

## Nonrandom sexual reproduction and diapausing egg production in a *Daphnia* hybrid species complex

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### Abstract

Sexual reproduction in *Daphnia* results in the production of diapausing eggs, which are enclosed in a structure called ephippium. Ephippia, accumulated by sedimentation, can be preserved for decades and offer the opportunity for microevolutionary studies as well as the study of former pelagic populations. In a Swiss subalpine lake (Greifensee), we studied the genetic structure of the pelagic *Daphnia galeata* × *hyalina* hybrid species complex. We examined sexual females, males, and ephippia production. Eggs from ephippia were hatched, and the genotypes and taxa composition of all daphnids were determined using four polymorphic allozyme loci, two of which are each diagnostic for *D. galeata* and *D. hyalina*. We found significant differences between the genetic composition and the backcross level of pelagic asexual females, sexual females, males, and ephippial eggs (*Daphnia* hatchlings). The asexual daphnids were dominated by hybrids. In contrast, sexual females, especially *Daphnia* hatched from ephippial eggs, are dominated by *D. galeata*. We conclude that hybrid *Daphnia* have a lower sexual reproductive success than the parental *D. galeata*. The recent hybrid dominance suggests that *D. galeata* that hatch from diapausing eggs are not able to alter the pelagic population. The genotypic class composition of the diapausing eggs does not reflect the extant pelagic population; therefore, *Daphnia* diapausing egg banks do not always represent the past lake taxa structure.

Lake sediments are ideal archives to examine climatic changes (e.g., Lotter et al. 1998), ancient industrialization (Branvall et al. 2001), or changes in species patterns through time. Diverse organic remains (seeds, diatoms, pollen, and remains of cladocera; Lotter et al. 1998) enable the investigation of processes in the past. The remains of cladocera have proved useful for paleolimnological studies (Frey 1974), reconstructing lake plankton communities of 10,000 yr ago (Duigan and Birks 2000). Evolutionary changes in species composition and morphology or possible hybridization events can be documented (e.g., Hofmann 1991) using residuals of mucrons, postabdominal claws, and diapausing eggs. Recently, researchers have begun to examine the diapausing eggs of zooplankton by hatching them (Hairston et al. 1999; Kerfoot et al. 1999) and to study their genetic makeup by directly genotyping the eggs (Duffy et al. 2000; Reid et al. 2000; Limburg and Weider 2002).

Genetic analysis of sexually produced diapausing eggs of *Daphnia*, either directly or indirectly (by genotyping the hatchlings), enables the study of past genetic changes in plankton populations. Sexually produced diapausing eggs are encapsulated in a protective envelope called an ephippium.

After a dormant phase, eggs hatch if they are exposed to proper hatching stimuli. Although the hatching process is not fully understood, light and elevated temperature may play a role in breaking dormancy (Schwartz and Hebert 1987). Ex-ephippial hatchlings from lake sediments have shown that the genetic composition of populations can change with time (e.g., Weider et al. 1997). Limburg and Weider (2002) found significant shifts between the genetic makeup of recent ephippia and those produced >150 yr ago in the *Daphnia longispina* group. In contrast, Cousyn et al. (2001) studied a relatively young *Daphnia magna* diapausing egg bank using microsatellites and found no genetic shift in time. The advantage of analyzing eggs directly is that postfertilization selection influences (e.g., selective hatching or mortality) can be avoided. Such an approach allows the study of genetic changes for much longer time frames. On the other hand, life-history experiments can be conducted on ex-ephippial hatchlings (Hairston et al. 2001).

An important question remaining is how well diapausing egg banks represent former pelagic populations. A general mismatch between egg banks and extant populations of cyclical parthenogens has been found for rotifers (Gomez and Carvalho 2000) and daphnids (e.g., Weider et al. 1997; Cáceres 1998); however, Weider et al. (1997) used only one species-specific genetic marker. Such an approach neglects the complex system of hybridization and backcrossing in hybrid *Daphnia* swarms. In many European midland lakes, parental species and hybrids (F<sub>1</sub> hybrids and backcrosses) co-occur (Spaak 1996). In some populations, F<sub>1</sub> hybrids may numerically dominate one or both parental species (e.g., Schwenk and Spaak 1995). The accurate classification of individuals is difficult in such complex systems with many generations of hybridization and backcrossing. Morphological, nuclear, and allozyme species-specific markers have been developed and combined to try to solve this problem (e.g., Gießler 1997). Campton and Utter (1985) described a

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method to classify parental and hybrid taxa using genetic data without the assumption of unique alleles. Anderson and Thompson (2002) used a combination of diagnostic and non-diagnostic genetic markers to compute the probability of the misclassification of particular individuals.

The cyclical parthenogenesis of *Daphnia* in large European midland lakes offers the possibility for year-long clonal propagation, thus reducing the effect of sexual reproduction. However, Spaak (1997) found that hybrids exhibit high levels of genetic variation, and Jankowski (2002) showed that hybrids produce fertile sexual individuals. Nonetheless, parental species remain distinct, even when hybrids and parental species occur together (Spaak 1996). Little is known about the fate of hybrid or backcrossed diapausing eggs compared with intraspecific ones, although Schwenk et al. (2001) found differences in hatching success between experimental intra- and interspecific crosses, as well as backcrosses between *Daphnia galeata* and *Daphnia cucullata*.

We investigated the processes that determine the genetic composition of diapausing eggs in hybridizing *Daphnia* species. We examined whether the various hybrid classes engage equally in sexual reproduction and how well diapausing eggs and ex-ephippial hatchlings from deep sediment reflect former *Daphnia* populations in hybrid species complexes. We analyzed the genetic composition of pelagic sexual and asexual *Daphnia* in detail, as well as the genetic composition of ex-ephippial neonates hatched from recently produced ephippia. Finally, we compared these data with the genetic pattern of laboratory hatchlings from various sediment depths, to determine whether hybrid taxon dominance in recent populations is represented in egg banks.

## Material and methods

**Study site**—The study was done in a eutrophic Swiss subalpine lake (Greifensee). Greifensee has a surface area of 8.5 km<sup>2</sup>, a volume of 150 × 10<sup>6</sup> m<sup>3</sup>, and an average depth of 17.7 m, with a maximum depth of 33 m. Total phosphorus values in the lake in February 2000 were 79.3 μg L<sup>-1</sup> and have declined sharply the past 25 yr (>400 μg L<sup>-1</sup>; 1975 Keller et al. 2002). Two rivers (Aa-Bach and Möchaldorfer-Aa) flow into the lake, and one lake outlet (Glatt) drains the system. Only the larger Aa-Bach seems to create a noticeable flow-through toward the lake outlet (Keller et al. 2002). *Daphnia galeata*, *D. hyalina*, and their interspecific hybrids occur in Greifensee (Spaak et al. 2001).

**Field methods**—Quantitative *Daphnia* samples were collected weekly (every other week during winter) beginning in February 1999 with a 95-μm net and preserved in 95% ethanol. Samples were taken from three distinct stations (all in the deepest part of the lake), to prevent the sampling of zooplankton patches. Adult asexual females without parthenogenetic eggs, adult females with parthenogenetic eggs, juveniles, ephippial females, and ephippia were enumerated separately, to calculate *Daphnia* densities in the lake. Qualitative *Daphnia* samples were taken with a 250-μm plankton net, to collect adult females for genetic analyzes. Sexual females (arrows in Fig. 1A) and males (if present) also were collected, to get an adequate number of individuals for ge-

netic analyzes. Samples were stored in liquid nitrogen for later analyzes.

Floating ephippia, just released into the water column from sexual females, were qualitatively sampled by carefully skimming wide areas of the water surface with a 100-μm plankton net. Ephippia were stored in the dark at 4°C (for 155–783 d) until they were exposed to hatching stimuli. Sediment cores of Greifensee (1999, 2000) were sampled and sliced into 1.4-cm-thick slabs, and ephippia were removed with forceps and stored in the same way as the floating ephippia. Keller et al. (2002) provides further details about the sediment cores.

**Ephippia hatching**—After the cold incubation in the refrigerator, all ephippia from the sediment cores and subsamples from floating ephippia (numbers ranged from 398 in May 2001 to 1,530 in August 2001) were counted and placed in six-well culture plates filled with filtered (0.45 μm) lake water and exposed to a long-day photoperiod (16 h light: 8 h dark) at 12°C, to break diapause (Weider et al. 1997). Ephippia were monitored daily for 4 weeks and thereafter every second day for another 4 weeks. Hatched daphnids were transferred to separate culture jars (50 ml) and individually raised as clonal cultures.

**Hatching success**—Damaged ephippia (broken in two parts), intact ephippia, and viable ephippial eggs (dark green in color) were counted in subsamples using a stereo microscope. Ephippia were opened if eggs were not visible from the outside, and the total number of viable ephippial eggs per sample was calculated as  $V/M \times 100$ , where  $V$  is the number of total viable eggs: the sum of hatchlings and the remaining unhatched viable eggs (olive green in color), and  $M$  is the number of maximal possible eggs: number of intact ephippia × 2, because one ephippium can contain a maximum of two diapausing eggs. The percentage of total viable eggs that hatched (hatching success) was calculated as  $H/V \times 100$ ; where  $H$  is the number of hatchlings and  $V$  is the number of total viable eggs (see above).

**Allozyme electrophoresis and statistical analyzes**—Four polymorphic enzyme loci were screened for asexual females, sexual females, males, and ex-ephippial hatchlings using cellulose acetate electrophoresis: aldehyde oxidase (AO; enzyme commission number [EC] 1.2.3.1.), aspartate amino transferase (AAT; EC 2.6.1.1), phosphoglucose isomerase (EC 5.3.1.9), and phosphoglucomutase (EC 5.4.2.1). Two of these loci, AAT (Wolf and Mort 1986) and AO (Gießler 1997), have diagnostic alleles for distinguishing between *D. galeata* and *D. hyalina*. Using two diagnostic markers, six possible genealogical classes can be identified (for a discussion on characterization, see Nason and Ellstrand 1993). This raw classification reflects only an incomplete estimate of natural frequencies of genealogical classes. Misclassification, especially in F<sub>2</sub> hybrids with nine possible genotypes, is unavoidable (Table 1). To reduce misclassification, genealogical class identification was done using the computer program of Anderson and Thompson (2002), which uses Bayesian statistical methods to calculate the probability that an individual belongs to various hybrid categories. Because of the

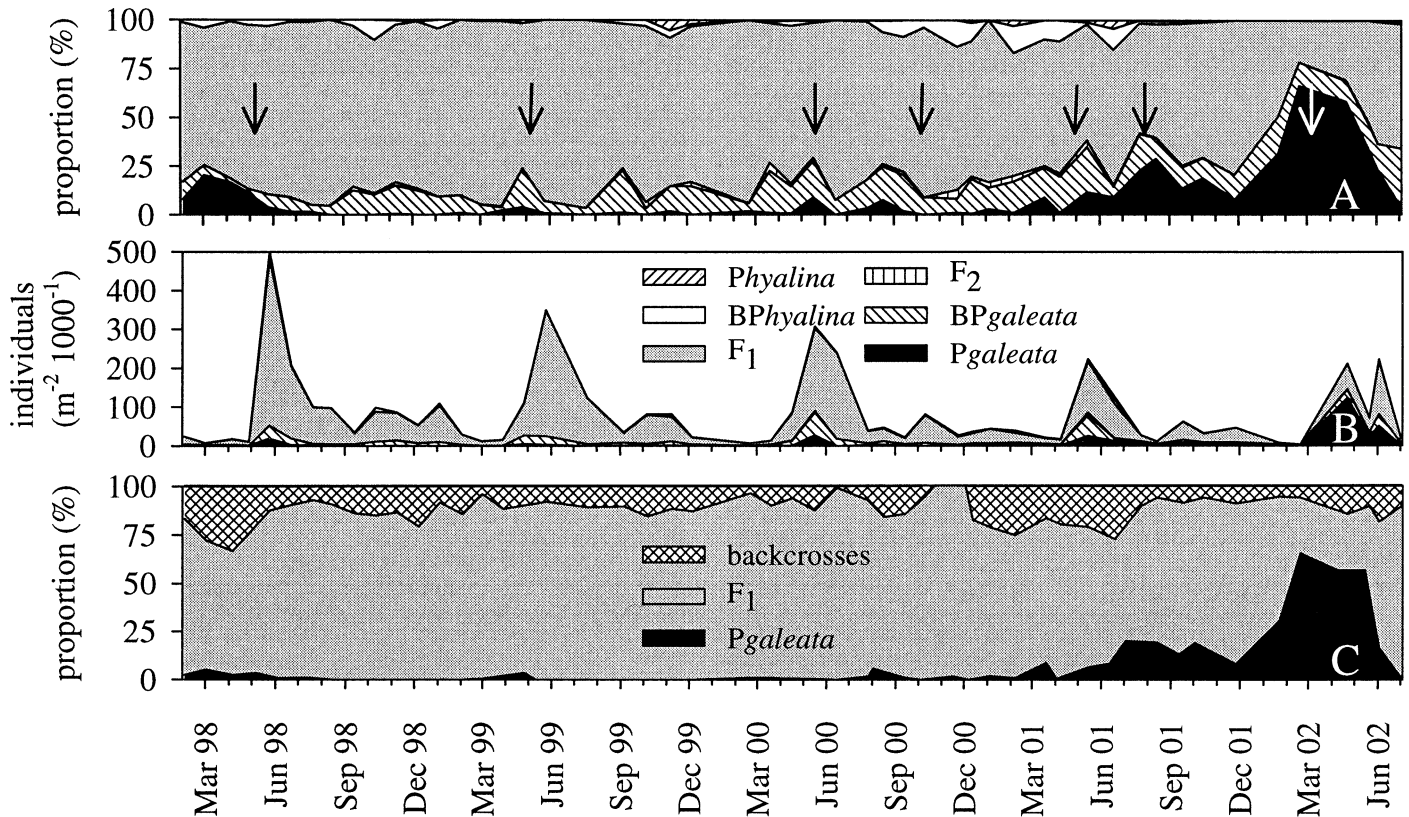


Fig. 1. Genealogical classes in asexual daphnids. (A) Proportional distribution according to Nason and Ellstrand (1993) (*P. galeata*, *P. hyalina*,  $F_1$ ,  $F_2$ , backcrossed [BP] *galeata*, and BP *hyalina*), with arrows marking dates when sexual females could be sampled. (B) Effective abundance based on quantitative sampling counts and genealogical class proportions (see panel A). (C) Proportion of three genealogical classes according to Anderson and Thompson (2002).

low number of available loci (four), we could only distinguish among one parental species (*D. galeata*),  $F_1$  hybrids (probability >95%), and all others. These remaining individuals could not be classified and were pooled as “backcrosses” (probability <95%). Thus, the group “backcrosses” is a mixed category. Each individual was analyzed three times with a distinct number of “sweeps” (100,000, 200,000, and 700,000; for a definition of this method, see

Table 1. Possible genotype classes after two generations of hybridization with two species-specific markers and the genealogical class interpretation based on the method of Nason and Ellstrand (1993).

Genotype	Genealogical class interpretation
AABb	*BP <i>galeata</i> , $F_2$
AaBB	*BP <i>galeata</i> , $F_2$
Aabb	*BP <i>hyalina</i> , $F_2$
aaBb	*BP <i>hyalina</i> , $F_2$
AaBb	* $F_1$ , BP <i>galeata</i> ; BP <i>hyalina</i> , $F_2$
AAbb	* $F_2$
aaBB	* $F_2$
AABB	*P <i>galeata</i> , BP <i>galeata</i> , $F_2$
aabb	*P <i>hyalina</i> , BP <i>hyalina</i> , $F_2$

\* Genealogical class to which each of nine possible genotype categories was classified.  
BP: backcrossed genotype, P: parental genotype.

Anderson and Thompson 2002) to check whether differences in calculated probabilities were <0.01 between the different sweeps (E. C. Anderson pers. comm.).

Tests of differentiation were used to determine differences in genetic structure for date, examining sexual and asexual females, asexual, sexual females and males, and asexual, sexual females and hatchlings (Goudet 2001). FSTAT analyzes the overall loci G-statistic to classify tables by randomizing multilocus genotypes between each pair of samples.

Correlations between hatching success and numbers of viable eggs in floating ephippia sampled during spring and fall were tested with STATISTICA software (Statsoft), to verify the relationship between egg quality and reproductive success. The effect of storage time in the sediment on hatching success also was tested, because Weider et al. (1997) had previously found a decreased hatching success with increasing ephippia age. Cáceres and Tessier (2003) found an increased degeneration of laboratory-stored diapausing eggs; we therefore tested whether storage time in the laboratory was correlated with the number of viable eggs in floating ephippia.

Results

Using the approximate six-genotype classification of Nason and Ellstrand (1993), asexual daphnids (collected during

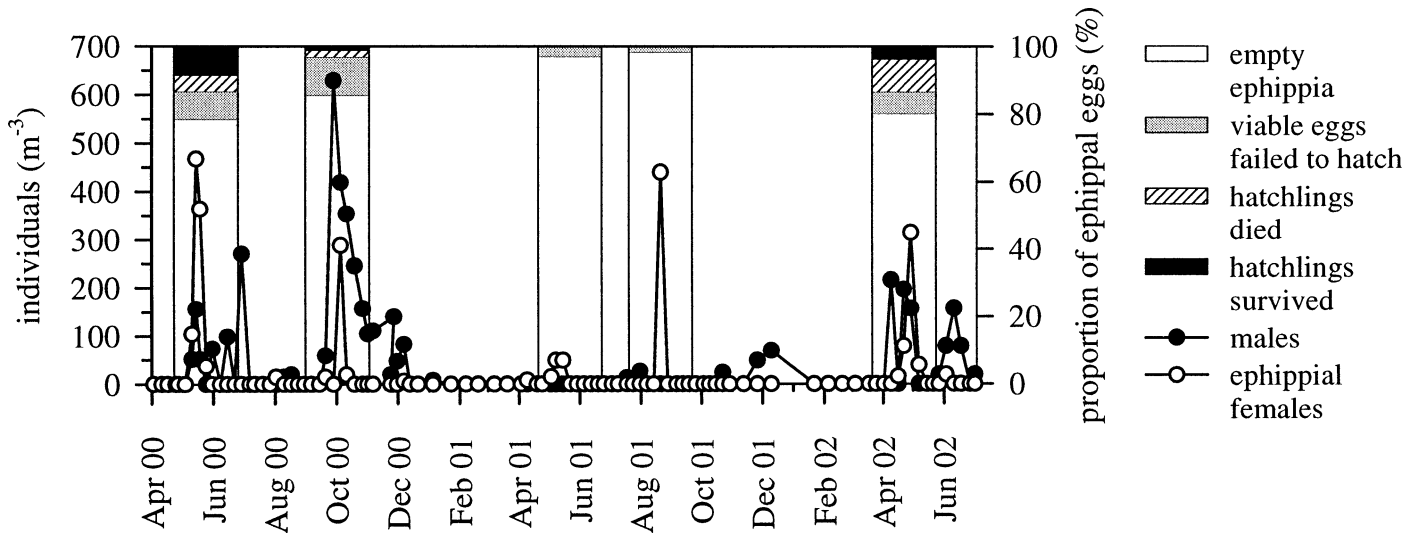


Fig. 2. Abundance of sexual females and males in Greifensee (April 2000–July 2002) and proportion of ephippial eggs from floating ephippia (successful hatchlings, hatchlings died after hatching, viable ephippial eggs that did not hatch, and empty ephippia). Empty ephippia were used to calculate the maximal number of sexual eggs ( $2 \times$  the number of intact ephippia). Viable ephippial eggs were calculated as the sum of hatchlings of floating ephippia and unhatched, olive green, diapausing eggs.

1998–2002) were dominated by  $F_1$  hybrids at all dates ( $\sim 80\%$ ; Fig. 1A), except those from April 2002, when the parental species, *D. galeata* (*P. galeata*) dominated the population ( $\sim 70\%$ ). Individuals with a backcrossed *D. galeata* and *D. hyalina* genotype occasionally occurred in proportions up to 20%, whereas the animals with a  $F_2$  hybrid and parental *D. hyalina* (*P. hyalina*) genotype never made up more than  $\sim 5\%$  of the whole population (Fig. 1A). The density fluctuations ( $500,000$  individuals  $m^{-2}$  in May 1998 to  $3,000$  individuals  $m^{-2}$  in February 2002) did not affect the distribution pattern of genotype classes described above (Fig. 1B). Using the statistically more rigorous three-genotype classification of Anderson and Thompson (2002), we observed a pattern comparable to that of the six-genotype classification (Fig. 1A,C). The model of Anderson and Thompson classified 1,096 more individuals as  $F_1$  hybrids and 174 fewer individuals as *P. galeata* (Table 2). All animals with a *P. hyalina* and  $F_2$  hybrid genotype could not be classified by the Anderson and Thompson model and were added to the backcrossed group.

Periods during which sexual females were present in Greifensee (arrows in Fig. 1A) fluctuated from 1 week up to 1 month (B.K., pers. observation). Sexual reproductive periods could be determined during spring and fall by the presence of sexual females, males, and newly produced floating ephippia (Figs. 1A, 2). Males occurred in the absence of sexual females on several occasions (Fig. 2). The viable egg proportion of floating ephippia varied from 21% (May 2000) to 2% (August 2001; Fig. 2). The proportion of viable eggs in floating ephippia and the hatching success was highly correlated ( $r^2 = 0.883$ ,  $P = 0.005$ ). Most hatching events occurred in May 2000 (62% of all viable eggs) and April 2002 (67%). The storage time of floating ephippia in our refrigerator ranged from 155 d (April 2002) to 738 d (October 2000) and was not correlated with hatching success ( $r^2 = 0.017$ ,  $P = 0.81$ ).

We compared the sexual and asexual females for the six dates on which they were sampled simultaneously using the three genealogical classes of Anderson and Thompson (2002) (Fig. 3). The genetic and genealogical class composition differed significantly between sexual and asexual females on all dates except during spring 2002 (Fig. 3). *P. galeata* tended to be more abundant in the sexual females (five of six samples). No clear pattern was observed for  $F_1$  hybrids. They occurred in higher proportion in asexual females on four dates (spring 1998 and 2000 and autumn 2000 and 2001), but, on the two remaining dates, the distribution was opposite.

In October 2000, we collected a sufficient number of males ( $n = 20$ ) to include them in our genetic analysis. Comparing males with asexual and sexual females, the most obvious difference was the complete absence of *P. galeata* in the group of males and asexual *Daphnia*, whereas its proportion was  $>25\%$  in the sexual females (Fig. 4).  $F_1$  hybrids dominated all reproductive stages—94% in the asexual fe-

Table 2. Classification of genetically analyzed asexual females (1998–2002), according to Nason and Ellstrand (1993, six genealogical classes) and Anderson and Thompson (2002, three genealogical classes). For further explanation, see the text.

Nason's classification	Anderson's classification			Total
	<i>P. galeata</i>	$F_1$	Backcrosses	
<i>P. galeata</i>	823	1	183	1,007
$F_1$	0	8,506	14	8,520
BP <i>galeata</i>	10	962	619	1,591
<i>P. hyalina</i>	0	0	48	48
BP <i>hyalina</i>	0	147	468	615
$F_2$	0	0	83	83
Total	833	9,616	1,415	11,864

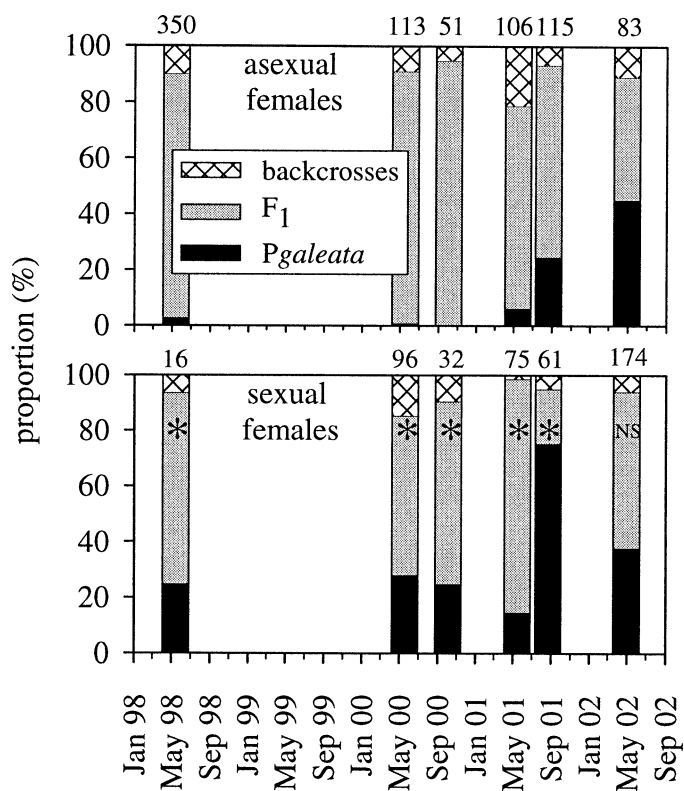


Fig. 3. Proportion of three genealogical classes (Anderson and Thompson 2002) in the asexual (top) and sexual females (bottom) of a hybridizing *Daphnia* complex in Greifensee. The top of each bar indicates the number of analyzed individuals. Significant differences were based on the pairwise test of differentiation between sample pairs (FSTAT version 2.9.3.1; Goudet 2001) \*Significant differences after Bonferroni correction (1,000 permutations,  $P = 0.05$ ). NS: not significant.

males, 80% in the males, and 66% in the sexual females (Fig. 4).

Hatchlings of floating ephippia that survived to maturity exhibited a different genealogical class distribution than the asexual and sexual pelagic females for the dates when ephippia were collected (May 2000 and April 2002). *P. galeata* dominated the hatchlings from floating ephippia (75% in 2000 and 71% in 2002), compared with the asexual and sexual females (Fig. 5).

The oldest ephippial egg that hatched from the sediment was ~40 yr old. The proportion of viable eggs varied from 23% to 7% (Fig. 6B). Hatching success was correlated weakly with sediment depth ( $r^2 = 0.42$ ,  $P = 0.043$ ). *P. galeata* and the backcrosses dominated the sediment hatchlings (Fig. 6A), as was the case with hatchlings from recently produced ephippia (Fig. 5). We could not identify F<sub>1</sub> hybrid genotypes with a probability >95%, but, most likely, they are hidden in the backcrossed group.

## Discussion

Our data show that the genetic composition of sexual females and males, as well as hatchlings from both recent (pelagic) and old (sediment) ephippia, is significantly different

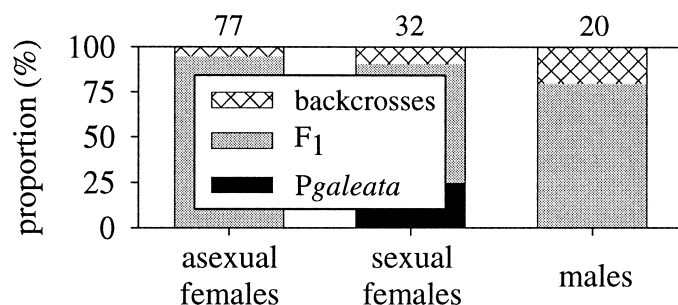


Fig. 4. Proportion of three genealogical classes (Anderson and Thompson 2002) in the sexual females, asexual females, and males of the hybridizing *Daphnia* species complex in Greifensee, October 2000. The top of each bar indicates the number of analyzed individuals. All groups are significantly different from each other (after Bonferroni correction; 3,000 permutations,  $P < 0.017$ ; pairwise test of differentiation between sample pairs; FSTAT version 2.9.3.1; Goudet 2001).

from the genetic composition of pelagic asexual *Daphnia*. Of interest, the F<sub>1</sub> hybrids dominate the pelagic population (Fig. 1A) but are nearly absent in the recently produced ephippia, as well as in the ephippia found in the diapausing egg bank. This finding is remarkable, because sexual hybrids were found frequently in the pelagic population (Figs. 3, 4), which indicates they should be capable of producing ephippia with the hybrid genotype. The genetic pattern of daphnids hatched from recent ephippia is comparable to those hatched from the sediment. Thus, we presume that comparable processes may have led to these similar genetic compositions over time. The high hatching success of floating ephippial eggs (Fig. 2) and the dominance of *D. galeata* in hatchlings (Fig. 5) leads us to speculate that the remaining unhatched viable eggs also belong to *D. galeata* and most probably not to F<sub>1</sub> hybrids.

Individuals with a F<sub>1</sub> hybrid genotype dominated the asexual females in Greifensee over the past 4 yr, and backcrossed hybrids (mainly backcrossed *D. galeata* genotypes) were present at a rate of 10–20% throughout the study period (Fig. 1A). This is in contrast to Lake Constance (Jankowski 2002), in which the same hybridizing taxa co-occur. Jankowski (2002) found no hybrid dominance and a much higher proportion of backcrossed *D. hyalina* using the same classification method of Nason and Ellstrand (1993) that we used. These contradictory findings suggest an opposite introgression pattern of alleles within the two species in Greifensee and Lake Constance. Spaak et al. (2001) suggested that, in Greifensee, pure *D. hyalina* probably never have been present, which might explain this dissimilar pattern. Another reason may be that the two lakes exhibit differences in environmental conditions that may favor different genealogical classes. Taxonomic differences can be influenced by the ability of different genealogical classes to cope with alterations in food composition (Repka et al. 1999), fish predation (Spaak et al. 2000), or parasites (Wolinska et al. 2004).

Genealogical class analyses of closely related hybridizing and backcrossing species, as in the *D. galeata* × *hyalina* complex, without clearly morphological distinguishable characters and with only a limited number of diagnostic ge-

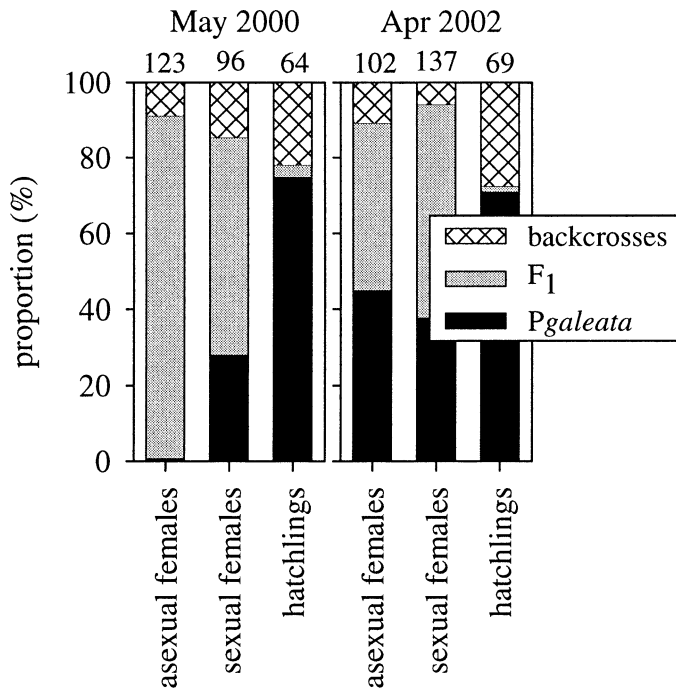


Fig. 5. Proportion of three different genealogical classes (Anderson and Thompson 2002) in the sexual, asexual females, and ephippial hatchlings of a hybridizing *Daphnia* complex in Greifensee on May 2000 and April 2002. The top of each bar indicates the number of analyzed individuals. All groups are significantly different from each other in both year (after Bonferroni correction; 3,000 permutations,  $P < 0.017$ ; pairwise test of differentiation between sample pairs; FSTAT version 2.9.3.1; Goudet 2001), except asexual and sexual females in April 2002.

netic markers, can lead to misclassification problems. Most allozyme studies on hybridizing *Daphnia* populations have been based on one (e.g., Wolf and Mort 1986; Weider et al. 1997) or two (e.g., Jankowski 2002) diagnostic markers. The comparison of the classification models from Nason and Ellstrand (1993) and Anderson and Thompson (2002) has shown that the overall taxa pattern does not change (Fig. 1A,C), although several individuals were classified differently. But the number of available loci (four) we used in computing the Anderson and Thompson (2002) model is too low to distinguish more detailed patterns; therefore, all *D. hyalina* and F<sub>2</sub> hybrids were lumped together in the backcrossed group (Table 2).

We found differences in the genealogical class composition between asexual and sexual generations, as was also reported by Jankowski (2002). *D. galeata* was significantly more abundant in the sexual females, which suggests a higher level of "sexual activity" for *D. galeata*. Jankowski (2002) found a high proportion of sexual *D. galeata* (90%) but no sexual *D. hyalina* during early summer in Lake Constance. In autumn, the pattern was reversed. Hybrids reproduced sexually during both seasons, but in low proportions. Such seasonal alteration can result in the reproductive isolation of both parental groups, as Spaak (1995) described in Tjeukemeer (The Netherlands). In Greifensee, we did not observe a genealogical class switch of sexual daphnids be-

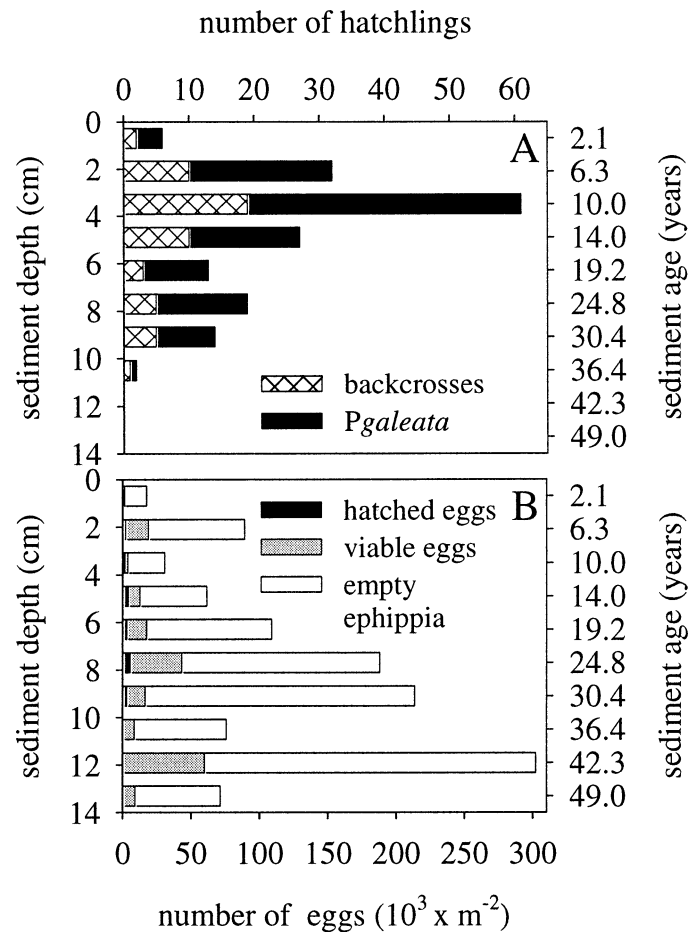


Fig. 6. (A) Genealogical class distribution (Anderson and Thompson 2002) and abundance of ex-ephippial hatchlings in the sediment of a hybridizing *Daphnia* complex in Greifensee (total number of matured neonates). (B) The distribution of ephippial eggs (successful hatchlings, hatchlings that died after hatching, viable ephippial eggs that did not hatch, and empty ephippia). Empty ephippia are equated to maximal possible eggs, which were calculated as  $2 \times$  the number of intact ephippia.

tween spring and autumn. Additionally, we found much higher proportions of sexual hybrids in Greifensee.

The discrepancy in taxa composition of ephippial females and floating ephippial hatchlings (Fig. 5) could be explained by pre-mating intolerances (e.g., assortative or failed mating or the asynchronous presence of males and sexual females) or by post-mating intolerances (e.g., cytological fertilization difficulties, discriminating embryo degeneration, selective hatching, and a biased juvenile survival rate). The genealogical class differences between asexual and sexual generations (Figs. 3, 4), in combination with the low number of individuals engaging in sexual reproduction (Fig. 2), suggest non-random (assortative) clonal participation in sexual reproduction. Assortative mating cannot completely explain the near absence of hybrids in the pelagic and sediment hatchlings (Figs. 5, 6), because a large portion of F<sub>1</sub> hybrids were present in both sexual females and males (Figs. 3, 4). The presence of hybrids and backcrosses in Greifensee indicate that hybrids are able to hatch. The large proportion of empty

floating ephippia (Fig. 2) is difficult to explain. The temporal separation of males and sexual females may be important (Fig. 2), but failed mating cannot be excluded. Biased cytological fertilization difficulties or discriminating embryo degeneration may also be important factors, but we have very little knowledge of this for *Daphnia*. Boersma and Vijverberg (1995) found no difference in egg mortality between *D. galeata* and *D. galeata* × *cucullata* hybrids in parthenogenetic eggs, but Schwenk et al. (2001) described failed artificial reciprocal crosses between *D. galeata* and *D. cucullata*. If sexual females are fertilized and diapausing eggs are successfully produced, clonal or species-specific selective hatching or biased juvenile mortality might occur. Schwenk et al. (2001) found that hatching and juvenile survival rates of artificially produced *D. galeata* × *cucullata* hybrids were relatively low compared with parental species. Because we applied only one set of hatching stimuli (12°C and a long-day photoperiod), we cannot exclude the loss of some hatchlings that might not have been able to hatch under these conditions. The high hatching success rate (>60% on May 2000 and April 2002; Fig. 2) and the significant positive correlation ( $r^2 = 0.883$ ) between hatching success and the number of viable eggs suggests that this effect might not be strong. Moreover, Cáceres and Tessier (2003) described an increased senescence rate of unhatched sexual eggs accompanied by increased hatching success under laboratory conditions compared with natural conditions. We did not observe increased degeneration in our laboratory-stored floating ephippia, as indicated by the significant correlation of the percentage of viable eggs with hatching success ( $r^2 = 0.883$ ,  $P = 0.005$ ) and a lack of correlation between storage period and viability of eggs ( $r^2 = 0.017$ ,  $P = 0.81$ ). However, we cannot exclude processes that might affect species and clones differently and thus influence the genealogical class composition of hatchlings. Finally, the low proportion of F<sub>1</sub> hybrid hatchlings compared with the sexual F<sub>1</sub> hybrid females (Fig. 5) strongly suggests the presence of pre- or postmating barriers. This is also supported by the low proportion of viable eggs that we found in the floating ephippia, which were produced when mainly hybrid sexual females were abundant in the lake (Figs. 2, 3, 4).

In our study, genotype class patterns drawn from floating ephippia hatchlings (Fig. 5) were comparable with those from the sediment (Fig. 6A), and both differed from the asexual pelagic females (Fig. 1C). Similar to our findings, Weider et al. (1997) found large differences between the asexual pelagic daphnids and ex-ephippial hatchlings from the diapausing egg bank in Lake Constance. No *D. hyalina* and only a few hybrid individuals hatched from sediment layers dating from years during which those taxa were present in the lake (Weider and Stich 1992). Weider et al. (1997) suggested, on the basis of high hatching success (up to 100% of the viable eggs) and analogous allele frequencies in hatchlings and corresponding pelagic population, that hatchlings are a random subsample of the diapausing egg pool. They hypothesized that differences in taxa composition may result from taxon-specific buoyancy or the inability of *D. hyalina* to produce viable diapausing eggs. Jankowski (2002) found shifts in various taxa hatched from sediments in littoral and deep water. This suggests taxon-specific buoyancy of ephip-

pia. In Greifensee, the low frequency of recent F<sub>1</sub> hybrid hatchlings (floating ephippia) and the simultaneous presence of sexual F<sub>1</sub> hybrids (Fig. 5), compared with the absence of hybrids in sediment hatchlings (Fig. 6A), suggest that very few viable hybrid ephippia were produced in the past. However, we cannot conclude from our data that hybrids did not occur in the lake during this time.

Our findings—the F<sub>1</sub> hybrid dominance in the pelagic asexual females, the low abundance of sexual individuals, the genealogical class differences between asexual, sexual generations and ephippial hatchlings, and the ephippia quality dissimilarities—strongly suggest that clonal reproduction is the more efficient short-term strategy for the *Daphnia* hybrid swarm in Greifensee. Clonal reproduction is characterized by its inflexibility to react to changes in environmental conditions, through a lack of recombination. Clonal reproduction also risks the accumulation of deleterious mutations (e.g., Palsson 2001) or easier adaptations of parasites (Hamilton 1980; Hurst and Peck 1996). Our results indicate that selection seems to occur against sexually reproducing hybrids. This may explain why *P. galeata* and hybrid taxa stay distinct and co-occur in Greifensee. In addition, our results suggest that the *Daphnia* diapausing egg bank may not represent the past population in the lake. No statement can be made about the former taxon distribution of hybrid swarms analyzing diapausing egg banks; our results show that the absence of taxa in the sediment does not indicate their absence in past populations. The low percentage of hatchlings in several sediment layers must therefore be explained by the presence of high numbers of degenerated hybrid-diapausing eggs, whereas ephippia from time horizons in the sediment with high hatching success may have been produced by *D. galeata*.

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