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Food quality triggers the reproductive mode in the cyclical parthenogen *Daphnia* (Cladocera)

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Abstract Cyclic parthenogenesis (heterogony) is a widespread reproductive mode found in diverse taxa such as digenean trematodes, gall wasps, gall midges, aphids, cladocerans and rotifers. It is of particular interest as it combines the advantages of asexual reproduction (rapid population growth) and sexual reproduction (recombination). Usually sexual reproduction is initiated when, or slightly before, environmental conditions deteriorate, and often results in the production of resting stages. The optimal timing of diapause induction must thus be under strong natural selection. Using the cladoceran *Daphnia* as a model system, we show here for the first time that the switch from parthenogenetic to sexual reproduction in a cyclical parthenogenetic organism can be influenced by the chemical composition of food. Under crowding conditions *Daphnia* reproduced parthenogenetically with subitaneous eggs when fed the algal species *Cryptomonas* sp., but started the production of resting eggs when fed with the green algal species *Scenedesmus obliquus*. Supplementation experiments with lipids and especially proteins showed that the induction of resting egg production in two clones of different *Daphnia* species was due to a dietary deficiency in the green alga. Hence, the low food quality induced a switch in the reproductive mode that may contribute to optimal timing of the sexual reproduction of *Daphnia* in nature. Furthermore, our results have two other major implications: first, they suggest that protein compounds should be added to the list of diet constituents potentially limiting or influencing *Daphnia* reproduction. Second, we show that the role of food quality goes far beyond the up to now documented effects of food quality on somatic growth and trophic transfer efficiency of herbivores: due to its effects on sexual reproduction and the production of resting eggs, food quality might influence genetic diversity and long-term persistence of *Daphnia* in lakes.

Keywords Cyclical parthenogenesis · Resting egg production · *Daphnia* · Food quality · Sexual reproduction

Introduction

Within sexually reproducing species and populations the production of male gametes results in a factor of 2 reduction of fitness, as males themselves do not produce offspring, and energetic resources are required for their production (see Maynard Smith 1978). Cyclical parthenogenetic animals have evolved a reproductive mode that largely overcomes this cost while maintaining the benefits of sex, i.e. genetic reshuffling (Peck et al. 1999). In many cyclical parthenogenetic organisms, sexual production confers an additional benefit: the production of resting eggs. For example, sexually produced eggs of aphids are able to resist freezing (Simon et al. 2002) and hence are of key importance for the persistence of aphid populations in

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colder regions. In the key herbivore *Daphnia*, sexually produced resting eggs (ephippia) are resistant to freezing and desiccation (Schwartz and Hebert 1987). Additionally, ephippia can survive for several decades and hence are important for dispersal in space (Louette and De Meester 2005) and time (Hairston and Kearns 2002).

Like most other cyclical parthenogenetic animals, *Daphnia* often start to reproduce sexually when environmental conditions deteriorate (Stross and Hill 1965; Hobæk and Larsson 1990; Kleiven et al. 1992). During most of the season *Daphnia* reproduces parthenogenetically with subitaneously developing eggs, and sexual reproduction is restricted to specific times of the year, e.g. during short periods in early summer or autumn and winter (Cáceres 1998; Jankowski and Straile 2004). This indicates that the high costs associated with sexual reproduction and resting egg production have favoured the evolution of an inducible rather than a fixed switch from parthenogenetic to sexual reproduction. Accordingly, sexual reproduction in *Daphnia* has been shown to be inducible by a multitude of environmental factors such as population density, food concentration, photoperiod and fish kairomones (Stross and Hill 1965; Hobæk and Larsson 1990; Kleiven et al. 1992; Slusarczyk 1995), as well as by maternal environmental conditions (Alekseev and Lampert 2001; LaMontagne and McCauley 2001). This ensures that the onset of sexual reproduction is coupled to deteriorating abiotic and biotic environmental conditions for parthenogenetic reproduction.

The role of food quality in determining the reproductive mode in cyclical parthenogenetic organisms is not well understood. However, in the polymorph rotifer *Asplanchna* it has been shown that the presence of dietary α-tocopherol (vitamin E) leads to the production of mictic female morphotypes (Gilbert and Thompson 1968; Gilbert 1981), which in turn produce resting eggs. Hence, in this system, the influence of food quality on the reproductive mode is indirect, as resting egg production depends on the female morphotype, the development of which is vitamin E dependent (Gilbert 2007). In aphids food quality has also been suggested to be important for the reproductive mode. However, up to now strong evidence for a role of food quality in producing resting eggs could not be found (Dixon and Kundu 1998; Dixon et al. 1993).

A major factor that determines growth and reproduction of *Daphnia* in nature is the composition of its food, which undergoes pronounced seasonal changes due to the succession of algal species and seasonally varying ratios of algal to detritus biomass (Sommer 1985). In early spring, lakes and ponds develop an algal maximum composed of small diatoms and fast-growing flagellates, i.e. *Cryptomonas* sp. that are of high food quality for *Daphnia*, while in summer other phytoplankton taxa, among them green algae, occur and lead to a lower food quality. During early summer, i.e. during the clear-water phase, food quality seems to be especially low (Wacker and Von Elert 2001) presumably due to a low contribution of algae to seston relative to detritus (D. Straile, unpublished data). Early summer is a preferred time for sexual reproduction in *Daphnia* (Jankowski and Straile 2004). This has been suggested to be due to high *Daphnia* densities (e.g. Stross and Hill 1965; Hobæk and Larsson 1990; Kleiven et al. 1992) and/or declining food availability (LaMontagne and McCauley 2001). Up to now, food quality has not been considered as a cue contributing to the decision of *Daphnia* to switch its reproductive mode in early summer. However, the strong change in food quality observed during this time period (Wacker and Von Elert 2001), suggests that a decrease in food quality may at least contribute to ensure the proper timing of the switch from parthenogenetic to sexual reproduction. To test the influence of the biochemical composition of food algae on *Daphnia*'s reproductive mode, we fed two clones belonging to two different *Daphnia* species (*Daphnia galeata* and *Daphnia pulex*) in laboratory experiments with two algal species that are known to be of minor (*Scenedesmus obliquus*) and superior (*Cryptomonas* sp.) food quality for *Daphnia* (Ahlgren et al. 1990, Weers and Gulati 1997).

Materials and methods

Experimental animals and set up

The study was conducted with one clone of *D. galeata* and one clone of *D. pulex*, both with a high allocation to ephippia production. The *D. galeata* clone is a sexual clone established from an ephippium which was taken from a sediment core sampled in Lake Constance, a large and deep pre-alpine lake in central Europe. The obligate parthenogenetic clone of *D. pulex* was originally isolated from a pond in northern Germany. Both *Daphnia* clones were cultured for many generations under laboratory conditions. The selected clones are particularly suitable for experiments on induction of resting egg production as they produce high amounts of ephippia even under slight crowding conditions, making additional treatments to enhance the usually very low ephippia production unnecessary. Experiments were run under continuous dim light at 20°C and with non-limiting food concentrations (2 mg C l^{-1}) in jars with 200 ml filtered (0.45-μm membrane cartridge; Sartorius) lake water, including ten animals each, to simulate moderate crowding conditions. Previous experiments have shown that this density results in ephippia production in both clones. Mothers of the experimental animals were cultured under non-limiting food conditions (*S. obliquus*, 2 mg C l^{-1}) for at least two generations. Each treatment was run with four replicates. Experimental animals were collected within 12 h after birth. Only third-clutch individuals were used. Ephippia production experiments lasted from birth to 16 days of age. During this time period, the animals could produce three parthenogenetic/subitaneous or two ephippial broods. During the experiment, the animals were fed in different treatments with the following types of food: *S. obliquus* (2 mg C l^{-1}); *Cryptomonas* sp. (2 mg C l^{-1}); *S. obliquus* (2 mg C l^{-1}) + protein beads (30 μl 200 ml^{-1}), *S. obliquus* (2 mg C l^{-1}) + protein beads (30 μl 200 ml^{-1}) loaded with *Cryptomonas* sp. lipid. The animals were transferred daily into fresh medium (food suspension), whereby released ephippia and neonates were counted and separated from the experimental animals. This procedure excludes the possibility of ephippia fertilization by males, and resulted in empty ephippia for the sexual clone. Allocation to sexual reproduction/resting egg production was measured as number of released ephippia. Allocation to parthenogenesis/subitaneous eggs was measured as total number of released neonates. In the following we use "parthenogenesis" for subitaneous developing offspring numbers and "ephippia production" for resting egg numbers of both clones. Male production was not recorded, as it could only be expected in the *D. galeata* clone. Furthermore, the induction of males is reported to be presumably induced by levels or combinations of cues slightly different from those inducing ephippia production (Hobæk and Larsson 1990). Hence, the role of food quality for male production in *Daphnia* needs additional study.

Algae cultures

The green alga *S. obliquus* (SAG 276-3; Stammsammlung für Algen, Göttingen, Germany) was grown in batch culture in Cyano medium (Jüttner et al. 1983) (20°C, illumination 146 μmol quanta s^{-1} m^{-2}). The cryptophyte *Cryptomonas* sp. (SAG 28.80) was grown in semi-continuous culture (dilution 0.2 day^{-1}) in WC medium with vitamins (Guillard 1975). P supply for algae in both media was non-limiting, thus preventing the possibility of P-limitation of *Daphnia.*

Algae sedimentation, a potential means of food limitation in the jars after 24 h, was analysed once in a test using a CASY particle counter. These measurements suggested that food limitation at the end of the 24 h period, if any, was only minor. In fact, only large *D. pulex* in combination with sedimentation were able to suppress food availability at the end of the experiment to below 0.5 μg C l^{-1}. However, *D. pulex* as a pond species is likely to feed also on sedimented algae. Hence, besides crowding, all other known stimuli for resting egg production were excluded, i.e. strong food limitation, fish kairomones or changes in photoperiod.

Supplementation experiments

To get more information about the chemical nature of the substance preventing the switch to ephippia production, we supplemented *S. obliquus* with extracted *Cryptomonas* sp. lipid attached to protein (bovine serum albumin; BSA) 6-μm-diameter beads (Micromod, Rostock, Germany), according to Von Elert and Wolffrom (2001). For lipid extraction, 30 mg C of *Cryptomonas* sp. algae was filtered onto pre-combusted glass fibre filters (Whatman GF/F). Lipids were extracted with dichloromethane/methanol (2:1; v:v), dried and dissolved in 10 ml absolute ethanol for storage. Protein beads were stored as stock solution in absolute ethanol (20 mg ml^{-1}). For supplementation with pure protein beads, 30 μl jar^{-1} of the stock solution was diluted in 30 ml absolute ethanol, dried and suspended in filtered lake water prior to the addition to algal food suspension. For protein beads loaded with *Cryptomonas* sp. lipid, 30 μl jar^{-1} of BSA beads was diluted in 30 ml absolute ethanol, 400 μl of the *Cryptomonas* sp. lipid extract (equal to the lipid amount of 1.2 mg C of *Cryptomonas* sp.) was added and both were dried and dissolved in filtered lake water.

Estimation of *Daphnia* growth rates

Daphnia performance on the different diets was further analysed by determining juvenile somatic growth rates (g) and population growth rates (r) calculated with Euler's equation. The latter provides a reasonable estimation of fitness for *Daphnia* (Stearns 1992) and is influenced directly by food quality and also indirectly due to switches in reproductive mode, i.e. from parthenogenetic to resting egg production. Juvenile g is often used as a surrogate for r (Lampert and Trubetskova 1996), but it will only be influenced directly by food quality as it is determined before reproductive decisions take place. The juvenile g was calculated from standardized experiments (Wacker and Von Elert 2001) using the equation:

$$g = [\ln(W_5) - \ln(W_0)]t^{-1}$$

where W_0 is the individual dry mass at the beginning of the experiment and W_5 is the individual dry mass after a growth period of 5 days. Dry masses were estimated as mean values of 10–15 individuals weighed on an electronic microbalance (Mettler UMT 2) and recorded to the nearest 0.1 μg.

r was calculated using the Euler–Lotka equation:

$$1 = \sum_{x=0}^{n} l_x m_x e^{-rx}$$

with age-specific survival probabilities, l_x, and fecundities, m_x. Data were analysed using one-way ANOVA with post hoc comparisons (Tukey's HSD), and repeated measures (RM) ANOVA. Normality of data was checked using the Shapiro-Wilk's test and homogeneity of variances using Cochran and Bartlett's test. As numbers of ephippia produced per individual were not normally distributed we used ANOVA on ranks for this (e.g. Conover and Iman 1981).

Finally, we conducted a literature search using the Web of Science data base (Thomson Cooperation) to determine the food quality applied in earlier experimental work on *Daphnia* ephippia induction. For details see Electronic supplementary material Appendix 1.

Results

r of *Daphnia* fed with the high-quality food *Cryptomonas* sp. were increased significantly for both *Daphnia* species compared to *S. obliquus* (Fig. 1a, c; Tukey's HSD $P < 0.001$, following one-way ANOVA, $F_{3,11} = 49.956$; $P < 0.001$ for *D. galeata*; Tukey's HSD $P < 0.001$, following one-way ANOVA, $F_{3,10} = 88.771$; $P < 0.001$ for *D. pulex*). This was, though to a smaller extent, also reflected in the juvenile g (Fig. 1b, d; Tukey's HSD $P < 0.05$, following one-way ANOVA, $F_{3,8} = 47.044$; $P < 0.001$, for *D. galeata;* Tukey's HSD $P < 0.001$, following one-way ANOVA, $F_{3,12} = 16.054$; $P < 0.001$ for *D. pulex*).

However, the estimation of fitness according to Euler is based on parthenogenetic or subitaneously developing offspring and is hence sensitive to switches from parthenogenetic to sexual reproduction, i.e. resting egg production. Low population growth on *S. obliquus* coincided with substantially reduced numbers of parthenogenetic offspring for both *Daphnia* species when compared to animals fed *Cryptomonas* sp. (Fig. 2b, d; Tukey's HSD $P < 0.001$, following one-way ANOVA, $F_{3,12} = 37.484$; $P < 0.001$ for *D. galeata;* Tukey's HSD $P < 0.001$, following one-way ANOVA, $F_{3,11} = 32.744$; $P < 0.001$ for *D. pulex*). Overall offspring numbers were higher for *D. pulex* due to its larger size. The reduction of parthenogenetic offspring by the low-quality food *S. obliquus* was due to the onset of resting egg production: *Daphnia* fed with *Cryptomonas* sp. did not produce any ephippia, whereas ephippia production on *S. obliquus* was high for both *Daphnia* species (Fig. 2a, c; Tukey's HSD $P < 0.001$, following one-way ANOVA, $F_{3,12} = 28.941$; $P < 0.001$ for *D. galeata;* Tukey's HSD $P < 0.001$, following one-way ANOVA, $F_{3,10} = 22.966$; $P < 0.001$ for *D. pulex*). Hence, *Daphnia*'s reproductive allocation depended strongly on algal species, i.e. food quality.

In order to confirm that the induction of resting egg production in *Daphnia* by *S. obliquus* was due to the absence of a compound that was a constituent of *Cryptomonas* sp. and that was responsible for the higher fitness of both *Daphnia* species on *Cryptomonas* sp. than on *S. obliquus*, we supplemented *S. obliquus* with protein (BSA) beads, and for additional lipid supplementation, with protein beads that had been loaded with *Cryptomonas* sp. lipids.

In both *Daphnia* species the supplementation of *S. obliquus* with protein lead to a substantial reduction in ephippia

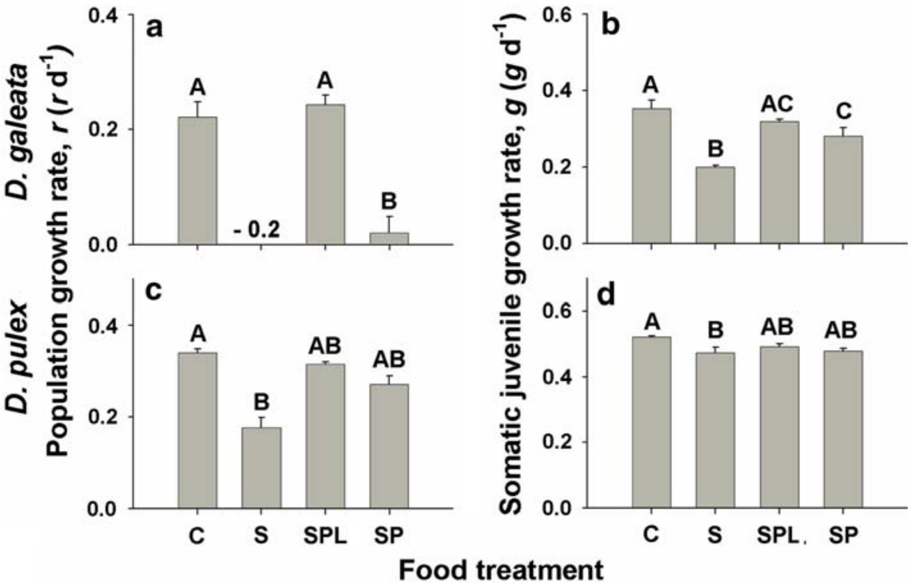

Fig. 1 Population growth rate (r) and somatic juvenile somatic growth rate (g) for the two experimental clones belonging to *Daphnia galeata* and *Daphnia pulex* on four food treatments: *Cryptomonas* sp (*C*), *Scenedesmus obliquus* (*S*), *S. obliquus* + protein + *Cryptomonas* sp. lipid (*SPL*), *S. obliquus* + protein (*SP*), means of four replicates ± SE. Average r of *D. galeata* on *S. obliquus* was negative (−0.2, not shown). *Bars* labelled with *different letters* are significantly different based upon Tukey's HSD, at least $P < 0.05$, following ANOVA

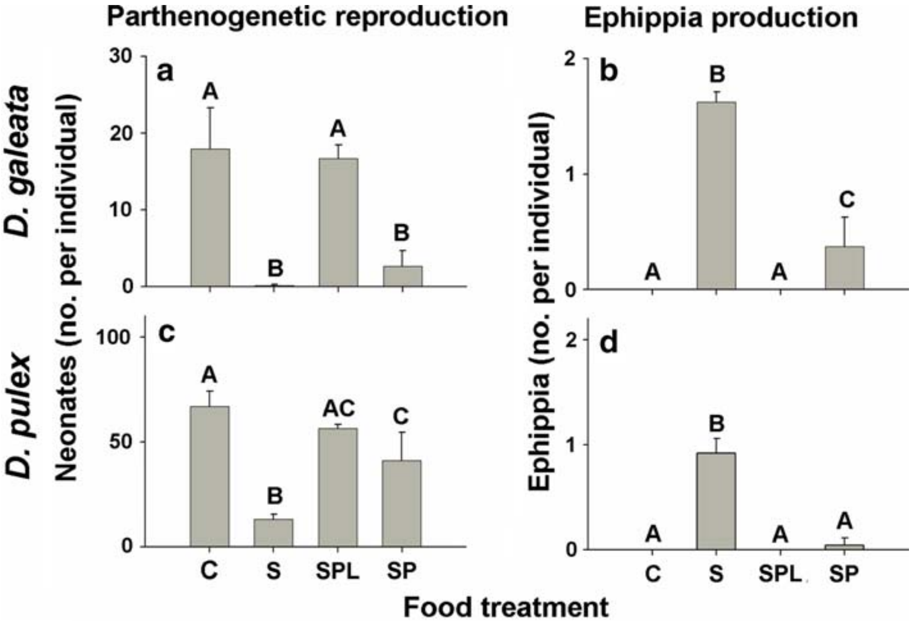

Fig. 2 Parthenogenetic reproduction (expressed as neonate production) and ephippia production in the two experimental clones belonging to *D. galeata* and *D. pulex* on four food treatments: C, S, SPL, SP; means of four replicates ± SE. Neonate and ephippia numbers are shown as sum per individual over 16 days after birth. First reproduction was on day 7 or 8. *Bars* labelled with different *letters* are significantly different, based upon Tukey's HSD, at least $P < 0.05$, following ANOVA. For abbreviations, see Fig. 1

production (Fig. 2), demonstrating that a dietary deficiency and not the presence of toxins or inhibitors in *S. obliquus* induced ephippia production. However, only when the low-quality food, *S. obliquus,* was supplemented with both protein and *Cryptomonas* sp. lipids, was the same reproductive allocation as on pure *Cryptomonas* sp. observed, i.e. only parthenogenetic reproduction (Fig. 2; Tukey's HSD $P < 0.001$, following one-way ANOVA, $F_{3,12} = 28.941$; $P < 0.001$ for *D. galeata;* Tukey's HSD $P < 0.001$, following one-way ANOVA, $F_{3,10} = 22.966$; $P < 0.001$ for *D. pulex*, comparison to *S. obliquus*).

In addition, protein supplementation caused a significant increase in juvenile *g* in *D. galeata* (Tukey's HSD $P < 0.05$, following ANOVA, $F_{3,8} = 47.044$, $P < 0.001$), whereas this effect was less clear in *D. pulex.* A similar difference between the respective clones of each species, was observed with respect to *r*. Furthermore, the two species/clones differed also in the effect of lipid supplementation on *r*: only lipid supplementation, not protein supplementation, significantly enhanced *r* in *D. galeata*.

Figure 3 shows the time course of reproduction in both *Daphnia* species. On a *Cryptomonas* sp. diet both species produced three parthenogenetic clutches during the experiment. On *S. obliquus* the first clutch of both species consisted of an ephippium. While *D. galeata* continued ephippia production throughout the experiment, most *D. pulex* switched to parthenogenetic production after releasing one ephippium. Hence, the much stronger reduction in *r* of *D. galeata* relative to *D. pulex* (Fig. 1) results from *D. pulex's* but not *D. galeata's* switch in reproductive mode with age (RM ANOVA: time × treatment × species interaction: $P < 0.001$). Supplementation with proteins caused a strong reduction in ephippia numbers, i.e. already in ephippial females for the first clutch in both *Daphnia* species. Some *D. galeata* still produced an ephippium with their second clutch. The numbers of parthenogenetically produced neonates remained, however, low in both clutches for *D. galeata* and *D. pulex*. Additional supplementation of lipids increased neonate numbers and resulted in a complete switch towards parthenogenetic reproduction in both *Daphnia* species. Thus the reproductive decision of *Daphnia* depended on a clonal-specific complex interplay between age and food quality.

Discussion

The role of food quality for *Daphnia* growth and consequently for the trophic coupling between phytoplankton and the key herbivore of many lake ecosystems is of considerable theoretical and applied interest (Müller-Navarra et al. 2000). Up to now, polyunsaturated fatty acids (Müller-Navarra et al. 2000; Von Elert 2002), sterols (Von Elert et al. 2003) and the mineral (P) content (Urabe et al. 1997) of algae have been considered as important facets of biochemical food quality for *Daphnia*. The algal species used in this study, *Cryptomonas* sp. and *S. obliquus*, are known to differ in their food quality for *Daphnia*. These food quality differences were thought to be particularly due to the fatty acid composition (Ahlgren et al. 1990), especially to the higher content of eicosapentaenoic acid (C20:5ω3) in *Cryptomonas* sp. However, there is evidence that the cryptophyte *Rhodomonas* sp., a close relative of *Cryptomonas*, also shows a higher total content of amino acids compared to *S. obliquus* (Ahlgren et al. 1992). The importance of lipids for *Daphnia* is also evident in our

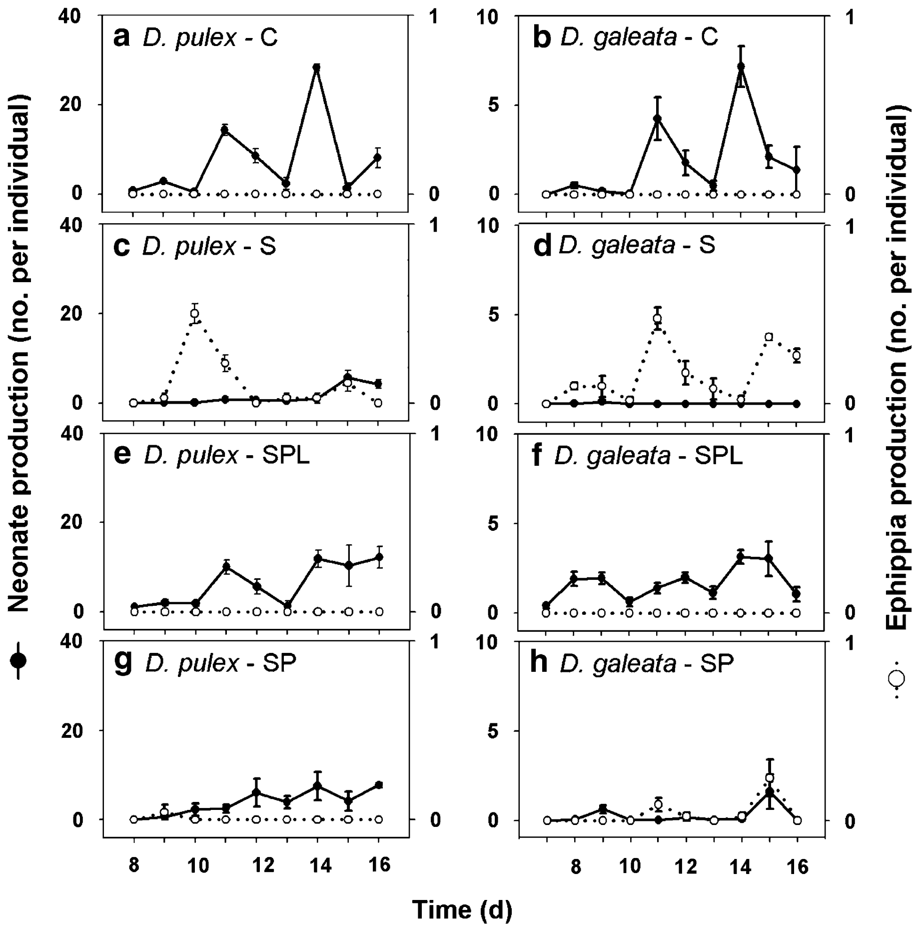

Fig. 3 Parthenogenetic reproduction (expressed as neonate production) and ephippia production (number released per individual) in the two experimental clones belonging to *D. galeata* and *D. pulex* on four food treatments: **a**, **b** C; **c**, **d** S; **e**, **f** SPL; **g**, **h** SP; means of four replicates ± SE. *Left-hand axes* Neonates per individual, *right-hand axes* ephippia per individual. For abbreviations, see Fig. 1

experiments: especially in *D. galeata*, lipid supplementation strongly increased neonate production and accordingly *r*. However, our results suggest that additionally proteins can be important for *r* and *g* of *Daphnia* and its persistence in ecosystems. While previous studies did not report an effect of proteins (BSA) on *Daphnia g* when supplemented with a cyanobacterial diet (e.g. Von Elert and Wolffrom 2001), we present evidence that proteins can increase *g* at least when provided as a supplement to *S. obliquus*. The effect of BSA supplementation on *g* was especially evident in *D. galeata*, while no clear effect could be demonstrated for *D. pulex*. However, *g* of *D. pulex* on *S. obliquus* was only slightly below its *g* on the high-quality food *Cryptomonas* sp., suggesting that *D. pulex* in contrast to *D. galeata* was hardly affected by the lower food quality of *S. obliquus*. Consequently, protein + lipid supplementation further increased the population increase in *D. galeata* but not in *D. pulex*. However, additional experiments with several *D. galeata* and *D. pulex* clones are needed to test if the differences observed are indeed differences at the species level or just clonal ones. Nevertheless, protein supplementation in both species/clones clearly resulted in a reduced ephippia and increased neonate production, i.e. in a switch between reproductive modes.

The food quality-mediated switch from subitaneous to resting eggs due to the protein content will clearly decrease *Daphnia* population growth and consequently also trophic transfer from algae towards higher trophic levels. On the other hand, this switch may increase *Daphnia* genetic diversity, secure *Daphnia* long-term survival within a lake or increase dispersal between lakes. All other things being equal, our results predict higher rates of *Daphnia* sexual reproduction in lakes and ponds with a low food quality. This would increase the chance for microevolutionary adaptation to low food quality, which has been suggested to have occurred in Lake Constance during eutrophication (Hairston et al. 1999). Likewise, increased ephippia production in lakes with low quality food would increase the possibility for dispersal into better food quality habitats. Altogether, this suggests that the role of proteins as a component of *Daphnia* food quality may be as important for lake functioning and *Daphnia* population dynamics as the food quality components considered up till now (see above). Clearly, there is an urgent need for more detailed studies on the specific components responsible for the protein effect in this study.

In nature only a few percent of female *Daphnia* carry ephippia—even during times of maximal ephippia production. Hence, we chose crowding conditions and two *Daphnia* clones which invest strongly in ephippia production to enhance the incidence of ephippia in our experiments. Crowding conditions often prevail in situ, when *Daphnia* produce ephippia (Cáceres 1998; Jankowski and Straile 2004), and there is genetic variability in the propensity of *Daphnia* clones to invest in sexual reproduction (Deng 1996). Possibly, this variability arises at least partially from clonal variability in the sensitivity to food quality. One of our clones, the *D. galeata* clone, originates from an ephippium collected in the deep and large pre-alpine Lake Constance. In this lake, the seasonal period of *D. galeata* sexual reproduction coincides with the seasonal minimum of food quality (compare Wacker and Von Elert 2001 with Jankowski and Straile 2004). This suggests that this clone's parental clones were indeed adapted to use food quality as a trigger for their change in reproductive allocation. In contrast, our *D. pulex* clone was isolated from a pond. The similar induction of resting egg production upon low food quality in *D. pulex* suggests that food quality may be also an important trigger for obligate parthenogens in pond ecosystems.

It should be noted that although *S. obliquus* clearly is of lower food quality than *Cryptomonas* sp., *S. obliquus* also is a rather high-quality food for *Daphnia*. In fact, many studies use *S. obliquus* as a standard food when studying the physiology or life history of *Daphnia*. Not surprisingly, *S. obliquus* was also used in the majority of studies analysing resting egg production in *Daphnia*: a literature review revealed that 59.4% of all studies retrieved ($n = 32$) used *S. obliquus* as food. Furthermore, an additional 31.2% used other green algae, leaving only 6.25% using non-green algae or mixtures of different algal species, whereas only one recent study (3.1%) used *Cryptomonas* sp. or other cryptomonads (see Electronic supplementary material, Appendix S1 for methods and references). This suggests that the vast majority of studies on *Daphnia* resting egg production were conducted with a hidden treatment, i.e. low food quality. Thus it is unclear if the quantity and quality of cues used to induce ephippia production in these studies would be sufficient to also induce ephippia production when *Daphnia* is fed food of a better quality, e.g. *Cryptomonas* sp. Consequently, the results of these studies with regard to the rate of ephippia production in response to other biotic and abiotic stressors should be re-evaluated. Only one study within our literature research also used, besides *Scenedesmus obliquus*, *Cryptomonas* sp. as a food source (Abrusán et al. 2007). The results of this study show that polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA), are also important for the formation of resting eggs. Using strongly limiting food concentrations as an inductor for resting egg formation, Abrusán et al. (2007) showed that supplementation with EPA or even a *Cryptomonas* sp. diet of mothers, enhanced ephippia production in the offspring generation compared to a pure *S. obliquus* diet. This suggests that under severe food limitation EPA acts as a resource enabling the production of resting eggs, whereas with higher food availability, proteins act as a cue inducing a switch towards resting egg production. Hence, food quantity might cause a reversal in the food quality effect on ephippia production in *Daphnia*.

Nevertheless, our study and numerous other ones using *S. obliquus* as a food source (see Fig. S1) show that under less severe food limitation, the quality of *S. obliquus* is clearly sufficient to produce resting eggs. This suggests that *Daphnia* do not postpone resting egg production in situ until periods of severe food limitation, but instead use environmental cues such as density, photoperiod, and also food quality to initiate resting egg production in time.

In conclusion, our study suggests that food quality can trigger the reproductive mode in *Daphnia*. Supplementation experiments demonstrated that the absence of lipids and especially proteins triggered the switch to sexual reproduction and resting egg production. The observation that periods of minimal food quality coincide with the time of preferred ephippia production (sexual reproduction) in natural habitats suggests that a change in food quality is a major trigger for the onset of sexual reproduction/resting egg production in *Daphnia* in nature and enables *Daphnia* to fine-tune the optimal timing of sexual reproduction/resting egg production. The timing of sexual and resting egg production must be under strong natural selection, as diapause is an essential component of the life cycle in *Daphnia* and many other animals and plants.

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