

## A comparative population genetic study on calanoid freshwater copepods: Investigation of isolation-by-distance in two *Eudiaptomus* species with a different potential for dispersal

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### Abstract

We examined patterns of genetic isolation-by-distance in two sister species of freshwater copepods, *Eudiaptomus graciloides* and *Eudiaptomus gracilis*, using the polymorphism of microsatellite markers. We assumed an enhanced dispersal potential of the species possessing diapausing eggs (*E. graciloides*) compared with *E. gracilis* with no diapause. On a longitudinal gradient spanning from northern Germany to Russia, we sampled 17 *E. graciloides* populations and 16 *E. gracilis* populations in order to investigate the scale dependence of isolation-by-distance from 0.4 to 1,340 km distance. In the 100–1,000 km range, no isolation could be detected in either *Eudiaptomus* species, suggesting either similar moderate gene flow over large geographic distances or persistence of historic patterns after postglacial recolonization. Our hypothesis was partly supported at the small-scale level (100 km range) where differences in population structure among species existed. We found isolation-by-distance in *E. graciloides*, the species with resting eggs, whereas *E. gracilis* exhibited a pattern of persistent founder effects. Further, in *E. graciloides* we found significant isolation-by-distance in the 1,340 km range with significantly reduced gene flow at distances >1,000 km. In *E. gracilis*, isolation-by-distance at distances >1,000 km could not be detected with our data because this species was not found in the Russian lakes. Our results suggest that short- and long-distance dispersal in *Eudiaptomus* are due to different processes, with diapausing eggs only being advantageous for short-distance dispersal. We also argue that different spatial scales must be sampled to understand the geographic partitioning of genetic variance at marker loci.

The study of patterns of genetic variation and differentiation at neutral marker loci has considerably advanced our knowledge on gene exchange in many species, especially those where direct measurement of the movement of individuals is impossible. In passively dispersing freshwater zooplankton, such studies often revealed high genetic differentiation even among nearby populations, indicating restricted gene flow (Boileau et al. 1992; Lynch and Spitze 1994; De Meester 1996). These results, achieved with genetic approaches, were at odds with the broad opinion that zooplankton organisms are frequently dispersed over large distances. At present, there is no general consensus to explain the discrepancy between genetic differentiation and dispersal capacity (Boileau et al. 1992; De Meester et al. 2002; Bohonak and Jenkins 2003). Boileau et al. (1992) suggested that these discrepancies were due to “persistent founder effects” where zooplankton populations, which developed from a few individuals and grew rapidly after colonization, remained stable against the invasion of newly arriving individuals. De Meester et al. (2002) expanded this theory to the “monopolization hypothesis” by including the buffering effect of resting propagule banks and the consequences of rapid local adaptation. Both of these factors

should increase the persistent founder effect. The above hypotheses were based on high dispersal capacity but reduced gene flow. On the contrary, Bohonak and Jenkins (2003) concluded that passive dispersal is not generally frequent and widespread in freshwater invertebrates. Thus, they suspected that the monopolization hypothesis formulated by De Meester et al. (2002) could be applied for freshwater invertebrates in general (Bohonak and Jenkins 2003). This controversy of genetic differentiation versus dispersal capacity underlines the fact that our understanding of the general mechanisms that regulate dispersal and gene flow—and therefore the population genetic structuring in zooplankton organisms—is still poor and partly is the result of a lack of empirical data to validate competing hypotheses. The motivation of the present work was to provide empirical data using high-resolution microsatellites for measuring genetic differentiation, and hence genetic structure across a range of spatial scales.

Studies on population genetics of zooplankton organisms have so far concentrated on parthenogenetic cladocerans and rotifers, but investigations on obligately sexual freshwater species, especially copepods, are still rare (De Meester et al. 2002). In this study we investigated the population genetic structure of two calanoid copepods *Eudiaptomus graciloides* and *Eudiaptomus gracilis*. Both species are closely related but exhibit distinct life-cycle patterns with diapausing eggs occurring only in *E. graciloides* (Santer et al. 2000). Diapausing eggs are known to be transported via several vectors such as wind, rain, or waterfowl and therefore possess a high potential for passive dispersal (Stemberger 1995; Bilton et al. 2001; Havel and Shurin 2004). Thus, we hypothesized an enhanced dispersal potential of *E. graciloides* when com-

### Acknowledgments

We are grateful to Siegfried Voss, Harald Deiwick, Victor Alekseev, Andrzej Mikulski, Adam Petrussek, Peter Kasprzak, and Kestutis Arbaciauskas for sampling assistance, and Silke Carstensen, Ilka Dankert, Nadine Ryk, and Tanja Sonntag for their laboratory assistance. We thank Nicole Aberle-Malzahn for helpful comments on an earlier version of the manuscript, and two anonymous reviewers for their constructive contribution to our work. T.B.H.R. was partly funded by DFG (Re 1108/4).

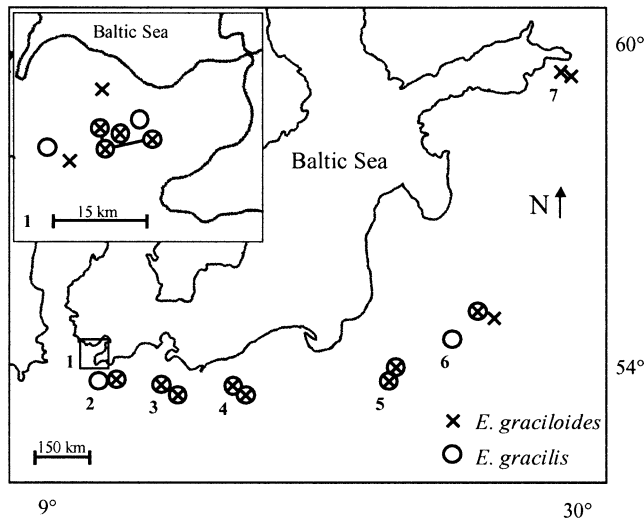


Fig. 1. Sampling sites of *Eudiaptomus* in the southern range of the Baltic Sea. Numbers indicate the sampling regions: Regions 1–3 are located in Germany, Regions 4 and 5 in Poland, Region 6 in Lithuania, and Region 7 in Russia. Regions 1–7 = 1,340 km range, Regions 1–6 = 1,000 km range, Regions 1 and 2 = 100 km range. The inset shows detail of Region 1. Solid lines between lakes indicate a connection by waterway.

pared with its sister species *E. gracilis*. The between-species variation on the one hand and their relatedness and co-existence in many European freshwaters on the other hand (Kiefer 1978; Nauwerck 1980) provided an excellent system to investigate the population genetic structure of planktonic copepods and to compare these between species with different dispersal capacities. For *E. graciloides*, we hypothesized effective exchange of individuals over short distances and increasing genetic differentiation over long distances due to effective but decreasing dispersal via diapausing eggs. In contrast, we expected higher genetic differentiation but no relationship with geographical distance for populations of *E. gracilis* due to reduced gene flow and higher importance of genetic drift.

## Methods

**Study species**—The calanoid copepods *E. graciloides* and *E. gracilis* are small zooplankton organisms (1.0–1.5 and 1.0–2.0 mm body length, respectively; Einsle 1993) that are widely distributed in continental European freshwater ecosystems (Kiefer 1978; Einsle 1993). *E. graciloides* and *E. gracilis* co-occur in a number of lakes in northern Europe (Kiefer 1978; Nauwerck 1980). Both species are phylogenetically closely related, morphologically similar, and reproduce obligately sexual (Einsle 1993).

**Sampling sites and sampling scheme**—Zooplankton samples were taken from lakes located on a longitudinal gradient spanning from northern Germany to Russia to look for scale dependence of genetic differentiation due to isolation-by-distance (Fig. 1). The landscape in this area was formed after the last ice age about 11,000 yr ago, and lakes located here have the same age and geological history. We chose seven

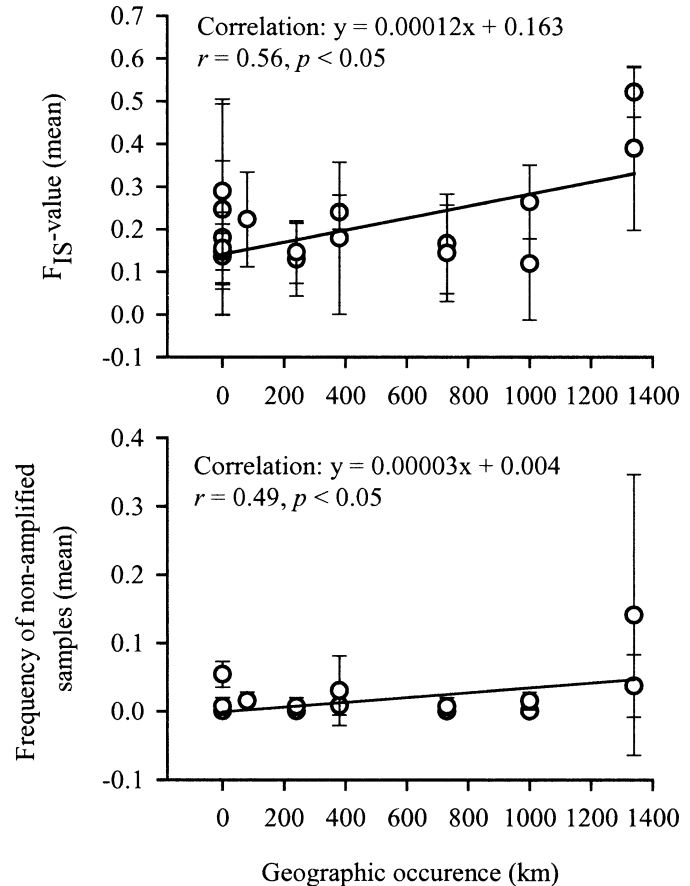


Fig. 2.  $F_{IS}$ -values and frequencies of nonamplified samples of *E. graciloides* populations as indicators for null-alleles along the sampled transect from northern Germany to Russia. Populations are shown in order of their geographic location from west (W) to east (E): distance 0 = northern Germany populations, distance 1,340 = Russian populations. On the y-axis the means of  $F_{IS}$ -values and frequencies of nonamplified samples, respectively, of each microsatellite loci (EGO2, 7, 10) are shown. Error bars: standard deviation.

regions within this area on a logarithmic spatial scale and sampled populations of two lakes per region in order to investigate population genetic structure on a large spatial scale. Distances between lakes varied from a few hundred meters up to a maximum of 1,340 km. Additionally, in northern Germany (Region 1; Fig. 1) we sampled a total of six populations of each *Eudiaptomus* species. These consisted of four populations from lakes where both species co-occurred and two populations of each species from lakes where the other *Eudiaptomus* species was missing. For our investigation of genetic differentiation on a small spatial scale (100 km range), we used the data of the above populations together with the data from populations of that lake in Region 2 where both species co-existed (Regions 1 and 2 = 100 km range; Fig. 1). Names and positions of studied lakes are available in the Web Appendix 1 ([http://www.aslo.org/lo/toc/vol\\_51/issue\\_1/0117a1.pdf](http://www.aslo.org/lo/toc/vol_51/issue_1/0117a1.pdf)).

We took zooplankton samples with a plankton net and stored them in 70% alcohol until further processing.

**DNA extraction and microsatellite analysis**—For the population genetic studies on *Eudiaptomus* species we used the polymorphism displayed by 10 microsatellite markers that we had developed previously: seven polymorphic loci for *E. gracilis* (EGI1, 3, 8, 12, 13, 17, and 35; Genbank accession numbers AY547395–547401) and three polymorphic loci for *E. graciloides* (EGO2, 7, and 10; Genbank accession numbers AY547392–547394; Zeller and Reusch 2004). These markers represented a total of 127 alleles of *E. graciloides* and 314 alleles of *E. gracilis* in the studied populations. For further information on library construction and primer sequences see Zeller and Reusch (2004).

For the genetic analysis, we isolated 45 females per lake and species from the zooplankton samples. We chose only females for our investigation, because it was difficult to distinguish between males of the two species. Prior microsatellite analysis of males and females originating from lakes where only a single *Eudiaptomus* species occurred did not show any sex-specific differences in the used markers.  $F_{ST}$ -values calculated between males ( $n = 46$ ) and females ( $n = 46$ ) of one population were not significantly different from zero in a total of two populations of each species that we investigated. In this study we extracted deoxyribonucleic acid (DNA) from the isolated copepod females using the Invisorb DNA Tissue HTS 96-Kit/C (Invitex). We amplified microsatellite loci in polymerase chain reactions (PCRs). Loci of *E. gracilis* were amplified together in multiplex PCRs as follows: EGI1 + 13 + 17, EGI8 + 12, and EGI3 + 35 in a total reaction volume of 20  $\mu\text{L}$  using 2  $\mu\text{L}$  template DNA. Loci of *E. graciloides* were amplified in single reactions of 10  $\mu\text{L}$  with 1  $\mu\text{L}$  template DNA. Further PCR contents were the following: 0.1% bovine serum albumin (BSA); 250 pmole  $\mu\text{L}^{-1}$  each dinucleotide triphosphate (dNTP); 1  $\mu\text{L}$  forward primer (5 pmole  $\mu\text{L}^{-1}$  stock), and 1  $\mu\text{L}$  reverse primer (5 pmole  $\mu\text{L}^{-1}$  stock) for EGI3 1.5  $\mu\text{L}$ , and for EGI17 2  $\mu\text{L}$  of each primer (5 pmole  $\mu\text{L}^{-1}$  stock); 1 $\times$  buffer (Promega); 1U Polymerase (Promega); 2.25 mmole  $\mu\text{L}^{-1}$   $\text{MgCl}_2$  (EGI1, 8, 12, 13, 17, EGO7), and 1.5 mmole  $\mu\text{L}^{-1}$   $\text{MgCl}_2$  (EGI3, 35, EGO 2, 10), respectively. An initial denaturation step at 94°C for 3 min was followed by 30 cycles of 94°C for 50 s, 56.5°C (55.5°C for EGO10) for 30 s, 72°C for 30 s, and a final extension step of 72°C for 20 min. Amplified microsatellite loci were electrophoretically separated and size scored on an ABI-3100 capillary sequencer (Applied Biosystems) using Rox 350 as the internal standard. The data were analyzed with GENESCAN and GENOTYPER (Applied Biosystems). We excluded from further analysis individuals that resulted in no PCR product for any primer. If only single primer pairs did not amplify, we repeated PCRs up to two times using only one failed primer pair per reaction, with a total reaction volume of 20  $\mu\text{L}$ . If necessary we increased cycling steps from 30 to 34. Where PCR products were still missing, we interpreted these as homozygote null alleles.

**Data analysis**—Null alleles: Null alleles are alleles that do not amplify during PCR because of mutation events changing the DNA sequence in the primer binding region, which causes the primer no longer to bind to the DNA during the PCR (Callen et al. 1993). This results in either no PCR prod-

Table 1. Null alleles in *Eudiaptomus* species. Null allele 1: Frequency of nonamplified samples. Null allele 2: frequency of calculated null alleles (Brookfield 2) with programme Microchecker 2.2.1 (Van Oosterhout et al. 2004); Std. dev., standard deviation; EGO, *E. graciloides*; EGI, *E. gracilis*. Inbreeding coefficients ( $F_{IS}$ ) and calculated frequencies of null alleles are shown as mean of all 17 *E. graciloides* populations and 16 *E. gracilis* populations, respectively. Primer pairs that were used for F-statistics in *E. gracilis* are printed in bold.

Primer	Null allele 1	Null allele 2	Std. dev.	$F_{IS}$	Std. dev.
EGO10	0.005	0.064	0.079	0.103	0.035
EGO7	0.008	0.150	0.103	0.274	0.036
EGO2	0.043	0.184	0.145	0.260	0.028
<b>EGI1</b>	<b>0.003</b>	<b>0.061</b>	<b>0.065</b>	<b>0.120</b>	<b>0.033</b>
EGI3	0.033	0.207	0.087	0.267	0.028
<b>EGI8</b>	<b>0.003</b>	<b>0.057</b>	<b>0.050</b>	<b>0.121</b>	<b>0.021</b>
EGI12	0.043	0.362	0.140	0.577	0.036
EGI13	0.029	0.240	0.109	0.325	0.032
<b>EGI17</b>	<b>0.006</b>	<b>0.052</b>	<b>0.050</b>	<b>0.110</b>	<b>0.030</b>
EGI35	0.010	0.259	0.141	0.446	0.059

uct, if the null allele is homozygote, or in false homozygote individuals, if the locus is heterozygote. Thus, an excess of homozygote individuals, as found for both *Eudiaptomus* in our investigation (Web Appendix 2, [http://www.aslo.org/lo/toc/vol\\_51/issue\\_1/0117a2.pdf](http://www.aslo.org/lo/toc/vol_51/issue_1/0117a2.pdf)), could be caused by the presence of null alleles or by a real biological phenomenon. Available software for estimating null allele frequencies calculate them on the basis of heterozygosities, making it difficult to decide whether inbreeding or the presence of null alleles causes the excess of homozygote genotypes. Therefore, to assess the frequency of null alleles in our study we did not want to rely on calculations of null allele frequencies only, but rather on a combination of information about calculated null allele frequencies (Brookfield 2, Microchecker 2.2.1; Van Oosterhout et al. 2004),  $F_{IS}$ -values, and frequencies of nonamplified samples (Table 1).

Further, we tested for correlations between the above parameters (means of information from microsatellite loci for each population) and their geographic occurrence to examine whether the frequency of null alleles was biased with distance. Populations in northern Germany were defined to be located at distance zero with all other populations oriented along an east-west transect.

**Hardy-Weinberg equilibrium and inbreeding:** We calculated the population genetic parameters observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ), linkage disequilibrium, and F-statistics with the software GENETIX v. 4.01 (Belkhir et al. 1998).  $F_{IS/IT}$ -values for *E. gracilis* were only calculated with information from three primer pairs, because it was likely that null alleles were abundant in the remaining four pairs (see Results). We performed a Hardy-Weinberg equilibrium test in GENEPOP v. 3.3, which estimates exact  $p$  values using the Markov chain method (Raymond and Rousset 1995).

**Isolation-by-distance and species comparison:** We performed an isolation-by-distance investigation for *Eudiapto-*

*mus* species, including geographic distances ranging from 0.4 to >1,000 km. The purpose was to address exchange of individuals on distinct spatial scales and to compare the population genetic structure between both species considering their different abilities for dispersal.

To test for isolation-by-distance we calculated pairwise  $F_{ST}$ -values with GENETIX v. 4.01 (Belkhir et al. 1998), which calculates the estimator  $\theta$  (Weir and Cockerham 1984). We plotted these estimates of pairwise population differentiation against the geographic distances separating each pair of populations. With the help of maps, geographic distances were measured as the shortest direct lines. Subsequently, we tested for a significant relationship between the matrix of pairwise  $F_{ST}$ -values ( $\theta$ ) and the matrix of geographic distances by performing Mantel tests of matrix correlations, calculated as reduced major axis (RMA) regression with the program IBD v. 1.52 (Bohonak 2002).

In order to investigate the population genetic structure on a large geographic scale, we performed Mantel tests including data from all 17 populations of *E. graciloides* and all 16 populations of *E. gracilis*, respectively. We used data of 12 populations where species co-occurred for species comparison. Matrices of geographic and genetic distances including all populations are available in the Web Appendix 3 ([http://www.aslo.org/lo/toc/vol.51/issue\\_1/0117a3.pdf](http://www.aslo.org/lo/toc/vol.51/issue_1/0117a3.pdf)). To compare the pairwise genetic differentiation between species, we performed a Mantel test between the matrix of pairwise  $F_{ST}$ -values for *E. graciloides* and the matrix of pairwise  $F_{ST}$ -values for *E. gracilis* (12 populations, program IBD v. 1.52; Bohonak 2002). An additional pairwise *t*-test was performed with the program Statistica v. 5 (StatSoft).

For short geographic distances, we used a data subset that contained information about seven investigated populations of each species from lakes located in northern Germany (Regions 1 and 2 = 100 km range; from Region 2 we included only populations of one lake where both species co-existed; Fig. 1). Three of these lakes were connected by waterways providing three out of 21 pairwise comparisons on genetic differentiation. We neglected stream connections and measured geographic distances as shortest direct lines, since the number of data points were low and populations were partly dispersal-limited (see Results). Mantel tests were performed, this time comparing the geographic and genetic distance matrix for each species separately using the software IBD v. 1.52 (Bohonak 2002).

## Results

**Null alleles**—Three of the seven primer pairs for *E. gracilis* provided low  $F_{IS}$ -values as well as low calculated frequencies of null alleles and low frequencies of nonamplified samples (loci EGI1, 8, 17; Table 1). For the remaining four primer pairs the occurrence of null alleles was very likely. Therefore, we calculated  $F_{IS/IT}$ -values for population genetic analysis with information from the loci EGI1, 8, and 17 only. For assessing isolation-by-distance we included the information of all primer pairs, because the occurrence of null alleles was not biased with distance (Brookfield 2,  $r = 0.05$ , not significant;  $F_{IS}$ -values,  $r = 0.15$ , not significant; fre-

quencies of nonamplified samples,  $r = -0.02$ , not significant). Thus, calculations of pairwise  $F_{ST}$ -values should be unaffected by the occurrence of null alleles. For *E. graciloides* we did not exclude information of any primer pairs for the calculations of  $F_{IS/IT}$ -values, but noted that null alleles might be abundant (Table 1). Calculated frequencies of null alleles were not biased with geographic distance in *E. graciloides* (Brookfield 2,  $r = 0.46$ , not significant).  $F_{IS}$ -values and nonamplified samples were positively correlated with increasing geographic distance to northern Germany ( $F_{IS}$ -values,  $r = 0.56$ ,  $p < 0.05$ ; frequencies of nonamplified samples,  $r = 0.49$ ,  $p < 0.05$ ; Fig. 2). These correlations were driven by one of the Russian samples (Lake 7.1), which had high null allele frequencies (data not shown).  $F_{IS}$ -values and nonamplified samples were not correlated with distance when this population was excluded from the analysis ( $F_{IS}$ -values,  $r = 0.35$ , not significant; frequencies of nonamplified samples,  $r = 0.15$ , not significant). Thus, in *E. graciloides* null alleles were not correlated with distance; rather, more null alleles existed in one of the Russian populations (Lake 7.1) compared with the other investigated populations.

**Hardy-Weinberg equilibrium and inbreeding**—All loci were in linkage equilibrium for both species. Expected heterozygosities ( $H_E$ ) and observed heterozygosities ( $H_O$ ) for all loci and all populations, as well as the inbreeding coefficients ( $F_{IS}$ -values) and deviations from Hardy-Weinberg expectations, can be found in the Web Appendix 2. Calculations of *F*-statistics over all populations resulted for *E. gracilis* in  $F_{IS}$ -values of 0.12 (0.11–0.12),  $F_{IT}$ -values of 0.16 (0.13–0.23), and  $F_{ST}$ -values of 0.05 (0.01–0.13) based on information of primers EGI1, 8, and 17. For *E. graciloides* we found  $F_{IS}$ -values of 0.22 (0.10–0.27),  $F_{IT}$ -values of 0.26 (0.17–0.29), and  $F_{ST}$ -values of 0.05 (0.03–0.08).

**Isolation-by-distance and species comparison**—On a large spatial scale (1,340 km), a high proportion of the pairwise genetic differentiation between all 17 *E. graciloides* populations could be explained by their geographic distances ( $r = 0.63$ ,  $p < 0.001$ ; Fig. 3A). However, the scatter plot pointed to differences in the relationship of genetic and geographic distance. Genetic variation increased with geographic distance for populations located in a 100-km range, but no obvious relationship between genetic and geographic distance existed for populations separated by ~100 to 1,000 km. We observed a further increase in genetic differentiation for populations separated by more than 1,000 km (Fig. 3A). In contrast, we could not find a significant correlation between genetic and geographic distances for the sister species *E. gracilis* (total data set, Fig. 3B;  $r = 0.26$ ,  $p = 0.056$ ). Note that information for populations separated by more than 1,000 km are missing in this data set, because *E. gracilis* was absent in samples from Russian lakes.

To compare the population genetic structure between *E. graciloides* and *E. gracilis* at a geographical range of 0.4–1,000 km, we reduced the data set to the 12 populations of both species originating from the same lakes. Neither population of *E. graciloides* nor *E. gracilis* were isolated by distance (*E. graciloides*,  $r = 0.32$ ,  $p = 0.065$ ; *E. gracilis*,  $r = 0.25$ ,  $p = 0.118$ ; Fig. 3, filled circles). The pairwise  $F_{ST}$ -

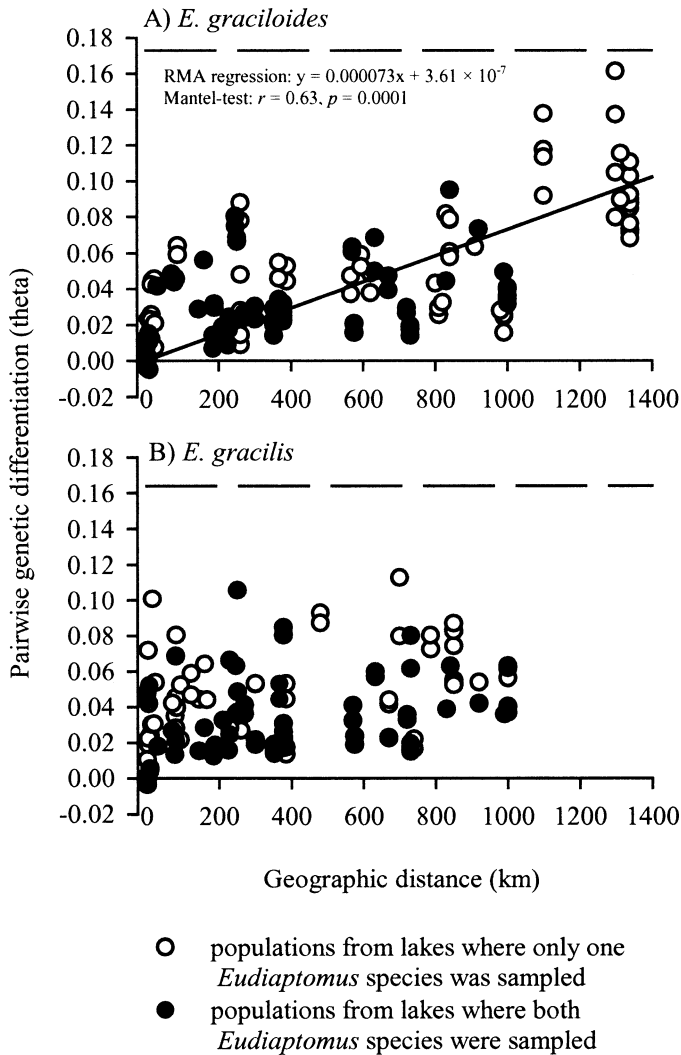


Fig. 3. Isolation-by-distance over the complete range (1,000–1,340 km). Shown are all pairwise comparisons among 17 populations of (A) *E. graciloides* and 16 populations of (B) *E. gracilis*. Solid line: reduced major axis (RMA) regression calculated with IBD v. 1.52 for all populations (Bohonak 2002). Dashed lines: expected maximal value of genetic differentiation (Hedrick 1999).

values for these co-occurring populations of both species were positively correlated ( $r = 0.45, p < 0.05$ ; Fig. 4) and did not differ between species (paired  $t$ -test,  $t = -0.438, df = 130, p = 0.662$ ).

At a small spatial scale (<100 km distance), we found differences between both species. At this scale we observed higher variability for *E. gracilis* than for *E. graciloides* (Fig. 3A,B). For example, at a 50-km distance, pairwise  $F_{ST}$ -values between *E. gracilis* populations ranged from 0 to 0.1 (Fig. 3B), whereas pairwise  $F_{ST}$ -values between *E. graciloides* populations only attained 0 to 0.05 (Fig. 3A). Figure 5 shows the pairwise  $F_{ST}$ -values from seven populations of lakes located in northern Germany plotted against the geographic distances (100 km range). We found a significant relationship between genetic differentiation and geographic distance in *E. graciloides* ( $r = 0.81, p = 0.004$ ), but not in

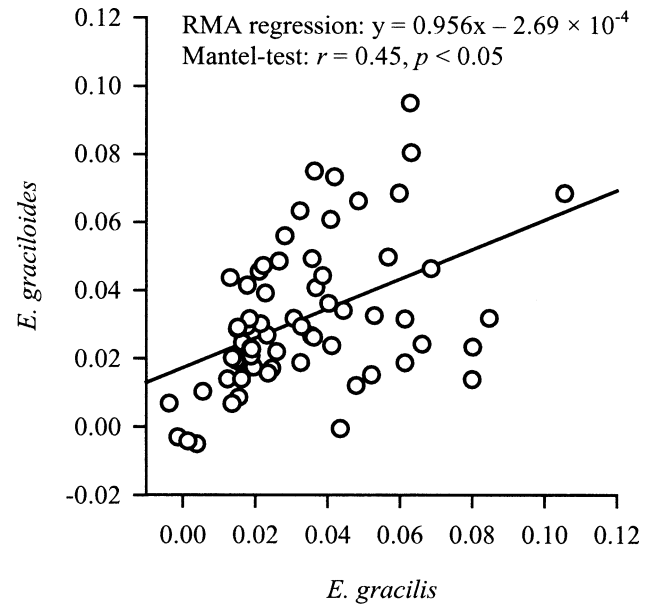


Fig. 4. Cross-correlation of pairwise genetic differentiation (theta) between 12 populations of *E. graciloides* and 12 *E. gracilis* populations originating from the same lakes (corresponding to filled circles in Fig. 2). Solid line: RMA regression calculated with IBD v. 1.52 (Bohonak 2002).

*E. gracilis* (Fig. 5;  $r = -0.08$ , not significant), although the latter species had a larger range of variation. Three out of the 21 pairwise genetic comparisons resulted from population pairs of lake locations that were connected by streams (Fig. 5, filled circles). Pairwise  $F_{ST}$ -values for one population pair from interconnected lakes (Lakes 1.3 and 1.4) differed significantly (geographic distance = 8.5 km):  $F_{ST} = 0.01$  ( $p < 0.05$ ), and  $F_{ST} = 0.006$  ( $p < 0.05$ ) for *E. graciloides* and *E. gracilis*, respectively (see Web Appendix 3;  $p$ -values not shown). Since only a few data points were affected and populations were already partly dispersal-limited, we neglected lake connectivity as an influencing factor in our study.

## Discussion

Isolation-by-distance is expected between populations if gene flow is prevalent but dependent on geographical distance, because the exchange of individuals should be more frequent among nearby populations than among populations located further away (e.g., Wright 1946; Slatkin 1993; Hutchison and Templeton 1999). However, we predicted to observe no isolation-by-distance when gene flow is very low or if current patterns of gene flow are largely independent of any spatial dependence of the vector for propagules. Our results were, at least in part, consistent with the above hypotheses. We could only detect isolation-by-distance for *E. graciloides* producing diapausing eggs, but not for *E. gracilis*. Genetic and geographic distances of populations of *E. graciloides* were positively correlated on a large spatial scale (1,340 km) and on a short spatial scale (<100 km). Surprisingly, we could not find isolation-by-distance in the 1,000 km range in either *Eudiaptomus* species, but rather an irregular pattern of similar and genetically differentiated popu-

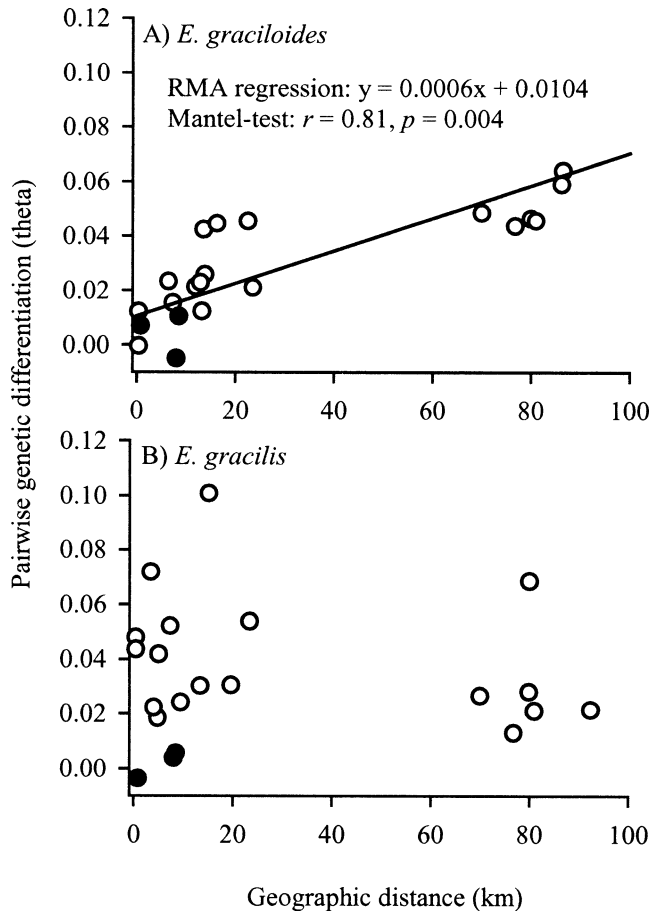


Fig. 5. Isolation-by-distance (100 km range). Pairwise comparisons of seven populations of (A) *E. graciloides* and (B) *E. gracilis*. Solid line: RMA regression calculated with IBD v. 1.52 (Bohonak 2002). Filled circles: pairwise comparisons between populations from interconnected lakes.

lations. This pattern might be due to past imprints of recolonization patterns after the last ice age, or, at least in the case of *E. graciloides*, to a different process of genetic exchange that was unaffected by distance. However, on a small spatial scale species-specific differences did occur. In *E. gracilis* genetic differentiation between populations was partially higher compared with *E. graciloides* and seemed to be randomly distributed with respect to geographic distance. Thus, we found our hypothesis to be true for a smaller geographic scale than originally expected. Due to our nested sampling design, we were able to detect these species-specific differences and to find for *E. graciloides* that isolation-by-distance (100 km range) was hidden in a larger pattern of genetic differentiation that seemed to be unaffected by geographic distance (1,000 km range). These results underline the importance of both the chosen sampling scheme for isolation-by-distance investigations in general and the necessary care that must be taken in the interpretation of studies addressing only a single spatial scale.

We can only speculate on the possible causes for the apparent dissimilarities in the patterns of genetic differentiation we observed at different spatial scales. The irregular patterns

of population differentiation in both *Eudiaptomus* species between distances of 100 and 1,000 km may indicate a similar moderate exchange of individuals over large distances for both species, for example through migratory waterfowl (Charalambidou and Santamaría 2002; Figuerola et al. 2003). Alternatively, this pattern does not reflect contemporary gene flow, but rather an average historic differentiation after a rapid recolonization after the last ice age. Within this matrix of moderate population differentiation due to founder effects, cells of isolation-by-distance developed in *E. graciloides*, whereas small- and large-scale patterns were congruent in *E. gracilis*. If we assume contemporary gene flow as shaping the observed patterns, it implies that the occurrence of diapausing eggs in *E. graciloides* does not enhance its dispersal ability in terms of long-distance dispersal compared with its sister species *E. gracilis*.

On small geographic scales (100 km range), however, populations of *E. gracilis* differed substantially in their genetic structure even at nearby locations. This pattern of high genetic differentiation between nearby populations is known in a variety of freshwater ecosystems (Boileau et al. 1992; De Meester 1996; Nies and Reusch 2005). De Meester et al. (2002) explained such patterns with persistent founder effects (Boileau et al. 1992), which are enhanced by resting propagule banks and local adaptation. Persistent founder effects should be largely independent of geographical distance and should be stronger in *E. graciloides* compared with *E. gracilis* due to the existence of resting propagule banks (De Meester et al. 2002). Thus the observed species-specific differences at short distances were probably caused by differences in short-distance dispersal and current gene flow, respectively, rather than by population history alone. Possible vectors for short distance dispersal may include both wind and rain (Cáceres and Soluk 2002; Cohen and Shurin 2003) or waterfowl (Green et al. 2002). There is some evidence that at small distances zooplanktonic organisms are in fact highly effective dispersers; however, animals may be a less important dispersal vector than wind (Cáceres and Soluk 2002; Cohen and Shurin 2003).

For the total investigative range of 1,340 km, we were not able to draw comparisons about genetic differentiation between the sister species, since we could only find individuals of *E. graciloides* in the Russian lakes. The increasing genetic differentiation of *E. graciloides* on distances above 1,000 km indicated restricted gene flow that might be caused by restricted dispersal. However, dispersal events must not necessarily lead to successful gene flow. The dispersers are not always as well adapted to the environmental conditions of the new habitat as the residents; therefore, they are not consequently able to reproduce or survive (De Meester et al. 1996; Bohonak and Jenkins 2003). In our study population, pairs with geographical distances greater than 1,000 km always included one of the two investigated *E. graciloides* populations sampled in the vicinity of St. Petersburg, Russia. These populations were not only located at the most easterly regions of this study, but also at a higher latitude (59–60°N) than all other investigated populations. At high latitudes, the summer season is shorter and lakes are covered with ice for at least 6 months of the year (V. Alekseev pers. comm.). Local adaptations are an expected phenomenon if popula-

tions are faced with very distinct environmental conditions. *E. graciloides*, for example, is only able to exhibit one new generation per year at this high latitude of 60°, whereas populations from lower latitudes are able to produce two or more generations per year (Nauwerck 1963; Ekman 1964; BosseLMANN 1975). Furthermore, the timing of reproduction and diapause should match with the seasonal changes in the environment to ensure survival (Hairston and Olds 1984; Santer 1998). Thus, gene flow between Russian and northern German populations might additionally be restricted by selection and local adaptation.

An alternative explanation for the identified pattern of genetic differentiation over the whole geographic range in *E. graciloides* might be source and sequence of postglacial recolonization. Different glacial refugia existed during the last ice age in Europe, from where species recolonized habitats after deglaciation (Hewitt 1996; Pamilo and Savolainen 1999). Lakes in northern Germany might be recolonized by *E. graciloides* spreading from other glacial refugia, rather than those colonizing the Russian lakes. However, information from other molecular markers that allow a coalescence-based reconstruction of colonization, such as single nucleotide polymorphisms (SNPs) (Morin et al. 2004) or mitochondrial DNA (mtDNA) (Hewitt 1999), would be necessary to adequately answer questions about different recolonization routes in *Eudiaptomus* species. We have to admit that the correlation of genetic differentiation and geographic distance in *E. graciloides* at the large spatial scale (1,340 km) might have partly been driven by the frequent occurrence of null alleles in one of the two investigated Russian populations. Null alleles are suggestive of mutations in the nonrepetitive regions of microsatellites (Callen et al. 1993). However, one may argue that the enhanced occurrence of null alleles in Russian populations is just another manifestation of diverging alleles, and thus of increasing genetic divergence. In that respect, our main conclusions are strengthened.

Our data represent the first population genetic analysis for planktonic freshwater copepods based on microsatellite polymorphism. We gave first insights into the population genetic structuring of two important members of zooplankton communities in European freshwaters and provided a new set of data in the recently discussed field of the dispersal gene flow paradoxon in freshwater organisms. We showed the importance of the sampling scale for detecting whether populations were isolated by distance. We found that a species with diapausing eggs exhibited distance-correlated genetic exchange (isolation-by-distance) over short spatial scales. In the other species lacking resting eggs, patterns seemed to confirm persistent founder effects with no current gene flow.

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Received: 17 May 2005

Accepted: 9 September 2005

Amended: 27 September 2005