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ORIGINAL ARTICLE

Do genetic differences in growth thermal reaction norms maintain genetic variation in timing of diapause induction?

Erlend I. F. Fossen[1,2] | Joost A. M. Raeymaekers[1,3] | Sigurd Einum[1]

[1]Department of Biology, Centre for Biodiversity Dynamics, NTNU, Norwegian University of Science and Technology, Trondheim, Norway

[2]Animal Ecology, Department of Ecology and Genetics, Uppsala University, Uppsala, Sweden

[3]Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway

Correspondence
Erlend I. F. Fossen, Animal Ecology, Department of Ecology and Genetics, Uppsala University, Uppsala 752 36, Sweden.
Email: erlend.fossen@ebc.uu.se

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Abstract

1. An optimal timing for diapause induction through the sexual production of dormant propagules is expected in organisms with temporary populations. Yet, empirical studies often find high within-population genetic variation in the sexual production of such propagules, suggesting that this is a common feature of such organisms.
2. Here, we hypothesize that genetic variation in the propensity to produce dormant propagules, P_d, is maintained by a genotype-by-environment interaction in clonal reproductive rates, where fast-growing genotypes within an environment should delay diapause relative to slow-growing genotypes. From this, we derive two predictions. First, if reaction norms of clonal reproduction cross between two environments, the genetic correlation of P_d between these environments should be negative. Second, the correlation between plasticity values of clonal reproduction and P_d should be negative.
3. We tested these predictions by quantifying ephippia production in genotypes of a population of the facultative sexual cladoceran *Daphnia magna* at two temperatures. The population biomass at the onset of ephippia production was used as a measure of P_d, whereas juvenile somatic growth rate was used as a proxy for clonal reproductive rate. Plasticity for both measurements was derived from thermal reaction norms.
4. Our results did not support either prediction, as neither the genetic correlation of P_d between environments, nor the correlation between plasticity values of growth and P_d were found to be significant.
5. Our results suggest that genetic variation in the timing of diapause is not maintained by genetic differences in thermal clonal reproduction reaction norms. We propose as an alternative hypothesis that if there is variation across years in how the environment deteriorates over a season, fluctuating selection may favor genotypes with different P_d between years.

KEYWORDS

diapause, genotype-by-environment interaction, reaction norm, resting eggs, temperature

1 | INTRODUCTION

The timing of reproduction is a crucial life history trait (Stearns, 2000). This is particularly true for many organisms living in temperate environments, where conditions for successful growth and reproduction are limited to certain time horizons (Gotthard, 2001). These time horizons can be interspersed by periods preventing survival (e.g. winter or dry seasons), in which case production of dormant propagules is required. Examples of these dormant propagules are seeds in plants and diapause eggs in animals like killifish (Murphy & Collier, 1997), cladocerans (Frey, 1960), rotifers (García-Roger et al., 2017), copepods (Holm et al., 2018) and some insects (e.g. silkworms, Tirelli, 1946). For these organisms, the timing of the switch from somatic growth and/or asexual reproduction to the production of dormant propagules has strong fitness consequences, and there is an optimal timing of sexual reproduction (Cohen, 1976). Delaying onset of sexual reproduction allows for more time to grow and higher reproductive output per reproductive event, but comes at the cost of reducing the number of reproductive events or risking reproductive failure (Furness et al., 2015; Weis et al., 2014). Organisms with facultative parthenogenesis, which can switch between asexual and sexual reproduction (e.g. cladocerans [Taylor & Gabriel, 1993; Gerber et al., 2018], rotifers [Serra et al., 2008; García-Roger et al., 2017] and aphids [Simon et al., 2002]), represent a special case. For these, the optimal timing involves an allocation trade-off between investing in biomass through repeated events of clonal reproduction versus ensuring production of dormant propagules before the growing season ends.

Genetic variation in the timing of flowering and subsequent seed production is commonly observed in plants (e.g. Bourion et al., 2002; Franks et al., 2007; Hara & Ohsawa, 2013). Similarly, for facultative parthenogenetic animals which typically switch from asexual to sexual reproduction when environmental conditions deteriorate (but see Serra et al., 2004 for alternative explanations where the timing of sexual reproduction matches favorable environmental conditions and optimal male-female encounter rates), there is genetic variation in the propensity to produce dormant propagules (P_d) in a given environment, suggesting that genotypes vary in their environmental cue thresholds (e.g. Carmona et al., 2009; Deng, 1996; Gilbert, 2002; Roulin et al., 2015; Yampolsky, 1992). In a seasonal environment, such variation in cue thresholds should translate into variation in timing of the switch. Thus, given the predicted optimal switch time within a population, explaining the maintenance of such genetic variation in P_d is a challenge. Whereas diversified bet-hedging may produce phenotypic variation within genotypes as an adaptation to unpredictable environmental variation (Botero et al., 2015; Tufto, 2015), it should not maintain variation among genotypes. Environmental variation may however contribute to maintaining genetic variance non-adaptively through imposing temporally fluctuating selection and hence preventing fixation of optimal alleles (Sasaki & Ellner, 1997). Thus, one possible explanation for the genetic variation in P_d is that the way by which the environment deteriorates throughout the season varies across years, which in turn may cause the optimal P_d to vary.

An alternative hypothesis for maintaining genetic variation in P_d is that different genotypes are adapted to different environmental conditions that occur during different parts of the season, and thus show different responses to environmental gradients in their ability to accumulate biomass (i.e. genotype-by-environment [G × E] interactions in somatic growth or clonal reproduction, e.g. Carvalho, 1987; Fossen et al., 2018; Kingsolver et al., 2004). If this G × E interaction is strong, it can generate an "ecological crossover" where different genotypes are superior in different environments (Ellner & Hairston, 1994; Gillespie & Turelli, 1989; Higginson & Reader, 2009; Turelli & Barton, 2004). This should lead to corresponding differences in P_d across environments, and different optimal timing of making the switch from asexual to sexual reproduction in an environment that declines in quality towards the end of the season (Figure 1). For example, a genotype with low clonal reproduction rate in cold environments would benefit from having a higher P_d when exposed to a low temperature, compared to a genotype that is able to maintain a high clonal reproduction at that low temperature. Similar patterns have been observed for other genetically correlated traits, where these show corresponding G × E interactions (e.g. Prati & Schmid, 2000; Stinchcombe et al., 2004; Mills et al., 2007). For example, Prati and Schmid (2000) showed a genetic tradeoff between flowering and rooting (i.e. between sexual reproduction and clonal growth), where flowering showed a G × E interaction that corresponded with a G × E interaction in rooting. Such a trade-off might also apply in the case of clonal versus sexual reproduction in facultative parthenogenetic animals, because the same female reproductive organs are used for both types of reproduction. Thus, according to this hypothesis, two predictions can be derived regarding the patterns of genetic variance in P_d (Figure 2). First, if there is ecological crossover in clonal reproductive rates (Figure 2a), genotypes have similar reproduction rates in an intermediate environment. From an optimality perspective this also means that they should have similar values of P_d in the same intermediate environment (Figure 2b). Moving in one direction away from this intermediate environment, genotypes that increase clonal reproduction relative to other genotypes should simultaneously reduce their relative values of P_d. This in turn should lead to a negative genetic correlation between P_d across environments (Figure 2c). Second, there should be a negative genetic correlation between the plasticity value of P_d and the plasticity value of clonal reproduction (Figure 2d). The plasticity value is here defined as the slope of the trait reaction norm. The second prediction is based on the assumption that for a given genotype, the direction of change in P_d across environments should be opposite of the direction of change in clonal reproduction, while the relative magnitude in change should be similar (e.g. a genotype with a steeper positive clonal reproduction slope compared to other genotypes is predicted to have a steeper negative P_d slope than other genotypes). Considering that clonal reproduction and P_d are under a trade-off to maximize final dormant propagule production, having steeper slopes

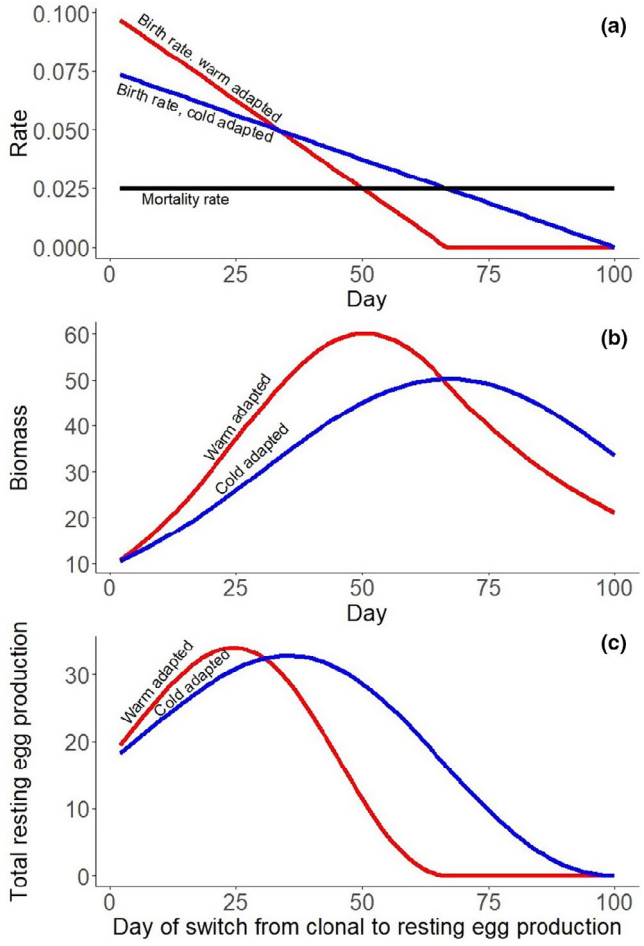

FIGURE 1 A modelled example of the temporal dynamics towards the end of the season (i.e. under declining temperatures) for an organism that switches from asexual (clonal) to sexual reproduction (production of dormant propagules). The population consists of two genotypes that (a) differ in their response of birth rates to the declining temperature due to a strong G × E interaction (i.e. ecological crossover). (b) In the absence of switching, the two genotypes reach their peak biomass at different days. (c) The total (cumulative) production of dormant propagules by the end of the season as a function of the day that the genotype makes the switch. For these latter calculations, no asexual production occurs after the switch is made, and hence population biomass declines according to the mortality rate. The following models and parameter values are used (a) Birth rate cold adapted = 0.075 − 0.00075 × Day, Birth rate warm adapted = 0.1 − 0.0015 × Day, Mortality rate = 0.025, (b) $\text{Biomass}_{i,t+1,} = \text{Biomass}_{i,t} \times (1 + \text{Birth rate}_{i,t} - \text{Mortality rate})$, where *i* indicates genotype, $\text{Biomass}_0 = 10$, (c) Dormant propagule production $_{i,t}$ = Biomass $_{i,t}$ × Birth rate $_{i,t}$ if t ≥ switch day, otherwise 0

implies investing a lot in one or the other trait, depending on the environment.

To increase the understanding of what maintains genetic variation in the timing of diapause induction we tested these predictions by studying a population of the facultative sexual cladoceran *Daphnia magna*, a species that is known to harbor pronounced genetic variance in dormant propagule production (Roulin et al., 2015; Yampolsky, 1992). The population used originates from a pond at its northern distribution limit, where production of dormant propagules during autumn is expected to be crucial for overwintering fitness. Using a set of ten genetically distinct genotypes, we ran population growth experiments at different temperatures and quantified production of dormant propagules through ephippia counts. Population density is one important cue that *Daphnia* use to switch to ephippia production (Gyllström & Hansson, 2004; Kleiven et al., 1992). The population density, measured as population biomass, required to trigger this switch was therefore used as an inverse measure of P_d at the different temperatures. Furthermore, thermal reaction norms of juvenile somatic growth rates for the same clones (Fossen et al., 2018) were used to test for a genetic correlation between environmental cue thresholds and clonal reproduction. For *Daphnia*, there is a close covariance between juvenile somatic growth rate and clonal reproduction rate across temperatures and food rations (e.g. r^2 = 0.95 in Rinke & Petzoldt, 2003; r^2 = 0.55 − 0.99 in Lampert & Trubetskova, 1996). Thus, thermal reaction norms of somatic growth rate represent a good proxy for reaction norms of clonal reproduction.

2 | METHODS

2.1 | Study animals and husbandry of stock cultures

Ephippia of *Daphnia magna* Straus, 1,820, which contain up to two sexually produced dormant propagules, were collected in November 2014 from the surface sediment of a shallow pond at Værøy Island (Sandtjønna, 1.0 ha, 67.687°N 12.672°E), northern Norway. This pond freezes over during winter and its mean daily water temperatures during summer fluctuates between 10–20°C (Figure 2 in Fossen et al., 2018). Ten genotypes, hatched from separate ephippia in December 2014, were cultured separately under common garden conditions for 2–3 years (>50 asexual generations). These genotypes, hereafter referred to as clones, vary genetically in thermal plasticity of life-history traits (Fossen et al., 2018). Daphnids were kept in 250 ml jars containing a modified ADaM medium (Klüttgen et al., 1994, SeO_2 concentration reduced by 50%, sea-salt increased to 1.23 g/L) at 17°C with a 16L:8D photoperiod, and the medium was changed weekly. Cultures contained five female adults per 250 ml jar and were fed three times a week with Shellfish Diet 1,800 (Reed Mariculture Inc.) at a final algae concentration of 4×10^5 cells m/L.

2.2 | Experimental design

Daphnia use several environmental cues to switch from clonal growth and reproduction to production of dormant propagules, including population density/food abundance, photoperiod, and temperature (e.g. Deng, 1996; Gyllström & Hansson, 2004; Kleiven et al., 1992; Slusarczyk & Rybicka, 2011). Of these, we kept photoperiod constant (16L:8D, representing autumn conditions in the

population's native environment), while experimentally manipulating temperature. The experiment (January – June 2017, where experimental populations were started across 23 dates) consisted of two temperature treatments (12 and 22°C). These temperatures were chosen based on an exploratory study (Appendix S1) that showed that they are 5°C below and above the temperature that triggered a change in mean ephippia production (Figure S1), and should enable testing of our predictions.

A total of 163 replicate populations were studied, with 7–9 replicates per clone per temperature (Table S1). Clones were kept in replicated lines at their experimental temperatures for two or more asexual generations prior to experiments to ensure acclimation. A single female juvenile (<24 hr old from the second clutch) was used to initiate each replicate experimental population. All temperature treatments (acclimation and experiment) were created by placing 250 ml jars in climate cabinets (IPP 260plus; Memmert). Throughout the acclimation and experimental period, animals were fed temperature-specific food concentrations every second day (concentrations: 12°C, 2.00×10^5 cells m/L; 22°C, 3.24×10^5 cells m/L) and the medium was refreshed by sieving every 8 days at 12°C and every 4 days at 22°C. This food regime represents ad libitum concentrations when the population size is low (Appendix S2), and ensures comparable starting conditions across temperatures. The same food regime was used in Fossen et al. (2018) to obtain somatic growth rates, thus making these two studies directly comparable. An alternative food regime with equal food concentrations and medium refreshments across temperatures was considered an option, but would result in suboptimal starting conditions for growth at one of the temperatures, less comparable water quality across temperatures, and results that are less comparable to the somatic growth rate estimates from Fossen et al. (2018). Considering that the predictions are tested based on the pattern of genetic variance within temperatures (see Statistical Analyses section), different food regimes across temperatures should not affect the conclusions.

Each experimental population was checked daily until both the second clutch was born and until the shedding of the first ephippium. During this phase, we obtained the timing and number of offspring of the two first clutches, and the timing of the onset of ephippia production. After this phase, and at the onset of ephippia production, we recorded a six seconds video of each population at every second medium change (camera: Panasonic DMC-TZ25, Full HD, 1,920 × 1,080 pixels). For the video, all animals of a population were transferred to a transparent glass baking dish, which was put on top of a light table to obtain a high contrast between animals and the background. These videos were later used to estimate population sizes and body size distributions over time using the R-package *trackdem* v. 0.3.1 (Bruijning et al., 2018). Videos made by us ($N = 153$) under the same conditions as described above were used by Bruijning et al. (2018) to evaluate the accuracy of *trackdem*. This analysis revealed highly accurate and unbiased estimates of the population size (Figure 3 in Bruijning et al., 2018). *Trackdem* uses automated particle tracking to keep track of the number of moving particles (here: individual daphnids) of potentially different size, in addition to outputting the size of the particles (number of pixels). By taking videos of animals that were not from the experiment, but raised under the same conditions, we obtained an equation for the relationship between dry mass (DM, mg) of individual animals and their particle size ($DM = -0.00635 + 0.00100 \times$ particle size, $n = 21$, $R^2 = 0.953$). The dry mass of these animals was first estimated from their carapax length (CL, mm) using a known length – dry mass relationship for our population ($DM = 0.00535CL^{2.72}$, Yashchenko et al., 2016). Thus, using videos from the experiment and estimated individual particle sizes, we could estimate total population size, the number of adults (animals >0.075 mg dry mass, based on size at maturity in Fossen et al., 2018) and the total biomass at any time a video was recorded. During each medium change, all shed ephippia were counted and removed (to prevent dormant propagules from hatching during the experiment). The experiments were terminated on day 128 at 12°C and on day 56 at 22°C, based on the results from the exploratory study (Appendix S1). This corresponds approximately to twice the time it took populations to reach peak population density (where ephippia production also peaked) in the exploratory study.

2.3 | Trait measurements

2.3.1 | Ephippia production

Ephippia production in *D. magna* involves production of dormant propagules that require fertilization by males to be viable. Thus, for a given clone, producing few ephippia could potentially be compensated by predominately producing males (that fertilize receptive females from other clones), resulting in a negative correlation between production of males and production of ephippia. In the presence of such alternative reproductive tactics, a high production of males could be an alternative way to achieve genetic contribution to the diapause stage at the population level. Our design that kept clones separately could then potentially introduce noise when using ephippia production as a measure of P_d. However, a previous study showed no such correlation among clones within populations of *D. magna*, and rather a positive correlation among populations (Roulin et al., 2015). Furthermore, we tested for this possibility in our own population by calculating the proportion of males of each population at the end of the exploratory study (Appendix S1). Although the proportion of males (mean [*SD*] = 0.025 [0.037]) differed significantly among clones (logistic GLM with clone as fixed effect, $p < 0.001$), there were no significant genetic correlations between the proportion of males and total ephippia production at any temperature (range: [−0.24, 0.23], $p > 0.5$ for all temperatures). This is a common finding for *Daphnia* (e.g. Lampert et al., 2012; Roulin et al., 2015; Yampolsky, 1992). Finally, the observation that an approximately 50:50 sex ratio is attained in populations under strong stimuli by production of alternating male and female clutches within females (Hobæk & Larsson, 1990), suggests that the presence of

alternative reproductive tactics is unlikely. Thus, we only consider ephippia production in further analyses.

To get a measurement of ephippia production that is comparable across temperatures, we calculated ephippia produced per generation for each population as follows: $\text{ephippia per generation} = \frac{\text{total number of ephippia}}{\text{duration/generation time}}$, where duration is the duration of the time series, and the average time to first clutch of each temperature was used as a proxy for temperature-specific generation time. The total number of ephippia is given in Figure S2a.

2.3.2 | Estimating P_d

Population density and food availability are known cues used by *Daphnia* to switch from growth to ephippia production (Gyllström & Hansson, 2004; Kleiven et al., 1992). Thus, population biomass, which should better represent food availability than population density, should also be a valid cue for the switch. Thus, population biomass at the onset of ephippia production (Figure S2b) was used as an inverse measure of P_d (i.e. a high population biomass required to trigger the switch to ephippia production represents a low P_d). To make this comparable across temperatures (which differed in food rations) we divided this measure with the temperature specific food rations. By making this normalization we assume that food availability, rather than biomass per se, is the cue that triggers ephippia production. While this may not completely control for differences in food ration, we note that all populations became food limited when reaching high population density and produced ephippia. Consequently, P_d could be estimated for each population. Furthermore, the pattern of genetic variance (e.g. ranking of clones) within temperatures is not affected by this normalization of P_d. Although we did not have biomass measurements in the exploratory study (Appendix S1), a correlated measure (population density at the onset of ephippia production, Figure S3a,b) was positively genetically correlated across exploratory versus main experiments (Figure S3c,d), indicating that this measurement is repeatable across experiments.

2.3.3 | Clonal reproduction

We used clone-specific estimates of thermal reaction norms for juvenile somatic growth rate, obtained from Fossen et al. (2018), as a proxy for thermal reaction norms of clonal reproductive rates (see Introduction). An ecological crossover in somatic growth rate was found by Fossen et al. (2018), where reaction norms of somatic growth rate crossed at 14°C (Figure S4). Clone-specific slope estimates of growth performance across temperatures were used to test our second prediction (as in Figure 2a,d).

2.4 | Statistical analyses

All analyses were conducted in R v.3.3.1 (R Core Team, 2014).

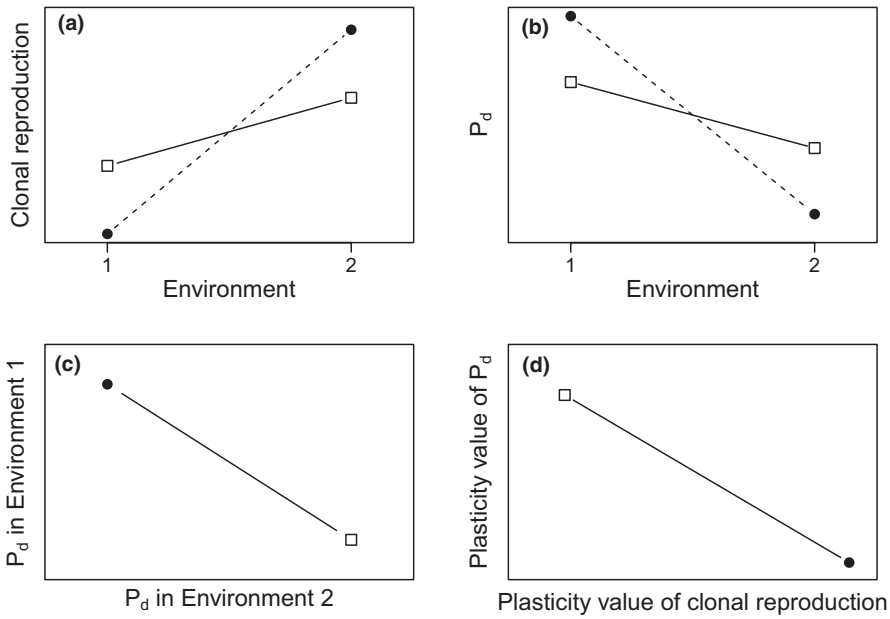

FIGURE 2 Predictions following the hypothesis that variation in reaction norms of clonal reproduction can maintain genetic variation in the propensity to produce dormant propagules (P_d). If there is a strong G × E interaction with ecological crossover in clonal reproduction (a, two genotypes represented by different symbols), reaction norms for P_d are expected to also show ecological crossover (b). Consequently, a negative genetic correlation is predicted between P_d in the two environments (c). Furthermore, we predict a negative genetic correlation between the plasticity values of these traits, where the plasticity value is defined as the slope of the trait reaction norm (d). This follows from the hypothesis that genotypes that experience a stronger decrease in clonal reproduction when going from environment 2 to environment 1 should also have a higher increase in P_d

2.4.1 | Total ephippia production – temperature response, genetic variance and correlation with P_d

We used linear mixed-effect models with the package lme4 (v. 1.1–7, Bates et al., 2015) to test for genetic variance in total ephippia production across the two temperatures. Ephippia per generation was the response variable, and temperature was included as a categorical predictor variable. Clone and start date (of experimental populations) were used as random effects. An interaction between clone effect and temperature effect was assumed in the full model (i.e., among-clone variance could vary with temperature), as well as a clone effect on the intercept. Start date was assumed to only affect the overall intercept. Akaike information criterion corrected for small sample sizes (AICc) were used in model selection and to access significance of effects (factors that only showed up in models >2 ΔAICc were considered non-significant). Full models with different random effect were first compared using restricted maximum likelihood (REML), and then the best random effect structure was used when comparing models with different fixed effects using maximum likelihood. Lastly, parameter estimates were obtained from the best model fitted with REML. Pseudo-R^2 values were calculated as the squared correlation coefficient between fitted values from the model and observed values.

To test if having a smaller P_d resulted in lower total ephippia production, we calculated genetic correlations between the two traits within each of the two temperatures, using temperature specific clonal means.

2.4.2 | Prediction 1: G × E interactions and a genetic correlation between P_d across environments

To estimate genetic variance and test for G × E interactions in P_d, we used mixed effect models with biomass per food abundance at the onset of ephippia production as the response variable. The same models and model procedures were applied as for ephippia production (see above). We then proceeded with testing for a negative genetic correlation between P_d across the two temperatures (as in Figure 2c). We note that even if the estimated biomass per food abundance does not completely control for differences in food ration across temperatures, the mean-standardized genetic variance and order of clones within temperatures do not change due to the normalization of P_d, and neither does the genetic correlation across temperatures (data not shown).

2.4.3 | Prediction 2: Genetic correlation between the plasticity values of P_d and of clonal reproduction

Our second prediction was that there should be a negative genetic correlation between the plasticity value of P_d and the plasticity value of clonal reproduction (Figure 2d). We quantified the plasticity value of P_d as the negative of the slope (i.e. slope multiplied with −1) between biomass per food abundance at the onset of ephippia production and temperature, such that a clone with a large positive slope-value requires a relatively stronger population-density cue at low temperatures than at high temperatures. As for prediction 1, since genetic variance within temperatures is not affected by the normalization of P_d, neither is the genetic correlation between plasticity values affected (even though the reaction norm slopes change).

2.4.4 | Evolutionary potential

To get an estimate of the population's evolutionary potential that is comparable across traits, populations and species, the broad sense evolvability (clonal variance/mean^2) was calculated within temperatures (Hansen et al., 2003, 2011; Houle, 1992). This was done for both ephippia production and P_d. Evolvability is a measurement of the expected percentage change in a trait per generation under a unit strength of selection, and is (in contrast to heritability) independent from environmental variance (Hansen et al., 2011).

3 | RESULTS

3.1 | Total ephippia production – temperature response, genetic variance and correlation with P_d

Ephippia production was on average about eight times larger at 12 than 22°C (Figure 3). We found a significant G × E interaction in ephippia production (Table S2), with higher broad sense evolvability at 12°C (clonal variance [V_{clone}] = 51.34 [ephippia per generation]^2, evolvability = 14.27%) than at 22°C (V_{clone} = 0.08, evolvability = 2.74%). Variance due to start date was 12.67 (ephippia per generation)^2, the residual variance was 19.49, and the model pseudo-R^2 was 0.86. Furthermore, the variation in ephippia production among clones was highly repeatable across exploratory versus main experiments at 12°C (Figure S1b), but not at 22°C (Figure S1c).

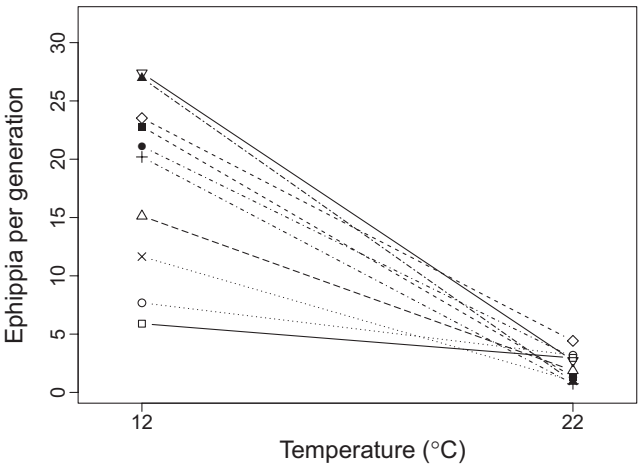

FIGURE 3 Ephippia production per generation across temperature for 10 clones of a population of *Daphnia magna*. For procedures on calculation see Methods. Each point is the mean of a clone, represented by different symbols

Ephippia production at 12°C increased for all populations after about 60 days (Figure S5), shortly after peak population density (Figure 4). At 12°C but not at 22°C, P_d was positively genetically correlated with ephippia production (Figure S6).

3.2 | Prediction 1: G × E interactions and a genetic correlation between P_d across environments

P_d showed a significant G × E interaction with temperature, with some clones having a particularly low propensity to produce dormant propagules at low temperatures (Figure 5a, Table S2). The overall broad sense evolvability was higher at 12°C (clonal variance [V_{clone}] = 1664.4 [ng/[cell ml^{-1} day^{-1}]]^2, evolvability = 35.45%) than at 22°C (V_{clone} = 2.2, evolvability = 0.08%). The model pseudo-R^2 was 0.75, variance due to start date was 117.0 (ng/[cell ml^{-1} day^{-1}])^2 and the residual variance was 414.5. However, in contrast to the prediction of a negative genetic correlation between P_d at 12 versus 22°C, the genetic correlation of the trait across temperature was not significant (Figure 5b; r = 0.41, t_8 = 1.26, p = 0.243).

3.3 | Prediction 2: Genetic correlation between the plasticity values of P_d and the plasticity of clonal reproduction

While we expected the plasticity value of P_d to decrease with increasing plasticity value of somatic growth rate, the relationship between the traits was not significant (Figure 6; r = 0.29, t_8 = 0.855, p = 0.417).

4 | DISCUSSION

To test if genetic differences in thermal clonal reproduction reaction norms can maintain genetic variation in the timing of diapause induction, we ran population growth experiments using clones from

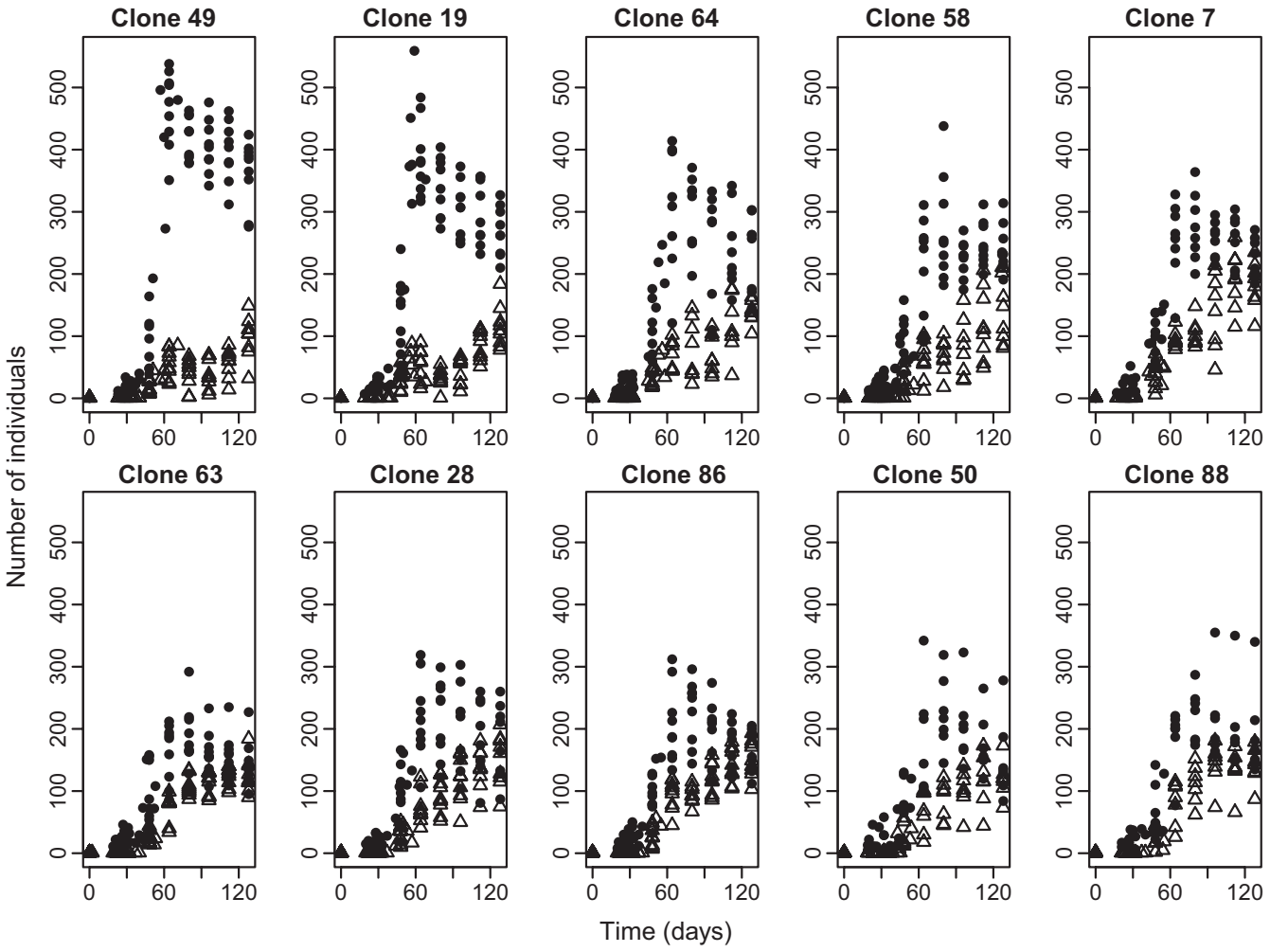

FIGURE 4 Time series showing an association between ephippia production (panels sorted by increasing ephippia production from top left to bottom right) and age structure in 10 clones of *Daphnia manga* at 12°C. Filled circles represent the total population size, triangles the number of adults. There are multiple observations per time point, because 7–9 replicates were used per clone

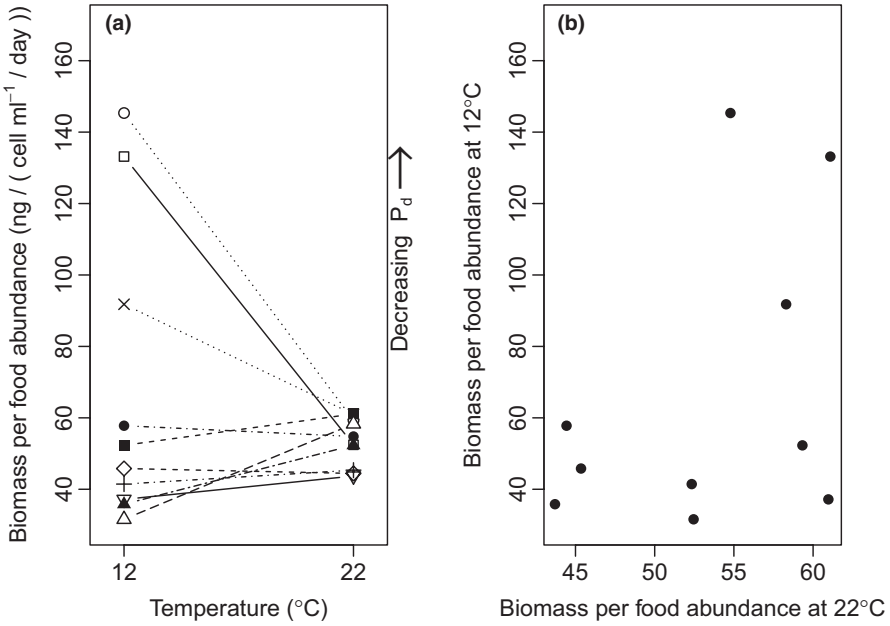

FIGURE 5 Propensity to produce dormant propagules (P_d) for 10 clones of a population of *Daphnia magna*. P_d was measured as population biomass per food abundance at the onset of ephippia production, where a low biomass represents a high P_d. There is a significant G × E interaction (a), but no significant genetic correlation across temperatures (b, $r = 0.41$, $t_8 = 1.26$, $p = 0.243$)

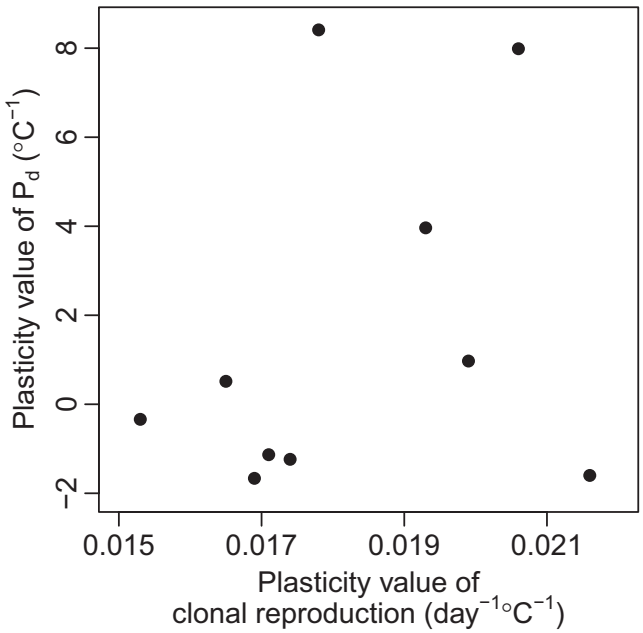

FIGURE 6 No significant genetic correlation ($r = 0.29$, $t_8 = 0.855$, $p = 0.417$) between thermal plasticity in the propensity to produce dormant propagules (P_d) and a clonal reproduction trait (somatic growth rate). Each point shows the plasticity value of the traits, representing the mean change in the trait per °C for a given clone of *Daphnia magna*. The plasticity value of clonal reproduction was measured as the slope of the traits reaction norm, whereas the plasticity value of P_d was measured as the negative of the slope between biomass per food abundance at the onset of ephippia production and temperature

a single population of *Daphnia magna*. We quantified relationships between ephippia production, the propensity to produce dormant propagules (P_d), and a growth performance trait that is closely correlated with clonal reproductive rates in *Daphnia* (juvenile somatic growth rate) across two temperatures. Our results showed that there was high genetic variance in P_d, and P_d was positively genetically correlated with total ephippia production at 12°C. Whereas both traits showed higher evolvability (>10% at 12°C) than the mean of that found in wild populations (about 1% for life history traits, Hansen et al., 2011), particularly at 12°C, the estimates were well within the range of previously reported values (<0.001% to >100%). The observed differences among clones at 12°C were also highly consistent with results from a separate exploratory study. Having found such large differences among clones in P_d, we tested a hypothesis derived from the observation that genotypes within the same population can be adapted to different seasons (e.g. Carvalho, 1987; Grosberg, 1988), i.e. that genotypes should match genotype-specific environmental variation in P_d with corresponding variation in their asexual production. We tested two predictions derived from this hypothesis. First, we predicted a negative genetic correlation between P_d across a temperature range that encompass ecological crossover in asexual production. Second, we predicted a negative genetic correlation between the thermal plasticity value of clonal production and the thermal plasticity value of P_d. Our results, showing no significant correlations, did not support either of the predictions. This lack of statistical support is unlikely to be due to low statistical power since the signs of the correlations were opposite of what was predicted from the hypothesis. This indicates that genetic differences in growth thermal reaction norms do not maintain genetic variation in P_d.

Although we failed to provide support for our predictions, certain conditions may have prevented this. For instance, if the temperature treatments were too similar, this could prevent detection of a negative correlation between P_d across temperatures (prediction 1). The low genetic variation in P_d at 22°C suggest that this may have been the case and that measuring P_d at higher temperatures could have shown a clearer ecological crossover. However, if there is a trade-off between clonal reproduction (and hence juvenile somatic growth) and ephippia production, growth and P_d should show similar crossover

points (see Introduction). Considering that thermal reaction norms for somatic growth rate cross at a temperature intermediate to our two experimental temperatures, P_d is also predicted to cross at an intermediate temperature. Moreover, ephippia production, which showed large differences at the tested temperatures and a similar pattern across temperature as P_d, also showed similar levels of phenotypic and genetic variation at 22°C compared to >22°C, suggesting that the temperature treatments should be sufficiently different to detect any genetic correlation between P_d across temperatures. Alternatively, if juvenile somatic growth rate was a poor measure of asexual population growth, a genetic correlation between the plasticity value of somatic growth and the plasticity value of P_d would not be predicted. This does however seem unlikely considering that this measure of somatic growth rate has previously been strongly linked to the intrinsic rate of population increase in our study species (Lampert & Trubetskova, 1996). Overall, for both predictions to fail simultaneously, two independent conditions would need to occur (too similar test-environments and using an inappropriate trait as a measure of clonal reproduction), which seems unlikely.

An alternative explanation for the maintenance of high genetic variation in P_d is that there is strong fluctuating selection (across years) on this trait. This can occur if there is variation across years in to what extent temperature regimes deviate from a smooth seasonal trend of spring increase and subsequent fall decrease. In years with high levels of stochastic deviation from such a trend, short periods of low temperatures are likely to be followed by subsequent increases that allows for continued high asexual growth under low population densities. This will lead to lost opportunities for genotypes that have a high P_d, and hence rapidly switch to ephippia production in the absence of strong competition (i.e. at low population density). Selection should then favor clones that have a low P_d and require stronger population density cues to commence ephippia production during such years. At the other extreme, for years with no stochasticity a decline in temperature is always followed by a further decline. In this situation, selection should favor clones that do not wait and see whether temperature conditions improve following a decline, but that have a high P_d and rapidly engage in ephippia production when exposed to a certain decline in temperature. Such maintenance of genetic variation due to temporally fluctuating selection is similar to other cases where the optimum trait value fluctuates over time (e.g. timing of diapause in copepods, Hairston & Dillon, 1990; morph color and pattern in stick insects, Nosil et al., 2018). These strategies may also explain the low genetic variance at 22°C compared to at 12°C. If temperature conditions are favorable, all clones should invest in growth until their growth rate is constrained to a certain level due to high competition, resulting in all clones requiring a similar and high population density threshold to initiate ephippia production. It is important to note that this potential explanation relates to predictability throughout the season within single years. Thus, it is different from variability and predictability in mean conditions (i.e. season length) across years. At this latter scale, an unpredictable environment should select for conservative bet hedging strategies (Starrfelt & Kokko, 2012), where genotypes with high P_d can be considered to be generalists and should be favored by selection due to their relative stable fitness across years, and hence high geometric mean fitness. Assessing the role of conservative bet hedging in evolution of P_d is beyond the scope of the current study, as this would require a comparison among populations with different degrees of among-year predictability in the environment (where higher P_d is expected in populations with more unpredictable environments).

Declines in temperature have been suggested as a cue for the onset of winter and for inducing diapause in freshwater zooplankton in temperate areas (Gyllström & Hansson, 2004; Slusarczyk & Rybicka, 2011). Our finding of ephippia production being much higher at 12 than at 22°C supports this. Yet, the median biomass per food abundance needed to induce ephippia production was similar at the two temperatures. This may seem surprising, but can be explained by the fact that ectotherms at higher temperatures have higher metabolic rates and therefore also higher food demands (also shown for our *Daphnia* clones, Fossen et al., 2019). This explanation is supported by the observation that populations were able to maintain a much higher mean biomass per food abundance after the first ephippium had been produced at 12°C (mean ± SE = 162.0 ± 3.3 ng/[cell ml^{-1} day^{-1}]) than that at 22°C (mean ± SE = 65.6 ± 1.5).

In this study, we tested if a strong G × E interaction in clonal reproduction can maintain genetic variation in the propensity to produce dormant propagules. Despite finding high genetic variance in P_d, and using a clonal reproduction trait (somatic growth rate) that showed a strong G × E interaction, we found no support for this hypothesis. P_d showed no genetic correlation across temperature (prediction 1), and there was no genetic correlation between the plasticity value of clonal reproduction and the plasticity value of P_d (prediction 2). We propose that genetic variance in P_d, and hence the timing of a switch from clonal to sexual reproduction in natural populations, can be maintained by fluctuating selection, favoring genotypes that respond either quickly or more slowly in producing dormant propagules when experiencing declines in temperature, depending on how stochastically the temperature changes throughout the season. It can be expected that the stochasticity of seasonal temperature change will vary from year to year, particularly for temperate populations, and even more so with increases in extreme climatic events because of climate change. Consequently, variation in the relative success of these strategies across years may explain genetic variation in timing of diapause induction for a wide range of annual organisms. Moreover, this variation may be beneficial for populations to survive increases in extreme climatic events.

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DATA AVAILABILITY STATEMENT

Data is archived in Dryad Digital Repository (https://doi.org/10.5061/dryad.0k6djhb19). The code for the model of Figure 1 is available upon request.

ORCID

Erlend I. F. Fossen https://orcid.org/0000-0002-5687-2743
Joost A. M. Raeymaekers https://orcid.org/0000-0003-2732-7495
Sigurd Einum https://orcid.org/0000-0002-3788-7800

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