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Coexisting overwintering strategies in *Daphnia pulex*: A test of genetic differences and growth responses

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Abstract

We tested whether clones of *Daphnia pulex* in a mesotrophic lake differ with respect to overwintering strategies: either surviving the winter as parthenogenetic females with reduced metabolism in pelagic waters, or as sexually produced dormant embryos in ephippia. During winter 2007, we established two groups of clonal laboratory lines from females collected in the lake and neonates hatched from ephippia. Genetic relatedness and differentiation of the two clonal groups were studied with microsatellite markers, and compared to the genetic structure of a field reference population. Flow-through experiments designed to measure the specific juvenile growth rate (g_j) of individual clones (a good proxy of fitness) at low and at high food concentrations show that differences in g_j indicate physiological adaptation of the different starting populations to improving food conditions in spring. Although g_j differed significantly among individual clones, both below and above the incipient limiting food concentration, we found no significant differences between the clonal groups. There was considerable clonal variation for reaction norms of g_j, but neither the group means of the slopes of reaction norms nor of the threshold food concentrations for growth differed significantly. The lack of differences in group means so far does not support the hypothesis that clones are specialized for either parthenogenetic or for dormant overwintering. It rather suggests a mixed strategy of individual females producing resting stages as insurance and then trying to survive the winter in an active state.

Winter conditions in temperate lakes are unfavorable for herbivorous zooplankton. Due to vertical mixing of the water column or ice cover, light availability for phytoplankton is poor and primary production is low (i.e., food conditions for herbivores deteriorate). Low water temperatures near 4°C slow down metabolism, growth, and development, and offspring production is reduced or lacking. Under ice, oxygen conditions can deteriorate, particularly in shallow lakes and ponds. This will negatively affect survival rates of zooplankton, despite reduced respiration rates at low temperatures. On the other hand, reduced metabolism at low temperatures enhances longevity at limiting food conditions. Also, due to the sluggish behavior of planktivores, fish predation is considerably reduced during winter. Low predation-driven mortality and enhanced physiological longevity can result in survival of individuals through the winter despite a complete absence of reproduction. However, depending on the varying winter conditions in different waterbodies and the predictability of catastrophic events, planktonic organisms must have evolved different overwintering strategies.

Daphnia play a major role as grazers in food webs of many lakes and ponds (Haney 1973), but only during the warm season. Due to their dual reproduction mode, *Daphnia* can use different overwintering strategies in temperate waterbodies: Parthenogenetic females can survive with reduced metabolism in pelagic waters. Sexually produced dormant 'resting stages' can, enclosed in a durable ephippium on the sediments, remain dormant in an early embryonic stage until conditions improve in spring.

The occurrence of the two strategies varies between waterbodies. Only the ephippial strategy can persist in arctic ponds that freeze to the bottom (Stross and Kangas 1969) or in waterbodies that go regularly anoxic during ice cover. Dormancy seems to be the most successful strategy in alpine ponds that are inhabited by winter-active predators or become anoxic in some years, although even there, part of the population survives in active state (Larsson and Wathne 2006). Larger lakes can harbor both strategies simultaneously. Slusarczyk (2009) describes two coexisting forms of *Daphnia pulicaria* in a fish-free, ultra-oligotrophic high mountain lake that represent extremes. One of them overwinters exclusively in ephippia after two summer generations, while the other one lives for 13–16 months, reproduces in the second year after overwintering as parthenogenetic females, and produces only few ephippia at the end of its first year.

The prevalence of the two strategies will be dependent on advantages and disadvantages under the given conditions. Survival as parthenogenetic females has an advantage if the probability of catastrophic events (e.g., oxygen depletion) is low. Although in temperate lakes *Daphnia* abundances decrease very much during winter (Rellstab and Spaak 2009), surviving females are large and ready to start reproduction as soon as conditions improve in spring. Their offspring are born into the algal spring bloom (Sommer et al. 1986). In contrast, hatching of neonates from diapause eggs in response to an environmental cue needs time, and the hatchlings must first grow and mature, before they can contribute offspring to the *Daphnia* population, which gives the offspring of overwintering

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females a clear advantage (Hanazato 1989; George and Hewitt 1999). Also, only a small proportion of ephippia hatch in a deep lake (Caceres and Tessier 2003; Jankowski and Straile 2004). Both factors bear considerable costs. However, a mixed strategy is possible under more relaxed conditions because females can continue to reproduce parthenogenetically after shedding an ephippium. Hence, an individual female may contribute to the ephippia bank but it may try to overwinter anyway.

Overwintering strategies are not only important for the fitness of genotypes; they also have implications for ecosystem functions. Hanazato (1989) showed experimentally that reducing the density of overwintering parthenogenetic females in favor of hatching from ephippia has a striking effect on species composition of zooplankton and phytoplankton, and grazing in spring. This can also provide an indirect mechanism affecting spring plankton composition by climate warming (Chen and Folt 1996).

Our goal was to investigate whether *Daphnia* clones within a lake are specifically adapted to one of the overwintering strategies and whether selection leads to differences in life-history between clones that overwinter parthenogenetically or as sexually produced dormant stages in ephippia. The overarching hypothesis to test is: Different life-cycle strategies coexist in a lake as some clones are selected for long-term parthenogenetic existence, while others are selected for an annual cycle with sexual reproduction. The alternative hypothesis would be: Clones are not selected for specific life-history strategies. Each clone alternates between parthenogenetic and sexual reproduction (i.e., contributes to the ephippia bank and tries to survive the winter parthenogenetically).

We formulated some specific hypotheses to test the two alternatives comparing clones of *Daphnia pulex* staying in the water column during winter (W) or resting in ephippia (E): (1) Individual females within one or both groups are genetically more closely related than between the two groups; (2) In order to achieve better survival, W clones show higher growth efficiencies at low food concentrations and lower threshold concentrations for growth; (3) Because sexual reproduction and ephippia production bear physiological and demographic costs, E clones are selected for higher growth rates at favorable food conditions (in spring), and they invest more into parthenogenetic reproduction to compensate part of the costs. Support for any of the specific hypotheses must be interpreted as support for the overarching hypothesis of differentiated strategies.

Methods

Origin of the clones—We studied *Daphnia pulex* populations of Myravann, a small (area 61,500 m^2, mean depth 7.6 m, max. depth 18 m) mesotrophic lake located in the southern outskirts of the town of Bergen, Norway (Jensen et al. 2001). The lake freezes regularly in winter but ice cover is variable. Oxygen depletion under ice occurs near the bottom but not in the upper layers. Earlier studies of the plankton in Myravann have shown that larvae of phantom midges (*Chaoborus*) were abundant, and that coexisting with the smaller *D. longispina* a population of large *D. pulex* persisted in the lake due to very low planktivore fish predation (Knudsen et al. 2006). Both overwintering strategies were found; hence, the lake was well-suited to test our hypotheses, and the results may have general relevance.

Clones of *D. pulex* were isolated in the winter of 2006–2007. Overwintering adult females were collected in December by vertical tows with a plankton net near the deepest point and transferred to the laboratory. Twenty individuals were kept in glass jars in membrane-filtered Myravann water at 20°C with *Scenedesmus obliquus* added as food. After populations had been established, we selected 12 clones at random for further maintenance in 1-liter glass jars. These 'winter' clones were labeled 'W'. Eleven of them survived until the end of the experiments after 2 yr.

Ephippia were collected in early March from lake sediments. Because the Myravann sediments are very soft, coring proved to be unsuitable; hence, we sampled surficial sediments with a plankton net (i.e., the ephippia were young). In the laboratory, 32 ephippia were opened with fine needles under a dissecting microscope. The dormant eggs were transferred into small petri dishes and incubated at 20°C under continuous fluorescent light. The majority (80%) hatched within 5 d. Hatchlings were transferred individually to 50-mL glass jars and treated like the parthenogenetic females. If both eggs from a single ephippium hatched, only one of them was kept. These 'ephippial' clones were labeled 'E'. Ten of the 12 selected clones survived until the end of the experiments.

Genetic analysis—Genotyping of the clones was performed to test Hypothesis 1. Deoxyribonucleic acid (DNA) was extracted using the Qiagen DNeasy blood and tissue kit following the manufacturer's recommendations. Twelve microsatellite loci were used for genotyping: Dp91, Dp122, Dp321, Dp463, Dp512, Dp513, Dp514, Dp520, Dp521, Dp523, Dp525alt (Colbourne et al. 2005), and Dp655 (Cristescu et al. 2006). Fragment amplification was done in a 10-μL polymerase chain reaction (PCR) reaction volume containing 1× EuroTaq buffer, 1.5 mmol L^{-1} $MgCl_2$, 5% dimethyl sulfoxide (DMSO), 0.025U EuroTaq, 0.05 mmol L^{-1} forward primer (with M13 tail), 0.2 mmol L^{-1} reverse primer, 0.2 mmol L^{-1} M13 primer with IRD800 fluorescent label. PCR cycling consisted of 5 min initial denaturation at 94°C, 40 cycles of 30 s at 94°C, 30 s at primer specific annealing temperature (Dp91: 48°C, Dp122: 42°C, Dp321: 48°C, Dp463: 44°C, Dp512: 45°C, Dp513: 50°C, Dp514: 50°C, Dp520: 40°C, Dp521: 55°C, Dp523: 50°C, Dp525alt: 50°C, Dp655: 45°C) and 30 s elongation at 72°C, and 5 min at 72°C to end the reactions. Fragment size was determined with a Licor4300 sequencer using a 6.5% denaturing polyacrylamid gel matrix (KB+ Licor). Individuals were genotyped using the Saga2 software (Licor). As a reference sample for allelic frequencies we genotyped 102 additional individuals from the water column of Lake Myravann. These individuals were sampled as adults in September 2007 (n = 38) and in February 2008 (n = 64). The sampling dates were about 10 months after the establishment of the experimental

clones, and we expected that the pooled sample contained genotypes hatched during spring 2007, as well as those that survived the following winter.

Food suspension—Green algae, *Scenedesmus obliquus*, proven to be good food for *Daphnia* in many experiments, were grown in chemostats (Ebert 2005) in an attempt to maintain constant food quality over extended periods of time. Algae were harvested by centrifugation. Concentrated algal stocks were diluted to the final concentration using a preestablished calibration curve of light (800 nm) extinction in a photometer vs. algal dry weight. Dry weight was converted to carbon using 50% carbon content of algal dry weight (W. Lampert unpubl.).

Growth experiments—A flow-through system (Lampert et al. 1988) was established to grow *Daphnia* of all clones to maturity. It consisted of 33 individual glass tubes (250 mL) sealed with a 100-μm gauze at the bottom, and kept in a temperature-controlled (20°C) water bath. Food suspension (filtered lake water plus the appropriate amount of *Scenedesmus* was pumped through the vessels at a rate of 1.5 L d^{-1}. Food suspension was kept in stirred 5-liter glass reservoirs each serving three flow-through vessels. The suspension was prepared fresh every day. Each flow-through vessel contained seven females of an individual clone and each clone was represented in three vessels. Clones were distributed at random in the water bath and staggered in time, depending on the availability of juveniles, until three measurements per clone had been completed. Hence, the individual vessels were considered replicates. For logistic reasons, five separate experiments were carried out at long intervals, three (in Jun 07, Jun 08, and Sep 08) at high food level (1.0 mg C L^{-1}) to test Hypothesis 3, and two (in Mar and Sep 09) at low food levels (0.12 and 0.15 mg C L^{-1}, respectively) to test Hypothesis 2. To make sure that the long intervals did not affect the experimental results (due to long-term changes in the clones or food conditions) we performed the first three experiments under identical conditions. Only when an analysis of variance (ANOVA; cf. Table 1) showed that the factor 'experiment' had no significant effect, we ran one experiment each at the low concentrations. The incipient limiting concentration (ILL) for *Scenedesmus* is $\sim$ 0.4 mg C L^{-1} (Lampert 1987); hence, the high food level was far above the ILL and the low food levels far below.

The experiments started with 2-d-old juveniles. Nine females of each clone carrying at least the third clutch of eggs were equally distributed to 300-mL glass beakers with fresh food suspension. If a beaker contained neonates the next morning, all females were transferred to a fresh vessel while the neonates remained in the vessel for about 48 h to reduce neonate mortality and make transfer to the flow-through vessels easier. Depending on the number of 2-d-old juveniles that were available, they were randomly distributed into one or two small glass jars containing seven individuals each (treatments), and one jar containing the remaining 7–20 individuals used as controls. The treatment individuals were transferred into flow-through vessels, while the controls were killed with a drop of formaldehyde, washed in distilled water, counted, loaded as a group into small, preweighed aluminum containers, dried, and weighed to the nearest 0.1 μg on an electronic microbalance. This resulted in the initial, individual dry weight for later calculations.

Table 1. Three-way ANOVA testing for the effects of 'group' (W and E clones), clone (nested in group), and experiment (expt.), and their interactions on specific juvenile growth rate (g_j) at high food concentration. Asterisks indicate significant results at α = 0.05. MS = mean square.

Effect	df	MS	*F*	*p*
Group	1	1.454×10^{-6}	0.00	0.990
Clone (group)	17	9.649×10^{-3}	5.88	<0.001*
Expt.	2	3.844×10^{-3}	0.82	0.449
Group×expt.	2	6.857×10^{-4}	0.15	0.864
Clone (group)×expt.	34	4.686×10^{-3}	2.86	<0.001*
Error	132	1.640×10^{-3}	—	—

Both, during the preparatory phase and in the flow-through system, *Daphnia* were kept under continuous light, which effectively prevented the production of males. Females in the flow-through vessel were harvested when they deposited their first clutch into the brood pouch. This required 4 (occasionally 5) d at high food and 6 (5) d at the low food levels. Mortality in the flow-through system was negligible (i.e., seven mature females were usually harvested from each replicate). They were immediately killed, and checked under a dissecting microscope. After washing in distilled water, all females from a single vessel were pooled to determine their average dry weight (including eggs) as described for initial weights. The average final dry weight per individual was calculated for each replicate.

Mean initial and final dry weights per individual provided the basis for the calculation of the specific juvenile growth rate (g_j), a good proxy for the instantaneous population growth rate (r), and hence, a measure of fitness (Lampert and Trubetskova 1996) if egg mortality can be neglected (Trubetskova and Lampert 2002). It was calculated according to

$$g_j = (\ln W_t - \ln W_0)/t \ (d^{-1}) \quad (1)$$

where W_0 and W_t are initial and final individual dry weights, respectively, and t is the time in days spent in the flow-through vessel (Lampert and Trubetskova 1996). Because each vessel resulted in one independent measurement of g_j, we had a final number of three replicates per clone and experiment.

For the two low food concentrations, reaction norms for all clones were plotted using clonal means (n = 3) of g_j at both concentrations. Slopes of all reaction norms were calculated, and linear equations of all reaction norms were solved for g_j = 0 to estimate the 'threshold food concentration for growth' (Lampert and Schober 1980) of the individual clones.

Statistics—We used separate three-way ANOVAs to test for differences in g_j at the high and at the low food levels. Because there was only one food concentration but three

separate experiments at high food, group (W or E), and experiment (1–3) were considered fixed factors and clone was a factor nested in group. At the low food levels, we used food as a fixed factor instead of experiment, because there were two food concentrations but only one experiment each. Identical labels were assigned to the clones that could not be discriminated by microsatellite analysis. Group means for slopes of reaction norms and threshold food concentrations for growth were compared by *t*-test. These analyses were run with NCSS 2000 (Hines 2000).

To test the power of the microsatellite loci to distinguish between different *Daphnia* clones we calculated the probability of identity using the program Gimlet version 1.3.3 (Valiere 2002). Genetic distances between clones (Cavalli–Sforza and Edwards chord distance) were estimated using the program microsatellite analyzer version 4.05 (Dieringer and Schlötterer 2003). Distances were used to construct a genotype network using the program Splitstree v.4 (Huson 1998). To test for population structuring based on individual genotypes we performed a principal component analysis (PCA), using the program PCA-GEN (Goudet 1999) and also a Bayesian clustering approach using BAPS 4 (Felsenstein 1989).

As a measure of physiological similarity between clones, we calculated the differences of g_j for high food and both low food concentrations, as well as for the slope of the (low food) reaction norms and the food threshold for all pairs of clones. The resulting matrices were compared with the matrix of genetic similarity by a Mantel test (Liedloff 1999). The *p* levels were adjusted using the Bonferroni correction for multiple testing.

Results

Genetic relationships—The microsatellites used in this study showed a maximum of four alleles and six different genotypes in a single locus. Resolution was enhanced by the usage of 12 different loci, which led to a combined probability of identity of $2.6e^{-6}$. Interestingly, using only the four most informative loci (Dp512, Dp523, Dp463, Dp122) was sufficient to discriminate all genotypes of the E group. As expected due to their sexual origin, all 10 clones in this group differed. On the contrary, among the 11 clones of the W group we found two pairs of clones that did not separate even with 12 polymorphic loci (i.e., we had to consider the pairs as identical clones). Fifty-two different clones were found in the additional 102 individuals of the reference sample. None of the W or E genotypes were recollected in this field sample 10 months after the establishment of the laboratory clones.

Neither the E nor the W clones showed any pattern of genetic relationship within or among groups. BAPS found no evidence of a population substructure, neither separating the E- from the W-clones nor either of the two groups from the reference sample (data not shown). The PCA analyses found no significant segregation of genotypes (Fig. 1); neither did the genotype network reveal any patterns of relatedness in the data set (Fig. 2). Genetic distances (means ± 1 SD), however, were larger within E clones (0.37 ± 0.098) than within W clones (all individuals: 0.31 ± 0.123; only individual genotypes: 0.32 ± 0.111). Genetic distances between E and W clones were about as high as genetic distances within the E clones (0.38 ± 0.096) and as genetic distances of the E and W clones compared to the reference sample (0.38 ± 0.117; Fig. 3). The slightly lower genetic distance within the W clones can also be seen in the shorter branches of the genotype network (Fig. 2).

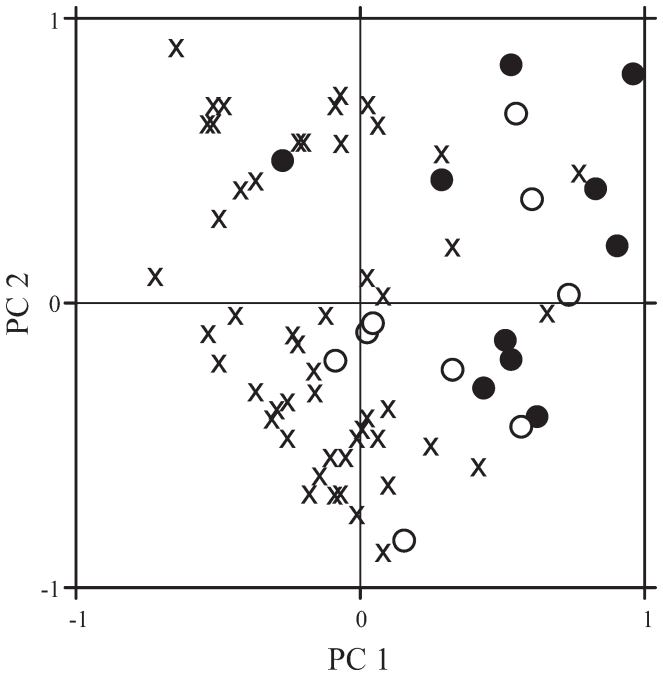

Fig. 1. Principal component analysis based on individual genotypes of *Daphnia pulex*. Full circles: laboratory E clones (overwintering in ephippia); open circles: laboratory W clones (overwintering as parthenogenetic females); crosses: reference field sample taken 10 months later.

For the five parameters tested the Mantel test found no significant correlation of genetic distance and difference in physiological performance, which is not surprising because microsatellites are assumed to be neutral markers and not related to any physiological trait.

Juvenile growth rates—Mean specific growth rates of clonal groups are plotted in Fig. 4. The three-way ANOVA for the high-food conditions (Table 1) depicts significant differences of g_j among clones, but not between groups and experiments. There is a significant interaction between clone and experiment, which means that clones do not differ systematically in all three experiments. Group means of g_j (d^{-1}) pooled for all experiments (±1 SD) are 0.545 ± 0.051 ($n = 90$) for E, and 0.545 ± 0.057 for W.

Although the difference between food concentration at the low level is not very large (0.12 vs. 0.15 mg C L^{-1}), the ANOVA shows a clearly significant response of g_j (Table 2). All clones grow faster at the higher food concentration. However, as at the high food level, there are significant differences among clones but not between the groups. The group means (±1 SD) of g_j at 0.12 mg C L^{-1} are 0.156 ± 0.023 ($n = 30$) for E, and 0.155 ± 0.030 ($n = 32$) for W. At 0.15 mg C L^{-1}, they are 0.235 ± 0.030 ($n =$

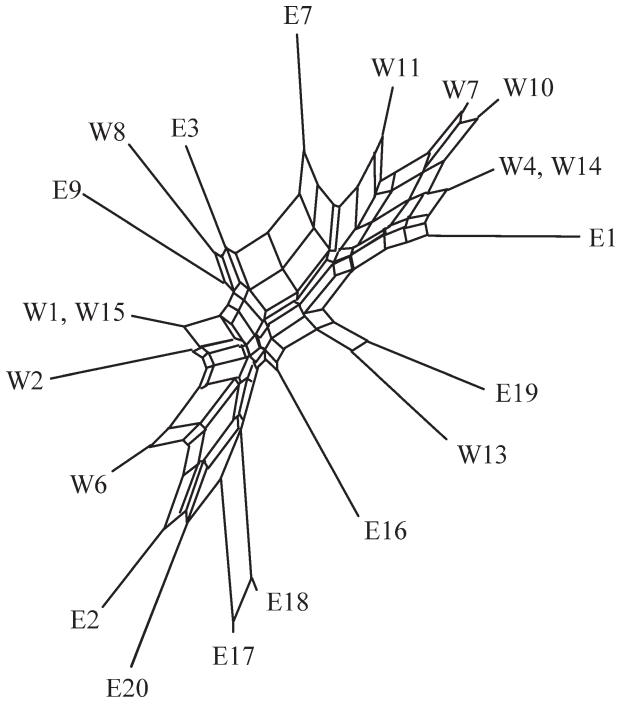

Fig. 2. Splitstree genotype network based on individual genetic distances for W and E clones.

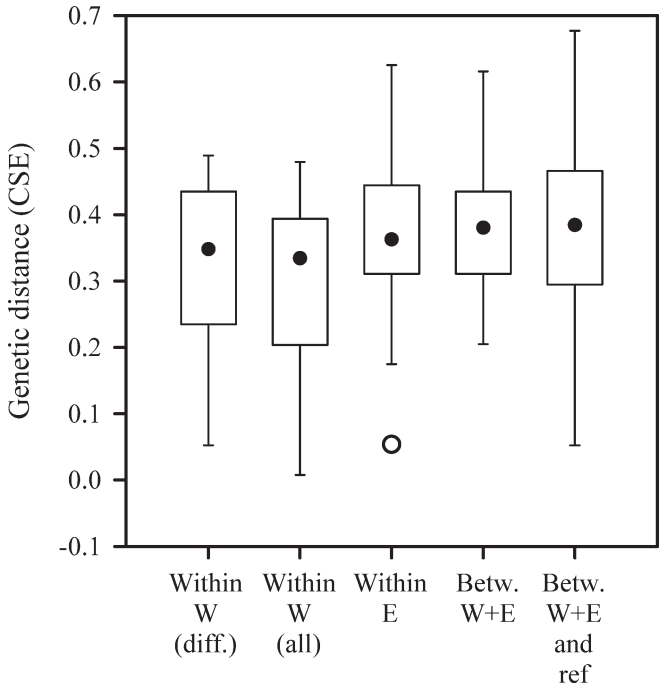

Fig. 3. Box plots of genetic distance (Cavalli–Sforza and Edwards chord distance [CSE]) within and between W clones and E clones and between W and E clones and the reference (ref) sample. Full circles: medians; boxes: 25–75% percentiles; bars: range, excluding outliers; open circle: outlier. W clones were analyzed considering only genetically distinct genotypes (diff.; n = 9) and including all investigated lines (all; n = 11).

30), and 0.259 ± 0.046 (n = 33), respectively. A significant interaction between clones and food indicates varying responses to improved food concentration among clones.

This variation is depicted in the reaction norms for g_j of the individual clones (Fig. 5). The mean characteristics of reaction norms and the mean estimates for threshold food concentrations are summarized in Table 3. Note that all clones (11 for W) are included in this analysis to represent a picture of the population subsamples, not just of different genotypes. Reaction norms of clones in both groups are rather variable but the variation is greater in group W. Due to this variability, none of the characteristics differ significantly between groups (slope: t-test, $t = -1.46$, df = 19, $p = 0.169$; elevation: t-test, $t = 1.30$, df = 19, $p = 0.234$; threshold: t-test, $t = -0.96$, df = 19, $p = 0.351$).

Discussion

Our first goal for the genetic analyses was to test whether all clones used in the experiments were genetically distinct. As expected, all ephippial (E) clones were genetically different because they had been founded by hatchlings from sexual eggs. On the other hand, we found two pairs among the 11 clones isolated from the parthenogenetic population during winter (W) that could not be discriminated with 12 polymorphic loci. This suggests that the probability of collecting identical genotypes from the winter population is higher than from the ephippial bank, a possible hint of clonal erosion in overwintering populations. However, the number of about 10 genotypes per group is too low for reliable population genetic conclusions. Hence, we compared our clonal lineages with a large reference sample collected from the lake 10 months later than the laboratory clones. The proportion of unique genotypes in the reference sample was much lower (~ 50%) than in the W group (80%), and none of the laboratory genotypes was detected in the reference sample. The latter is not surprising for the E clones, because we removed the

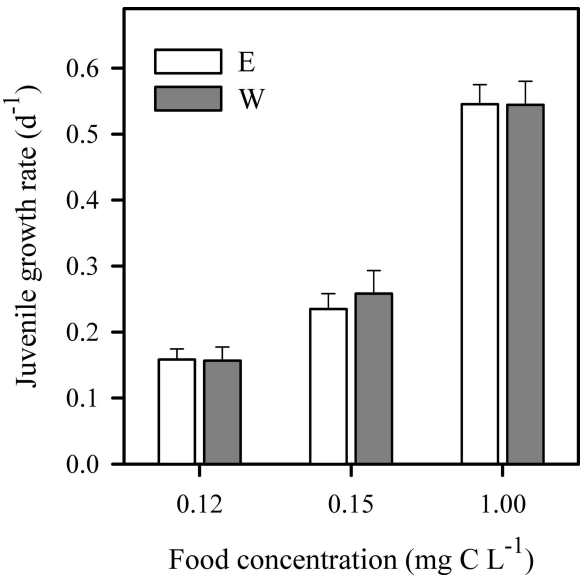

Fig. 4. Mean (±1 SD) specific juvenile growth rates (g_j) for the two clonal groups (E, W) at differing concentrations of food.

Table 2. Three-way ANOVA testing for the effects of 'group' (W and E clones), clone (nested in group), and food concentration (food), and their interactions on specific juvenile growth rate (g_j) at low food concentrations. Asterisks indicate significant results at $\alpha = 0.05$. MS = mean square.

Effect	df	MS	F	p
Group	1	4.406×10^{-3}	2.63	0.124
Clone (group)	17	1.678×10^{-3}	1.96	0.022*
Food	1	0.263	134.85	<0.001*
Group×food	1	4.979×10^{-3}	2.55	0.129
Clone (group)×food	17	1.935×10^{-3}	2.29	0.007*
Error	87	1.640×10^{-3}		

unique dormant eggs from the sediments, and the probability of finding an identical genotype in the sexually produced ephippia bank is very low. On the other hand, the low percentage of identical genotypes in the W group may suggest that the genetic diversity in the overwintering population is still high, and the probability of finding a particular genotype in a sample of 100 after 10 months is low.

The small difference in mean genetic relatedness between the E and the W clones could be considered as a hint of clonal erosion, although there was no visible pattern of genetic relationship in the total population. Neither the genotype network nor a PCA showed significant clustering of clones according to the groups. This lack of genetic differentiation suggests that clones of both groups originate from the same population, and that there is no permanent effect of winter selection.

The juvenile growth rate of *Daphnia* is a good proxy of fitness in the absence of predation (Lampert and Trubetskova 1996). It shows a close correlation not only with fecundity but also with the population growth rate (r) calculated from abbreviated life tables (Stearns 1992). Hence, it is useful to estimate clonal fitness at varying environmental conditions. Our growth-rate measurements consider different scenarios. Growth at high food reflects conditions during the spring algal maximum (Sommer et al. 1986). If E clones showed higher growth efficiency at high food levels, they would be able to compensate some of the costs of a delayed population build-up due to the start with hatchlings. In fact, Arbaciauskas and Lampert (2003) found higher growth rates before maturation for ex-ephippio hatchlings of *D. magna* compared with parthenogenetic neonates. However, the difference between groups disappeared in the first offspring generation; hence, any effect in the field must be short-lasting. Our ex-ephippio clones had been propagated parthenogenetically in the laboratory for many generations (i.e., the physiological differences were no longer visible).

Table 3. Mean ± 1 SD characteristics of the reaction norms for g_j presented in Fig. 3. The threshold for growth (mg C L^{-1}) was calculated for $g_j = 0$. (E: $n = 10$; W: $n = 11$).

Characteristic	E	W
Slope	2.553±1.050	3.331±1.310
Elevation	−0.148±0.137	−0.238±0.176
Threshold	0.044±0.042	0.060±0.036

Slopes at low food concentrations are relevant at times of improving food conditions (e.g., during the spring period before the algal maximum). A steep slope of the functional response curve indicates an advantage (fast response) at improving food conditions. Although the mean reaction norm characteristics of the groups do not differ significantly, it appears that the variation is larger in the W group (cf. Table 3) despite the slightly greater genetic relatedness (based on neutral markers). We found significant differences of slopes among clones, but the significant clone × food interaction makes it impossible to estimate the contributions of genetic variability and experimental error. Because a similar problem occurred at the high food concentration where the clone × experiment interaction was significant, we may assume some clone-specific error between the experiments that had been performed about 6 months apart. The responsible factor is most likely related to the size of juveniles varying between different batches when they were introduced to the flow-through

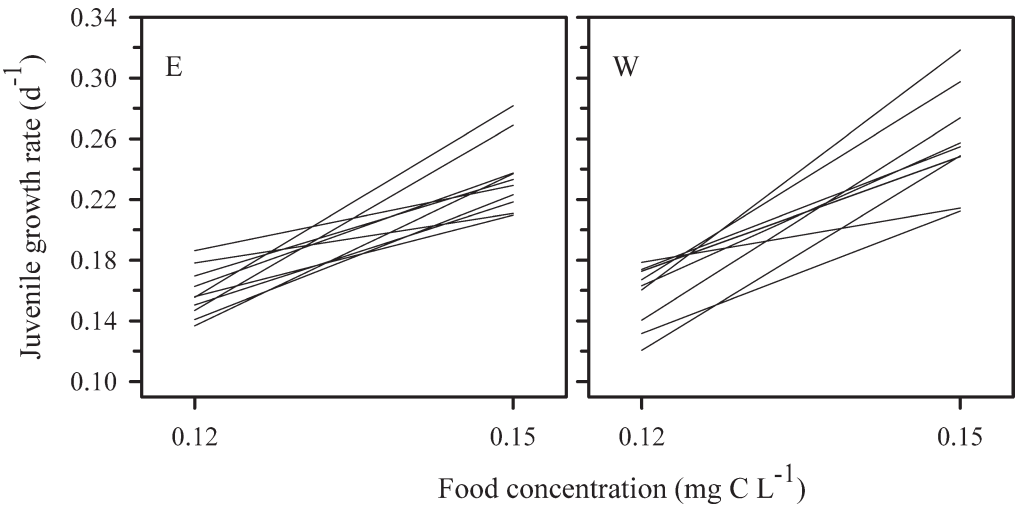

Fig. 5. Reaction norms of individual clones in the two groups at low food concentrations.

vessels (due to the 24-h sampling interval). In fact, individual dry weights of the initial controls varied between 8 μg and 15 μg. However, for our hypothesis, it is more relevant that there was no significant difference between the groups.

The calculated threshold food concentration for growth is a measure of starvation resistance and relative competitive ability (Gliwicz 1990; Gliwicz and Lampert 1990; Kreutzer and Lampert 1999) during periods of extremely low food concentrations (winter and clear-water phase; Sommer et al. 1986). If W clones were better adapted to live in the food-diluted open water than E clones, we would expect them to have a lower threshold (Hypothesis 2). Our data do not support this hypothesis. There was no significant difference in thresholds between groups.

This does not necessarily mean that survival of the two groups could not differ under natural winter conditions. We were not able to test whether reaction norms are differently affected by very low temperatures (i.e., W clones are cold-adapted and have a better growth efficiency at temperatures near 4°C). Under realistic winter conditions, when temperature is extremely low and food is scarce, the only sensible life-history trait to be measured is longevity. Growth is very poor and reproduction nearly absent. The size structure of the *Daphnia* population is strongly biased toward large, old females under these conditions. Performing experiments as described here under realistic winter conditions would be technically improbable because a measurable growth response would require several months in a flow-through vessel. Therefore, Rellstab and Spaak (2009) focused at survival in batch experiments at 5°C with low food. Although they calculated population growth rates, these were negative because only 25 neonates were born in all treatments during 4 months.

Some recent studies reported adaptation of *Daphnia* to overwintering conditions. Larsson and Wathne (2006) monitored *Daphnia umbra* in a small high-mountain pond in Norway. Although the extremely harsh winter conditions should favor dormant overwintering, part of the population remained active. *D. umbra* females accumulate large amounts of lipids in their body to survive the winter. The production of an ephippium may require a considerable part of the available resources; hence, lipid production would be reduced. Being relatively costly, ephippia production may be disfavored compared to lipid accumulation under these conditions, but the relative success of the strategies cannot be assessed by the field work.

Rellstab and Spaak (2009) studied clonal differences in survival of *D. hyalina* and *D. hyalina* × *galeata* from three deep, prealpine lakes of different trophic state (oligotrophic to eutrophic) under winter conditions. Because they considered ephippial hatching irrelevant in these large lakes, they used only clones isolated from the open water. The ANOVA detected strong differences between clones and a significant clone × food interaction, which resembles our results for the growth rate. Lake origin and *Daphnia* taxon were factors statistically equivalent to our groups, and they were not significant. However, a significant lake × food interaction indicated that clones of different origin responded differently to the addition of food (i.e., they were probably locally adapted to the trophic state of the lakes). Although the work of Rellstab and Spaak (2009) differed from our study with respect to experimental design, methods, and *Daphnia* species, there are remarkable similarities in the results. This may indicate that temperature does not strongly affect the differences between clones.

Slusarczyk (2009) presented field evidence for specialized overwintering strategies of two *D. pulicaria* color morphs in an ultra-oligotrophic high-mountain lake. These nearly represent the two alternatives requested in our general hypothesis: one type overwinters exclusively in ephippia, the other one as parthenogenetic females. It is exciting to learn that such a situation really exists, but unfortunately, this seems to be a very special case that cannot be used to interpret our results. So far, no common garden experiments have been performed to measure the expression of traits under differing conditions. The author proposes that the two types each consist of a single clone, which requires all ephippial eggs in the lake to be produced asexually (obligate parthenogens). If the existence of only two clones can be confirmed, there is no potential for evolution in these populations, but different scenarios for coexistence can be developed.

In conclusion, we found no support for the specific hypotheses 1–3. Although the W clones were genetically slightly more related to each other, the two groups could not be separated with microsatellite markers, and the groups did not differ in growth rates at high and low food concentrations or in food thresholds for growth. At the present state of knowledge we must conclude that both groups belong to a single population. It seems that a variety of clones contribute to the ephippia bank, and then continue to reproduce parthenogenetically and overwinter as adult females. Trying to produce as many parthenogenetic offspring as soon as possible in spring and to rely on the ephippia bank as insurance seems to be the most profitable strategy.

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