some, but not all ecological and evolutionary processes.	Bohonak and Jenkins (2003)

dispersed, asexual microorganisms and for sexually reproducing microorganisms with much lower passive dispersal capacity. For the latter, the processes resulting in biogeographic patterns may be more comparable to those of macroorganisms, although at much reduced spatial and temporal scales.

Unknown rates of dispersal and survival

Local diversity in any habitat depends on the size of the reservoir community (metapopulation) from which new immigrants originate; if this is large, even physically identical habitats will harbour different communities (Curtis and Sloan 2004). The characteristic features of metapopulations (reviewed by Hanski 1999) have been summarized in Table 2. Metapopulation structure has been demonstrated for protist species with patchy distributions, which may be caused by physical factors or pronounced predator–prey cycles (Holyoak and Lawler 1996; Holyoak 2000; Montagnes et al. 2002). It is a fact that there are rare soil and aquatic protist species which will not be encountered permanently in each suitable habitat (Foissner et al. 2002; Foissner 2006, 2007). To some extent, this may reflect inadequate sampling techniques. With the sampling gear and counting methods typically applied in taxonomical and ecological studies, it is difficult to detect species with abundances that are 3 or more orders of magnitude lower than those of frequent species. Furthermore, the fixation techniques and enrichment cultures used for estimating the abundance and species composition of protists are all more or less selective (e.g., Bloem et al. 1986; Modigh and Castaldo 2005; Foissner 2005). However, in spite of these caveats it remains that some taxa occur in presumed suitable habitats, if at all, only at very low levels.

There is no doubt that many wide-spread protist species comprise vast population sizes. 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, for instance, harbour 5×10^{15} ciliates of a common species (assuming a mean abundance of 1 ciliate ml^{-1}) and 5×10^{17} flagellates of a common chysomonad species. Similarly, 1 g of soil may contain some 10,000–100,000 active ciliates and similar abundances of naked amoebae and flagellates (Foissner 2005). On longer time scales (decades to centuries), dispersal of most free-living protists may result in their world-wide distribution, due to passive drifting by water currents, turbulence and mixing, and by transportation via wind, waterfowl and other migrating animals (reviewed by Kristiansen 1996c), and even more exotic vectors such as ballast water of ships (Hallegraeff and Bolch 1992; Hülsmann and Galil 2002). It is important to note that a flagellate or ciliate has no chance to travel over a long distance as an active cell, simply because the lifespan of most protists is in the order of hours to days and active cells are vulnerable to desiccation during overland transport. It must therefore be assumed that long distance dispersal is mediated via resting stages such as cysts known from many soil protozoa, oligotrich freshwater ciliates or chrysophyte flagellates (reviewed by Corliss and Esser 1974; Cronberg and Sandgren 1986; Foissner 1987; Ekelund and Rønn 1994; Müller 2000). Cysts of soil protozoa from temperate and arid environments are generally assumed to be able to withstand harsh environmental conditions for decades (Ekelund and Rønn 1994; Foissner 2005), but resting stages of soil protozoa produced in more or less permanently moist rainforests may survive droughts for only a few weeks (Foissner 2005, 2006). Foissner (2007) concluded that cyst viability is likely much more restricted than widely assumed. Cysts of the oligotrich freshwater ciliate species *Meseres corlissi* were highly resistant to adverse conditions including inorganic and organic solvents (Foissner et al. 2005). Another recent study with the same species revealed that encystment and

excystment of two temperate strains from Austria were triggered by the presence of soil extract (Müller et al. 2006), while temperature was the primary factor inducing cyst formation in a tropical strain of the same species (Weisse 2004). Cysts of another oligotrich freshwater ciliate, *Pelagostrombidium* sp., lost their viability rapidly when stored under cold (1–6°C) and dark conditions (Müller 2002).

The above examples illustrate that the factors controlling encystment, excystment and viability of cysts may differ among closely related protist species and probably even between clones of the same species. Further, the few experimental studies currently available do not allow to generalize; the significance of resting stages as part of the protist life strategy and the viability of cysts remain at present unknown for >99% of all contemporary protist species.

Investigations of airborne dispersal date back to Antony van Leeuwenhoek and C. G. Ehrenberg; the latter was probably the first who demonstrated the occurrences of unicellular algae in wind and dust in the mid 19th century (reviewed by Gregory et al. 1955; Corliss 1996). Quantification of viable microbes composing the 'aeroplankton' is difficult. Rogerson and Detwiler (1999) used a filtration sampler to collect particles in the 2.0–20.0 μm size range in near-surface urban air and determined the number of viable protist cysts after enrichment cultivation in soil extract medium. Abundances of 25 different morphotypes ranged from below detection (<0.05 m^{-3}) to 1.08 cysts m^{-3}, with an overall mean of 0.25 m^{-3}. Cysts of flagellates and naked amoebae were the commonest found; ciliates were rare. A similar recent study confirmed the generally low cyst numbers in near-surface air (Feldmann 2007); only amoebal cysts were found, with a mean abundance of 0.05 m^{-3}. It is important to note that these seemingly low numbers suggest *a tremendous potential significance of airborne dispersal* in at least some protist taxa.

Terrestrial studies on immigration and succession of soil organisms in newly colonized habitats demonstrated that immigration by air is characteristic for protists such as testate amoebae, flagellates, and small ciliates (e.g., Wanner et al. 1998; Wanner and Dunger 2002; Wanner and Xylander 2005). Similarly, the few studies that used artificial microcosms to investigate the between-habitat dispersal rate of aquatic protists (Warren 1996a, b; Östman et al. 2006) assumed that airborne dispersal of protists may play a major role in natural environments. However, the rates of dispersal of common protist taxa across disconnected habitats are virtually unknown.

In theory, rates of dispersal can be inferred from estimates of gene flow between (meta)populations. However, common measures of gene flow estimate dispersal followed by successful establishment of immigrants. Allozyme and molecular DNA markers have been particularly useful to decipher rates of dispersal of freshwater cladocerans (primarily *Daphnia*), rotifers, and insects, but all gene flow estimates depend on models with underlying assumptions (e.g., equilibrium conditions) that may not be met in the field (Boileau et al. 1992; Bohonak and Jenkins 2003). The latter authors concluded from their literature review that for freshwater invertebrates (including many small crustaceans and rotifers <1 mm in size) the currently available evidence contradicts the notion that dispersal in most freshwater taxa is frequent and widespread on relatively short time scales. Freeland and co-workers (Freeland et al. 2000, 2001) reported negligible to low levels of spatial gene flow between (meta)populations of asexually propagating bryozoans, but low to high levels of within-population genetic diversity; these authors concluded that temporal gene flow via resting stages (statoblasts) at a single site is more important than spatial gene flow across different habitats. In contrast, De Meester et al. (2002) reported that high dispersal capacity of both freshwater animals (cladocerans, rotifers and bryozoans) and macrophytes contrasts with the abundant evidence of pronounced genetic differentiation

among neighbouring populations in many pond-dwelling organisms. Since the size, mode of dispersal and life strategy, with intermittent production of sexual, asexual and resting stages, of larger protists are similar to that of rotifers and small cladocerans, I conclude that many populations of freshwater protists in neighbouring habitats may also be genetically different. High genetic differences, in the SSU rDNA and ITS regions, have been reported recently for neighbouring populations of the dinoflagellate *Peridinium limbatum* from Northern Wisconsin freshwater bodies (Kim et al. 2004). However, compared to cladocerans and rotifers, the study of population genetics of protists is still in its infancy (Weisse 2006b). In conclusion, population genetic studies with both common and rare free-living protist species are urgently needed to measure their effective dispersal rates; this is a key issue for the current debate about protist biogeography.

Dispersal and immigration are neither random nor neutral processes

Physical processes such as wind and water currents are primarily responsible for the uneven distribution of soil and aquatic microbes. Similar to migrating birds, the physical processes are non-random. Like water currents, airborne dispersal is uneven in areas with a prevailing wind direction. Even in areas that are permanently subjected to high wind stress, there will be some sheltered places of refuge with lowered chances of passive invasion.

Immigration and community assembly are non-neutral processes; the more similar an invader is to the resident population, the more it is inhibited and, in most cases, prevented from establishing a new population in the same habitat (Fargione et al. 2003). The strength of biological interactions such as competition, predation and parasitism are all density-dependent and tend to peak in climax communities. Accordingly, colonization favours generalists while immigration into already colonized habitats favours specialists within a seemingly uniform guild (i.e., a population, a species, a functional group such as, e.g., bacterivores). Each event of immigration may lead to a shift in the gene pool of the population (microevolution), thus promoting directional selection. Directional selection may lead to adaptive speciation sensu Gavrilets (2005), i.e., to "a speciation process in which genetic changes underlying divergence and reproductive isolation are driven by selection (as opposed to changes driven by mutation and random genetic drift)". Under which circumstances natural selection is strong enough to enhance the intraspecific genetic differences between different populations by selective sweeps remains at present unknown.

This important aspect, that every population that is everywhere is at some phenotypic and, most likely, also at some (functional) genotypic level different from each other has received little attention in the biogeography debate. In fact, phenotypic plasticity may be even a prerequisite of global distribution of protist species. Although random dispersal may be supported by the 'unified neutral theory of biodiversity and biogeography' (Bell 2001; Hubbel 2001), we cannot ignore the significance of biological interactions for everything that is smaller than 1 mm. In the neutral theory, there is no feedback from a resident population to invaders. This is a reasonable assumption in newly colonized habitats such as inland dunes or reclaimed opencast mining areas, which are characterized by a lack of biotic interactions among invading soil protists and where typical 'pioneer populations' dominate (Wanner et al. 1998; Wanner and Xylander 2005). If the assumption holds that probabilities of per capita reproduction, mortality, and migration to a neighbouring site are all identical, there is no difference in fitness among the individuals of a population and even among a species. Then, and only then indeed, are dispersal and, in consequence, distribution of species reduced to a size-related, strictly stochastic process.

Diversity of free-living protists

Protist speciation—is parapatric speciation the rule?

There is increasing evidence that allopatric speciation is not the sole speciation process in free-living protists. Within-habitat variation, a prerequisite of sympatric speciation, has already been demonstrated for freshwater bacteria and was attributed to the isolation of water by lake shoreline features such as bays or narrow constrictions (Yannarell and Triplett 2004). Peripatric speciation has been suggested as "a subset of allopatric speciation in which a peripherally isolated population diverges to become a new species" (Losos and Glor 2003). Parapatric speciation, the intermediate case between allopatric and sympatric speciation, with limited migration or dispersal between divergent (sub-)populations, has recently been assumed as the most general mode of speciation (Gavrilets et al. 2000; Gavrilets 2003). In contrast to peripatric speciation, parapatric speciation results from divergent evolution of adjacent populations (Losos and Glor 2003). Parapatric, as well as sympatric speciation require that there is some, although limited gene flow between the subpopulations. Recently, Amato et al. (2007) reported reproductive isolation among sympatric cryptic species in marine diatoms.

The mode of speciation is ultimately linked to the occurrence and extent of sexual reproduction in a population. For some taxonomic groups, such as diatoms, regular sexual reproduction will be the rule. This is, because due to their peculiar mode of cell division, average cell size of diatoms decreases in the course of continued asexual reproduction; for most diatoms, sexual reproduction, with auxospore formation, is "the only way out of this miniaturization trap" (Amato et al. 2007). For other taxa, such as ciliates, sexual reproduction has been studied in detail in the laboratory (reviewed by Dini and Nyberg 1993), but data on the frequency with which sexual reproduction occurs in situ are rare and controversial. While Doerder et al. (1995) reported that pond dwelling *Tetrahymena thermophila* mate often, Lucchesi and Santangelo (2004) found that mating among ciliates in marine temperate sandy shores is quite low. These authors concluded that conjugation is an erratic sexual phenomenon.

Clonal selection, local adaptation and unknown significance of sexual reproduction

Local adaptation results in improved fitness of individuals in a resident population relative to their invading congeners. Adaptation can be measured as increased survival and reproduction rates of the residents in response to the ambient environmental conditions. To increase the fitness of individuals, the genetic shift must affect quantitative traits; neutral (non-functional) changes in allele frequencies do not lead to improved performance. Local adaptation is a dynamic process, mainly resulting from mutation, natural selection and (limited) gene flow. If large population sizes are typical for most protist species, the absolute number of beneficial mutations in a population is presumably higher than in most macroorganisms. The opposite holds true for genetic drift, which is of minor importance in large populations. However, if the population structure is largely clonal, effective population size may be low (for quantification of the degree of clonality consult De Meester et al. 2006). Natural selection can only lead to a shift in beneficial allele frequencies in a population if gene flow is limited. If new immigrants are permanently swamping the gene pool of a resident population, local adaptation to a specific habitat is hindered.
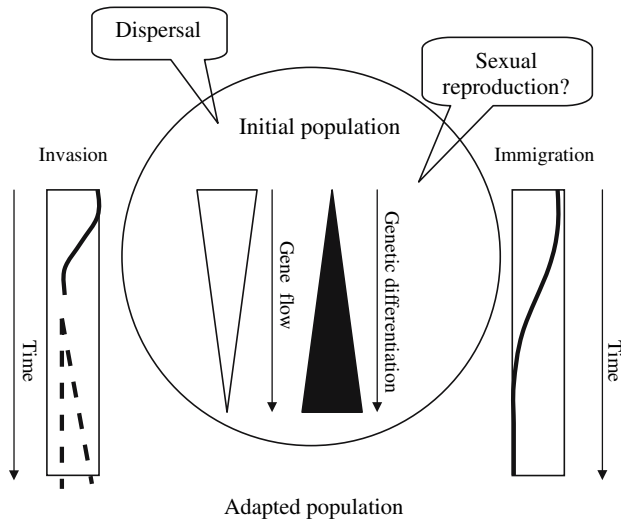
Fig. 1 A conceptual model showing the transition from an initial, unadapted population towards a population adapted to the local habitat conditions. Invasion (left, solid line) may be high at the beginning, but must then decline to reduce immigration (right) and gene flow (central, left). Invasion may remain low or increase again during the later stages of the adaptation process (left, dashed lines). Reduced gene flow and directional selection will lead to increased genetic differentiation and increased local adaptation (central, right). The speed and extent of local adaptation depend on the rate of dispersal (top, left) and the frequency of sexual reproduction

The conditions, under which local adaptation is likely to develop, and the major uncertainties in our current understanding of the protist population structure can be summarized in a conceptual model (Fig. 1). The initial population in Fig. 1 can be a founder population or any population in a given habitat at an arbitrary time zero. The gene pool of this population will be affected by a dynamic interplay of mutations, gene flow and natural selection. Marked shifts in the allele frequencies, leading to genetic differentiation towards a better adapted state of the population (with increased fitness), require that gene flow is reduced (Fig. 1, central left). Accordingly, there should be a minimum in the rate of invasion during the initial steps of the adaptation (Fig. 1, left). Invasion may stay low or increase again during the later stages of the adaptive process; irrespective of the rate of actual dispersal, the rate of immigration may continuously decline (Fig. 1, right) in response to the increased genetic differentiation (Fig. 1, central right). This is a consequence of continuously reduced competitive ability (and, therefore, lowered fitness) of the new invaders, relative to the residents, which become better and better adapted with time (De Meester et al. 2002). Note that genetic differentiation in this model does not denote genetic variation of the gene pool (which will be reduced in the course of adaptation), but the relative deviation from the initial, unadapted state. If the initial population of Fig. 1 were clonal, it should represent a 'general-purpose genotype' with broad ecological tolerances (see Vrijenhoek 1998 for review) that would slowly evolve towards a more specialized genotype, provided that the environment remains relatively stable. This conceptual model is similar to the 'Monopolization Hypothesis' (MH) proposed for pond-dwelling organisms (De Meester et al. 2002), according to which early colonists develop large, rapidly adapting populations which prevent immigration by new genotypes. The MH emphasizes the importance of numerical effects of effective dispersal, which may be

reinforced in some taxa such as *Daphnia* or bryozoans (Freeland et al. 2000, 2001) by dormant stages hatching in this habitat.

There are two major unknowns in the conceptual model outlined in Fig. 1. First, invasion, directly, and immigration, indirectly, depend on the overall rate of actual dispersal, which includes release of new individuals into the habitat from excysting resting stages that were deposited earlier. Secondly, the frequency of sexual reproduction is also unknown for >99% of all free-living, extant protist species. If occurring, recombination and non-random mating in the course of sexual reproduction may largely affect the gene pool of a population and the process of local adaptation. Regular sexual reproduction, which can be inferred from relatively close agreement of allele frequencies with the Hardy–Weinberg equilibrium, will prevent a constant reduction in genetic variation in each generation. Accordingly, while sex increases the efficacy of natural selection (Goddard et al. 2005), continued recombination will prevent local adaptation by clonal lineages with improved fitness. It is the combination of sexual and clonal reproduction (cyclical parthenogenesis) that is very powerful to generate local adaptation (De Meester et al. 2002). Frequent sexual reproduction at the beginning of the growing season or during the initial stages of colonization will generate a high genetic diversity. If sexual reproduction is then replaced by clonal reproduction and diversifying clonal selection, this will efficiently promote local adaptation (De Meester et al. 2002). In obligate asexually propagating organisms the increasing average fitness of the residents with time may result from strong clonal selection, favouring the clones of the initial clone pool or new invaders that were already best (pre-)adapted to the conditions in the new habitat, rather than from adaptive mutational changes within one or a few clones (De Meester et al. 2002).

To my knowledge, no evidence is available that would allow an estimate of the relative significance of the processes enhancing or impeding local adaptation among free-living protist species. Among aquatic organisms, small-scale changes in (neutral) allele frequencies (microevolution) have been reported mainly for freshwater zooplankton, in particular for cladocerans and rotifers (reviewed by De Meester 1996; De Meester et al. 2002). It was demonstrated primarily with *Daphnia* that changes in an adaptive, quantitative trait may occur over relatively few generations (Hairston et al. 1999; Cousyn et al. 2001). The invasion resistance of resident populations has been measured experimentally with crustacean and rotifer species (Korpelainen 1986; Shurin 2000). In Shurin's (2000) study, a surprisingly high proportion, >91% of the species introduced, immediately became extinct. If the knowledge that has been gained primarily with cyclical and obgligate parthenogens (*Daphnia*, rotifers) is valid for obligate or facultative asexual protist species, I infer that (some degree of) measurable local adaptation may be the rule, rather than the exception in most protist populations.

In conclusion, it appears at present impossible to unify all different aspects of genotypic variation and phenotypic plasticity for a given protist species. Pertinent questions for future research on protist diversity are, (1) how much variability beyond phenotypic plasticity and flexibility is necessary to establish a new species, and (2) how do we compare and rank the various levels (molecular, morphological, physiological, ecological) of variation for species designation. In order to understand natural protist diversity, it is more important to analyse the phenotypic properties of individuals, clones, populations and species than to characterize their genetic potential. From an ecological point of view, I suggest that a species is undergoing (parapatric or peripatric) speciation if (i) local adaptation has progressed to an extent that immigration into a particular habitat by its congeners is no longer possible, and if (ii) ecophysiological criteria (thresholds) can be defined that do not overlap between the different populations.

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