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Evolutionary stoichiometry: The role of food quality for clonal differentiation and hybrid maintenance in a *Daphnia* species complex

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Abstract

Interspecific hybridization is a common phenomenon among *Daphnia* species (Crustacea: Anomopoda); interspecific hybrids and parental species have been shown to be ecologically differentiated and often co-occur in the same lake. Food quantity, temperature, and the level of predation (by juvenile fish) have been identified as the main environmental factors determining the fitness of *Daphnia* taxa. Here we tested another environmental factor, food quality, which is known to shape fitness in *Daphnia*. We conducted life-history experiments with clones of *Daphnia galeata, Daphnia cucullata,* and their interspecific hybrids and measured fitness-related life-history traits at two food quality conditions (phosphorus [P]-rich and P-limited algae). *D. galeata* × *cucullata* hybrids show highest fitness values in some traits at low food quality conditions, relative to the parental species, whereas *D. galeata* was superior in P-rich conditions. These results, based on single-clone life-history studies, were confirmed by a multiclone experiment. Large natural variation in food quality and the observed differential response of clones and taxa to P variation indicates that variation in food quality represents an additional factor, besides fish predation and food quantity, explaining hybrid maintenance.

It has long been thought that interspecific hybridization among animal species represents a rare phenomenon (Mayr 1963). Recent studies, however, have shown that interspecific hybridization is in fact fairly common and contributes significantly to evolutionary changes (Harrison 1990; Seehausen 2004). The origin and establishment of hybrid lineages can occur rapidly within a few generations, which allows evolutionary biologists to study a number of basic ecological and genetic processes under natural conditions (i.e., reproductive isolation, ecological differentiation, and speciation). Consequently, interspecific hybridization has become a major research field in evolutionary biology and molecular ecology (Harrison 1990; Seehausen 2004). Well-isolated populations on islands or in lakes and ponds offer a unique opportunity to study the consequences of interspecific hybridization in syntopic populations that lack any geographic isolation or gradients. In particular, the analysis of ecological differentiation among these hybridizing taxa allows the analysis of exogenous factors responsible for the origin and maintenance of hybrid lineages (e.g., Grant and Grant 1994). Cyclic parthenogenetic organisms are especially suited to the study of hybridization processes, as their reproductive mode allows for the experimental differentiation between exogenous and endogenous selection. Studies on interspecific hybridization among several cladoceran taxa, mainly *Daphnia* species, showed that parental taxa co-occur over large areas, populations are well isolated, and interspecific hybrids are found frequently in syntopy with at least one parental species (Wolf 1987; Hebert et al. 1989; Schwenk and Spaak 1995). However, species are genetically well differentiated despite frequent interspecific hybridization and backcrossing (Schwenk et al. 2000).

Most attempts to explain the origin and maintenance of interspecific hybrids can be classified as derivatives of two different types of models. First, the *tension zone models* explain the formation of hybrids by a balance between dispersal of parental species into a hybrid zone and the subsequent selection against interspecific hybrids. This selection against hybrids is mainly attributed to endogenous factors, such as genetic incompatibilities between parental genomes. In contrast, *cline or ecotone models* are

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based on exogenous factors (i.e., environmental gradients), which determine the origin and fate of interspecific hybrids (e.g., Arnold 1997). Both types of models have been used to explain hybrid maintenance in many plant and animal taxa (e.g., Arnold 1997; Rieseberg 1997; Seehausen 2004).

Among species of the microcrustacean genus *Daphnia*, a number of studies described that under certain environmental conditions, interspecific hybrids exhibited a higher relative fitness (e.g., intrinsic rate of increase) than did parental species (Spaak and Hoekstra 1995; Repka et al. 1999*b*; Declerck and De Meester 2003). This phenomenon motivated Spaak and Hoekstra (1995) to propose the *temporal hybrid superiority* (THS) model. This model assumes higher fitness for interspecific hybrids only during certain periods of the year, when several environmental conditions are met. This model is a derivative of a cline model (i.e., bounded hybrid superiority model) that defines fitness among parental species and interspecific hybrids based on exogenous factors (Moore 1977). The THS model is supported by recent field data, which demonstrated temporal dominance of interspecific hybrids during a season (Declerck and De Meester 2003). In addition, a number of life-history studies provided evidence for different environmental factors that are responsible for hybrid maintenance, such as variation in fish predation (Declerck and De Meester 2003), temperature (Weider and Wolf 1991), and food quantity (Boersma and Vijverberg 1994*a*,*b*). Here we tested the question of whether food quality differences, which often occur during a season in lakes (Kreeger et al. 1997), help to explain the maintenance of hybrid lineages.

Although low food quality is known to affect fitness in *Daphnia* adversely (Boersma 2000), and although food quality plays an important role in explaining the community structure and population dynamics of zooplankton (Sterner and Elser 2002), the effect of food quality has so far not been tested in the framework of studies on interspecific hybridization. One of the best studied determinants of food quality in freshwater environments is phosphorus (P) content of food particles, since it is essential as a component of proteins, nucleic acids, lipids, and energetic nucleotides (Sterner and Elser 2002). It represents one of the limiting factors for growth in freshwater zooplankton species (Scheffer 2001), and several studies indicated that *Daphnia* species responded to a decrease in P content of algae with a reduction in fitness (Weers and Gulati 1997; Boersma 2000; Becker and Boersma 2003). It is known that above a critical carbon (C) to P ratio (C : P ratio) of about 225–375, growth is limited in *Daphnia*; this value is one that can be found in a substantial subset of lakes (Brett et al. 2000), although recent evidence indicates that the use of these thresholds in field situations should be handled with care (DeMott and Tessier 2002).

The general aim of our study was to investigate the life-history variation of the two *Daphnia* species *Daphnia galeata* and *Daphnia cucullata* and their interspecific hybrids at two food quality treatments (P-rich and P-limited algae) in order to assess the potential contribution of food quality variation to hybrid maintenance. Specifically, we addressed the following question: Do *Daphnia* species and their interspecific hybrids differ in their response to variation in food quality?

Materials and methods

Life-history experiments—Three clones of *D. galeata*, two clones of *D. cucullata*, and three clones of their interspecific hybrid, *D. cucullata* × *galeata*, were subjected to three different experiments. All interspecific hybrids and one *D. galeata* clone originate from laboratory crosses (Schwenk et al. 2001). We used the *D. galeata* clones G100 (isolated from Lake Tjeukemeer, The Netherlands) and G44 (Grote Brekken, The Netherlands) and one clone, GL5, which resulted from an intraspecific cross between G100 and G44. *D. cucullata* was represented by the two clones C33 (Lake Tjeukemeer) and V50 (Lake Vechten, The Netherlands). Interspecific hybrids originate from laboratory crosses of G100 and C33, resulting in clone X1 and X3, and a cross between V50 and G100 resulted in clone GCL1. The offspring of all crosses were verified using several genetic markers (Schwenk et al. 2001).

Semicontinuous cultures of *Scenedesmus obliquus* were established in Z/4 medium (Zehnder and Gorham 1960) with full phosphorus or with reduced phosphorus content in a method similar to that of Becker and Boersma (2003), resulting in algal cells with a molar C : P ratio of 70–80 for P-rich cells (P^+) and a ratio of about 1,000 for P-limited algae (P^-). Every day, 700 mL (total volume: 1.5 liters) of culture medium was replaced with fresh medium: Algae were centrifuged at 2,700 *g* for 10 min and diluted in P-free medium (Aachener Daphnien Medium, AdaM; Kluttgen et al. 1994) to remove traces of dissolved P of algal culture media. C content of the cultures was established photometrically using a calibration curve for both culture conditions. The calibration curve was established by measuring the extinction of different diluted algae suspensions at 800 nm using a spectrophotometer (U-2000, Hitachi). For each dilution, C content was measured subsequently by filtration of algae onto precombusted 24-mm–diameter glass-fiber filters (Whatman GF/C), and C content was quantified by a CHN-analyzer (Perkin Elmer). P content of algae was measured spectrophotometrically after digestion with potassium persulfate (Langner and Hendrix 1982).

All experiments and cultures were kept at 18°C with a light : dark cycle of 16 : 8 h. Before starting the experiments, daphniids of each clonal lineage were adjusted to ADaM medium for at least five generations. Juvenile animals were collected from stock cultures and placed into 250-mL jars filled with ADaM medium and fed 1 mg C L^{-1} of P^+ algae to guarantee a food supply above the incipient limiting level (Lampert 1987). Neonates, born within 24 h, were subjected to three different experiments. First, newborns were kept individually in 250-mL beakers at P^+ and P^- conditions at 1 mg C L^{-1} until they reached maturity and reproduced. Medium and algae were replaced every day. Size of the newborns (JUS) as well as size of the females at reproduction (SAR) were measured under a microscope to the nearest 0.02 mm. The number of newborns (NJU) and days to reproduction (DTR) under

both conditions (P^+ and P^-) were counted. In a second experiment, 10 newborns of each clone were kept at P^+ and P^- conditions (in 250-mL beakers) for 4 d to measure their somatic growth rate (SGR). The initial size of juveniles was measured using 10 newborns per clone, and the size of the experimental animals after 4 d was measured to the nearest 0.02 mm under a microscope. The size of the animals was obtained by measuring the maximum length, excluding the caudal spine. SGR was calculated following the formula $SGR = \ln(L_t/L_0)/t$, where L_0 is the average initial size of a clone and L_t is the size of the animals at time t (Sterner and Elser 2002). To test for the susceptibility of a taxon to variation in food quality, we estimated the relative growth rate (RGR) using the formula: $RGR = \mu/\mu_m$, as described in Sterner and Elser (2002), where μ_m represents the somatic growth rate under P-rich conditions and μ the somatic growth rate under P-limited conditions.

In order to investigate if results from life-history experiments of individuals can be extrapolated to population experiments, we carried out a third experiment. We determined the effect of different food sources on population growth rates of different clones of *Daphnia* species and hybrids in a multiclone experiment. To that end, we set up a flow-through system with six 15-liter containers. Three containers were supplied with P^+ algae medium and three containers with P^- medium. Media and algae were replaced once a day using a flow-through system comprising a peristaltic pump that continuously replaced the old (vs. the new) media. Each container was initially inoculated with 10 juveniles of each clone. Once a week each container was cleaned of algae and bacteria growing at the glass walls. After 6 weeks, the experiment was terminated, and all animals (per replicate) were counted after they had been preserved in 70% ethanol. In addition, we subjected a random sample of 35 animals per replicate to genetic analyses to determine the abundance of clones and taxa in each container.

Genetic analysis—DNA preparation of the individuals from experiment III was conducted following a standard protocol (Schwenk et al. 1998). For each individual, a polymerase chain reaction (PCR) was accomplished using the primers ITS2-5.8S and ITS-18S to amplify an ~1,500–base pair segment consisting of ITS2, 5.8S, ITS1, and a part of 18S ribosomal DNA (Billiones et al. 2004). A subsequent restriction fragment length polymorphism (RFLP) analysis was conducted to determine taxon affiliation following the protocol of Billiones et al. (2004).

In order to analyze the clonal affiliation of each individual (eight different clonal lineages), we conducted microsatellite analyses in combination with mitochondrial DNA PCR-RFLP analyses. Since two hybrid clones (generated by different maternal species) showed the same genotype at all microsatellite loci, we applied PCR-RFLP of maternally inherited mitochondrial DNA. We amplified mitochondrial DNA using the primers S1 and S2, as described in Schwenk et al. (1998), and conducted a RFLP analysis with the restriction enzyme *Rsa* I. Mitochondrial DNA analysis was used to identify the two hybrid clones X3 and GCL1, since they originate from different maternal lineages (G100 and C33). Thus, it was possible to assign each individual to one of the eight clonal lineages and to determine the relative frequencies of taxa and clones used in the multispecies/multiclone experiment.

A subsample of each replicate ($n = 35$) from experiment III was subjected to microsatellite analysis using six microsatellite loci (Dove et al. unpubl. data). Fragments were separated on polyacrylamide gels with the help of an automatic DNA sequencer (Automatic Length Fragment Analyser, ALF; Amersham Pharmacia). Allelic variation at each microsatellite locus was combined to diagnostic multilocus genotypes.

Numerical analyses—Experiments I and II: Rates of population increase (r) for each taxon and treatment were calculated using the Euler–Lotka equation (Stearns 1992). Since our cultures consisted of individual animals, no age-specific survivorship (l_x) could be determined; therefore, l_x was set to a value of 1. To estimate the variance for r, two random combinations between the three replicates per clone and treatment were drawn to calculate an average and standard deviation to obtain a rough estimate of variation.

Because of an unbalanced experimental design (three clones for *D. galeata* and the interspecific hybrids and two clones of *D. cucullata*), we used a General Linear Model (GLM). Clones were nested within taxon and were implemented as random factors within the model; all other effects were treated as fixed factors [statistical model: *taxon* + *condition* + *clone*(*taxon*) + *taxon* × *condition* + *clone*(*taxon*) × *condition*]. The F values and the accompanying degrees of freedom (df) were calculated according to the methods described in Satterthwaite (1946). Differences between the taxa were tested a posteriori with a Fisher least significant differences test using type III GLM. In addition, we conducted a nested GLM analysis for each life-history trait using taxon and clones (nested within taxon) as two levels to assess the proportion of total variance for this life-history trait.

For intraspecific comparisons (among clones), two-way analyses of variance (ANOVAs) were performed, and one-by-one comparisons between taxa and clones were analyzed using Mann–Whitney U-tests.

Total variance of a trait and taxon (σ^2_T) was decomposed by a one-way ANOVA into clonal variances (σ^2_G). Broad-sense heritabilities (H^2) for each trait and taxon were calculated following the approach described in Lynch and Walsh (1998) for clonal organisms ($H^2 = \sigma^2_G/\sigma^2_T$, where σ^2_G = genetic variance, σ^2_T = total variance).

Experiment III: A comparison of taxon and clonal composition among replicates and treatments was conducted using a multiple comparison procedure (Holm–Sidak method; Sidak 1967; Holm 1979). For the analysis of the clonal composition, we excluded the taxon *D. cucullata*, since only a relatively small number of *D. cucullata* individuals were detected (0–11% of total).

Results

Daphnia clones of each taxon reared at high concentrations (1 mg C L^{-1}) of P-rich and P-limited green algae (*S.*

Table 1. Results of the General Linear Model (GLM) analysis of variation in six life-history traits among eight clones of *D. galeata*, *D. cucullata*, and *D. cucullata* × *galeata*. Taxon written in brackets indicates that clones were nested within taxa. Clone(Taxon) and Clone(Taxon)*Condition have been treated as random factors; all others were set as fixed factors. SGR, somatic growth rate; NJU, number of juveniles; DTR, days to reproduction; SAR, size at reproduction; JUS, size of juveniles; *r*, rate of population increase. Significant results are emphasized in bold letters ($p < 0.05$). Degrees of freedom (df) are nonintegers as a result of the special type of calculation (*see Materials and Methods section*).

		Sum of squares	df	Mean square	*F*	*p*
SGR	Taxon	0.015	4.994	0.004	1.735	0.268
	Food quality (P^+/P^-)	0.076	4.978	0.001	83.928	**<0.001**
	Clone(Taxon)	0.022	5.000	0.001	4.815	0.055
	Taxon*Condition	0.009	4.971	0.001	5.060	0.063
	Clone(Taxon)*Condition	0.005	124.000	0.001	1.407	0.226
	Error	0.080				
SAR	Taxon	1.729	5.189	0.026	33.492	**0.001**
	Food quality (P^+/P^-)	0.077	5.282	0.021	3.729	0.108
	Clone(Taxon)	0.137	5.000	0.022	1.250	0.406
	Taxon*Condition	0.067	5.237	0.021	1.607	0.286
	Clone(Taxon)*Condition	0.109	73.000	0.006	3.390	**0.008**
	Error	0.471				
JUS	Taxon	0.773	6.143	0.055	7.053	**0.026**
	Food quality (P^+/P^-)	0.024	16.666	0.013	1.861	0.191
	Clone(Taxon)	0.337	5.000	0.010	6.693	**0.029**
	Taxon*Condition	0.019	14.749	0.013	0.760	0.485
	Clone(Taxon)*Condition	0.050	247.000	0.020	0.501	0.775
	Error	4.966				
NJU	Taxon	28.728	5.793	1.401	10.256	**0.012**
	Food quality (P^+/P^-)	18.860	5.918	1.466	12.869	**0.012**
	Clone(Taxon)	7.077	5.000	1.490	0.950	0.522
	Taxon*Condition	13.900	5.751	1.470	4.729	0.061
	Clone(Taxon)*Condition	7.452	75.000	1.232	1.210	0.313
	Error	92.396				
DTR	Taxon	6.277	5.282	23.346	0.134	0.877
	Food quality (P^+/P^-)	160.816	5.426	18.838	8.537	**0.030**
	Clone(Taxon)	123.552	5.000	20.012	1.235	0.411
	Taxon*Condition	23.864	5.350	19.027	0.627	0.569
	Clone(Taxon)*Condition	100.058	75.000	7.832	2.555	**0.034**
	Error	587.436				
r	Taxon	0.025	5.000	0.001	12.219	**0.012**
	Food quality (P^+/P^-)	0.040	5.000	0.002	18.645	**0.008**
	Clone(Taxon)	0.005	5.000	0.002	0.477	0.782
	Taxon*Condition	0.008	5.000	0.002	1.808	0.257
	Clone(Taxon)*Condition	0.011	16.000	0.001	1.577	0.223
	Error	0.022				

obliquus) differed significantly in their life-history traits and showed a reduced fitness at low P conditions. Somatic growth rate of all taxa was on average reduced by 45% (from 0.11 ± 0.03 to 0.06 ± 0.03; mean ± standard error [SE], based on the average across all clones $n = 8$); the number of juveniles declined by 29% (from 3.42 ± 1.47 to 2.44 ± 1.16); and individuals required 20% more time to reproduce (from 10.56 ± 2.46 d to 13.28 ± 3.74 d).

Using a nested statistical ANOVA, no significant interaction for SGR of taxa with experimental conditions was detected (Table 1), but interspecific hybrids were significantly different to both parental taxa at P^+ conditions and to clones of *D. cucullata* at P^- conditions (Table 2). For SAR, no effect of food quality was detected, but clones differed in their reaction to the different qualities (Tables 1, 2; Fig. 1). In addition, JUS was not altered by P-limited algae. NJU was different for all three taxa, but not among clones within taxa. For the NJU, a significant food quality effect was detected, and clones of *D. galeata* differed significantly in the number of juveniles at P-sufficient conditions. Furthermore, NJU varied much more at P^+ than at P^- conditions (Fig. 2). In addition, the time it took the taxa to reproduce was significantly longer when they were fed P-limited algae, and in addition, an interaction of clonal lineages with food quality was detected (Table 1). The taxa differed in their rate of population increase, which also was significantly negatively affected by P-limited algae (Fig. 2). In addition, the variation that emerges from the different analyses is mostly attributable to interspecific variation (more than 90%; Table 3); intraspecific (clonal) variation was low, except for the case of SGR (32% intraspecific variation of total).

In order to reveal the sensitivity of clones and taxa to the different experimental treatments we calculated the relative growth rate, the growth rate at low-P relative to high-P food (RGR). The clones of *D. galeata* showed the lowest

Table 2. Results of the post hoc comparison tests, derived from a General Linear Model (GLM) analysis of three life-history traits among eight clones of *D. galeata* (gal), *D. cucullata* (cuc), and *D. cucullata* × *galeata* (cg). SGR, somatic growth rate; SAR, size at reproduction; NJU, number of juveniles. Significant results are emphasized in bold letters ($p < 0.05$).

SGR	cg P−	cg P+	cuc P−	cuc P+	gal P−	gal P+
cg P−						
cg P+	**<0.001**					
cuc P−	0.222	**<0.001**				
cuc P+	0.125	**<0.001**	**0.011**			
gal P−	**0.003**	**<0.001**	0.133	**<0.001**		
gal P+	**<0.001**	**0.025**	**<0.001**	**0.001**	**<0.001**	
SAR	cg P−	cg P+	cuc P−	cuc P+	gal P−	gal P+
cg P−						
cg P+	**<0.001**					
cuc P−	**<0.001**	**<0.001**				
cuc P+	**<0.001**	**<0.001**	0.860			
gal P−	**<0.001**	**<0.001**	**<0.001**	**<0.001**		
gal P+	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.005**	
NJU	cg P−	cg P+	cuc P−	cuc P+	gal P−	gal P+
cg P−						
cg P+	0.925					
cuc P−	0.875	0.375				
cuc P+	1.000	0.960	0.874			
gal P−	0.976	1.000	0.520	0.988		
gal P+	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	

values for RGR (0.44 ± 0.05), the interspecific hybrid values were intermediate (0.55 ± 0.07), and clones of *D. cucullata* showed the highest values for RGR (0.77 ± 0.25). The parental species differed significantly in their RGR (Mann–Whitney *U*-test: $p = 0.0133$), indicating that parentals exhibit different levels of susceptibility to variation in food quality.

The rate of population increase (r) differed among taxa and among different treatments (Table 1), and all taxa showed a reduced level of population increase at P− conditions (Fig. 2). *D. galeata* showed the highest r at P-rich conditions, but at P-limited conditions, clones of the hybrid *D. cucullata* × *galeata* exhibited the highest values (Fig. 2), but differences were not significant (results not shown). Interestingly, one hybrid clone (X3) revealed a very shallow reaction norm compared to the other clones (i.e., no significant difference between rates of population increase at P-limited and P-rich conditions) (Fig. 2A).

Daphnia taxa showed marked differences in broad-sense heritabilities of various life- history traits (Table 4). For example, *D. galeata* showed the highest heritability values in SGR, whereas interspecific hybrids showed the highest heritability in NJU, DTR, JUS, and SAR. In general, *D. cucullata* showed for all traits the lowest broad-sense heritabilities compared to the other two taxa (Table 4).

Densities of individuals in multiclonal vessels of experiment III varied strongly after 6 weeks between both conditions; we found, on average, 1,429 ± 77.8 individuals at P+ conditions and 245 ± 96.1 individuals at P− conditions. Differences between both treatments were significant ($F = 15.073$, $p = 0.018$). Discrepancies between the individuals subjected to DNA analysis and the number of individuals given in Table 5 can be explained by a nonsufficient yield of DNA for genetic analysis. Relative abundances of taxa did not differ significantly among treatments (ANOVA, Holm–Sidak method: $F = 1.617$, $p = 0.239$). However, one replicate (L2) of the high-quality treatment contained much lower densities of animals (L1 = 1,484 individuals, L3 = 1,374 individuals, compared to L2 = 722 individuals). Based on this large deviation, we considered this value to be an outlier and calculated a comparison between the relative abundances without replicate L2. The reduced data set revealed a significant difference in taxon composition between treatments (ANOVA, Holm–Sidak method: $F = 4.394$, $p = 0.037$). In general, frequencies of *D. galeata* were higher at P-rich conditions than were those of interspecific hybrids (Fig. 3A). In addition, no individual of *D. cucullata* was detected at P+ conditions (Table 4). In contrast, interspecific hybrids dominated at P− conditions (Table 5; Fig. 3A). *D. galeata* and interspecific hybrid clones occurred in similar proportions at P+ conditions (Fig. 3B), except for clone X3, which was not found in any replicate. In contrast, the interspecific hybrid clone X1 was clearly dominant in all three replicates at P− conditions, including the extreme case of replicate R3, in which only clone X1 was found. Clonal frequencies differed significantly among treatments, and an interaction of clonal composition with treatment was detected (Holm–Sidak method: $F = 3.654$, $p = 0.016$).

Discussion

Our study shows that variation in food quality causes inter- and intraspecific variation in fitness-related life-history traits of *Daphnia* species and their interspecific hybrids. Clones and taxa showed a differential response to an exogenous factor, which is bound to determine the

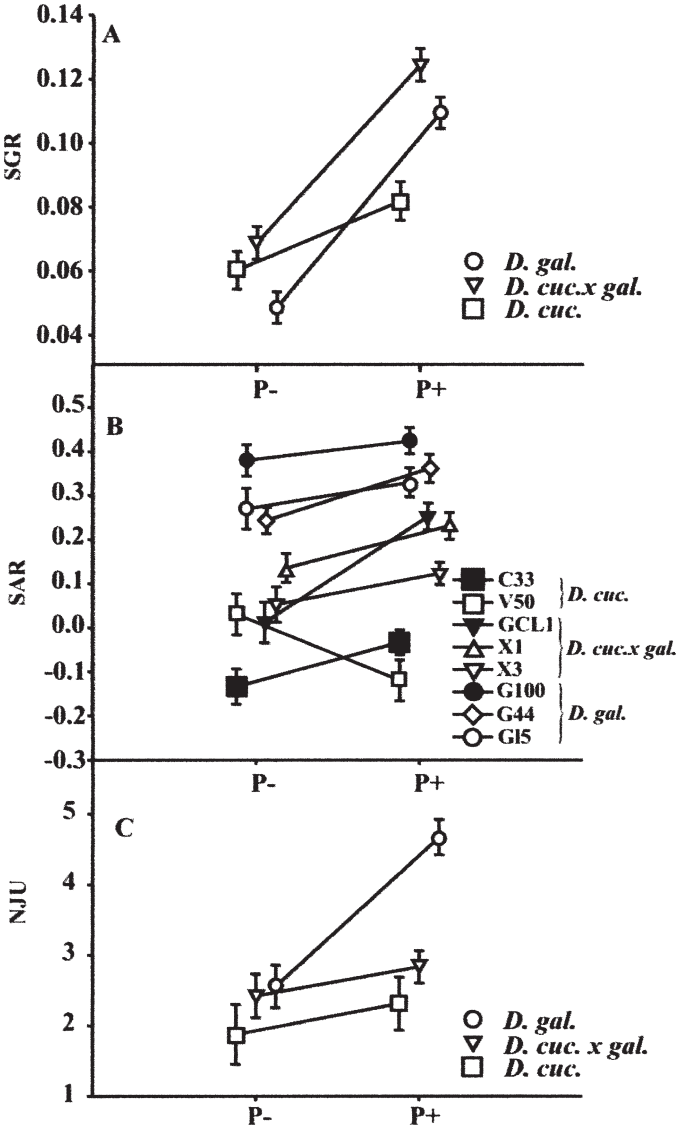

Fig. 1. Reaction norms of *D. galeata, D. cucullata*, and their interspecific hybrids *D. cucullata* × *galeata*, for three life-history traits. (A) Somatic growth rates (SGR) under two food quality conditions (P^+ and P^-), (B) size at first reproduction (SAR) presented on log-scale, and (C) number of juveniles of first clutch (NJU); error bars represent standard errors.

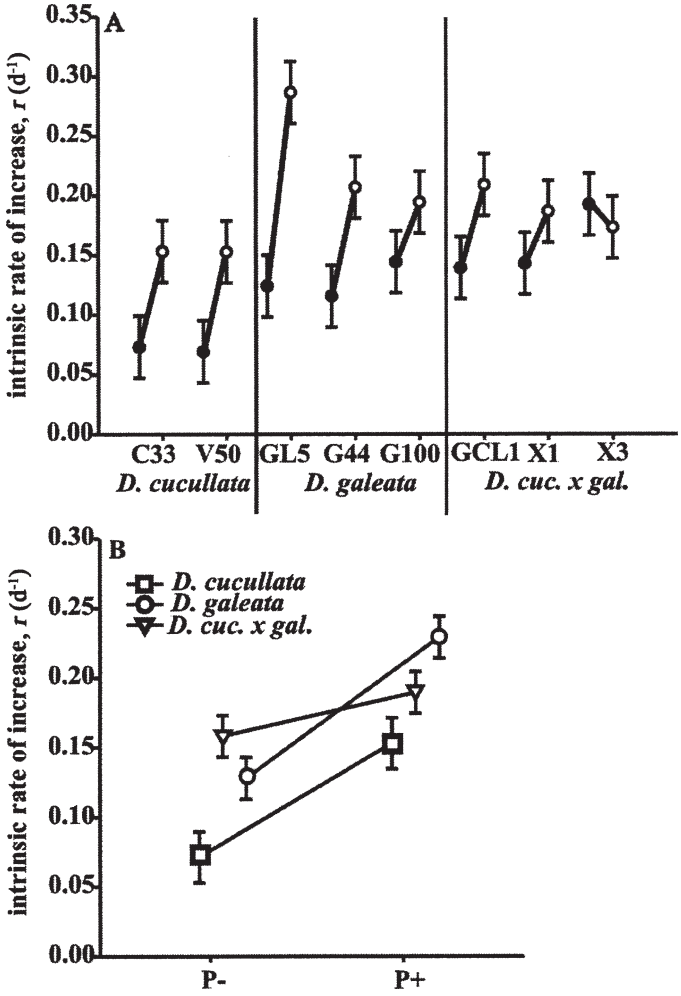

Fig. 2. Rates of population increase (r) for (A) each clonal lineage of *D. galeata*, *D. cucullata,* and their interspecific hybrids. Open circles represent P-rich conditions; closed circles represent P-limited conditions. Black lines are connecting the means of each clonal lineage under two food quality conditions (P^+ and P^-). (B) Reaction norms of the rates of population increase per taxon at both conditions. *D. cuc.* × *gal.* = interspecific hybrids. Error bars represent standard errors.

fitness of evolutionary lineages. All life-history traits (except juvenile size and size at reproduction) of *D. galeata, D. cucullata,* and their interspecific hybrids were negatively affected by P limitation (Table 1; Fig. 1). Several studies had already demonstrated that a chemical-unbalanced food source (such as P-limited algae) reduces fitness in *Daphnia* (e.g., Repka 1996; Weers and Gulati 1997; Repka et al. 1999*a*). At P-sufficient conditions, clones of *D. galeata* showed the highest values at several life-history traits (e.g., number of juveniles, which was significantly higher than in the other taxa). However, interspecific hybrids responded differentially, showing highest values in SGR at both food quality conditions (Fig. 1; Table 2), and they also dominated in our multiclone experiment at P^- conditions (Fig. 3). As was the case in some other studies (Grant and Grant 1996; Schluter 1996; Declerck and De Meester 2003), we found that hybrids are superior to their parental species in several life-history traits under certain environmental conditions.

In general, we observed a considerable amount of clonal variation for *D. galeata* and *D. cucullata* × *galeata*, but interactions between clones and experimental conditions were hardly found. *D. cucullata* clones differed in none of the life-history traits, whereas *D. galeata* and hybrid clones varied in 60% of their traits, but only two interactions with food quality were detected (results not shown). This pattern is in agreement with the results of previous studies, which showed that variation between *D. galeata* and interspecific hybrid clones is smaller than the variation between taxa (e.g., Weider 1993). Our results indicate that life-history variation under various food quality conditions is mainly

Table 3. Partitioning of variation in life-history traits between intraspecific (clone within taxon) and interspecific (taxon) components. Significant results are emphasized in bold letters ($p < 0.05$). SGR, somatic growth rate; NJU, number of juveniles; DTR, days to reproduction; JUS, size of juveniles; SAR, size at first reproduction; *r*, rate of population increase; MS, mean square.

		df	MS	Proportion of variance	*F*	*p*
SGR	Taxon	2	0.008	0.635	5.646	**0.004**
	Clone(taxon)	5	0.004	0.365	3.243	**0.009**
SAR	Taxon	2	0.866	0.968	92.782	**<0.000**
	Clone(taxon)	5	0.028	0.032	3.045	**0.014**
JUS	Taxon	2	0.697	0.909	35.039	**<0.000**
	Clone(taxon)	5	0.070	0.091	3.518	**0.004**
NJU	Taxon	2	19.690	0.917	12.099	**<0.000**
	Clone(taxon)	5	1.775	0.083	1.091	0.372
DTR	Taxon	2	2.759	0.147	0.259	0.773
	Clone(taxon)	5	15.994	0.853	1.500	0.199
r	Taxon	2	0.012	0.924	3.752	**0.038**
	Clone(taxon)	5	0.001	0.076	0.307	0.904

based on the differentiation between taxa rather than on differentiation between clones, although the selection of clones was not representative for natural variation of field populations. In this study, we investigated only one family (all interspecific hybrids that originate from the same paternal and maternal clone), and, hence, we potentially underestimated clonal variation among hybrids and thus overestimated between-species effects. Thus, field hybrids might exhibit a much larger variance in response to food quality variation than was observed in our experiments. However, the clonal variation of one parental species (*D. galeata*) was comparable to the variation among interspecific hybrids (Table 3); thus, hybrid variation is at least in the range of clonal variation within species. Hence, we conclude that the consistent differential response of *Daphnia* species and hybrids to variation in food quality may facilitate coexistence and niche differentiation.

Our results from single-clone life-history experiments were supported by a multiclone experiment: *D. galeata* showed the highest rate of population increase and the highest relative frequencies during the multispecies experiment under P-sufficient conditions. In contrast, *D. cucullata* × *galeata* dominated in all three replicates at P-limited conditions. *D. cucullata* was only detected at P-limited conditions, indicating a higher ability to compete with *D. galeata* and the interspecific hybrids at those conditions. At the clonal level we found a more complex pattern: the single-clone experiments did not exactly predict the most successful clone of our multiclone experiments; however, those clones that had a relatively high population increase were among the most frequent clones in the multiclone experiment. Based on the values for somatic growth rate and *r*, we had expected clone X3 to represent the most frequent clone at P^- conditions; however, clone X1 dominated in all three replicates. The differences between our expectations and what we observed in the population experiment might be the result of several processes: clonal interactions, stochastic effects, or a differential mortality rate during experimental conditions. The inoculation or the build-up of the X3 populations might have been different among replicates, which caused large variation of clonal abundances among vessels at P^+ conditions (outlier L2; Fig. 2). We excluded a feedback between grazing and nutrient recycling from the grazer onto its resource to be able to compare the single and multiclonal experiments. These feedback mechanisms are probably fairly important in nature, as they may alter the stoichiometry of the grazer and the resources (Andersen 1997) and, hence, should be included in future experiments.

Phenotypic plasticity has often been assumed to limit natural selection by buffering the effects of selection, but recent studies showed that phenotypic plasticity represents a fundamental component of evolutionary change (Behera and Nanjundiah 2004). Based on the considerable phenotypic plasticity of *D. galeata* and the *D. cucullata* × *galeata* hybrids and the environmental variability of food quality, we suspect a large potential for the establishment of new evolutionary trajectories. To understand how different phenotypes perform under different environmental conditions, we need to evaluate whether the phenotypic differences among populations and species represent the outcome of evolution by natural selection. Our results indicate that interspecific hybrids showed higher heritability estimates for most of their life-history traits than did parental taxa (except for the case of SGR: *see Table 2*),

Table 4. Broad-sense heritabilities (H^2) for the three different taxa *D. galeata*, *D. cucullata* × *galeata*, and *D. cucullata*, for five life-history traits. SGR, somatic growth rate; NJU, number of juveniles; DTR, days to reproduction; JUS, size of juveniles; SAR, size at first reproduction.

Trait	*D. galeata*	*D. cucullata* × *galeata*	*D. cucullata*
SGR	0.163	0.078	0.028
SAR	0.128	0.264	0.011
JUS	0.190	0.201	0.007
NJU	0.057	0.101	0.034
DTR	0.083	0.174	0.040

Table 5. Results of RFLP and microsatellite analysis of the multiclone experiment (experiment III). ΣT, number of individuals assigned to taxon level; ΣC, number of individuals assigned to each clone; $\Sigma\Sigma$C, total number of assigned individuals per replicate (L1, L2, L3 for P-rich conditions and R1, R2, R3 for P-limited conditions); $\Sigma\Sigma$T, total number of investigated individuals per replicate.

		D. cucullata	*D. galeata*					*D. cucullata* × *galeata*						
Replicate		ΣT	100	G44	GL5	ΣC	ΣT	GCL1	X1	X3	ΣC	ΣT	$\Sigma\Sigma$C	$\Sigma\Sigma$T
P^+	L1	0	11	3	5	19	20	2	3	0	5	13	24	33
	L2	0	2	0	1	3	3	8	8	0	16	27	19	30
	L3	0	4	3	5	12	14	0	6	0	6	10	18	24
													Σ	87
P^-	R1	0	1	0	2	3	7	3	12	1	16	23	19	30
	R2	4	3	0	8	11	12	5	12	0	17	18	32	34
	R3	3	0	0	0	0	0	0	21	0	21	23	24	26
													Σ	90

which may allow them to adapt more quickly to changes in food quality. Increased levels of heritability provide a greater scope for directional natural selection and thus adaptation (Grant and Grant 1994), resulting in fast adaptation of novel genotypes and phenotypes that may serve as the starting point of a new evolutionary trajectory. Increased heritability in interspecific hybrids is caused by greater genetic variation among hybrids due to additive effects (Graham 1992), new associations between nuclear and mitochondrial genomes (Ferris et al. 1983), and mutation (Woodruff 1989). However, since our experimental design did not allow for the comparison of heritability among hybrids of different families, heritability levels might be elevated as a result of within-family comparisons. Further studies on the quantitative variation in life-history traits are required to determine the phenotypic consequences of interspecific hybridization and the resulting potential for evolutionary change.

Several theories have been developed to explain hybrid maintenance based on tension zone or cline and ecotone models; a derivative, the THS model, by Spaak and Hoekstra (1995), assumes temporally fluctuating levels of exogenous selection among taxa. Since the THS represents a hypothesis rather than an explicit mathematical model, we used our data to qualitatively test assumptions of the THS model. First, we found that fitness in parental species and hybrids varies with environmental conditions (i.e., food quality) and, secondly, that interspecific hybrids show higher somatic growth rate at low food quality conditions. Therefore, we add food quality to the list of parameters, such as predation levels and food quantity, that contribute to the temporary superiority of interspecific *Daphnia* hybrids. Although the THS model seems closely related to models on species coexistence and maintenance of diversity (e.g., Chesson 2000), hybrid superiority, as described by the THS model, is solely based on fitness differences among taxa during the asexual phase of reproduction. This is largely caused by the focus of most studies on this phase: nearly all empirical data on fitness variation in *Daphnia* rely on measurements of the rate of population increase during the asexual phase. Further empirical studies of hybrid complexes should include the sexual phase of reproduction, as well as diapause and the 'storage effect,' thus providing data to rigorously test hypotheses on species coexistence.

Which exogenous factor—fish, invertebrate predators, food quality, or food quantity—represents the most important factor responsible for hybrid maintenance in *Daphnia* remains an open question for further research. Most likely, however, food quality has an important impact on population dynamics of *Daphnia* species and their intraspecific hybrids, in particular in syntopic populations. Our data indicate that food quality differences during a season (Kreeger et al. 1997) or between lakes might facilitate species and hybrid differentiation and that food quality certainly could play a role in determining the fate of hybrid lineages. Hybrids occur in high frequencies during certain periods of the growing season (Wolf 1987; Weider and Stich 1992), showing that hybrids show different fitness optima than their parental taxa. Wolf (1987) showed that the relative abundance of hybrids increased after the midsummer decline, when food quality (measured as C : P ratio) is usually low (Kreeger et al. 1997). In addition, several studies document large variation in food quality within and among seasons (Lampert 1988; Müller-Navarra and Lampert 1996). Thus, the functional link between differential ecological demands of taxa and large temporal variation in food quality might explain the co-occurrence of hybrids with their parental taxa. This might explain the observation made by Schwenk (1997) and Hessen et al. (1995), who observed associations of different *Daphnia* taxa with different environmental factors in lakes. However, further field studies that monitor food quality, food quantity, and taxon frequencies are necessary to test the hypotheses derived from our experimental study. In addition, this approach should be supplemented by multiclone life- history studies considering more than one potential factor in explaining hybrid maintenance.

In conclusion, we have shown that different taxa from the *Daphnia galeata hyalina* complex react differently to changes in food quality (measured as P content) conditions. This implies that elemental stoichiometry of the food is not only expected to influence the performance of individual species or hybrid lineages but also the composition of zooplankton communities in aquatic environments.

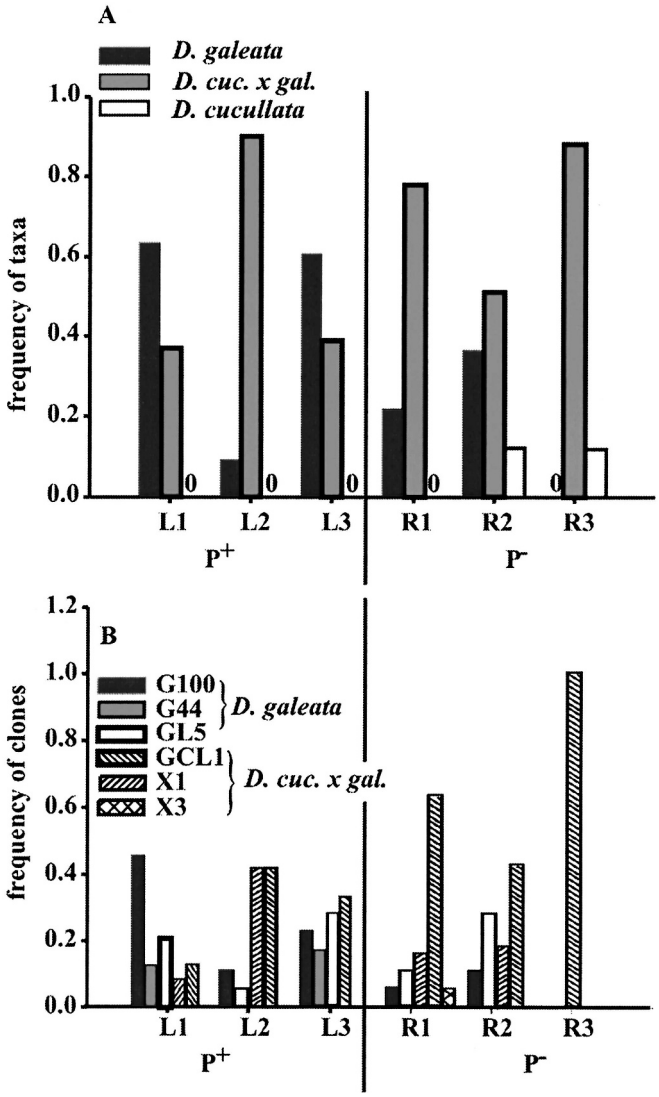

Fig. 3. (A) Relative abundance of *Daphnia* taxa per replicate (L1, L2, and L2 for P^+ conditions; R1, R2, and R3 for P^- conditions) of the multiclone experiment (experiment III). Zero numbers indicate frequencies of corresponding taxa not identified within the different replicates. (B) Relative abundances of multilocus genotypes per replicate (see Table 5). *D. cuc.* × *gal.* = interspecific hybrids. Clones of *D. cucullata* were excluded from microsatellite analysis because they occurred only in very low frequencies.

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