Allochronic differentiation among *Daphnia* species, hybrids and backcrosses: the importance of sexual reproduction for population dynamics and genetic architecture

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Abstract

Seasonal dynamics of the abundance, sexual reproduction and genetic architecture in a *Daphnia hyalina-galeata* hybrid complex were studied in the large and deep Lake Constance. We found evidence for the occurrence of first and second order hybridization. Our study revealed strong differences between the parental species not only regarding their seasonal dynamics, genetic architecture and diversity, but also their sexual reproductive behaviour. The overwintering *D. hyalina* showed low genetic diversity, no genetic differentiation during the season, and reproduced sexually in autumn, whereas *D. galeata* reached higher levels of genetic diversity, reproduced sexually in early summer, and exhibited changes in genetic structure during the season, but was only present from spring to autumn. However, in both species sexual reproduction was a rare event, and daphnids, including hybrids, reproduced predominantly asexually. This allows long-term persistence of hybrids as well without continuing hybridization events. Within all variables studied, F1 and F2 hybrids showed an intermediate pattern, whereas proposed backcross hybrids were more similar to their respective parentals. These differences in phenotype as well as significant differences in pairwise F_{st} values between parentals suggest that gene flow seems to be relatively low in the Lake Constance hybrid system. We found evidence for unidirectional introgression by backcrossing from *D. galeata* to *D. hyalina* and found a decrease in at least one of the proposed introgressed alleles in the *hyalina*-backcross while the season progressed. Our findings suggest allochronic differentiation within this hybrid population and different microevolutionary trajectories of the parental species, which will be discussed in the light of the ongoing reoligotrophication process of Lake Constance.

Introduction

Hybrid systems are often viewed as natural experiments that serve as windows on evolutionary processes (Harrison, 1993; Arnold, 1997). In particular, the highly recombinant genotypes found in hybrid systems offer a unique opportunity to dissect the architecture of gene flow between species. Recently, several studies showed evidence for introgression (for review see Arnold, 1997; Dowling & Secor, 1997) not only in plants, e.g. in *Populus*

(Martinsen *et al.*, 2001), *Helianthus* (Rieseberg *et al.*, 1999) and *Rorippa* (Bleeker & Hurka, 2001), but also in animals, e.g. in *Daphnia* (Schwenk & Spaak, 1995), *Bufo* (Malmos *et al.*, 2001) and in *Drosophila* (Noor *et al.*, 2001). In general, animal hybrids were suggested to be less fit than their parentals (Haldane' rule, Orr, 1997). However, hybrids have been shown to be fitter than their parents under certain circumstances, and the pursuit of this hybrid vigour has been important in agriculture and animal breeding for over a century (Darwin, 1876). In particular, hybridizing species might be better in quickly adapting to changing situations as they can pick up genes of both species. For example, Galápagos finch hybrids appear to have higher fitness than parental species during unusual stressful conditions (Grant & Grant, 1996). Most

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studies on the fitness of interspecific and intraspecific crosses have examined only the F1 hybrids, whereas effects of genetic mechanisms are typically most pronounced in F2 and backcross hybrids (Lynch, 1991). The genome rearrangements (e.g. in F2 and backcrosses) may disrupt epistatic interactions that confer fitness in specific environments (local adaptation), as well as gene interactions that are independent of the environment. Studies on the success and fitness of F2 and backcross hybrids in animals are sparse (Arnold, 1997; Edmands, 1999).

Here we present a study on the seasonal success, seasonal differentiation and timing of sexual reproduction of lineages within a *Daphnia* hybrid complex (subgenus *Hyalodaphnia*). *Hyalodaphnia* comprises several species complexes known to form hybrids across large geographic areas in Europe and North America (Taylor & Hebert, 1993; Schwenk & Spaak, 1995). Field data and laboratory crossing experiments have shown that hybrids are fertile and can successfully interbreed with parentals; mating probabilities suggest that interspecific hybridization and backcrossing may occur in similar frequencies (Schwenk, 1997). Furthermore, there is evidence for reticulate evolution through introgressive hybridization (Giessler *et al.*, 1999). However, a recent molecular phylogenetic analysis revealed a large sequence divergence of about 9% within *Hyalodaphnia*, and also that *Daphnia hyalina* and *D. galeata* represent two of 15 distinct species (Schwenk *et al.*, 2000). Hence, reproductive isolation in *Hyalodaphnia* seems to evolve significantly slower than genetic isolation (Schwenk *et al.*, 2000). In addition, parental species show strong ecological differentiation (Weider & Stich, 1992; Weider, 1993).

Sexual reproduction, the only possibility for hybrid formation, is a rare event in large lake daphnids. They reproduce parthenogenetically during most of the year and switch to sexual reproduction during specific, short periods. The gene pool of a population at the beginning of a growing season consists of parthenogenetically overwintering individuals and of individuals hatching in early spring from sexually produced resting eggs. Hence, the success of *Daphnia* lineages is determined by parthenogenetic as well as sexual reproduction and most of the genotype frequency changes during the season are because of clonal competition. Furthermore, hybrids, even when infertile, may still reproduce parthenogenetically and are therefore able to persist for a long time. Sexual reproduction is under both genetic and environmental control (Alekseev & Lampert, 2001) and is induced by environmental conditions like crowding, food limitation and day length (Kleiven *et al.*, 1992). The seasonal timing of sexual reproduction shows strong interspecific variability (Spaak, 1995; Deng, 1997). In addition to environmental control of sexual reproduction, environmental conditions determine the success of genotypes during parthenogenetical reproduction. Environmental conditions, i.e. food availability, parasitism and predation pressure as well as physical and chemical parameters, change dramatically between lakes and within a season (Sommer *et al.*, 1986; Bittner, 2001). Hence, both modes of reproduction have to be adapted to the special conditions of the respective habitats including their seasonal variability.

Here we analyse the population dynamics and population genetics of a 50-year-old *Hyalodaphnia* hybrid complex (Straile & Geller, 1998) in large and deep Lake Constance focusing on sexual reproduction of daphnids. Analyses using morphological characters, allozymes and ncDNA of planktonic females and resting stages suggest that *D. hyalina* and *D. galeata*, two hybridizing species, occur in syntopy in Lake Constance (Jankowski & Straile, 2003; K. Schwenk, pers. comm.). Additionally, we know that both species show differences in several traits, such as diel vertical migration (Stich & Lampert, 1981), with traits of F1 hybrids being more similar to *D. hyalina* (Weider & Stich, 1992). Using two species-specific markers allows us for the first time to provide a detailed field analysis estimating the seasonal dynamics, sexual reproduction, and success of F1, possible F2, and backcross hybrids compared with the parentals in this *Daphnia* hybrid complex. Specifically, we are interested in allochronic differentiation within this hybrid complex. We ask whether the *Daphnia* population represents one hybrid swarm or distinct genealogical classes? Do F1 hybrids reproduce sexually? Is there evidence for advanced generation hybrids and subsequent gene flow? If so, what are the dynamics of abundance and sexual reproduction of advanced generation hybrids compared with the parental species in a seasonally fluctuating environment? How important is clonal selection and hatching from sexually produced resting eggs for clonal diversity and population differentiation?

Methods

Study site and organism: *Daphnia* in Lake Constance

The mesotrophic, large (472 km^2) and deep (mean depth = 100 m) prealpine Lake Constance, located on the northern fringe of the Alps (9°18'E, 47°39'N), offers a unique system for studying the genetic architecture in the *Daphnia hyalina-galeata* hybrid complex. During eutrophication *Daphnia galeata* invaded Lake Constance in the 1950s, where originally only *Daphnia hyalina* occurred (Straile & Geller, 1998). Morphological data suggest massive hybridization during the 1970s (Einsle, 1983) and allozyme investigations showed that the hybrid ratio was between 20 and 30% in the late 1980s (Weider & Stich, 1992).

Sampling

Daphnia were sampled weekly to fortnightly in the central part of the fjord-like northwestern part of Lake

Constance (Überlinger See) from January to December 2000 with a Clark-Bampus sampler (mesh size 140 μm) by vertical hauls from 50 m depth. Living individuals (if possible 100 parthenogenetical females and all ephippia carrying females and males) were picked from the sample and were frozen in a −80 °C freezer for subsequent electrophoretic analysis. One net haul was taken and was fixed in formol for subsequent determination of abundance.

Allozyme analysis

The seasonal genetic architecture of the hybrid complex was determined using allelic variation. Four enzyme loci were screened for polymorphism in the *Daphnia* population of Lake Constance: aspartate amino transferase (AAT; E.C. 2.6.1.1.), aldehyde oxidase (AO; E.C. 1.2.3.1.), phosphoglucomutase (PGM; E.C. 5.4.2.2.) and phosphoglucose isomerase (PGI; E.C. 5.3.1.9.). Protocols for electrophoresis were as described elsewhere (Hebert & Beaton, 1993), except that electrophoresis was run at 300 V. Both, AAT (Wolf & Mort, 1986) and AO loci (Giessler, 1997) are considered to be diagnostic for *D. galeata* and *D. hyalina*. Using two marker loci it is possible to distinguish six possible genealogical classes: parentals (*D. hyalina* = P_{hya}, *D. galeata* = P_{gal}; with two homozygous species-specific alleles at each loci), first (F1; both loci heterozygous) and secondary (F2; both loci homozygous, but one for species 1 and the other for species 2) hybrids and first generation backcrosses (BP$_{hya}$, BP$_{gal}$; one locus is homozygous for one species and the other is heterozygous). This classification assumes only first and second order hybridization. Hence, it provides a conservative estimate of the relative frequency importance of higher order hybridization, including backcrosses, as, for example, 25% of the offspring of a parental × F1 hybrid cross can show the same genotype as the F1 hybrid class or the parental class, respectively (for discussion see: Nason *et al.*, 1992; Nason & Ellstrand, 1993). The two polymorphic loci PGI and PGM were used to differentiate between multi-locus-genotypes (MLG's) within each genotype class.

Data analysis

Multi-locus-genotype diversity was calculated for all classes. Samples were pooled monthly to get representative sample sizes, in particular for winter samples and rare genotype classes. Calculations were performed only at a sample size greater than five individuals per class. We used the genotype diversity measure, G_0:

$$G_0 = \frac{1}{\sum_{i=1}^{k} p_i^2}$$

where p_i is the relative frequency of the ith MLG and k is the number of genotypes. G_0 will vary from a minimum

of 1, where there is only a single genotype, to a maximum of k, when genotypes are evenly distributed (Carvalho, 1994).

F-statistics and deviation from Hardy–Weinberg equilibrium (HWE) were calculated with F-STAT (Goudet, 2000) and GENETIX (Belkhir, 2001). Within-population and subpopulation deviations from HWE were estimated by F_{is} values (small f). To determine the degree of genetic differentiation between genotype classes (all samples pooled) pairwise F_{st} (θ) values (Weir & Cockerham, 1984) were calculated. Additionally, pairwise F_{st} values were calculated for 'subpopulations' of three seasons differing in food availability for daphnids: S1 (samples of January to April), S2 (samples of May to July) and S3 (samples of August to December) within each class, to detect potential differences in the genetic architecture resulting from recruitment of sexual produced resting eggs (differences between S1 and S2) and clonal selection (S2 vs. S3). Probability values of the F_{is}- and pairwise F_{st}-values were adjusted for multiple table-wide test using Bonferroni correction to minimise type-I errors.

Results

When screening 1879 parthenogenetic females with two specific markers all six possible genealogical classes were found: both parentals (P$_{hya}$ and P$_{gal}$), the F1 hybrid as well as the F2 and backcross (BP$_{hya}$ and BP$_{gal}$) hybrids. Abundance of all genotype classes increased exponentially during spring (Fig. 1a), with P$_{gal}$ showing the fastest increase resulting in a relative abundance of more than 70% in May (Fig. 1b). After obtaining peak abundances P$_{gal}$ decreased in abundance and was absent from plankton samples from November onwards. In contrast, spring increase of P$_{hya}$ was slowest and the contribution of P$_{hya}$ to overall *Daphnia* abundance was lowest during May. However, P$_{hya}$ maintained high abundances until autumn. Seasonal dynamics of backcross hybrids were similar to the respective parentals. Hence, P$_{hya}$ and BP$_{hya}$ occurred during the whole year and contributed in winter and autumn more than 95% of the population size. F1 and F2 hybrids showed an intermediate pattern: their spring increase was lower than the spring increase of P$_{gal}$, and their drop in abundance during summer was intermediate between P$_{gal}$ and BP$_{gal}$ on one hand and P$_{hya}$ and BP$_{hya}$ on the other hand (Fig. 1a). Consequently, relative abundance of hybrids was highest from end of May to mid of August, and abundance of hybrids at the end of the year was intermediate between P$_{gal}$, BP$_{gal}$ and P$_{hya}$, BP$_{hya}$.

The occurrence of ephippial females and males was restricted to two distinct periods (early summer and autumn) with a tendency for the start of male production to be just before the peak of ephippial females (Fig. 2). Even within these two periods, ephippial females contributed <1% and males <5% to overall abundance. Using
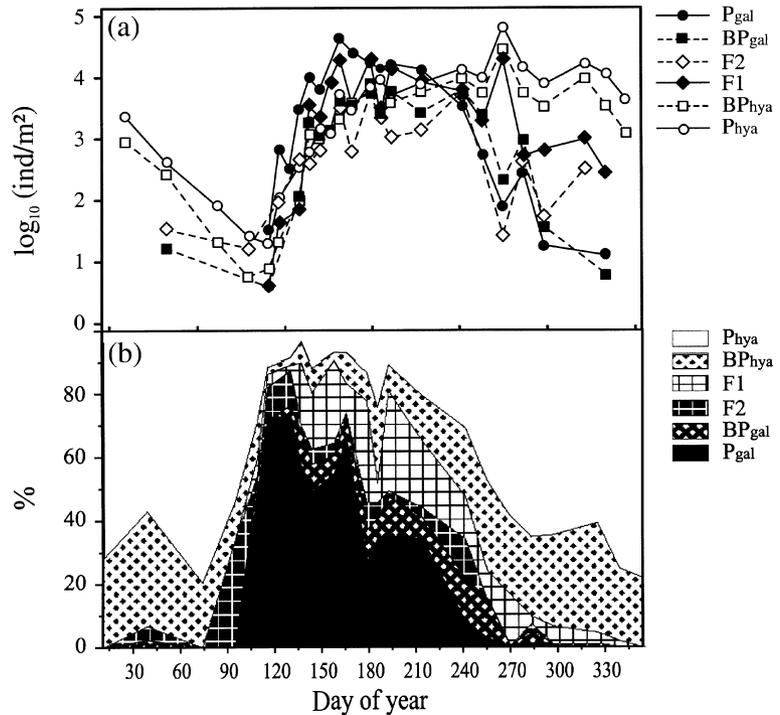
Fig. 1 Seasonal abundance (a) of partheno-genetical females of the six genotype classes (identified with the two marker loci AAT and AO) and (b) their contribution (%) to total *Daphnia* abundance in the *Daphnia* population of Lake Constance during 2000. Genotype classes represent parental species (P_{hya}, *D. hyalina*; P_{gal}, *D. galeata*) F1 hybrids and proposed F2 and backcross (BP_{hya}, BP_{gal}) hybrids.
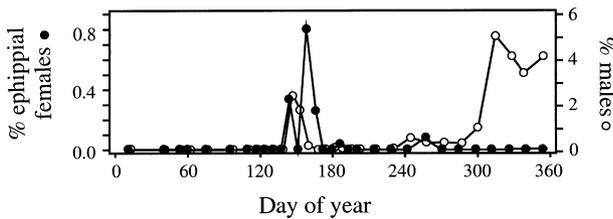
Fig. 2 Contribution (%) of sexual (ephippial) females (●) and males (○) to the *Daphnia* population in Lake Constance during the seasonal course.

allozymes we screened 65 ephippial females and 20 males in early summer and 30 ephippial females and 161 males in autumn, respectively. The timing of sexual reproduction differed between the two parental species (Fig. 3): *D. galeata* produced ephippia only after the spring peak, whereas *D. hyalina* produced ephippia only in autumn. Hybrids were observed to produce ephippia during both periods. The abundance of ephippial females was 10 times higher in spring than in autumn, i.e. *D. galeata* produced more ephippia than *D. hyalina*. Male production of *D. hyalina* was 10 times higher in autumn than in spring, and *D. galeata* produced males nearly exclusively in spring.

Genetic architecture and diversity

F_{st} values between the six genotype classes (all samples pooled) ranged from 0.001 to 0.245 showing significant differences between most of these classes (Table 1). Both parentals (P_{hya} and P_{gal}) were significantly different from all other classes, except from their backcrosses (BP_{hya} and BP_{gal}). The F1 hybrid differed significantly from all other classes.

Overall we found seven P_{hya}, eight BP_{hya}, 10 F1, 12 F2, 13 BP_{gal} and 23 P_{gal} MLG's. G_0 was highest for P_{gal} (maximum eight) and lowest for P_{hya} (maximum two) with intermediate diversities for hybrids and backcrosses (Fig. 4). G_0 of P_{gal} and the F2 hybrid were highest in May and June and decreased towards the end of the season (Fig. 5). G_0 of the other genotype classes exhibited no clear seasonal pattern. Pairwise F_{st} (θ) values calculated for S1, S2 and S3 (Table 2) revealed no significant differences between seasons for P_{hya}, F2 and BP_{gal}, while BP_{hya} populations differed significantly between S1 vs. S2 and S2 vs. S3, and F1 hybrid populations between S1 and S2. P_{gal} populations from all three seasons were significantly different from each other. F_{is} values varied from 0.250 to −0.453 and showed significant excess of heterozygotes in P_{gal} in all three seasons and in BP_{gal} in S2 (Table 2). High heterozygote deficiency was only observed in populations with small samples sizes.

Evidence for selection against introgressed individuals

The PGI allele '2' was only present in one parental population, but not in the other (Table 3). The frequency of this allele decreased from P_{gal} to P_{hya}. At the PGM locus
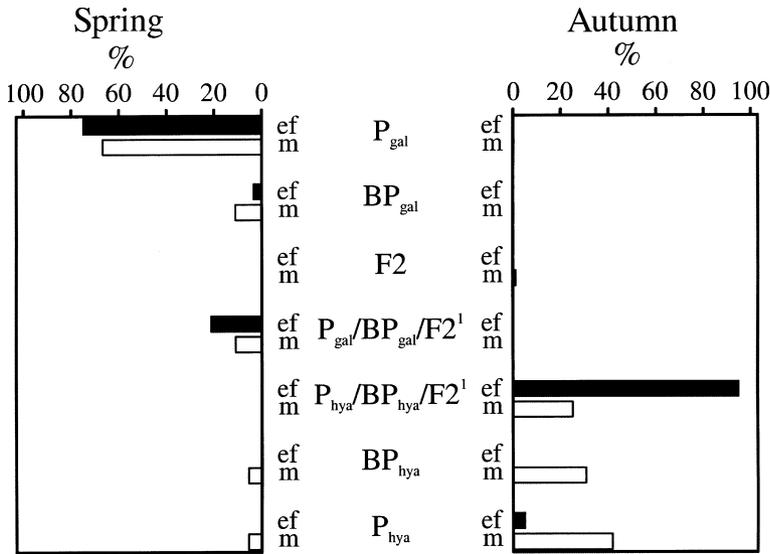
Spring %

Autumn %

Fig. 3 Contribution (%) of different geno-type classes to sexual (ephippial) females (ef = black) and males (m = white) in spring (January to July, left panel) and autumn (August to December, right panel). These two time periods were selected as no sexual individuals were observed during summer. Abbreviations of genotype classes as in Fig. 1. [1]Because of technical problems we failed to screen sexual (ephippial) females and males for one of the species-specific marker (AO) for some of the samples. But the AAT locus indicated that all of these ephippial females and males belong to the genotype classes P_{gal}, BP_{gal} or F2 hybrid in spring, and to P_{hya}, BP_{hya} or F2 hybrid in autumn.

Table 1 F_{st} values calculated for two polymorphic loci (PGI and PGM) between all genotype classes over all samples. Abbreviations of genotype classes as in Fig. 1.

	P_{hya}	BP_{hya}	F1	F2	BP_{gal}
BP_{hya}	<−0.001				
F1	0.045*	0.038*			
F2	0.127*	0.096*	0.066*		
BP_{gal}	0.246*	0.193*	0.159*	0.021	
P_{gal}	0.245*	0.202*	0.172*	0.060*	0.012

*Significant after Bonferroni correction to minimise type-I errors ($P < 0.003$); 1500 permutations.

two alleles showed a similar pattern (Table 3). Alleles '2' and '3' decreased from P_{gal} to P_{hya}. The relative frequency of *PGM* allele '3' within P_{gal}, PB_{gal} and BP_{hya} decreased from a maximum in late spring, i.e. after hatching, towards the end of the season (Fig. 6). In addition *PGM* '3' allele carrying individuals within the BP_{hya} class did not contribute to the overwintering population of BP_{hya} (Fig. 6).

Discussion

Our study revealed strong differences between the parental species regarding their seasonal dynamics, genetic architecture and diversity, and their sexual reproductive behaviour. Within all variables studied, F1 and F2 hybrids showed an intermediate pattern, whereas proposed backcross hybrids were more similar to their respective parentals. Significant differences in pairwise F_{st} values as well as differences in the timing of sexual reproduction suggest that gene flow seems to be relatively low in the Lake Constance hybrid system.
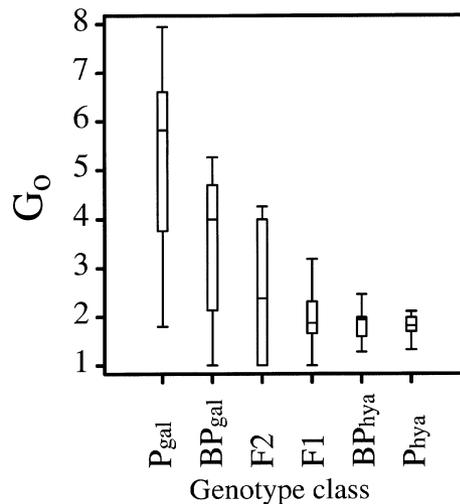
Fig. 4 Genetic diversity (G_0) calculated with the two polymorphic loci PGI and PGM for the six genotype classes. Shown are the mean, the 50% (box) and the 95% (bar) confidence limits of monthly-pooled samples over the year. Abbreviations of genotype classes as in Fig. 1.

Frequency of hybridization and introgression

Our results are in agreement with other studies on *Daphnia*, which show that hybrids live in syntopy with one or both parental species (Schwenk & Spaak, 1995). Furthermore, our results show that hybrids reproduced sexually during the same seasonal periods as their parental species, i.e. backcrossing and introgression was possible. Using two species-specific markers we identified six genotype groups, including BP_{hya} and BP_{gal}, which possibly represent backcrossed individuals of parental
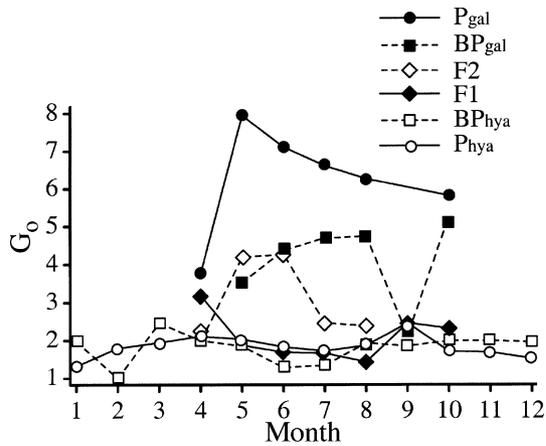
Fig. 5 Seasonal changes in the genetic diversity within the six genotype classes calculated as G_0 for monthly pooled samples. Abbreviations of genotype classes as in Fig. 1.

Table 3 Allele frequencies of the two polymorphic loci PGI and PGM over all samples. Abbreviations of genotype classes as in Fig. 1 (frequencies of proposed introgressed alleles are in bold).

Locus	Allele	P_{hya}	BP_{hya}	F1	F2	BP_{gal}	P_{gal}
PGI	1	1.000	0.995	0.984	0.900	0.789	0.686
	2	–	**0.004**	**0.015**	**0.100**	**0.210**	**0.313**
PGM	1	–	–	–	0.007	–	0.001
	2	**0.006**	**0.012**	**0.011**	**0.084**	**0.186**	**0.171**
	3	**0.017**	**0.026**	**0.129**	**0.184**	**0.214**	**0.293**
	4	0.852	0.840	0.850	0.669	0.570	0.517
	5	0.120	0.120	0.006	0.053	0.028	0.016
	6	0.003	–	0.002	–	–	–

Table 2 Population genetic characterization between seasons (S) within each genotype class. The left side shows the number of individuals (N), the number of multi-locus-genotypes (MLG's) and the F_{is} values for each season (S). The right side shows the pairwise F_{st} values calculated for two polymorphic loci (PGI and PGM) between three seasons. Abbreviations of genotype classes as in Fig. 1.

Class	S	N	MLG's	F_{is}	S	F_{st}
P_{hya}	1	110	7	0.004	1 vs. 2	−0.001
	2	72	4	−0.125	2 vs. 3	−0.003
	3	306	5	−0.058	1 vs. 3	−0.001
BP_{hya}	1	40	4	−0.152	1 vs. 2	0.045*
	2	60	4	0.125	2 vs. 3	0.043*
	3	145	7	−0.092	1 vs. 3	−0.006
F1	1	13	5	−0.220	1 vs. 2	0.056*
	2	151	7	−0.067	2 vs. 3	−0.001
	3	56	5	−0.095	1 vs. 3	0.021
F2	1	10	4	0.250	1 vs. 2	0.010
	2	39	11	−0.028	2 vs. 3	0.051
	3	16	6	−0.134	1 vs. 3	−0.010
BP_{gal}	1	5	5	0.200	1 vs. 2	−0.005
	2	65	11	−0.194*	2 vs. 3	<−0.001
	3	37	10	−0.193	1 vs. 3	−0.005
P_{gal}	1	57	10	−0.453 *	1 vs. 2	0.031*
	2	332	21	−0.199*	2 vs. 3	0.012*
	3	97	13	−0.233*	1 vs. 3	0.030*

*Significant difference after Bonferroni correction to minimise type-I errors ($P = 0.008$ for F_{is} and $P = 0.017$ for F_{st}); 1000 permutations; S = seasons: 1, 1–4 months; 2, 5–7 months; 3, 8–12 months.

species. Although daphnids belonging to genotype groups BP_{hya} and BP_{gal} can also result from sexual reproduction between hybrids, analysis of F_{st} values suggest that these taxa result from backcrossing between hybrids and parentals.

Significant differences in F_{st} values between parentals and hybrids suggest that the daphnid population does not represent one large and homogenous hybrid swarm. This conclusion is also supported when using a recent method based on Markov chain Monte Carlo simulations in a Bayesian setting to identify genealogical classes (Anderson & Thompson, 2002). Although this analysis supports the existence of second order hybrids in Lake Constance, their relative abundance was estimated to be only 4% of the total population (all F2 hybrids, no backcrosses), while first order hybrids were estimated to contribute 14% and parentals 48% (P_{hya}) and 34% (P_{gal}) to all screened animals. Our classification using two species-specific markers resulted in very similar relative abundances, i.e. 26% second order hybrids (4% F2's, 22% backcrosses), 19% F1, 30% P_{hya} and 25% P_{gal}. The lower frequency of second order hybrids and higher frequency of parentals estimated by the model compared with the classification of genealogical classes with marker allozymes was due to the slight genetic differentiation between parentals and backcrosses, which resulted in the assignment of backcrosses as parentals.

Several factors might contribute to the strong genetic differentiation between parentals and hybrids in the Lake Constance *Daphnia* hybrid system: first, hybridization is restricted because of the parentals' differences in timing of sexual reproduction; secondly, mating probabilities have been observed to be lower for interspecific hybridization and backcrossing as compared with intraspecific crosses (Schwenk, 1997); thirdly, the hybrid system may still be in an early phase of hybridization, as the two parentals have coexisted in Lake Constance for less than five decades (Jankowski & Straile, 2003); and fourthly, there is selection against hybrids, i.e. parthenogenetic reproduction of hybrids is less successful than parthenogenetic reproduction of parentals, resulting in low abundance of males and ephippia producing hybrids.

The latter is supported by a rather low hybrid frequency in Lake Constance, which never exceeded a relative frequency of 50% and was considerably lower through most of the season (Fig. 1b). In contrast, hybrids reached maximum frequencies of about 60 to >95% in
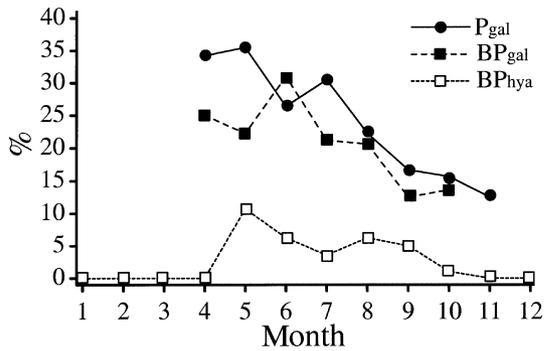
Fig. 6 Contribution (%) of individuals with the *PGM* allele '3' to the three genotype classes P_{gal}, BP_{gal} and BP_{hya} for monthly pooled samples. Abbreviations of genotype classes as in Fig. 1.

many other lakes (Table 4). 'Temporary hybrid superiority' (Spaak & Hoekstra, 1995) is thus restricted in Lake Constance to a short time period.

A possible selective advantage of parentals – at least of one parental species, *D. hyalina* – is suggested by the seasonal occurrence of possibly introgressed alleles (Table 3). We found evidence for selection against genotypes carrying proposed introgressed alleles at the PGI and PGM loci (Fig. 6). Earlier studies suggest that PGI and PGM loci might be important markers to characterize the genetic structure of *Daphnia* populations (Spaak, 1996; Weider *et al.*, 1997). Unfortunately, experiments concerning the fitness of daphnid backcrosses compared with parentals using life-history variants were not conducted up to now, and those experiments are also rare for other species (Arnold, 1997; Schluter, 2000).

Seasonal pattern of recruitment and clonal selection in parental taxa

The parental species showed strong differences in their seasonal occurrence and genetic architecture (Fig. 1a,b and 6; Table 2). These differences were not specific to the

year 2000 as similar patterns were observed in 1999 and 2001 (own unpubl. data). In contrast to other investigations (Wolf, 1987; Weider & Stich, 1992; Spaak, 1996) *D. galeata* was not found in late fall and winter. Hence, our allozyme study supports morphological data (Straile & Geller, 1998) indicating different overwintering strategies for the two parental *Daphnia* species: *D. galeata* seemed to rely more strongly on resting stages than *D. hyalina* to get through severe winter conditions, while for *D. hyalina* overwintering in the plankton is more important. These differences in the importance of resting stages for the seasonal cycle are supported by our data on the abundance of sexual individuals and additionally by the increase in G_0 during the hatching period within the *D. galeata* population, but not within *D. hyalina*. This increase in G_0 and the higher frequency of sexual reproduction indicates that hatching from resting eggs was more frequent for *D. galeata*. Furthermore, there was no significant difference in F_{st} values between the spring and autumn population of *D. hyalina*, suggesting that hatching from ephippia did not alter the *D. hyalina* clonal composition of individuals that were successful in overwintering. In contrast, seasonal changes in genetic structure and diversity were found for *D. galeata*, which probably results from the hatching of resting eggs and subsequent selection against introgressed genes (Fig. 6), as pointed out above.

Consequences of the differences in the timing of sexual reproduction for seasonal clonal selection and diversity

Differences in clonal selection and diversity between the two parental species can be at least partially explained by the differences in the timing and frequency of sexual reproduction, which is under both genetical and maternal control (Alekseev & Lampert, 2001). Because of species differences in sexual reproduction, the seasonal origin of the spring cohorts of *Daphnia* populations differed between *D. hyalina* and *D. galeata*. As *D. galeata* did not overwinter in the plankton and did not produce

Lake	Taxa	Hybrid percentage	Sampled	Ref.
Greifensee	hya, gal	h × g: 70–90%	Feb–Jul 1998	1
Tjeukemeer	gal, cuc	c × g: 10–70%	Apr 1989–Apr 1992	2
Lake Maarsseveen	(hya,) gal	h × g: 50–100%	May 1989–Aug 1991	3
Kellersee	hya, gal, cuc	c × g: 0–20%	May 1984–Feb 1985	4*
		h × g: 10–60%		
Schöhsee	hya, gal, cuc	h × g: 40–90%	May 1984–Feb 1985	4*
Lake Constance	hya, gal	h × g: 10–50%	Nov 1989–Oct 1990	5
Lake Constance	hya, gal	h × g: 10–40%	Jan–Dec 2000	This study

Table 4 Hybrid percentage (AAT locus) of the *Daphnia galeata-hyalina-cucullata* complex from different European lakes (hya = h = *D. hyalina*; gal = g = *D. galeata*; cuc = c = *D. cucullata*; taxa in parenthesis means taxa not found).

*Wolf (1987) sampled also several other lakes, but for this comparison we used only lakes, which were sampled during at least 1 year.
1, Spaak *et al.*, 2000; 2, Spaak, 1996; 3, Spaak & Hoekstra, 1993; 4, Wolf, 1987; 5, Weider & Stich, 1992.

resting eggs in autumn, the spring cohort stems from hatching of resting eggs produced after previous spring blooms, i.e. in May and June of previous years. Furthermore, this suggests that *D. galeata* clones which were successful in summer did not contribute to the next spring generation either because they failed to produce resting eggs and/or failed to survive winter conditions. Hence, we expect differences in genetic architecture between seasons for *D. galeata* populations. In contrast, the spring cohort of *D. hyalina* stems from the clones that either successfully produced resting eggs in autumn or successfully got through the winter in the plankton. That is, the *D. hyalina* population should be composed of rather generalist clones able to reproduce parthenogenetically throughout the season.

The timing of sexual reproduction should hence strongly contribute to different microevolutionary trajectories of the two parental *Daphnia* populations on an interannual scale. *D. galeata* clones should be selected which are able to cope with the benign spring conditions, i.e. high food concentrations and low predation pressure, whereas *D. hyalina* clones should be selected which can best cope with summer conditions, i.e. higher predation pressure and lower food concentrations. This line of argument is further supported by the relative decrease of *PGM* allele '3' during summer, which is most prominent in *D. galeata*. The frequency of this allele within various genotype groups decreases during summer (Fig. 6). As hatching from ephippia was only observed during spring (unpubl. data), this suggests that clonal selection might have decreased the relative contribution of *D. galeata* parentals and backcrosses during the growing season. Probably this allele is maintained in the population as its carriers produce a sufficient amount of resting eggs in late spring, i.e. before strong selection against this allele takes place. In contrast, *D. hyalina* parentals and backcrosses seemed to be better adapted to the conditions after the spring bloom. This is supported by life-history experiments, where *D. hyalina* grew and reproduced better at low food conditions than *D. galeata* (Stich & Lampert, 1984). As there seems to be a trade-off in daphnids between abilities to exploit high vs. low resource richness (Tessier *et al.*, 2000) adaptation of daphnids to spring conditions, i.e. to exploiting high resource levels, might even have detrimental effects for summer conditions, i.e. to exploiting efficiently low resource levels. It is worth noting that F1 and F2 hybrids seem to have rather intermediate success during both spring and summer conditions. In evolutionary terms, adaptation to spring vs. summer conditions might represent two adaptive peaks (Schluter, 2000). If this is the case, disruptive ecological selection might also contribute to the rather low hybrid ratio observed in Lake Constance. Hence, the timing of sexual reproduction in combination with increasingly unfavourable conditions in summer in Lake Constance with ongoing oligotrophication, might contribute to or even promote different microevolutionary pathways for the two parental *Daphnia* species.

Consequences of the differences in the timing of sexual reproduction for long-term clonal selection and persistence

Like many other European lakes, Lake Constance experienced strong changes in trophic status during the last century resulting from anthropogenic eutrophication and oligotrophication (Güde *et al.*, 1998). Currently, nutrient levels in the lake decrease strongly from year to year with subsequent changes of phytoplankton biomass and production and now approach levels typical for the time period when *D. galeata* invaded Lake Constance (Güde *et al.*, 1998). This immediately poses the question of how long *D. galeata* will be able to persist in the lake given a further reduction of nutrient concentrations. So far, changes in phytoplankton biovolume and production have only been observed during summer and autumn, but not in spring (Gaedke, 1998). This suggests that resting egg production early in the season seems to be a 'short-cut' of the seasonal dynamics which should allow *D. galeata* and hybrids to persist in the lake despite unfavourable summer conditions. The draw-back of this 'short-cut', however, is, that *D. galeata* will be less able to adapt to decreasing food concentrations during summer as clones which are successful in summer do not produce resting eggs in autumn and also fail to overwinter and hence do not contribute to the next spring generation.

The seasonal window of occurrence of *D. galeata* already seems to shrink in Lake Constance (Straile & Geller, 1998). However, persistence of this species is likely as long as population growth and subsequent resting egg production following the spring bloom of phytoplankton is possible. The timing of sexual reproduction events hence seems to have a major impact on genetic architecture, clonal selection and microevolutionary dynamics of *Daphnia* populations. In the case of *D. galeata* the strategy of resting egg production in early summer may represent an ecological short-cut which might allow persistence of this species also in a oligotrophic lake; however, it also presents to some extent an evolutionary 'one-way street' hindering an adaptation to reoligotrophied conditions.

Acknowledgments

References

Alekseev, V. & Lampert, W. 2001. Maternal control of resting-egg production in *Daphnia*. *Nature* **414**: 899–901.

Anderson, E.C. & Thompson, E.A. 2002. A model-based method for identifying species hybrids using multilocus genetic data. *Genetics*. **160**: 1217–1229.

Arnold, M.L. 1997. *Natural Hybridization and Evolution*. Oxford University Press, Oxford.

Belkhir, K. 2001. *GENETIX, logiciel sous WindowsTM pour la génétique des populations*. URL: http://www.univ-montp2.fr/%7Egenetix/genetix/genetix.htm.

Bittner, K. 2001. Parasitismus bei Daphnia im Bodensee. PhD thesis, University of Konstanz, Konstanz.

Bleeker, W. & Hurka, H. 2001. Introgressive hybridization in *Rorippa* (Brassicaceae): gene flow and its consequences in natural and anthropogenic habitats. *Mol. Ecol.* **10**: 2013–2022.

Carvalho, G.R. 1994. Genetics of aquatic clonal organisms. In: *Genetics and Evolution of Aquatic Organisms* (A. Beaumont, ed.), pp. 290–323. Chapman and Hall, London.

Darwin, C. 1876. *Cross and Self-Fertilization in Plants*. Murray, London.

Deng, H.-W. 1997. Photoperiodic response of sexual reproduction in the *Daphnia pulex* group is reversed in two distinct habitats. *Limnol. Oceanogr.* **42**: 609–611.

Dowling, T.E. & Secor, C.L. 1997. The role of hybridization and introgression in the diversification of animals. *Ann. Rev. Ecol. Syst.* **28**: 593–619.

Edmands, S. 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. *Evolution* **53**: 1757–1768.

Einsle, U. 1983. Die Entwicklung und Männchenbildung der *Daphnia*-Population in Bodensee-Obersee 1956–1980. *Schweizer Z Hydrologie* **45**: 321–332.

Gaedke, U. 1998. Functional and taxonomical properties of the phytoplankton community of large and deep Lake Constance: interannual variability and response to re-oligotrophication (1979–1993). *Arch. Hydrobiol. Advanc. Limnol.* **53**: 119–141.

Giessler, S. 1997. Analysis of reticulate relationships within the *Daphnia longispina* species complex. Allozyme phenotype and morphology. *J. Evol. Biol.* **10**: 87–105.

Giessler, S., Mader, E. & Schwenk, K. 1999. Morphological evolution and genetic differentiation in *Daphnia* species complex. *J. Evol. Biol.* **12**: 710–723.

Goudet, J. 2000. *FSTAT, a Program to Estimate and Test Gene Diversities and Fixation Indices (version 2.9.1)*. URL http://www.unil.ch/izea/softwares/fstat.html.

Grant, B.R. & Grant, P.R. 1996. High survival of Darwin's Finch hybrids: effects of beak morphology and diets. *Ecology* **77**: 500–509.

Güde, H., Rossknecht, H. & Wagner, G. 1998. Anthropogenic impacts on the trophic state of Lake Constance during the 20th century. *Arch. Hydrobiol. Advanc. Limnol.* **53**: 85–108.

Harrison, R.G. 1993. *Hybrid Zones and the Evolutionary Process*. Oxford University Press, Oxford.

Hebert, P.D.N. & Beaton, M.J. 1993. *Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis - a Practical Handbook*. Helena Laboratories, Beaumont, TX, USA.

Jankowski, T. & Straile, D. 2003. A comparison of egg-bank and long-term plankton dynamics of two *Daphnia* species, *D. hyalina* and *D. galeata*: Potentials and limits of reconstruction. *Limnol. Oceanogr.* **48**: 1948–1955.

Kleiven, O.T., Larsson, P. & Hobaek, A. 1992. Sexual reproduction in *Daphnia magna* requires three stimuli. *OIKOS* **65**: 197–206.

Lynch, M. 1991. The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution* **45**: 622–629.

Malmos, K.B., Sullivan, B.K. & Lamb, T. 2001. Calling behavior and directional hybridization between two toads (*Bufo microscaphus* × *B. woodhousii*) in Arizona. *Evolution* **55**: 626–630.

Martinsen, G.D., Whitham, T.G., Turek, R.J. & Keim, P. 2001. Hybrid populations selectively filter gene introgression between species. *Evolution* **55**: 1325–1335.

Nason, J.D. & Ellstrand, N.C. 1993. Estimating the frequencies of genetically distinct classes of individuals in hybridized populations. *J. Heredity* **84**: 1–12.

Nason, J.D., Ellstrand, N.C. & Arnold, M.L. 1992. Patterns of hybridization and introgression in populations of oaks, manzanitas, and irises. *Am. J. Botany* **79**: 101–111.

Noor, M.A.F., Grams, K.L., Bertucci, L.A., Almendarez, Y., Reiland, J. & Smith, K. 2001. The genetics of reproductive isolation and the potential for gene exchange between *Drosophila pseudoobscura* and *D. persimilis* via backcross hybrid males. *Evolution* **55**: 512–521.

Orr, H.A. 1997. Haldane's rule. *Ann. Rev. Ecol. Syst.* **28**: 195–218.

Rieseberg, L.H., Whitton, J. & Gardner, K.A. 1999. Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. *Genetics* **152**: 713–727.

Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.

Schwenk, K. 1997. Evolutionary genetics of *Daphnia* species complex – hybridism in syntopy. PhD thesis, University of Utrecht.

Schwenk, K. & Spaak, P. 1995. Evolutionary and ecological consequences of interspecific hybridization in cladocerans. *Experientia.* **51**: 465–481.

Schwenk, K., Posada, D. & Hebert, P.D.N. 2000. Molecular systematics of European *Hyalodaphia*: the role of contemporary hybridization in ancient species. *Proc. R. Soc. Lond. B* **267**: 1833–1842.

Sommer, U., Gliwicz, Z.M., Lampert, W. & Duncan, A. 1986. The PEG-model of seasonal succession of planktonic events in fresh waters. *Arch. Hydrobiol.* **106**: 433–471.

Spaak, P. 1995. Sexual reproduction in *Daphnia*: interspecific differences in a hybrid species complex. *Oecologia* **104**: 501–507.

Spaak, P. 1996. Temporal changes in the genetic structure of the *Daphnia* species complex in Tjeukemeer, with evidence for backcrossing. *Heredity* **76**: 539–548.

Spaak, P. & Hoekstra, J.R. 1993. Clonal structure of the *Daphnia* population in Lake Maarsseveen: its implications for diel vertical migration. *Arch. Hydrobiol. Advanc. Limnol.* **39**: 157–165.

Spaak, P. & Hoekstra, J.R. 1995. Life history variation and the coexistence of a *Daphnia* hybrid with its parental species. *Ecology* **76**: 553–564.

Spaak, P., Eggenschwiler, L. & Bürgi, H.R. 2000. Genetic variation and clonal differentiation in the *Daphnia* population of the Greifensee, a pre-alpine Swiss lake. *Verh. Internat. Verein. Limnol.* **27**: 1919–1923.

Stich, H.B. & Lampert, W. 1981. Predator evasion as an explanation of diurnal vertical migration by zooplankton. *Nature* **293**: 396–398.

Stich, H.B. & Lampert, W. 1984. Growth and reproduction of migrating and non-migrating *Daphnia* species under simulated food and temperature conditions of diurnal vertical migration. *Oecologia* **61**: 192–196.

Straile, D. & Geller, W. 1998. Crustacean zooplankton in Lake Constance from 1920 to 1995: response to eutrophication and re-oligotrophication. *Arch. Hydrobiol. Advanc. Limnol.* **53**: 255–274.

Taylor, D.J. & Hebert, P.D.N. 1993. Habitat-dependent hybrid parentage and differential introgression between neighboringly sympatric *Daphnia* species. *Proc. Natl. Acad. Sci. USA* **90**: 7079–7083.

Tessier, A.J., Leibold, M. & Tsao, J. 2000. A fundamental trade-off in resource exploitation by *Daphnia* and consequences to plankton communities. *Ecology* **81**: 826–841.

Weider, L.J. 1993. Niche breadth and life history variation in a hybrid *Daphnia* complex. *Ecology* **74**: 935–943.

Weider, L.J. & Stich, H.B. 1992. Spatial and temporal heterogeneity of *Daphnia* in Lake Constance; intra- and interspecific comparisons. *Limnol. Oceanogr.* **37**: 1327–1334.

Weider, L.J., Lampert, W., Wessels, M., Colbourne, J.K. & Limburg, P. 1997. Long-term genetic shifts in a microcrustacean egg bank associated with anthropogenic changes in the Lake Constance ecosystem. *Proc. R. Soc. Lond. B* **264**: 1613–1618.

Weir, B.S. & Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.

Wolf, H.G. 1987. Interspecific hybridization between *Daphnia hyalina*, *D. galeata*, and *D. cucullata* and seasonal abundances of these species and their hybrids. *Hydrobiologia* **145**: 213–217.

Wolf, H.G. & Mort, M.A. 1986. Inter-specific hybridization underlies phenotypic variability in *Daphnia* populations. *Oecologia* **68**: 507–511.