Hybridization in the *Daphnia galeata* complex: are hybrids locally produced?

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Abstract

Within the species complex of *Daphnia galeata*, *D. cucullata* and *D. hyalina* various combinations of hybrids and parental taxa occur in lakes throughout Europe. Since daphnids are cyclic parthenogens and mostly reproduce asexually, hybrid populations can be maintained by asexual reproduction and without recurrent hybridization events. Therefore, it is possible that hybridization events have been rare, with range expansion occurring by dispersal of hybrids.

Allozyme data from seven European populations were used to compare genetic variation within and between hybrid and parental taxa. An UPGMA cluster analysis of genetic distances showed that *D. cucullata* × *galeata* hybrids from different lakes grouped in different clusters according to the lake from which they were isolated, suggesting multiple hybridization events. Clonal diversity within hybrid taxa was comparable to parental taxa. Furthermore, evidence was found for introgression of the *Pgi-S* allele from *D. cucullata* to *D. galeata* in three lakes. These results indicate that multiple hybridization events within this species complex are likely, and that hybrid taxa can reproduce sexually.

Introduction

Interspecific hybridization is a common phenomenon in *Daphnia*. Hybrids occur in several species complexes in North America (Colbourne & Hebert, 1996; Hebert & Finston, 1996), Australia (Hebert & Wilson, 1994) and Europe (Schwenk & Spaak, 1995). The wide distribution of *Daphnia* hybrids may be caused by their reproductive mode. Since *Daphnia* reproduce through cyclic parthenogenesis, only one or a few hybridization events are needed to establish a population of hybrids. Since these hybrids can reproduce asexually, no further hybridization events are necessary to maintain a hybrid population; presuming that environmental conditions are such that hybrids may remain present throughout the year. However, hybrid taxa also produce males and sexual females (Carvalho & Wolf, 1989; Spaak, 1995). This creates the possibility of producing a myriad of backcrosses with parental and other hybrid taxa. Recent studies (Taylor & Hebert, 1993; Spaak, 1996)

have shown that backcrossing may be a more common process than previously believed (Wolf, 1987; Hebert et al., 1989).

Several genetic studies have shown clearly that the production of *Daphnia* hybrids is a continuing process (Taylor & Hebert, 1992; Müller & Seitz, 1995; Spaak, 1996). One argument to support this notion is that genetic variation within hybrids is comparable, or even higher, than within parental species. This would not be expected in cases where hybrids were formed a long time ago and populations persist only as parthenogenetic females. In that case, the only source for genetic variation would be the initially available variation supplemented by the variation added via mutations. As mutations also add variation to parental species, they offer an unlikely explanation for the relatively high genetic variability within hybrid populations. Furthermore, at least one hybrid was found to hatch from ephippia collected from the bottom of the Shösee in Northern Germany (Carvalho & Wolf, 1989), and

Schwenk (pers. comm.) recently succeeded in producing hybrids from laboratory crosses. However, these observations do not exclude the possibility that a substantial part of the hybrid lineages may be old.

One of the questions concerning hybridization in *Daphnia* is whether hybridizing taxa are separate species. Based on the 'Biological species concept' (Mayr, 1942) of genetically isolated taxa, one could argue that they are not if hybrids are regularly produced and if back-crossing occurs. On the other hand, several genetic studies (Taylor & Hebert, 1992; Spaak, 1996; Schwenk, 1997) have shown that *Daphnia* species that produce interspecific hybrids have unique combinations of alleles, suggesting some reproductive isolation among these groups. Furthermore, parental species are morphologically distinct from each other (Flößner, 1993) and not all possible intermediate forms are observed as expected in a randomly mating 'hybrid swarm'.

More evidence for local production of interspecific hybrids in *Daphnia* can be obtained through comparisons of the genetic structure of the hybrid populations from isolated lakes. Let us assume that hybrids are evolutionary old, dispersed from one or a few hybrid 'sources', and that no additional hybrids or backcrosses were produced. In this scenario, one would expect hybrid populations to be characterised by: (i) a lower number of clones compared to parental taxa; (ii) a lower genetic differentiation among populations of different lakes than among hybrids and parental taxa within lakes. If hybridization is a local phenomenon, however, one would expect relative large genetic distances among hybrid taxa from different lakes. In the present study, I use two allozyme loci to compare *Daphnia* populations of the *Daphnia galeata* species complex (*D. galeata*, *D. cucullata*, *D. hyalina* and all three of their possible hybrids) from seven European lakes. Genetic distances among taxa within and between lakes were compared using the unweighed pair-group (UPGMA) clustering algorithm. I tested the hypothesis that hybrid populations from different lakes will cluster with the parental taxa from the same lakes rather than with the hybrids of other lakes. Support for this hypothesis indicates local and likely recurrent hybridization events in the genus *Daphnia*.

Materials and methods

Data on the genetic structure of seven *Daphnia* populations were used in this study (Table 1). Tjeukemeer

is a shallow eutrophic lake in the northern part of The Netherlands, Maarsseveen is a mesotrophic lake in the centre of The Netherlands, Ringsjön is a eutrophic shallow lake situated in southern Sweden, Plußsee, Belauersee and Höftsee are situated in northern Germany, and the Bodensee is a large, deep mesotrophic lake in southern Germany. From all lakes except Ringsjön only September and October samples were used: Lake Tjeukemeer was sampled in 1989, 1990 and 1991, Maarsseveen in 1989 and 1990, Belauersee and Höftsee in 1994 and Plußsee in 1994 and 1995. Ringsjön was sampled on 16 June and 14 October 1994 by Eva Bergstrand. Data for the Bodensee were taken from Weider & Stich (1992); I limited the analysis of this data set to only September and October sampling dates. For sampling methods and dates in Tjeukemeer see Spaak (1994; 1996); the other lakes were sampled using vertical plankton net tows (see Spaak & Hoekstra, 1993).

Individual adult females were picked randomly and assayed using cellulose acetate electrophoresis following standard methods (Hebert & Beaton, 1989). Generally, 100 animals per sampling date were assayed for three enzyme loci: phosphoglucumutase (*Pgm*, EC 5.4.2.2), phosphoglucose isomerase (*Pgi*, EC 5.3.1.9) and glutamate oxaloacetate transaminase (*Got*, EC 2.6.1.1). *Got* is a diagnostic marker for discrimination between *D. galeata*, *D. cucullata* and *D. hyalina* (Wolf & Mort, 1986). *D. galeata* is fixed for the *F* (fast) allele, *D. cucullata* for the *S*⁻ (very slow) allele, and *D. hyalina* for the *S* (slow) allele. Interspecific hybrids can be identified as heterozygotes (*S*⁻*F*, *SF*, *S*⁻*S*) at *Got* (*D. galeata* × *hyalina* [*G* × *H*]: *SF*; *D. cucullata* × *hyalina* [*C* × *H*]: *S*⁻*S*; *D. cucullata* × *galeata* [*C* × *G*]: *S*⁻*F*). Variation at the *Pgi* and *Pgm* loci made it possible to identify a number of two-locus genotypes within each taxon.

Clonal diversity

Clonal diversity was calculated for all taxa in all populations. As a measure of clonal diversity, I used Simpson's (1949) index of concentration, $C = \sum p_i^2$, where p_i is the frequency of the i th clone (two-locus genotype) in the sample, and the summation is over all clones. Clonal diversity D was calculated as $D = -\log C$ (Pielou, 1975). This diversity index is a composite of abundance and evenness: it exhibits low values when a single clone is overwhelmingly predominant and high values when the number of clones is high and when clones tend to be equally abundant.

Table 1. Average frequencies of the different taxa of the *Daphnia galeata* species complex for the seven studied lakes. N is to the total number of *Daphnia* individuals analysed

Lake	Country	N	<i>D. cucullata</i>	<i>D. cuc.</i> × <i>gal.</i>	<i>D. galeata</i>	<i>D. gal.</i> × <i>hyl.</i>	<i>D. hyalina</i>	<i>D. cuc.</i> × <i>hyl.</i>
Tjeukemeer	Netherlands	1184	0.30	0.38	0.32			
Maarsseveen	Netherlands	316			0.14	0.86		
Plußsee	North Germany	743	0.36	0.20	0.15	0.16		0.13
Belauersee	North Germany	229	0.82	0.04			0.14	
Höftsee	North Germany	111		0.10	0.90			
Ringsjön	Sweden	275	0.61	0.11	0.28			
Bodensee ¹	South Germany	486			0.49	0.20	0.31	

¹ Data from Weider & Stich (1992)

Genetic distances

Unbiased genetic distances (Nei, 1978) among samples of the different taxa from each lake were calculated from genotype frequencies and the relatedness of the different samples was assessed using the unweighed pair-group (UPGMA) clustering program of BIOSYS-1 (Swofford & Selander, 1981). Because the number of samples was unequal for several lakes, analyses of genetic distances were based on mean frequency data per taxon per lake combining all available sampling dates. Analyses were restricted to *Pgi* and *Pgm* (*Got* genotypes were used to differentiate between the taxa). Höftsee was excluded from the analysis, since only 11 *C* × *G* individuals were found, leaving only one taxon for the analysis. For the same reason, *C* × *G* from Belauersee was excluded from the analysis, but the remaining two taxa in this lake (*D. cucullata* and *D. hyalina*) were included.

I also calculated mean genetic distances between several categories of populations to make comparisons among several classes of taxa. A population in this analysis was defined as the individuals that belong to the same taxon (hybrid or parental) in a specific lake. Comparisons were made among parental taxa (different lakes); within parental taxa (among lakes); within hybrid taxa (among lakes); and among hybrid taxa and their parental taxa in the same lake.

Results

Taxon frequencies

Based on their *Got* genotypes individuals were assigned to *D. galeata* (*FF*), *D. cucullata* (*S⁻S⁻*), *D. hyalina* (*SS*) or the interspecific hybrids: In all seven

studied lakes, two or more *Daphnia* taxa were found (Table 1). *D. cucullata*, *D. galeata* and their interspecific hybrid were the most common taxa. In three lakes, both *D. cucullata* and *D. galeata* were found together with their hybrid. In Bodensee, *D. galeata* and *D. hyalina* were found together with their interspecific hybrid. The *G* × *H* hybrid was also found in two other lakes, co-occurring with only one of the parental species (*D. galeata*). The *C* × *H* hybrid was found only in the Plußsee, along with one of the parental species (*D. cucullata*).

Allele frequencies

The most common *Pgi* allele found was *M* (Table 2) with a mean frequency of 72%. Both *D. galeata* and *D. hyalina* were almost fixed for this allele (frequency > 90%) in all but one population (the *F* allele had a frequency of 16% in the *D. galeata* population in the Höftsee). In *D. cucullata*, the *M* and *S* alleles were found at comparable frequencies. For this species, the Ringsjön population in Sweden was an exception with the *S⁻* allele rather than the *M* allele having a frequency of greater than 30%. As expected, the hybrid taxa showed intermediate allele frequencies, except for *C* × *G* in the Höftsee where no *S* allele was found. In three *D. galeata* populations (Maarsseveen, Höftsee, Bodensee), no *Pgi-S* allele was found. These were also the populations where *D. cucullata* was absent.

Four alleles were found at the *Pgm* locus (Table 3). The most common *M* and *F* alleles had an equal mean overall frequency of 40%. Allele frequencies of *Pgm* were less taxon-specific than for *Pgi* (Table 3). The *S* allele had the highest frequency in *D. cucullata*, whereas the *F* allele seemed to be more frequent in *D. galeata* and *D. hyalina*. No *Pgm-F⁺* allele was found in *D. cucullata*.

Table 2. Mean allele frequencies of *Pgi* averaged over all sampling dates for *D. cucullata*, *D. galeata*, *D. hyalina* and their interspecific hybrids in seven European lakes

Lake	<i>D. cucullata</i>				<i>D. cuc. × gal.</i>				<i>D. galeata</i>				<i>D. gal. × hyl.</i>				<i>D. hyalina</i>				<i>D. cuc. × hyl.</i>			
	S ⁻	S	M	F	S ⁻	S	M	F	S ⁻	S	M	F	S ⁻	S	M	F	S ⁻	S	M	F	S ⁻	S	M	F
Tjeukemeer	.04	.52	.42	.02	.00	.18	.80	.02	.00	.04	.95	.01												
Maarsseveen									.00	.00	1.0	.00	.00	.00	1.0	.00								
Plußsee	.13	.59	.28	.00	.17	.31	.49	.03	.01	.03	.92	.05	.01	.05	.94	.00					.04	.50	.44	.01
Belauersee	.00	.45	.55	.00	.00	.45	.55	.00									.00	.02	.98	.00				
Höftsee					.00	.00	.96	.04	.00	.00	.84	.16												
Ringsjön	.37	.56	.06	.01	.19	.33	.48	.00	.00	.01	.98	.01												
Bodensee ¹									.00	.00	.94	.06	.00	.00	1.0	.00	.00	.00	1.0	.00				

¹ Data from Weider & Stich (1992)

Table 3. Mean allele frequencies of *Pgm* averaged over all sampling dates for *D. cucullata*, *D. galeata*, *D. hyalina* and their interspecific hybrids in seven European lakes

Lake	<i>D. cucullata</i>				<i>D. cuc. × gal.</i>				<i>D. galeata</i>				<i>D. gal. × hyl.</i>				<i>D. hyalina</i>				<i>D. cuc. × hyl.</i>			
	S	M	F	F ⁺	S	M	F	F ⁺	S	M	F	F ⁺	S	M	F	F ⁺	S	M	F	F ⁺	S	M	F	F ⁺
Tjeukemeer	.24	.49	.27	.00	.10	.28	.62	.00	.08	.31	.61	.00												
Maarsseveen									.18	.33	.49	.00	.06	.33	.61	.00								
Plußsee	.38	.51	.11	.00	.18	.53	.25	.04	.01	.30	.62	.08	.18	.53	.25	.04					.05	.52	.06	.37
Belauersee	.06	.89	.05	.00	.00	.80	.20	.00									.00	.07	.82	.11				
Höftsee					.41	.49	.05	.05	.18	.42	.36	.04												
Ringsjön	.49	.46	.05	.00	.14	.72	.14	.00	.04	.64	.30	.02												
Bodensee ¹									.04	.38	.53	.05	.01	.02	.93	.04	.00	.00	.95	.05				

¹ Data from Weider & Stich (1992)

Clonal diversities

Clonal diversity for several lakes did not differ much for *D. cucullata*, *D. galeata* and the hybrid *C × G* (Table 4). Mean values for these taxa were all above 0.60. Clonal diversities were much lower in the *D. hyalina*, *C × H* and *G × H* populations (mean values 0.30 or less).

Genetic distances

Mean genetic distances were highest among populations of different 'parental species' (Figure 1) and lowest within these species (0.08). An average genetic distance of 0.13 was found for different populations of a given hybrid taxon. The mean genetic distance between hybrid populations and their parental taxa from the same lake was 0.12 and not significantly different from the value obtained in the species or within hybrids comparison.

The UPGMA cluster analysis of Nei's genetic distances separated the 20 taxon-lake combinations into two main groups (Figure 2). These two groups, which I will call the *D. galeata* and the *D. cucullata* group,

were separated by a distance of more than 0.67. In the *D. galeata* group, *D. hyalina* and *G × H* from Bodensee and *D. hyalina* from Belauersee were separated from the rest of the group by a distance of 0.09. The Tjeukemeer population of *C × G* was the only '*D. cucullata* like' hybrid that clustered in the *D. galeata* group. The *C × H* from Plußsee and the two populations of *C × G* clustered within the *D. cucullata* group. The Ringsjön population of *D. cucullata* was quite distinct from the rest of the *D. cucullata* group (Figure 2).

Discussion

Individual *Daphnia* taxa from the *D. galeata* species complex, sampled from geographic distant locations in Europe, showed large similarities in allele frequencies. Within species, populations from different localities showed only minor genetic differentiation among them, with the one exception being the *D. cucullata* population from Lake Ringsjön, Sweden (Figure 2). This population differed from the other *D. cucullata* populations by showing a high frequency (0.37) of the *Pgi-S⁻* and by the occurrence of a *Pgm-S* allele. Nev-

Table 4. Clonal diversity D , calculated as the negative logarithm of Simpson's index of concentration (see text) and averaged over all sampling dates, for all taxa of the *D. galeata* species complex in seven European lakes

Taxon	Bodensee ¹	Maarsseveen	Plußsee	Ringsjön	Tjeukemeer	Belauersee
<i>D. cucullata</i>			1.15	0.95	1.09	0.70
<i>D. cuc. × gal.</i>			0.54	0.60	0.79	
<i>D. galeata</i>	0.61	0.65	0.61	0.51	0.65	
<i>D. gal. × hyl.</i>	0.10	0.33	0.46			
<i>D. hyalina</i>	0.08					0.24
<i>D. cuc. × hyl.</i>				0.27		

¹ Data from Weider & Stich (1992)

Figure 1. Mean values of Nei's genetic distance among populations of different or the same taxa. Four categories were made: among parental species (different populations), within parental species (different populations), within hybrid taxa (different populations, all hybrid taxa averaged), among parentals and hybrid taxa (within the same lake). Error bars are 95% confidence limits of the mean.

ertheless, Ringsjön *D. cucullata* still clustered within the *D. cucullata* group.

Recently, several studies have shown that backcrossing and introgression are important processes in *Daphnia* hybrid species complexes (Taylor & Hebert, 1992; Spaak, 1996; Gießler, 1997; Schwenk, 1997). Spaak (1996) found that the population of hybrid $C \times G$ in Tjeukemeer probably consisted of a large number of backcrosses with *D. galeata*, and showed that $C \times G$ hybrids from Tjeukemeer clustered close to *D. galeata*. The data also suggested that the *Pgi-S* allele may be introgressed from *D. cucullata* to *D. galeata* through the $C \times G$ hybrid. The present study shows that the same process probably happened in Ringsjön and Plußsee. In both these lakes, *D. galeata* co-occurs

with *D. cucullata* and their interspecific hybrid, and the *Pgi-S* allele was found in *D. galeata* of both lakes in low frequencies (0.01 and 0.03 respectively; Table 2), whereas this allele was not found in the three other lakes (Maarsseveen, Höftsee, Bodensee) where *D. galeata* occurred, but did not co-occur with *D. cucullata*. Despite this suggestion for backcrossing and reticulated evolution, the Plußsee and Ringsjön $C \times G$ populations clustered in the *D. cucullata* group (Figure 2). This suggests that other processes (e.g. differences in sexual reproduction, selection, different ages of hybrid populations) play a more important role in causing the genetic distinction between populations than the occurrence of backcrosses.

Figure 2. UPGMA clustering of Nei's genetic distance calculated for *Pgi* and *Pgm* among pooled samples of *Daphnia* taxa. Samples originated from six different lakes (see text for explanation).

Of the $C \times G$ hybrids, three populations were investigated. One (Tjeukemeer) clustered within the 'galeata' group, whereas the other two populations (Plußsee and Ringsjön) clustered together in the 'cucullata' group. Also, the $G \times H$ hybrids showed strong differences between populations. The Bodensee population clustered very close to *D. hyalina*, whereas the Maarsseveen and Plußsee populations clustered closer to *D. galeata* than to *D. hyalina*. These observations support the hypothesis of multiple hybridization events within the *D. galeata* species complex. If hybridization would have occurred once with the hybrids afterwards being dispersed over different European lakes, one would expect a clustering comparable to the parental species. Furthermore, if hybrids were relatively old and reproduced only parthenogenetically, one would expect a relatively low clonal diversity within hybrid populations. The observed clonal diversity, however, is in the range of the parental species. This

also support the hypothesis that hybridization events still occur. Further support for this hypothesis can be derived from the mean genetic distances between taxa. In the case of 'old dispersing hybrids' one would expect a much lower differentiation within hybrid taxa compared to hybrids and parental taxa from the same lake. In fact no difference was found (Figure 1).

$C \times G$ hybrids showed a high clonal diversity in all three studied populations (Table 4), similar to the *D. cucullata* and *D. galeata* populations from the same lake. From my data it seems that sexual reproduction and hybridization events are more frequent in these taxa than in *D. hyalina* populations. This may be related with the type of habitats in which these taxa live (see Hrbáček, 1987). *D. cucullata* is a typical species of eutrophic lakes which displays high abundances in summer and autumn and disappears during winters, whereas *D. galeata* is frequent in lakes that freeze during winter. In contrast, *D. hyalina* is found in deep lakes

where they can over-winter more easily as parthenogenetic females. This may explain the low clonal diversity within *D. hyalina* and their hybrids, although the low clonal diversity may also be caused by the low numbers of 'hyalina like' taxa found in the present study. My observations are in agreement with earlier genetic studies (Taylor & Hebert, 1993; Müller & Seitz, 1995) which have shown that the production of *Daphnia* hybrids within this species complex is most likely an ongoing process. Clearly, further experimental and field studies are needed to solve the question why hybrid and parental taxa are still morphologically and genetically distinct.

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