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Genetic and environmental factors influence survival and hatching of diapausing eggs

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Abstract

To test the hypothesis that variation in hatching and survival of *Daphnia* dormant eggs is fostered by genetic differences among populations, rather than system-specific availability of environmental cues, I measured hatching and egg survival rates for *Daphnia* from 22 shallow, fishless ponds in the midwestern U.S.A. Although all eggs were incubated at a water depth of 0.75 m or less in their natal pond, hatching rates varied between 5% and 90% and survival rates of eggs remaining in the egg bank ranged from 7% to 72%. There was no significant relationship between hatching and environmental cues such as light, oxygen content, or conductivity, although a negative relationship with depth was observed. Reciprocal transplant experiments quantified genetic and environmental influences on dormancy and survival, revealing strong population-by-host environment interactions. Thus, plasticity to environmental cues and genetic or maternal effects likely interact to determine hatching and survival rates in the field.

For organisms living in seasonal or variable habitats, persistence depends on life history strategies that allow survival through unfavorable conditions. Dormancy is one common life history mechanism that allows propagules (e.g., seeds, diapausing eggs, statoblasts, quiescent adult stages) to ''escape through time,'' surviving in an inactive state until favorable conditions return. Yet, breaking dormancy can be risky: individuals may receive emergence cues and return to an active phase in a habitat that cannot sustain them long enough to reproduce. Given this risk, many taxa have strategies whereby some fraction of offspring does not break dormancy at the first opportunity (e.g., desert annuals, insects, crustaceans [Philippi and Seger 1989; Ellner 1997]). This ''prolonged dormancy'' spreads the risk across multiple generations, leading to the buildup of propagule or seed banks (Templeton and Levin 1979; Cáceres 1997). The optimal strategy is predicted to maximize the long-term geometric growth rate given the degree of unpredictability in the particular habitat (Cohen 1966). However, prolonged dormancy investment is one of multiple potentially coevolving life history strategies for individuals to maximize their long-term growth rate under variable conditions (e.g., dispersal among habitats and iteroparity [McPeek and Kalisz 1998]). Thus, whether a propagule breaks dormancy depends on the interaction of several factors including coevolution with other traits, optimal life history trade-offs (e.g., active and dormant survival probabilities), and exposure to the ecological conditions cuing emergence.

Early evolutionary models for dormancy assumed differences in survival in the dormant vs. the active states selected for specific germination rates of propagules (Cohen 1966, 1967). Further theoretical work incorporated density dependence (Bulmer 1984; Ellner 1985), spatial and temporal variability (Levin et al. 1984; Kalisz et al. 1997), and trade-offs with other important life-history traits such as dispersal, adult longevity, and propagule size (Venable and Brown 1988; Rees 1994; McPeek and Kalisz 1998). Particular theoretical attention has been paid to the relationship between optimal levels of prolonged dormancy and dispersal (Venable and Lawlor 1980; Levin et al. 1984; McPeek and Kalisz 1998). These models show a trade-off between dispersal and dormancy: as the level of dispersal into alternate habitats increases, the optimal germination fraction also increases. All this work suggests that prolonged dormancy contributes to optimizing long-term fitness in variable habitats, but may exhibit complex interactions with other traits. As a result, predicting the expected level of dormancy for organisms in a particular habitat is challenging.

Many aquatic organisms have some form of dormancy (Hairston and Cáceres 1996), and the observation of large egg banks for many taxa suggests that prolonged dormancy also occurs (Cáceres 1998; Brendonck and De Meester 2003). Eggs hatch in response to a variety of stimuli (e.g., predator chemicals, crowding, food quality, light, temperature [Gyllstrom and Hansson 2004]), and there is evidence for differential responses to hatching stimuli at the population level (Schwartz and Hebert 1987; De Meester and De Jager 1993; Zarattini 2004). In some cases, models developed for seed banks seem to fit aquatic organisms well. For example, Simovich and Hathaway (1997) showed that dormant cysts of anostrocan species living in ephemeral pools fit the conditions for a diversified bet-hedging strategy (Cohen 1966; Philippi and Seger 1989). However, for other habitats, prolonged dormancy may simply result from a lack of appropriate emergence cues (Brendonck 1996; Cáceres and Hairston 1998; Cáceres and Tessier 2003). As a result, the presence of egg banks may not represent an evolutionary strategy for persistence, but rather results from the local environment simply preventing all eggs from immediately hatching. Thus, depending on the nature of the ambient environment, both evolutionary and ecological factors may influence the hatching fraction of aquatic organisms.

Daphnia are common freshwater crustaceans that make dormant eggs, with populations often developing substantial egg banks (Cáceres and Tessier 2004). *Daphnia*

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dormant eggs typically hatch in response to light and temperature cues, and there is evidence that cue receptivity is partly under genetic control (Schwartz and Hebert 1987; Gyllstrom and Hansson 2004; Vandekerkhove et al. 2005). Previous work on lake-dwelling *Daphnia pulicaria*, however, found little evidence for genetic differentiation of hatching rates among populations in the field (Cáceres and Tessier 2003). These authors concluded that environmental conditions prevented access to the appropriate hatching stimuli, which limited hatching variation and effectively suppressed any observable genetic response or evidence of bet hedging. *Daphnia* in shallower habitats (e.g., temporary ponds), however, should have ready access to light and temperature hatching cues. Additionally, pond-dwelling *Daphnia* typically require annual re-establishment from the egg bank because of population crashes after pond drying or predation. Thus, although environmental cues likely continue to have strong effects on hatching rates, because those cues are more readily experienced in ponds, population-level differentiation in response to hatching cues may be more strongly expressed.

In this study, I examined natural variation in prolonged dormancy investment among *Daphnia* inhabiting small, fishless ponds in the midwestern U.S.A. Specifically, I addressed the following questions: (1) How variable are the hatching and dormant egg survival rates of *Daphnia* populations from shallow ponds? (2) To what extent is that variation controlled by environmental vs. genetic factors? (3) Do specific limnological variables or evolutionary trade-offs (e.g., with dispersal potential) influence hatching rates? To address these questions, I selected 22 ponds that varied in environmental characteristics. Ponds were temporary to semipermanent, but rarely dried before *Daphnia* dormant eggs were produced. In each pond, I surveyed the hatching fraction and dormant egg survival rate of the resident *Daphnia* population and used reciprocal transplant experiments in the field and artificial common gardens to explore genetic and environmental influences on hatching and survival.

Methods

Field experiments—To examine hatching and survival variation and estimate Cohen's (1966) hatching parameters for each population, I collected newly produced ephippia from five ponds in 2005 and 22 ponds in 2006 (Table 1). Allozymes and mitochondrial deoxyribonucleic acid suggest that *Daphnia pulex* dominated most ponds, although *Daphnia obtusa* was dominant in Center and Edge ponds and present in low numbers in Bridge South Pond (M. Allen unpubl. data). For each population, I used a 3-liter pitcher and 70-μm sieve to collect a 100+-liter live sample from the water column during peak ephippial production. Ponds were sampled between 20 May and 01 June 2005 and between 08 and 31 May 2006. In the laboratory, newly produced ephippia were sorted from live samples, dried, and stored at 4°C. After 4 months, dried ephippia were placed into six-well culture trays, which were covered with 200-μm mesh and sealed with a lid containing holes above each well (Cáceres and Tessier 2003). Trays contained 50–60 eggs (25–30 ephippia).

In fall 2005, two or three replicate trays were secured to their natal pond's sediment. Trays were placed approximately 0.3 m below the highest surface of the ponds (which were dry) and allowed to overwinter in the field. Additionally, enough eggs were available to place trays of Center Pond eggs into Edge and Top ponds, Edge Pond eggs into Center and Top ponds, and Top Pond eggs into Center Pond. Water covered the emergence trays by March 2006. Trays were removed in May 2006 and stored frozen until analysis. Once thawed, eggs were removed from their ephippium and scored as missing, present and viable, or present and inviable (Cáceres and Tessier 2003). Eggs were considered viable if they were solid green in color with an intact membrane and no interior degradation. The total number of ephippia recovered was often less than the total number placed in the field, most likely because ephippia were missed during visual inspection of the trays and the sediment therein under the microscope. In 2006, eggs collected from the 22 ponds were placed in two or three replicate hatching trays and returned to each natal pond in November or December, where they were allowed to overwinter. Those trays were removed in late May 2007 and again stored frozen until they could be scored.

In December 2006, I also established dual common gardens to better assess the extent of environmental vs. genetic control on hatching fraction. At both the Kellogg Biological Station Pond Lab (Michigan) and the Phillips Tract Natural Area (Illinois), three cattle tanks were filled with $\sim$ 0.5 m of well water. One tray from each pond (provided enough eggs were available) was secured to the base of each tank. Tanks were covered with mesh or chicken wire to prevent animals from disturbing the experiment. Because of the locations of the field stations, important hatching cues such as light intensity and temperature differed between the common gardens during April 2007 (e.g., mean daily April temperature: Urbana 4–16°C, Kalamazoo 2–13°C [National Climate Data Center 2009]). In late May 2007, trays were removed and stored with those from the field. If insufficient quantities of eggs were available for both common gardens, trays were placed in the common garden closest to the source of the eggs. In all, 14 populations were included in the Michigan common garden and 7 were included in the Illinois common garden. Five populations overlapped (Table 1).

Between 10 and 17 April 2007, I visited each pond once to measure dissolved oxygen, conductivity, and pH during the hatching period. Also, chlorophyll *a* content was estimated by filtering pond water through a 0.2-μm filter (Whatman GFF), extracting the chlorophyll in ethanol, and measuring the absorbance using a Turner Designs 700 fluorometer (Welschmeyer 1994). Water collected for total phosphorus content was analyzed following the molybdate–ascorbic acid extraction method (APHA 1980). Light was measured in the open, at and just below the pond surface, and at the depth of the hatching trays using a LI-185B photometer (Li-Cor). These light measurements were used to calculate a composite light variable, percentage ambient light reaching the hatching tray. Additionally, I measured the depth of the tray below the water surface, as this covariate could influence the hatching cues that the eggs experienced (i.e., light and temperature).

Table 1. Field locations and characteristics of populations included in the 2005 and 2006 studies. Variables: State, Illinois (IL), Indiana (IN), or Michigan (MI); Illinois common garden (ILCG) or Michigan common garden (MICG); Tray_d, depth of the hatching tray in meters; Light_tray, proportion of ambient light reaching the hatching tray; DO, mg L^{-1} dissolved oxygen measured by DO probe; Cond, conductivity in μS cm^{-1}; Chlorophyll *a* (Chl *a*) in the water column in μg L^{-1}; TP, total phosphorus in μg L^{-1}; Neighbors, number of ponds within 1 km during spring; Egg-filling, percentage of ephippial chambers containing eggs during initial collection. A (—) represents missing data. Edge Pond was not included in the 2006 study.

Pond Name	Pond ID	Latitude (N)	Longitude (W)	State	ILCG	MICG	Tray_d	Light_tray	DO	pH	Cond	Chl *a*	TP	Neighbors	Egg-filling
3 Rivers 2	3R2	41.84840	−85.75025	MI	No	Yes	0.57	0.219	9.3	7.1	108.0	2.946	81.6	8	100
Baby	Baby	42.58478	−85.41745	MI	No	Yes	0.28	0.790	8.44	7.2	348.0	0.418	43.7	7	100
Bridge South	BS	40.12212	−87.73674	IL	Yes	No	0.63	0.376	5.59	7.6	887.0	2.447	—	8	94
Busey	Busey	40.12868	−88.21310	IL	No	No	0.40	0.306	10.02	7.9	1005.0	6.002	126.6	3	86
Campground	Camp	42.32619	−85.33459	MI	Yes	Yes	0.28	0.370	5.60	7.6	334.0	3.878	295.1	7	92
Center	Center	40.13291	−88.14004	IL	Yes	No	0.20	0.850	8.10	7.5	545.0	0.732	103.8	1	93
Duffy Road 1b	DR1b	42.60260	−85.47924	MI	No	Yes	0.37	0.828	5.74	6.9	337.0	1.329	27.0	16	90
Duffy Road 2a	DR2a	42.60242	−85.47997	MI	No	Yes	0.50	0.409	6.27	8.2	510.0	0.323	278.8	16	100
Duffy Road 4	DR4	42.60443	−85.48932	MI	No	Yes	0.56	0.247	9.60	8.4	597.0	5.827	62.1	12	88
Edge	Edge	40.12945	−88.14165	IL	No	No	—	—	—	—	—	—	—	1	92
Engle	Engle	42.71543	−85.36863	MI	Yes	Yes	0.49	0.135	4.62	6.8	68.0	20.430	158.2	11	93
Erway 5b	Er5b	42.61503	−85.39925	MI	No	No	—	0.018	9.69	6.9	44.7	10.289	129.7	21	96
Fulton	Ful	42.10064	−85.31939	MI	No	Yes	0.25	0.285	7.24	7.4	260.0	1.891	325.2	5	86
Potato Creek 2	PC2	41.54020	−86.35744	IN	Yes	Yes	0.40	0.458	9.81	6.9	92.4	1.074	64.7	7	100
POVI	POVI	42.71878	−85.38793	MI	Yes	Yes	0.25	0.341	7.53	7.1	175.0	0.624	119.1	30	100
Rainbow	Rain	42.61642	−85.47557	MI	No	Yes	0.43	0.192	5.79	9.1	48.0	4.492	229.7	8	98
Robertson6	Rob6	42.74553	−85.42217	MI	Yes	Yes	0.25	0.423	5.95	7.2	268.0	1.582	41.1	14	92
Mallard	RWF2	41.71519	−89.18073	IL	No	No	0.44	0.688	9.74	9.6	205.0	1.603	86.7	8	98
Pothole	RWF5	41.70474	−89.19486	IL	No	No	0.70	0.341	7.36	7.6	12.7	5.829	271.4	7	92
Top	Top	40.24203	−87.78242	IL	No	No	0.38	0.655	5.75	7.6	360.0	1.242	56.2	6	94
West Gull	WG	42.41289	−85.43919	MI	No	No	0.69	0.078	9.96	8.3	129.0	7.769	138.8	3	100
Willow Slough C	WSC	40.96626	−87.52456	IN	No	No	0.40	0.230	10.38	7.1	154.0	1.007	66.1	3	89
Wildwood	WW	42.58858	−85.48839	MI	No	Yes	0.20	0.786	10.91	7.5	85.0	5.509	72.5	7	100

Data analysis—From the hatching data, I calculated two vital rates typically used in demographic models of egg bank dynamics: the hatching fraction and the dormant egg survival rate. Hatching fraction is the proportion of seeds or eggs that hatches in a given year (Cohen 1966). Typically, it is expressed as the number of hatched eggs (H_E) divided by two times the total number of ephippia recovered at the end of the experiment (two egg slots per ephippium) (T_E). However, because eggs are contained within an ephippium, I could not determine the exact number of eggs placed into the experiment. Initially, missing eggs result from a female shedding an empty or partially filled ephippium. For example, if 50 ephippia were placed into the field, there are at most 100 eggs since each ephippium can hold up to two eggs. However, some of those initial eggs may be missing. To correct for this, I dissected newly produced ephippia, collected concurrently with those placed in hatching trays, to quantify the proportion of initially missing eggs (M_I^*) in each population (18–25 ephippia or 36–50 egg slots examined per population). For ponds from 2005, these extra ephippia were preserved in ethanol before examination. Once dissected, I noted that the eggs were either present and appeared viable (greenish and whole) or were missing. As a result, I scored the eggs from the 2006 experiment as present or absent after storing them dry for 2 yr. On the basis of these data, I calculated the initial proportion of missing eggs as:

$$M_I^* = \frac{M_I}{M_I + V_I} = \frac{M_I}{T_I}, \quad (1)$$

where M_I is the number of egg slots that were empty in that initial screen, V_I is the count from the initial screen of eggs that were present and viable, and T_I is the total number of eggs that should be present if all ephippia contained two eggs. In the example with 50 ephippia, say that 90 eggs were present and 10 were missing. This would give an initial proportion M_I^* of 0.1. The proportion initially viable (V_I^*; Table 1) equals 1 minus the proportion initially missing, here, 0.9. In 2005, 90–98% of potential eggs were present, and in 2006 86–100% were present (Table 1).

Using this estimate, I determined a corrected count of hatched eggs at the end of the experiment (H_E) as the total number of missing eggs at the end of the experiment (M_E) minus the number of egg slots that were empty to begin with:

$$H_E = M_E - M_I^* \cdot T_E. \quad (2)$$

However, because the initial proportion of missing eggs in the trays (M_I^*) was an estimate, the corrected count of hatched eggs could be slightly negative if M_E was very low or M_I^* was high. This happened for 2 of 124 hatching estimates in 2006, and these counts were subsequently set to 0 (from -0.48 and -1.92). Finally, to determine the corrected hatching fraction, I divided the corrected count of hatched eggs by the sum of eggs that were viable, inviable, or hatched at the end of the experiment.

Of the eggs that do not hatch, some will survive in the sediment to hatch in subsequent years, whereas others will die. Mortality of these eggs may have occurred during or before egg development. The proportion surviving is calculated as the number of viable eggs recovered divided by the sum of the viable and inviable eggs recovered. Because some populations had very high hatching rates, the precision of these survival estimates is low because of the small number of eggs remaining dormant.

As hatching and survival rates of eggs from each population are proportions, I used generalized linear mixed modeling (GLMM) with binomial errors and a logit link function. Analyses were run in the GLIMMIX procedure in SAS 9.2 (SAS Institute). The procedure accepts positive noninteger values for counts and pools all the data from replicates within a cell (i.e., all replicate trays within a pond). The significance of each fixed variable was tested with type 3 *F*-tests with Satterthwaite degrees of freedom corrections. Significance of random effects (covariance parameters) was tested using likelihood ratio tests.

For both the 2005 and 2006 data sets, I had many more estimates of hatching and survival from ponds in the field than in the common garden experiments. As such, for each data set I used all of the data for populations incubated in their own pond to test whether there were differences in survival and hatching among populations (with pond as a random variable).

I used the 2006 data set to test the effect of environmental variables on hatching and survival rates. I first summed the hatched and total number of eggs across the within-pond replicates. This provided pooled count data for the binomial regression analysis with 22 replicates (one per pond). I used a generalized linear model (GLM) with an overdispersion correction to address four hypotheses for hatching: (1) increased conductivity reduces hatching (Spencer and Blaustein 2001), (2) increased light reaching the eggs increases hatching (Schwartz and Hebert 1987), (3) increased tray depth (a composite variable of light and temperature) decreases hatching, and (4) pH or dissolved oxygen concentration influences hatching (De Meester 1993; Brown 2008). For survival, I tested whether pH or oxygen concentration influenced egg survival rates (De Meester 1993). These variables showed little evidence of multicollinearity as all pairwise Pearson's $r < 0.7$ (McGarigal et al. 2000). I initially used multiple binomial regression with stepwise variable removal on all five hatching variables and their pairwise interactions to find the best-fit model using QAIC selection in R 2.9.2 (R Core Development Team) (there were not enough degrees of freedom to include higher-order interactions). However, as the best-fit model contained only one variable (the lowest QAIC and only significant variable), I present the results of univariate binomial regressions for each specific hypothesis. The best-fit model for survival contained both variables, but no interaction term. Given the range of the study, I also tested for a latitudinal effect on hatching and survival.

I used a two-way GLMM analysis to test the hypothesis that genetic-by-environmental interactions influenced survival and hatching of eggs. Using the reciprocally transplanted trays from Center, Edge, and Top ponds in 2005, I tested for interactions between environment and

population source for eggs incubated in the field. Population source and host pond were crossed and treated as random variables. Because of a missing cell (Top eggs hosted in Edge Pond), it was necessary to remove the interaction term from the survival analysis to achieve convergence. The 2006 common garden experiment was analyzed similarly using data from the five populations incubated in both common gardens. I treated the common gardens (CG) as a fixed variable because they represented the conditions at the ends of the region. Pond (P) and its interaction with CG were treated as random variables.

I used univariate binomial regression (GLM) to test the evolutionary hypothesis that dormancy and dispersal potential were negatively correlated (Venable and Lawlor 1980; Levin et al. 1984). I used the hatching data from the Michigan common garden, because I had 14 hatching estimates and the common garden provided more control of environmental variance than the field data. To measure the potential for dispersal among ponds, I calculated the total number of neighboring habitats within 1 km of each focal pond. Previous work has suggested that this distance is a good approximation of a local zooplankton dispersal kernel (Allen 2007). Neighboring habitat frequency was counted using both aerial photos and the National Wetlands Inventory (U.S. Fish and Wildlife Service 2006) in ArcGIS Desktop version 8.1 (ESRI), and by visual inspection on site. I also used the Michigan common garden data to examine other variables that might influence hatching and survival. For example, dormant egg quality might be influenced by the mother's health status. As such, I tested for relationships between hatching and chlorophyll *a*, total phosphorus, pH, and the egg-filling rate. Additionally, inbreeding might influence egg quality, so I tested for a relationship between habitat area (a potential proxy for inbreeding status) and hatching and survival rates. However, none of these relationships was significant, so I do not present the results here.

Results

Hatching rates in the field varied substantially among the ponds in both 2005 and 2006 (Figs. 1a, 2a). However, viable eggs remained in all populations after a year's incubation, suggesting prolonged dormancy (Figs. 1b, 2b). In 2005, hatching fraction ranged from 50% to 92% ($\chi^2 = 25.4$, $p < 0.0001$; Fig. 2a), whereas in 2006, between 5% and 90% of the viable eggs hatched ($\chi^2 = 521.7$, $p < 0.0001$; Fig. 1a). There was no latitudinal effect on the hatching rate ($F_{1,20} = 0.04$, $p = 0.85$). Additionally, there was no direct effect of conductivity ($F_{1,20} = 0.09$, $p = 0.77$), pH ($F_{1,20} = 0.32$, $p = 0.58$), percentage ambient light reaching the trays ($F_{1,20} = 2.08$, $p = 0.16$), or dissolved oxygen ($F_{1,20} = 0.13$, $p = 0.73$) on mean hatching fraction. However, trays further beneath the water surface had lower hatching rates (Fig. 3; $F_{1,19} = 4.43$, $p = 0.05$), and this variable had a significant negative correlation with percentage ambient light ($r = -0.54$, $p = 0.01$).

In 2006, dormant egg survival rates ranged from 7% to 72% (Fig. 1b; $\chi^2 = 95.09$, $p < 0.0001$). With only five ponds surveyed in 2005, survival did not differ ($\chi^2 = 0.26$, $p = 0.31$; Fig. 2b). However, estimates from populations with hatching fractions greater than $\sim$ 75% should be treated with caution, as hatching reduced the number of eggs contributing to estimates of viability past the first year to 10 or less. This was the case for most estimates in 2005. Oxygen concentration significantly reduced the likelihood of egg survival in the multiple regression, whereas pH had no effect (pH: $F_{1,19} = 2.13$, $p = 0.16$; oxygen: $F_{1,19} = 5.59$, $p = 0.03$).

The source of the eggs and the local environmental conditions influenced hatching and survival in the field reciprocal transplant experiment (Fig. 2c,d). The lower mean hatching rate of Top Pond eggs drove the main effect, as Center and Edge pond hatching fractions were not significantly different in the three environments. Both population ($\chi^2 = 2.88$, $p = 0.05$) and host environment ($\chi^2 = 2.71$, $p = 0.05$) significantly influenced hatching, but the interaction effect was not significant ($\chi^2 = 0.69$, $p = 0.20$). Survival rates were high in the field and similar among each of these populations. Thus, there was no effect of population ($\chi^2 = 1.04$, $p = 0.15$) or host environment ($\chi^2 = 1.30$, $p = 0.13$) on survival.

In the Michigan–Illinois common garden experiment, I found a significant interaction of population and environment for both hatching and survival (Table 2). Survival rates tended to be low in both common gardens, with the exception of Robertson6 (Fig. 4). The mean hatching rates were higher in the Illinois common garden (Fig. 4), but one population (Potato Creek 2 [PC2]) had the opposite trend. This population also had the lowest hatching rate in the field (Fig. 1a), with a high mortality of eggs (72% died rather than hatched or remained dormant). These high death rates were also observed in both common gardens (Illinois: 70%, Michigan: 67%). Higher death rates were generally observed in the Michigan relative to the Illinois common garden (contrast: $F_{5,19.38} = 27.09$, $p < 0.0001$). The Campground population had low egg death rates in both common gardens (Illinois: 0.14, Michigan: 0.37), which contributed to the higher hatching rates observed. Generally, the field-based estimates of hatching and survival were very different from and not correlated with those observed in the common garden (hatching: Spearman's $\rho = 0.27$, $p = 0.34$, survival: $\rho = 0.29$, $p = 0.31$; Figs. 1, 4).

Finally, I found a relationship between hatching and the number of neighboring habitats (potential for successful dispersal) in the field (Fig. 5; $F_{1,12} = 9.02$, $p = 0.01$). However, the relationship was in the opposite direction from that predicted by theory (Venable and Lawlor 1980; Levin et al. 1984; McPeek and Kalisz 1998). This relationship became nonsignificant when the pond with the most neighbors was excluded from the analysis ($p = 0.06$).

Discussion

The temporary populations in this study exhibited substantial hatching variability with fractions ranging from almost zero to near 100% in a single season. Additionally, viable eggs were recovered from every population after the

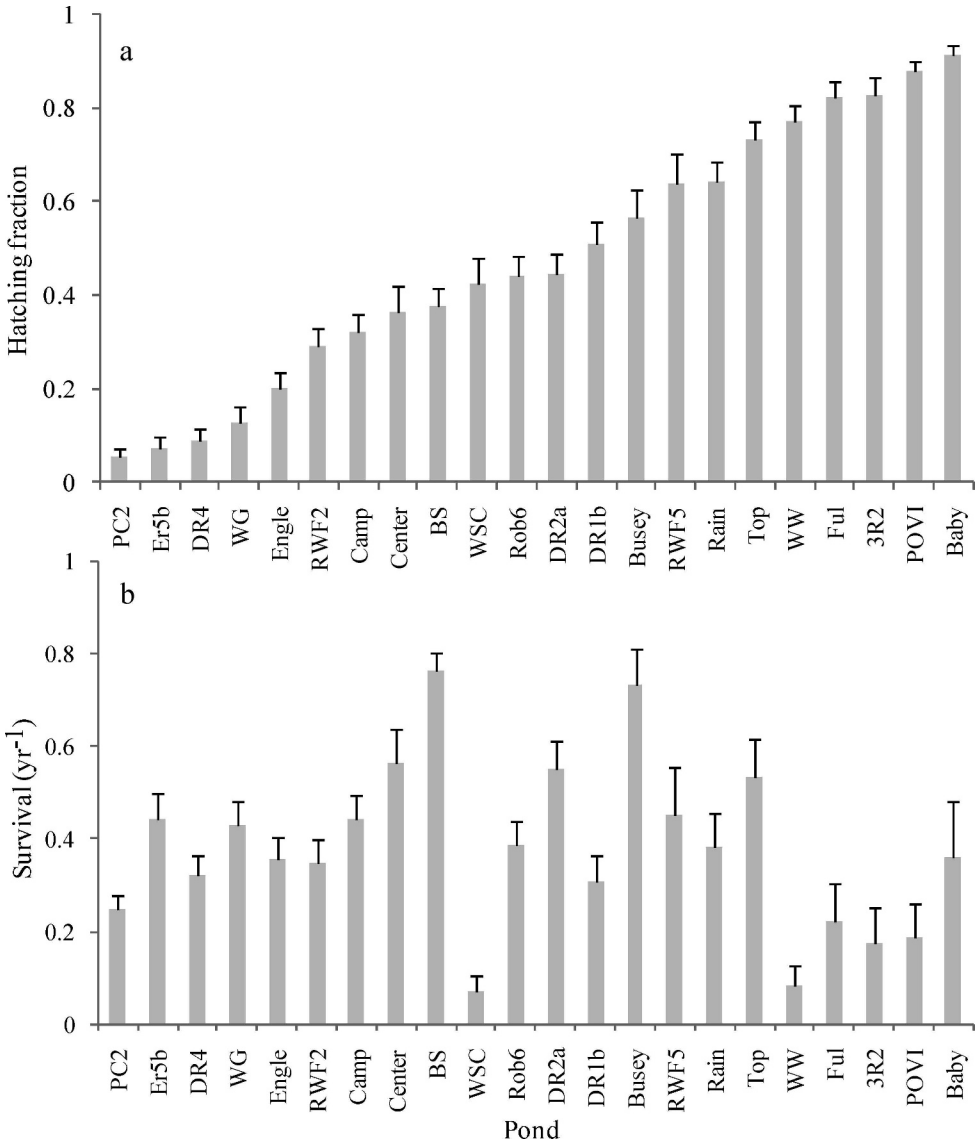

Fig. 1. Mean (a) hatching fraction and (b) dormant egg survival rate of field-collected eggs incubated in their own pond during 2006–2007. Both figures are sorted from lowest to highest hatching fraction. Error bars are the standard deviation of the predicted means from the statistical analysis. *See* Table 1 for legend of pond names.

hatching season, suggesting that eggs remain in the sediments in a prolonged dormant state. These experiments also suggest that a large proportion of variability could be attributed to environmental variation among sites. First, incubation depth—a composite variable partially accounting for temperature and light differences among incubation sites—was negatively related to hatching fraction. This suggests that eggs incubated farther below the pond surface experienced reduced exposure to hatching cues, a pattern also observed in lakes (Cáceres and Tessier 2003). Second, there were large differences in hatching rates between the two common gardens in 2006, with generally higher hatching and survival rates in the Illinois common garden. However, unlike previous work (Cáceres and Tessier 2003), the 2005 and 2006 data also provide evidence that genetic or maternal effects contribute to hatching variation. Populations incubated in common gardens had distinctly different mean hatching and survival rates from one another in many cases. Additionally, there was evidence for genetic- (or maternal) by-environmental ($G \times E$) effects in the 2006 experiment.

The reciprocal transplant and field experiments provide evidence that environmental factors act on hatching and survival. Previous work has shown that temperature and incident light cues are important for achieving maximum hatching rates (Schwartz and Hebert 1987; Pfrender and Deng 1998; Vandekerkhove et al. 2005). For shallow ponds, cues may be more readily accessible, but a variety of factors can influence relative cue exposure, including canopy cover, pond size, egg depth, sedimentation rate, and turbidity. Among-pond variation in measured environmental factors was substantial in this

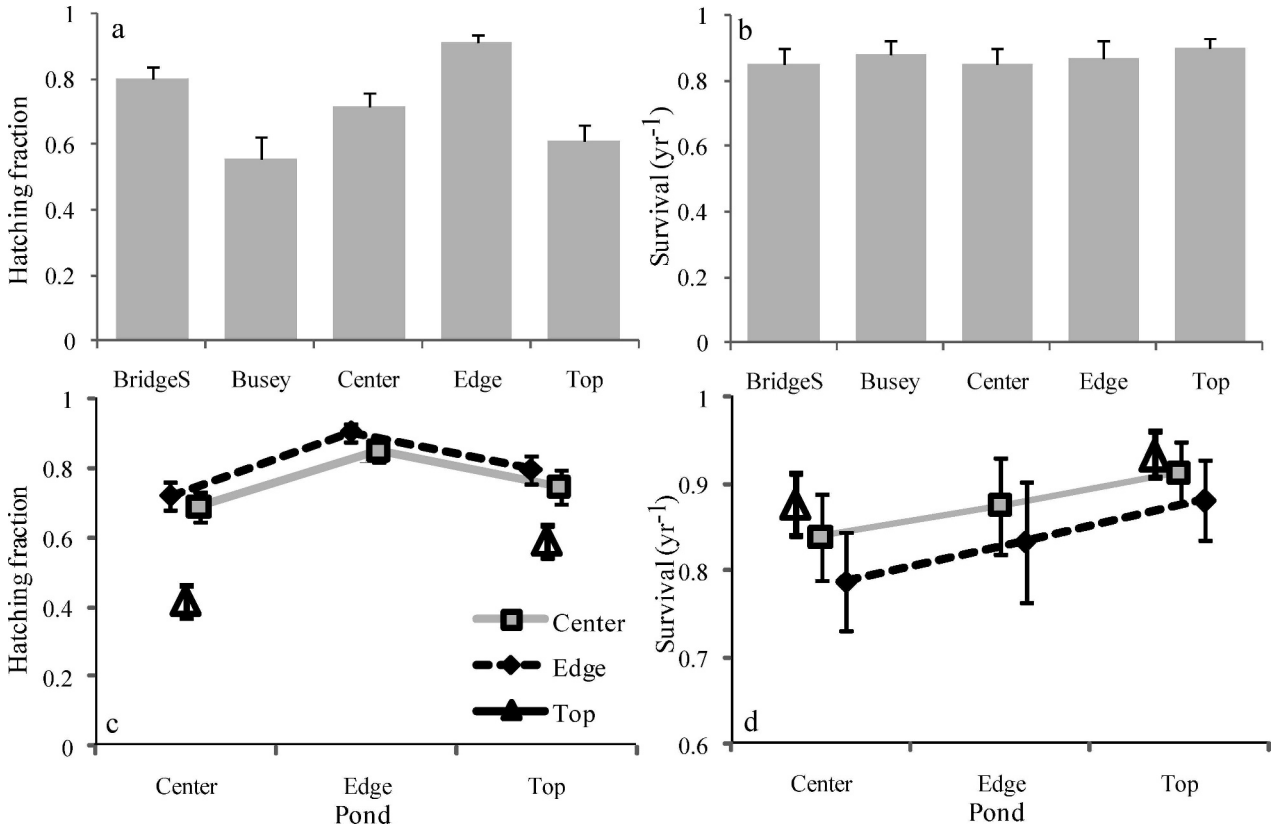

Fig. 2. Results from the 2005–2006 hatching and survival experiments. Mean (a) hatching fraction and (b) dormant egg survival rate of field-collected eggs incubated in their own pond. Hatching rates varied significantly among the ponds, but survival rates did not. Mean (c) hatching fraction and (d) dormant egg survival probability of field-collected eggs reciprocally transplanted among three ponds. Population and host environment significantly influenced hatching rate. There was no difference in survival among populations or environments. Error bars are the standard deviation of the predicted means from the statistical analysis.

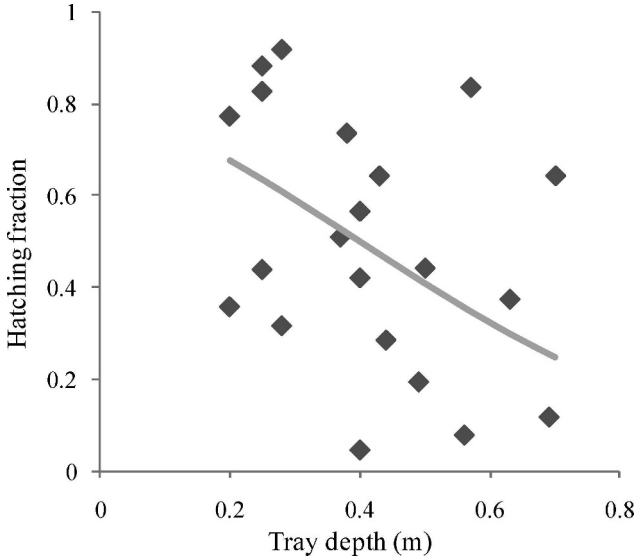

Fig. 3. Egg hatching fraction in response to tray depth for the 2006–2007 regional hatching experiment. Populations had significantly lower hatching rates when incubated farther below the surface of their host pond. The linear prediction was fit using a generalized linear model with binomial errors and a logit link function.

study. Yet, only tray depth explained any variation in hatching or survival among field populations (seasonal temperature data were not available). There are several possibilities for the lack of the expected direct response to incident light intensity. First, the environmental measurements at the hatching trays may be too coarse to capture the cues experienced by the eggs. Individual ephippia may experience different microenvironmental conditions, contributing to intrapopulation variation (e.g., the wells of some trays may fill with sediment). Second, because it was only possible to measure the environmental variables on one occasion, temporal variability in field conditions could not be accounted for. As a result, tray depth may be a better estimate of light conditions over time than the snapshot estimates of actual light intensity as measured in the present study. The observation that light intensity correlated with tray depth hints at such a relationship. Third, rather than light intensity, photoperiod may be a more important cue for hatching, and photoperiods were similar among ponds in the region. Alternatively, mean hatching temperature or temperature variation, or their interaction with light, may have been stronger environmental cues (Pfrender and Deng 1998; Arnott and Yan 2002; Vandekerkhove et al. 2005), as is the case for many anostrocan species in

Table 2. Reciprocal common garden experiment. Tests for the interactive effects of genetic (pond, P) and environmental (common garden, CG) variance on hatching and survival in the Illinois and Michigan common gardens. Fixed effects were tested by type 3 *F*-tests, and random effects [R] were tested with likelihood ratio tests. VC, variance component (standard error).

Effect	df	Error df	VC	F/χ^2	*p*
Hatching fraction					
CG	1	3.98	—	5.45	0.080
P [R]	1	—	0.43 (0.62)	0.59	0.22
P × CG [R]	1	—	0.69 (0.52)	48.39	<0.0001
Survival rate					
CG	1	3.65	—	1.31	0.32
P [R]	1	—	0.52 (0.88)	0.39	0.27
P × CG [R]	1	—	0.97 (0.91)	10.33	0.0007

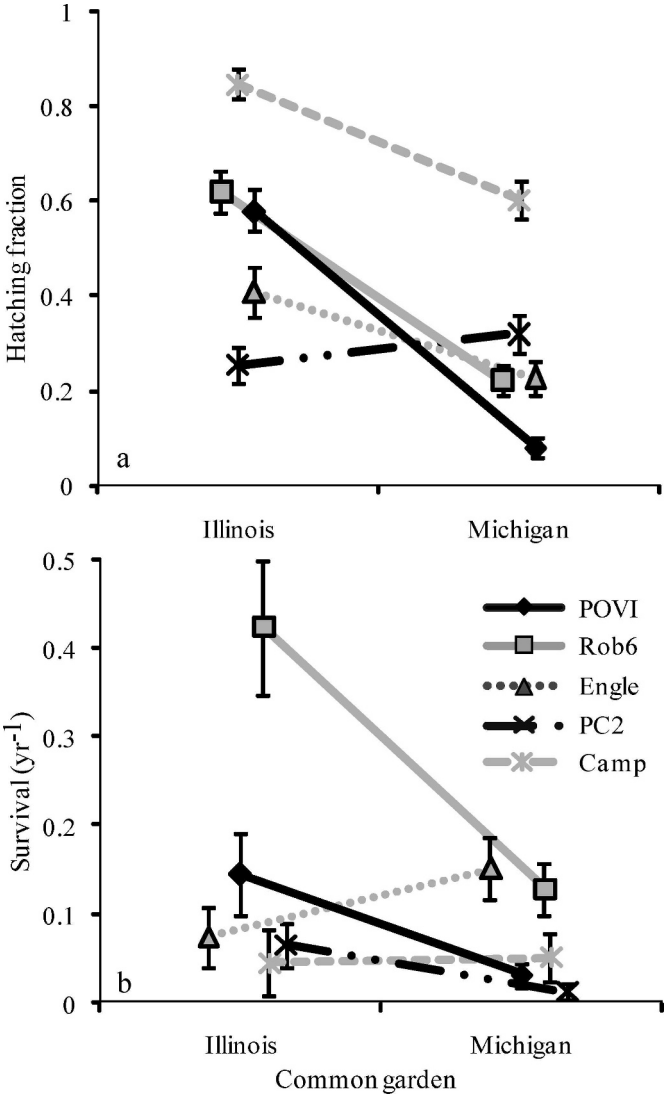

Fig. 4. Reciprocal transplant of field-collected eggs in two common gardens during 2006–2007. Mean (a) hatching fraction and (b) survival probability of dormant eggs. Error bars are the standard deviation of the predicted means from the statistical analysis. *See* Table 1 for legend of pond names.

similar environments (Brendonck 1996). Finally, genetic and G × E control of hatching behavior may confound the field observations. If underlying genetic differentiation sets different mean hatching fractions for each population, these values must be accounted for in any statistical analysis. Genetic-by-environmental interactions would further complicate the observed response in the field by altering the direction or magnitude of the environmental response. That I found evidence for G × E interactions in the common garden, but no relationship (either a direct relationship or rank correlation) between the hatching rates in the field and in the Michigan common garden, supports this proposition. Thus, although a lack of such a correlation does not mean genetic differentiation (or maternal effects) is absent, it does provide support for environmental conditions influencing the hatching rate of eggs, as populations hatched at different rates in each of three environments.

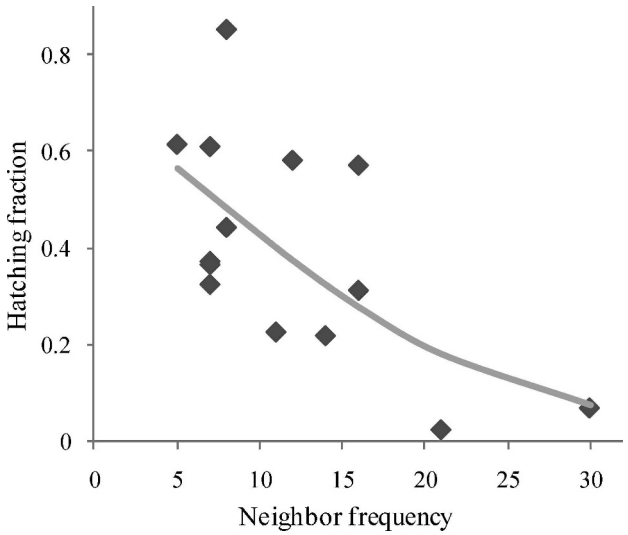

Fig. 5. Relationship between the number of neighbors within 1 km of a pond and the hatching fraction exhibited by that population in the Michigan common garden. The linear prediction was fit using a generalized linear model with binomial errors and a logit link function.

Estimates of dormant egg survival also varied widely among populations in both the field and in the common gardens. A variety of factors could cause this variation. First, survival was generally higher in the field than in the common gardens, suggesting harsher conditions in the common gardens. The measured pH of 9.3 ($\pm$ 0.3 SD) in the Michigan common garden exceeded the pH observed in most ponds (6.8–9.6), and high pH conditions are known to reduce viability of parthenogenetic eggs (Vijverberg et al. 1996). Second, ultraviolet (UV) light has been shown to alter survival rates and induce melanization in *Daphnia* clones (Hessen et al. 1999). Eggs in the common gardens may have been exposed to greater UV light intensity than those in the field, as deeper ponds, suspended or dissolved organic matter, and canopy cover may reduce UV light penetration. Third, dissolved oxygen concentrations were high in the Michigan common gardens (14.7 $\pm$ 0.9 SE mg L^{-1}). I found higher dissolved oxygen concentrations to reduce survival in the field, and others have shown them to increase hatching rate but decrease survival (De Meester 1993; Cáceres and Tessier 2003). Finally, many ponds in this study were small or isolated (e.g., PC2 is < 3 m^2). Such populations may exhibit varying levels of inbreeding, which also affects egg emergence and survival rates (although no relationship existed between these rates and pond size, a potential inbreeding correlate; data not shown) (De Meester 1993; Pfrender and Deng 1998).

Additionally, I found substantial differences in hatching and survival rates in the field for the four ponds incubated in both 2005–2006 and 2006–2007. Environmental conditions certainly varied among the years, as ponds filled much more slowly in spring 2006 relative to spring 2007 (M. Allen pers. obs.). This suggests that environmental variation among years also contributes to hatching and survival rates within populations. However, different genetic backgrounds of the eggs collected in 2005 and 2006 may also contribute to interannual variation in these rates.

Perhaps most interesting is the observation of genetic or maternal control of hatching variation among the populations. This result could be due to selection for an optimal germination strategy or a bet-hedging response (Cohen 1966; Philippi and Seger 1989), adaptive phenotypic plasticity or a selective response to particular environmental cues (e.g., ''predictive germination'' [Cohen 1967]), or inherent differences in initial egg quality among the populations (i.e., maternal effects [De Meester and De Jager 1993]). Additionally, in the 2005 reciprocal transplant, it is possible that species differences controlled the genetic effect as Center and Edge ponds contained *D. obtusa*, whereas Top Pond contained *D. pulex*.

The evolution of optimal germination strategies has been documented for several ephemeral systems (e.g., desert annuals, anostrocans) following a predicted relationship between the frequency of failed active stage reproduction (''catastrophes'') and dormancy emergence (Philippi 1993; Simovich and Hathaway 1997; Clauss and Venable 2000). Such strategies may evolve in response to true population crashes, or events that lead to interannual variation in reproductive success. For ephemeral pond species, variation in hydroperiod or the onset of pond drying is a strong selection pressure influencing the evolution of hatching rates (Belk 1977; Brendonck 1996; Simovich and Hathaway 1997). However, few ponds in this study ever dry before the ephippial production date (early to mid-May; S. Smith pers. comm.; M. Allen unpubl. data), suggesting that hydroperiod is an unlikely cause of catastrophes for most populations. Rather, if catastrophe frequency does influence the evolution of emergence, variation among populations in predator presence or identity, or temporal variability in the onset of predator dominance may influence hatching rates (Hairston and Dillon 1990), but such data are not available. Additionally, germination strategies may co-evolve with other traits (e.g., dispersal potential and propagule size [Venable and Lawlor 1980; Rees 1994; Ellner 1997]). When I investigated such a relationship, hatching rates in the common garden were negatively correlated with dispersal potential, the opposite direction predicted by theory. This suggests that (1) at the low levels of dispersal exhibited among these ponds, the predicted relationship no longer holds, (2) dispersal potential is correlated with another trait that is related to dormancy, or (3) the G $\times$ E interaction makes the hatching values inappropriate for the test. Given the observation of G $\times$ E interactions in the two common gardens and the high variability of hatching in the field, it is likely that any effects of spatial dispersal are complex and interact with other selected traits and stochastic environmental variation.

The interaction of genetic and environmental factors provides support for a role of phenotypic plasticity and genetic background on hatching variation. An interaction between adaptation and differential plasticity to hatching cues has been clearly demonstrated in desert annuals along a precipitation gradient. Clauss and Venable (2000) showed that populations responded differently to water in a common garden, whereby those experiencing less precipitation in the field were more sensitive to such events. Populations from wetter sites had lower germination rates in the common garden, but had higher germination success in the field due to greater overall precipitation. Such genetic-by-environmental variation in the germination response allows populations to grow when conditions are most favorable for survival, a form of predictive germination (Cohen 1967; Pake and Venable 1996; Clauss and Venable 2000). Pond dwellers may exhibit similar patterns. For anostrocans, there is considerable variation among species and habitats for response to hatching cues (Belk 1977). Some have attributed this variation to Cohen's (1966) model of differential probabilities of reproductive success during pond-filling events (Brendonck 1996; Simovich and Hathaway 1997), but there is ample evidence that hatching rates vary in response to a range of cues (Brown and Carpelan 1971; Al-Tikrity and Grainger 1990; Zarattini 2004). The observation of differential hatching responses in this common garden experiment fits such a scenario.

Among-population differences in hatching may also be driven by variation in the environment under which the

diapausing eggs were formed. Total phosphorus values varied by 10-fold and total chlorophyll values varied by greater than 100-fold across these ponds during the early spring. The variation in food availability is known to influence the mother's nutritional state, which can influence the fitness of her offspring (Brett 1993). Additionally, maternal effects can influence the hatching rate of her diapausing eggs (De Meester and De Jager 1993). Such maternal effects could be manifested as population-level genetic or G × E effects observed here.

I have shown substantial variation in hatching and survival rates of diapausing eggs, both incubated in the field and in common gardens. That there was no direct effect of a particular environmental hatching cue in the field, but environmental variation was pronounced among the common gardens, suggests that a variety of ecological or genetic factors (or both) interact to determine actual hatching rates. Although adaptive bet hedging may contribute to such variation, local adaptation to hatching cues, adaptive plasticity (predictive germination), maternal effects, and variable access to hatching cues provide alternative evolutionary and ecological explanations that can explain the observed patterns. Future work controlling for maternal effects, genetic background, and cue exposure will help elucidate the relative importance and interactions among these competing hypotheses for regional hatching variation.

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