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PRIMARY RESEARCH PAPER

Maternally derived variation in the early termination of dormancy in *Daphnia pulex*

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Abstract The timing and duration of dormancy can be influenced by many environmental cues, such as changes in light and temperature. This study examined the early termination of dormancy in the absence of any major changes in environmental cues. We examined maternal influence on the early termination of dormancy in *Daphnia pulex*, as well as the effects of early termination of dormancy on life-history traits. In a mesocosm experiment, we found that a substantial proportion of individuals emerged early, prior to experiencing a cold shock, with no significant change in environmental conditions from the maternal environment. We found that siblings from the same ephippium were more likely to emerge at the same time, even after dissection and separation, suggestive of a maternal effect. Additionally, we found that the time to first reproduction was significantly delayed in individuals that emerged early, and that early hatchers also produced fewer dormant embryos in subsequent asexual generations. We suggest that early dormancy termination may be an adaptive strategy influenced by maternal effects to generate variation in developmental timing when the length and harshness of the unfavorable season is unpredictable.

Keywords *Daphnia pulex* · Ephippia · Bet-hedging · Dormancy

Introduction

Dormancy is critical for the survival of organisms that live in harsh and ever-changing environments. Dormancy can occur in numerous forms depending on the severity of the environment, such as a period of arrested development (Hairston & Kearns, 2002; Lopes et al., 2004; Hand et al., 2016). Some organisms, such as killifish (Dolfi et al., 2019), rotifers (Ricci, 2001), and crustaceans (Brendonck et al., 1998) produce dormant embryos capable of surviving desiccation and freezing. Typically, dormant embryos resume development and emerge when environmental conditions return to being hospitable, at the beginning of the next growing season. However, variation in dormancy strategy can occur

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in response to heterogeneity in habitat quality over time (Garcia-Roger, 2014; ten Brink et al., 2020). In environments where habitat quality predictably and drastically declines and resumes, the timing of dormancy is strictly reinforced by selection (Wagner & Simons, 2009; Vanschoenwinkel et al., 2010). When habitat quality does not change drastically or varies unpredictably, the strict timing of dormancy is no longer enforced by selection, and consequently individuals can terminate dormancy early (Satake et al., 2001) or not enter dormancy at all (Hairston, 1996; Ensslin et al., 2018). Variation in the timing of dormancy can cause individuals to emerge at different times, and experience different environments (Gilbert, 2017; Postma & Ågren, 2022). We hypothesize that there is variation in dormancy termination that is maternally driven, and that there are further life-history consequences associated with emerging early from dormancy.

The cladoceran *Daphnia* is a prime example of an organism found in many changing environments, from permanent lakes subjected seasonally to near freezing temperatures (Macháček & Seda, 2013), to fully ephemeral ponds that frequently become dry (Lynch, 1983). Under favorable environmental conditions, *Daphnia* reproduce parthenogenetically. This form of asexual reproduction results in nearly identical clonal female offspring (Hebert & Ward, 1972; Flynn et al., 2017). However, in order to survive unfavorable environmental conditions, such as stress caused by freezing or drought, *Daphnia* produce dormant embryos that are deposited within an ephippium–a protective, drought-resistant envelope (Hiruta & Tochinai, 2014). Ephippia are typically produced in response to worsening environmental conditions (Alekseev & Lampert, 2001), and can be produced throughout the year (Cáceres, 1998). Ephippia usually hold up to two sexual embryos depending on mating success (Winsor & Innes, 2002), but in certain cases contain two asexually produced dormant embryos (Hebert, 1981). Ephippia are produced in place of a non-dormant asexual clutch, which would typically contain 2–40 offspring (Gliwicz & Lampert, 1994). Therefore, to produce an ephippium, *Daphnia* sacrifice immediate population growth to invest in future offspring (Gerber et al., 2018). This strategy, while costly in the short-term, ultimately allows *Daphnia* populations to propagate following the cessation of harsh conditions. Embryos within the ephippium can survive food scarcity, hypoxia, drought, and freezing temperatures until conditions for hatching become favorable again (Radzikowski et al., 2018). As such, it is not expected for dormant embryos to emerge prior to experiencing harsh conditions.

The cues required for the hatching of *Daphnia* ephippial eggs have been extensively studied. Laboratory experiments have found that stimuli such as light quantity (Ślusarczyk & Flis, 2019), temperature (Vandekerkhove et al., 2005), and ephippia decapsulation (Davison, 1969; Paes et al., 2016) affect the rate of dormancy termination. Exposure to light greatly increases ephippial offspring development, while continuous exposure to darkness decreases hatching greatly (Pancella & Stross, 1963). Numerous studies have found that incubation of the dormant eggs in low temperature and dark conditions, followed by exposure to warm temperatures and bright light, triggers the development of ephippial offspring (Davison, 1969; Schwartz & Hebert, 1987; Vandekerkhove et al., 2005; Shabani et al., 2012; Luu et al., 2020), and that the cues associated with terminating dormancy can be genetically derived (De Meester & De Jager, 1993) and locally adapted (Pfrender & Deng, 1998). Additionally, the cues involved may be separated into those that terminate dormancy, or those that trigger development (Ślusarczyk et al., 2019). Maternal signaling may prevent offspring from developing for a certain period, and the offspring may be able to survey the environment to determine whether conditions are suitable for growth. However, few studies have investigated the early termination of dormancy prior to experiencing harsh conditions in *Daphnia* (Cáceres, 1998), nor the life-history effects associated with early dormancy termination.

To investigate the phenomenon of early dormancy termination in *Daphnia*, we observed dormancy termination rates from two clonal isolates of *D. pulex* Leydig, 1860. This study investigated (i) the role of maternal effects on embryonic dormancy, (ii) the impact of early dormancy termination on offspring life-history traits, and (iii) the putative role of dormancy strategy on transgenerational reproductive effects. From this study, we demonstrate the existence of early dormancy termination in *D. pulex* and suggest that maternal effects are a major driving force in the timing of dormancy.

Materials and methods

Study population

We collected *D. pulex* from a semi-permanent pond in Dorset, England in the Kilwood Nature Reserve (50.642483° N, − 2.091652° W) in the spring of 2017. Pond ephemerality was determined using trail cameras, and multi-year survey of the genetic composition of *D. pulex* (Barnard-Kubow et al., 2022). Live *Daphnia* samples were transported back to the University of Virginia, where clonal lineages were established from wild female isolates. We maintained lineages at 18°C and 16:8 light:dark conditions in 250 ml glass jars where they underwent asexual reproduction. The jars were maintained in hard artificial pond water (ASTM) (Standard, 2007) and fed with lab-grown *Chlorella vulgaris* obtained from UTEX (strain #4223).

Mesocosm conditions

We established four 15 L mesocosms filled with ASTM in the spring of 2019, and inoculated each mesocosm with one of two *D. pulex* lineages (D8515, D8222, two replicates each), which were established from two separate field isolates of the same wild superclone lineage (superclone C, Barnard-Kubow et al., 2022). Clonal isolates D8515 and D8222 were nearly genetically identical, although there were a limited number of genetic differences that have arisen due to mutation accumulation and gene conversion (Barnard-Kubow et al., 2022). We seeded the mesocosms with ~ 60 adult females, and placed them in a Percival incubator (Model Number: I66NL) at 18°C 16:8 light:dark conditions using LED lights (~ 1500 lx, Royal Pacific 4311WH), and aerated for the duration of the experiment. We fed the mesocosms with 95,000 cells/ml *Chlorella vulgaris* Beijerinck every Monday, Wednesday, and Friday for the first five weeks, and increased the concentration of *C. vulgaris* to 142,000 cells/ml after week 5 due to increased population density. Ephippia production began in week 4 in response to the increased population density and continued until the end of the experiment (Barnard-Kubow et al., 2022). We sampled mesocosms weekly for 8 weeks to determine age and reproductive demographics. Sampling was conducted by homogenizing the mesocosms via stirring, then removing all the individuals in 1L of media. We fixed the individuals in the 1L sampled media in ethanol to be counted later.

Ephippial hatching

We collected ephippia ($N = 2615$) on weekly basis from mesocosms of both clonal lineages and stored individually in clear 96 well plates filled with ASTM at 18°C under long day conditions (16L:8D) for four weeks in a Percival incubator (Model # CU41L4, LED lights, ~ 1200 lx). To determine whether buoyancy influenced hatching, we labeled a subset of ephippia ($N = 574$) according to whether they were found to be floating on the surface ($N = 163/574$, 28.4%), or fully submerged. Immediately after collection, we manually decapsulated a portion of ephippia ($N = 402/2615$, 15.4%) using fine metal needles in order to provide a general estimate of the number of eggs (Paes et al., 2016). We placed the decapsulated embryos obtained from the dissected under the same hatching conditions as the non-dissected ephippia, with the exception that each dissected embryo being placed in its own well. We checked each plate every two to three days under lower light using a Leica S8 APO microscope to determine hatch rate.

We removed individuals from the plates as they developed, and isolated in 50 ml jars with ASTM to establish new isofemale lines. We marked individuals that emerged prior to experiencing the cold shock as “early”. We incubated any individuals that had not hatched after about four weeks for four more weeks in a cold treatment in complete darkness at 4°C. We topped off any wells that experienced evaporation after the 4 weeks at 4°C with fresh media. After the four weeks at 4°C, we placed plates once more under hatching conditions (18°C, 16L:8D) to develop for a further two weeks. After two weeks of hatching, we observed no more developing individuals. We marked the individuals that emerged after experiencing the cold shock as hatching “late”. We dissected all undissected ephippia at the end of the experiment, and counted embryos to determine the total number of embryos produced over the course of the experiment ($N = 1931$ embryos).

Jar censusing

We followed 1105 sexually produced ephippial hatchlings over the course of 11 weeks. Within this number, a total of 233 sibling pairs were followed to determine maternal effects, including 60 sibling pairs from ephippia that were dissected. We isolated all ephippial offspring that developed from 96 well plates in 50 ml jars with ASTM and checked weekly for five weeks to determine survival and reproduction. We marked any individuals that failed to reproduce by 5 weeks as sterile. After reproduction was established, we moved individuals to 250 ml jars, and further checked at 5, 8, and 11 weeks for male and ephippia production. As such, we did not measure males/ephippia production in each distinct asexual generation after, but whether the lineage itself produce ephippia/males over the duration of the 11 weeks (≤ 5 asexual generations). We moved a subset of individuals (~ 10–15) to new media at each time point to propagate the lineage. We collected and dissected any ephippia produced within isofemale lines generated due to crowding to establish the number of embryos produced, as the presence of filled ephippia could be used as an additional metric of the presence of males. We established the presence of males by random sampling of 9–12 sub-adult individuals from each jar, as males are smaller than females.

Sequencing

We analyzed previously published whole-genome sequence data for a subset of ephippial offspring ($N = 10$ lineages) and their parental lineages to determine whether the ephippial offspring were produced asexually or sexually. We used the VCF file generated by Barnard-Kubow et al. (2022) and tested for patterns of genotype segregation in the F1s of sites that are known to be heterozygous in the parental C clone.

Statistical analysis

Statistical analyses were performed using R version 3.6.2 (R Core Team, 2019). When the dependent variable was binary or a proportion (survival, hatch rate), we used a generalized linear model with binomial error structure (Models 1, 2, 3, 5, 6; Table 1). When the dependent variable was count based (number of ephippia produced), we used a Poisson error (Model 4; Table 1). We performed a Likelihood Ratio Test between models with and without each term (Chambers & Hastie, 1992) to determine the factors that affected early hatching, as well as to test whether early hatching affects other traits in the offspring.

Results

This study investigated the rate of early dormancy termination in lab-reared *D. pulex*. We characterized the role of maternal effects on the rate of early dormancy termination, as well as the effects of early dormancy termination on offspring's life-history traits. Of the ephippial hatchings that emerged from the mesocosm experiment, 37.4% ($N = 413/1105$) emerged from dormancy in the four weeks prior to experiencing a change in environmental cues (Fig. 1).

Maternal effects on early hatch rate

To assess whether maternal effects influenced the timing of hatching, we tracked 233 sibling pairs that developed from the same ephippia. By examining the genome-wide distribution of genotype frequencies, we show that these embryos are produced sexually from a selfed-cross of the C superclone (Fig. 2A). Because all of the fathers and ephippial mothers were from the same clonal lineage, embryos from different ephippia were effectively full-siblings. However, embryos from the same ephippia will share exposure to maternal cues that could affect hatching, and would likely cause embryos from the same ephippia to have a similar hatching behavior, barring any cues that mothers provide to embryos individually. We found that embryos from the same ephippium were more likely to emerge at a similar time: there were fewer embryos from the same ephippium where one hatched prior to cold treatment and one hatched after cold treatment than would be expected by chance ($df = 1$, $\chi^2 = 122.1$, $P = 2.195e{-}28$, Table 2). Moreover, the hatching dynamics of embryos from the same ephippia were similar regardless of whether we dissected ephippia ($N = 60$ pairs) or let embryos naturally hatch ($N = 173$ pairs), ruling out the possibility that the development of one embryo triggered the development of the other (Fig. 2B). We assessed whether the maternal environment affected the early hatching rate as a function of mesocosm age. We found that the rate

Table 1 Models and ANOVA results

	DF	Deviance	Resid. DF	Resid. dev	Pr(> Chi)
(Model 1) Proportion early hatching ~ Mesocosm age + Clone/replicate					
Mesocosm age	1	148	8	108	2.2E−16
Clone	1	30.3	7	78.0	3.71E−0.8
Clone:Replicate	2	17.2	6	60.8	0.000182
(Model 2) Survival to reproduction ~ Hatching time * Dissection status + Clone/replicate					
Hatching time	1	1.71	14	18.1	0.191
Dissection status	1	0.148	13	18.0	0.701
Clone	1	3.54	12	14.4	0.0599
Hatching time × dissection	1	4.33	11	10.1	0.0374
Clone:Replicate	2	1.24	9	8.84	0.537
(Model 3) Proportion reproduced ~ Hatching time * Jar age + Clone/replicate					
Hatching time	1	50.3	38	1080	1.30E−12
Jar age	1	22.8	37	1060	1.840E−6
Clone	1	0.074	36	1060	0.786
Hatching time × jar age	1	10.8	35	1050	0.00101
Clone:Replicate	2	92.9	33	955	< 2.2e−16
(Model 4) Ephippia production ~ Hatching time + Clone/replicate					
Hatching time	1	266	644	3420	< 2.2e−16
Clone	1	17.8	643	3400	2.48E−05
Clone:Replicate	2	37.3	641	3360	8.07E−09
(Model 5) Ephippia fill rate ~ Hatching time + Clone/replicate					
Hatching time	1	0.00	362	2873	0.998
Clone	1	0.0005	361	2873	0.981
Clone:Replicate	2	4.45	359	2868	0.108
(Model 6) Early hatching ~ Buoyancy + Clone/replicate					
Buoyancy	1	8.86	6	43.6	0.00291
Clone	1	27.8	5	15.8	1.31E−07
Clone:Replicate	2	2.39	3	13.4	0.303

of early hatching was significantly different between the sample weeks, increasing as the mesocosms aged (df = 1, $\chi^2 = 148$ $P = 2.2\text{E}{-16}$, Fig. 2C, Model 1).

Hatchling survival and reproduction

Early hatching did not significantly affect individual survival to reproduction (df = 1, $\chi^2 = 1.71$, $P = 0.191$, Fig. 3A, Model 2). Moreover, whether individuals were dissected or naturally hatched did not significantly affect survival (df = 1, $\chi^2 = 0.148$, $P = 0.701$, Fig. 3A, Model 2). Individuals that hatched early took a significantly longer amount of time to reach reproduction compared to those that hatched late (df = 1, $\chi^2 = 50.3$, $P = 1.30\text{E}{-12}$, Fig. 3B, Model 3).

Transgenerational effects of early hatching

To determine putative transgenerational effects, we measured the number of ephippia produced by hatchlings and their immediate offspring and assessed the ephippial fill rate as a proxy for male production and mating success. Lineages produced by offspring that emerged early produced significantly fewer ephippia than individuals that hatched out late (df = 1, $\chi^2 = 266$, $P = 2.2\text{e}{-16}$, Fig. 4A, Model 4). The two parental clonal isolates also differed in ephippia production (df = 1, $\chi^2 = 17.8$, $P = 2.48\text{e}{-05}$, Model 4). We did not detect any significant effect of hatching time on ephippial fill rate (df = 1, $\chi^2 = 0.00$, $P = 0.998$, Fig. 4B, Model 5) in the clonal generations after hatching.

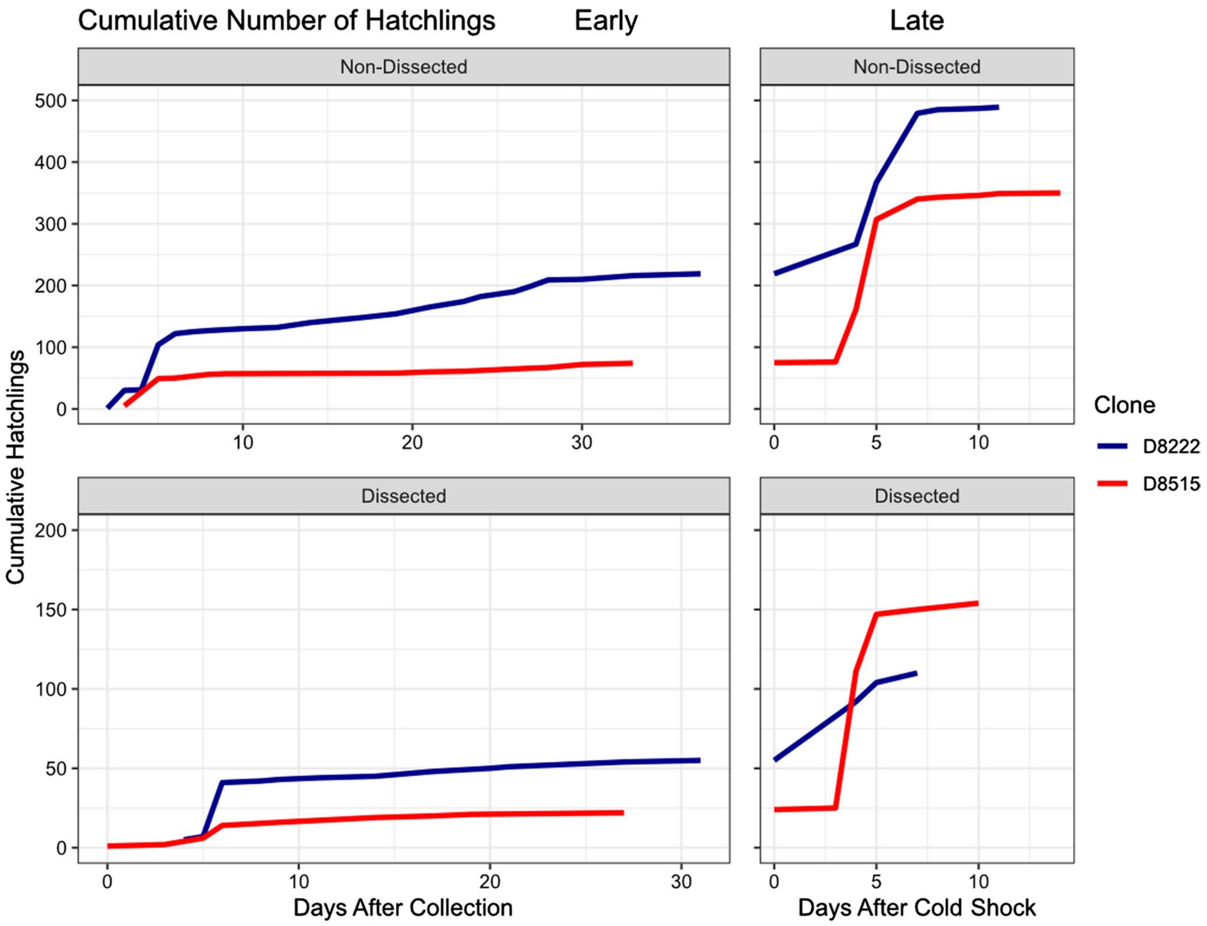

Fig. 1 Hatching dynamics of ephippial offspring: We observed a moderate increase in hatching immediately after collection, but early hatching continued for over 30 days. Early hatching ceased completely after 37 days. Over half of early hatching ($N = 203/370$, 55.1%) occurred after 5 days

Buoyancy

We found that while there was a significant effect of buoyancy on the timing of dormancy termination, individuals that were fully submerged when collected from the mesocosms were more likely to emerge early than expected by chance ($\mathrm{df} = 1$, $\chi^2 = 8.86$, $P = 0.00291$, Model 6, Fig. 5). However, there was a significant difference in hatching between clonal lineages (df 1, $\chi^2 = 27.8$, $P = 1.31\mathrm{E}{-}07$, Model 6). This effect appeared to be driven by D8515, where few individuals that were floating hatched early ($N = 8/107$, Fig. 5).

Discussion

In this study, we examined variation in the timing of dormancy, and assessed the effects of early hatching on life-history traits. We found that variation in the timing of dormancy termination in *D. pulex* is influenced by maternal effects. This study contributes to the existing literature of maternally derived variation in dormancy duration and provides insight into how early dormancy termination may drive some transgenerational effects on reproduction (Martínez-Ruiz & García-Roger, 2015).

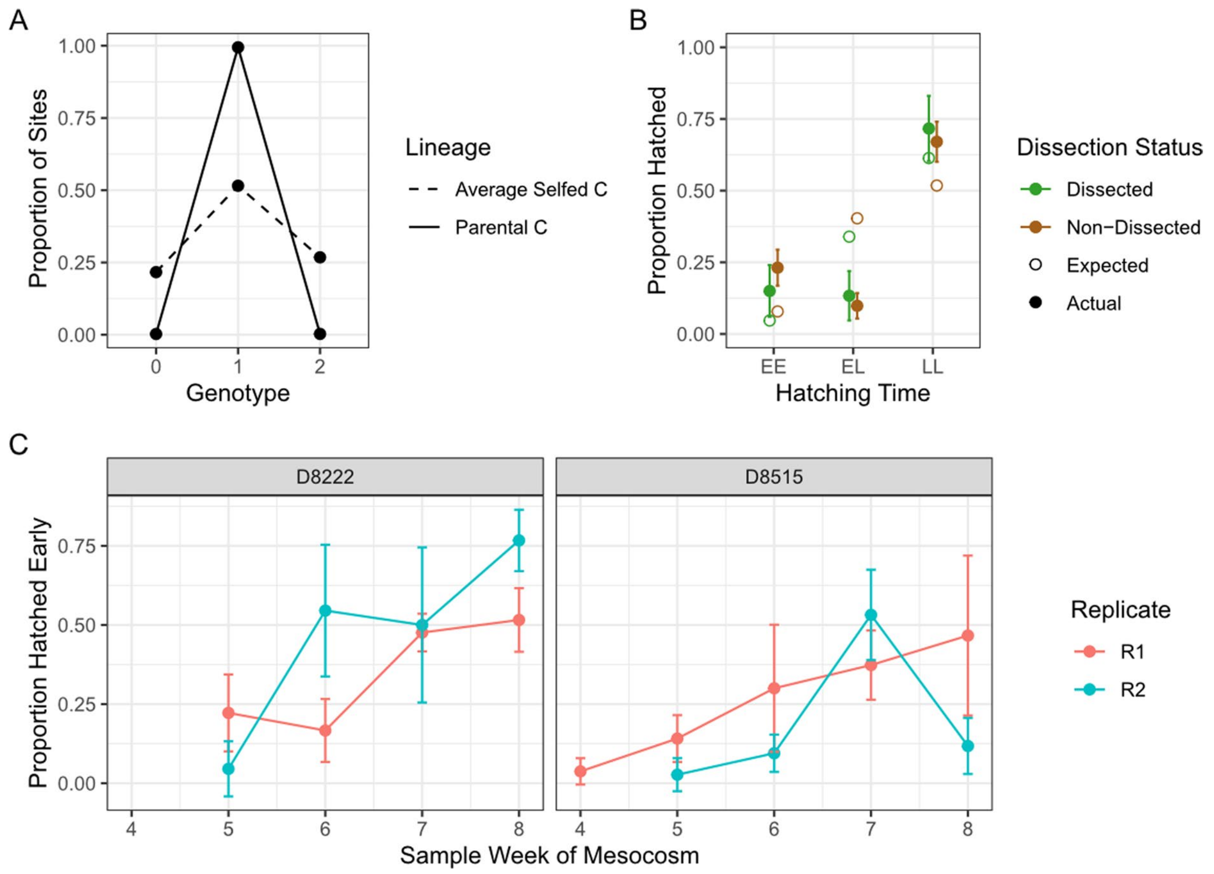

Fig. 2 Maternal environment and maternal cues affect early hatching. **A** Offspring from the mesocosms are full siblings produced from a selfed cross. Heterozygous alleles in the parental genotype segregated out according to Hardy–Weinberg in the F1 generation. The solid line are heterozygous sites found in the parental superclonal lineage. The dotted line is the average proportion of alleles in the sequenced F1 offspring ($N = 10$ lineages). **B** Proportion of sibling pairs that have hatched out based on treatment. EE—both siblings emerged early; EL—One sibling emerged early, the other late; LL—Both siblings emerged late. Error bars represent 95% confidence intervals calculated using standard error. Open circles represent expected values based on the marginal rate of early hatching; filled circles represent the observed values. **C** Proportion of individuals that hatched early over the course of the 8 week mesocosm. The rate of early dormancy termination was significantly different between the weeks (df = 1, $\chi^2 = 148$ $P = 2.2\text{E}{-}16$, Model 1), and increases as the mesocosms aged. Each line represents a clonal isolate, with error bars representing 95% confidence intervals calculated using standard error

Table 2 Individuals tend to hatch out with their immediate sibling in similar conditions (EE, LL) than expected by chance

	Number of sib. pairs	EE	EL	LL	X^2	*P* value
D8222 dissected	20	7	2	11	12.54	0.0003983
D8222 non-dissected	102	28	13	61	52.19	5.038e−13
D8515 dissected	40	2	6	32	3.951	0.04684
D8515 non-dissected	71	12	4	55	47.98	4.306e−12
Total	233	49	25	159	122.1	2.195e−28

There were fewer sibling pairs where one individual hatched early, and one had hatched late (EL) than expected by chance

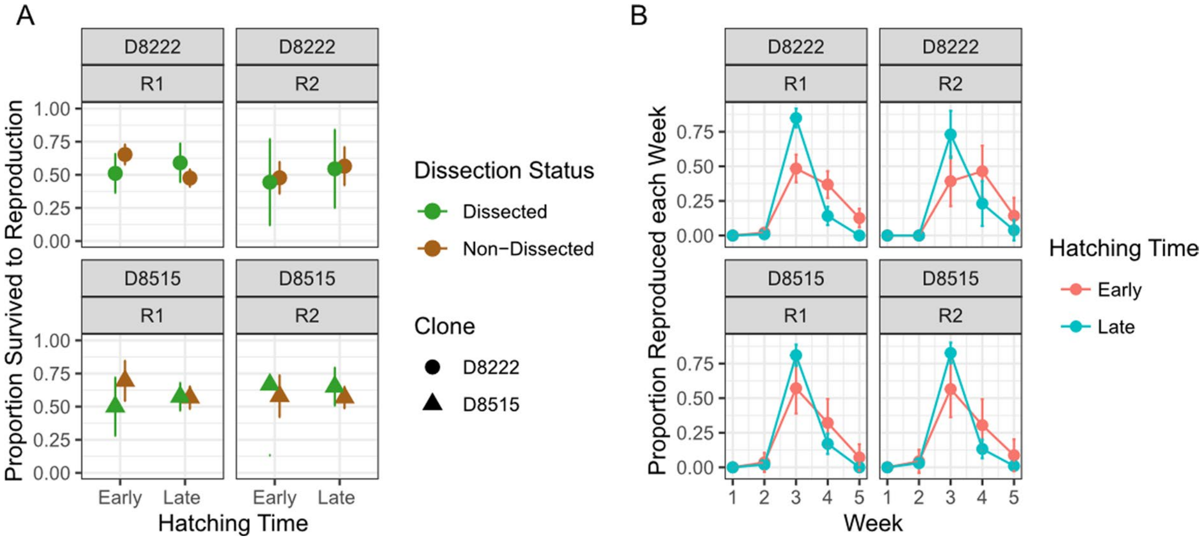

Fig. 3 Effects of dormancy duration on life-history traits. **A** There were no major differences in survival at the end of the first five weeks between individuals that hatched out early or late (df = 1, χ^2 = 1.71, *P* = 0.191, Model 2), nor did dissection affect survival (df = 1, χ^2 = 0.148, *P* = 0.701, Model 2). **B** Time to first reproduction differed significantly between treatments. The time to first reproduction was slightly, but significantly slower in individuals that had hatched early (df = 1, χ^2 = 50.3, *P* = 1.30E−12, Model 3)

Early dormancy termination

Our primary result is that a large proportion of hatchlings (*N* = 413/1105, 37.4%) of a lineage of *D. pulex* collected from a semi-permanent pond emerged from dormancy without any obvious changes in environmental cues (Fig. 1). Variation in dormancy strategy has been observed in many species living in temporally heterogeneous environments, such that individuals from the same population terminate dormancy at different times (Donohue, 2009; Martínez-Ruiz & García-Roger, 2015; Polačik et al., 2017).

Variation in the duration of dormancy has often been viewed as a bet-hedging strategy in both plants and animals (Brendonck et al., 1998; Evans & Dennehey, 2005; Simons, 2011; Gremer & Venable, 2014). Bet-hedging is a risk-management strategy that reduces variance in mean fitness, to increase fitness over time (Simons, 2011). Environmental variation may select for either conservative or distributive bet-hedging (Botero et al., 2015). In conservative bet-hedging, individual organisms sacrifice some immediate fitness to hedge against overall fitness loss. For example, flowering phenology could occur at an earlier point in time to hedge against an erratic end to the growing season even if it results in a lower seed set (Simons & Johnston, 2003). In distributive bet-hedging, risk-management takes the form of investment into multiple strategies: for example, a female may produce offspring with a wide range of phenotypes to prepare offspring for multiple possible environments (Evans & Dennehy, 2005). Likewise, organisms may vary in the timing of dormancy termination to increase the chances that some offspring emerge at the right time (Sasaki & Ellner, 1995; Brendonck et al., 1998; Vanoverbeke & De Meester, 2009; Pinceel et al., 2017). Multiple strategies can coexist within a population, and can be driven by either genetic (Alonso-Blanco et al., 2003) or maternal effects (Martínez-Ruiz & García-Roger, 2015). Additionally, the cues associated with dormancy termination may be separated into the maternal signaling associated with dormancy length and the ability of offspring to survey the environment to determine habitability (Ślusarczyk et al., 2019).

We propose that variable hatching in *D. pulex* is a mechanism to distribute risk and benefit in heterogenous environments. Obviously, dormancy is beneficial when the environment becomes inhospitable. But, what are the potential benefits of early hatching if there is a mild off-season? We envision

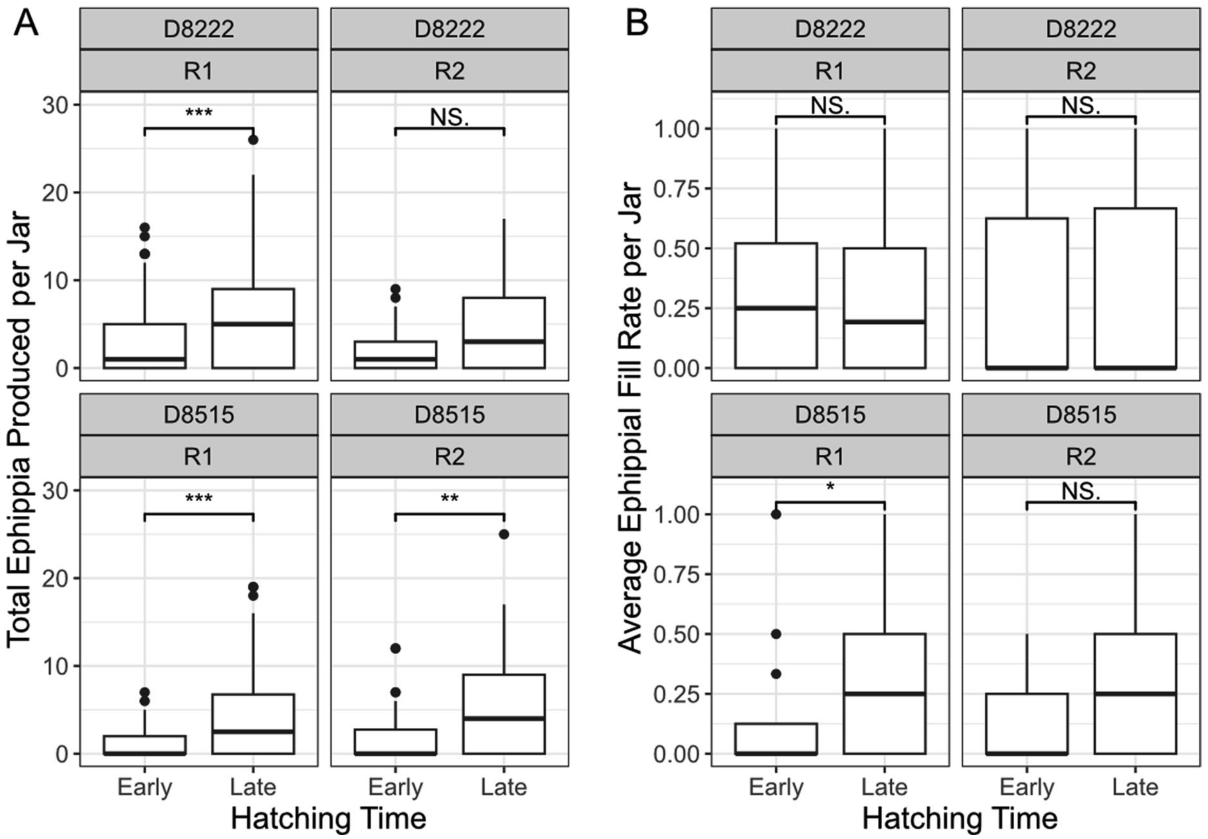

Fig. 4 Early hatching affects some offspring life-history traits. **A** Average ephippia per jar significantly increased in post-treatment individuals (df = 1, χ^2 = 266, P = 2.2e−16, Model 4). **B** Average ephippia fill rate did not change significantly between treatments in D8222, but did in one replicate of D8515

two possible benefits. First, as our study pond rarely dries completely, it would be advantageous for some *Daphnia* to emerge spontaneously. Individuals emerging early would then be able to survive as adults during the mild off-season. If individuals do not hatch out for a period of time, there is also the risk of being buried in sediment, which drastically decreases the rate of ephippial hatching (Radzikowski et al., 2016). Second, as ephippial offspring are typically sexually recombinant, early hatchers may add necessary genetic diversity to a mostly clonal population late in the season in permanent ponds. The increased rate of sexual reproduction in these clonal populations may provide novel gene combinations that can adapt to changes in the environment, such as increasing parasitism (Hamilton et al., 1990) and predation (Koch et al., 2020).

Maternal effects involved in early dormancy termination

Our data suggests that there is a strong maternal effect involved in dormancy variation (Fig. 2B), shown by the observation that siblings from the same ephippium tended to emerge around the same time, even after being separated. This result is consistent with prior studies linking embryonic dormancy to changes in the maternal environment. In plants such as *Campanula americana* Hort. ex Steud., germination time can vary as a function of the parental environments (Galloway, 2001)**.** Amongst invertebrates, maternal effects have been demonstrated to influence traits associated with an offspring's dormancy. Our results resemble a previous study in a rotifer population on the effects of the maternal environment, and subsequent maternal control, on the variation in dormancy timing (Martínez-Ruiz & García-Roger 2015). In

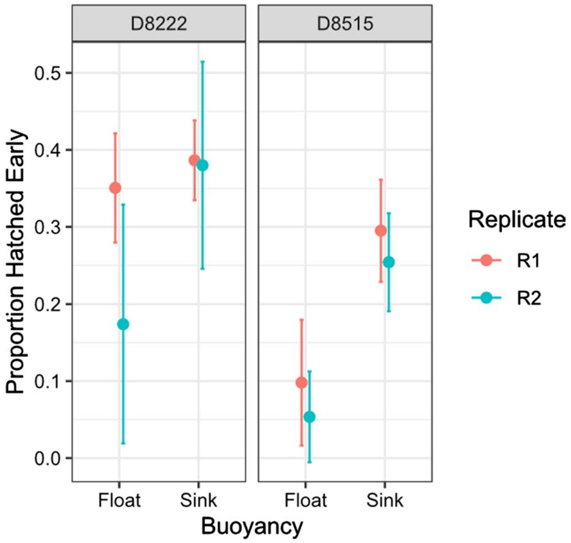

Fig. 5 Floating ephippia does not account for early hatching. The rate of early hatching differed between ephippia found floating or sinking (df = 1, $\chi^2 = 8.86$, $P = 0.00291$, Model 6). Contrary to our initial hypothesis, ephippia that were found sinking had a higher rate of early dormancy termination than those found floating in D8515

these rotifers, the length of dormancy was correlated with maternal age, and eggs produced by younger mothers had a higher chance of being late hatchers that required environmental cues to break dormancy. Our results demonstrate a similar phenomenon in *Daphnia*. Individuals produced in the early stages of the mesocosm experiment, when mothers were younger, had a lower rate of early hatching (Fig. 2C).

Although we demonstrate that dormancy termination is likely under maternal control, we cannot rule out genetic variation as an additional source of variation. Lineages were maintained under laboratory conditions for two years prior to starting the mesocosm cultures and mesocosms were initiated with multiple females from each clonal lineage (Barnard-Kubow et al., 2022). This might have been ample time for novel mutations to appear prior to the start of the mesocosm or during the multigeneration mesocosm experiment itself (Keith et al., 2016; Flynn et al., 2017). In principle, these newly arisen mutations could affect dormancy duration and therefore be misinterpreted as maternal effects. We suggest that this scenario is unlikely for several reasons. First, it would suggest that the mutational target size for dormancy termination is large. Although many genes have been implicated in dormancy in invertebrates (Schwarzenberger et al., 2020), it would be difficult to imagine that it is so easy to genetically alter dormancy duration. Second, most new mutations are recessive and if a mother carrying a single copy of a new mutation affecting dormancy duration mated with a wild-type male, none of her offspring would manifest the phenotype. Therefore, we consider the model of genetic variation within the clonal mesocosm affecting dormancy termination rate unlikely.

We should also consider the dysregulation of dormancy due to inbreeding depression. As mentioned previously, individuals were the product of sexual reproduction within each clonal lineage, such that mothers and fathers were the same genetically. Overall, survival to five weeks after hatching was 56.0% for these selfed offspring (Fig. 3A), and so individuals may be suffering from inbreeding depression (Innes, 1989; De Meester, 1993). On the other hand, a pedigreed survey of a wild metapopulation of *D. pulex* demonstrates that some common clonal lineages are the product of selfing, suggesting that not all selfed clones suffer from inbreeding depression (Barnard-Kubow et al., 2022). Although inbreeding depression could in principle influence our hatching timing results, there is no evidence to support it. For instance, we assayed hatching timing in 16 outbred clones between the A and C superclone as identified in Barnard-Kubow et al. (2022); these individuals were not included in our analysis because of the small sample size. Of these 16 clones, 56.3% emerged early, suggesting that the early termination of dormancy was not directly caused by inbreeding depression.

Buoyancy as a possible alternative

Daphnia ephippia can either float to the surface or remain submerged in the water column depending at least partially on behavior of the mother (Ślusarczyk & Pietrzak, 2008). It is generally believed that floating ephippia are a mechanism of dispersal (van de Meutter et al., 2008), or that they aggregate on the edge of the pond where they are more likely to receive a hatching cue than ephippia that sink (Pietrzak & Ślusarczyk, 2006). One concern of our experiment was that the positively buoyant ephippia would hatch immediately upon submersion in the 96 well plates, after desiccation on the surface. However, we observed that individuals found floating on the surface of the mesocosms were less likely to hatch early than expected (Fig. 5). Thus, ephippia floating on the surface did not appear to drive the early termination of dormancy we observed. In fact, this result suggests that positively buoyant ephippia may have an additional requirement to emerge from dormancy compared to negatively buoyant ephippia, preventing them from emerging before they disperse or experience a change in environmental cues.

Life history traits

Our analysis showed that early hatching had no significant effect on offspring survival (Fig. 3A). However, individuals that emerged early took slightly longer to reach reproductive age than individuals that emerged late (Fig. 3B). Coupled with the reduction in the number of sexual eggs produced in subsequent generations (Fig. 4A), we hypothesize that individuals that emerged early may invest more in growth than reproduction. However, these results should be considered speculative. The early and late hatchers being separated in time may have caused minor batch effects, despite our attempts to keep the environmental conditions the same. However, our findings reveal a decrease in the quantity of sexual eggs across successive asexual generations, like previous studies in cyclic parthenogens (Schroder & Gilbert, 2004).

For organisms that emerge from dormancy at different times, the environment that they emerge into may favor alternative life-history strategies. As ephippia are typically produced late in the growing season (Altermatt & Ebert, 2008) when population density is high, any dormant eggs that emerge early would likely experience these same conditions. To survive harsh conditions, individuals that emerge into an end-of-season environment would likely be favored if they are more focused on growth/survival, rather than rapid reproduction (Mueller & Ayala, 1981). Alternatively, organisms that terminate dormancy in response to strict environmental signals, emerging later, likely experience an environment that is more hospitable with a low density of intraspecifics (Cáceres, 1998). Under low intraspecific competition, organisms that reproduce rapidly would have an advantage (Pianka, 1970). Similar trade-offs associated with size and time to reproduction have been documented as being both genetically (Reznick, 1983) and maternally (Arnold et al., 2018) derived.

Conclusion

Our results demonstrate that *D. pulex* ephippial offspring can hatch without experiencing changing environmental cues. The timing of emergence from dormancy also appears to be maternally derived, with siblings from the same ephippia more often hatching at the same time even after separation. We observe putative transgenerational effects on reproduction dependent on when individuals emerged from dormancy, with individuals that emerged early delaying reproduction. Further research must be conducted in multiple lineages and populations to determine whether early hatching affects population dynamics in ponds, and whether similar results are found in additional outbred populations.

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Data availability The datasets generated during and/or analyzed during the current study are available in the Bergland-Lab-Mesocosm-2019 repository, https://github.com/rjp5nc/BerglandLab-Mesocosm-2019.

Declarations

Conflict of interest The authors have no relevant conflicting or competing interests to declare.

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