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REPORT

Differentiation in sex investment by clones and populations of *Daphnia*

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Abstract

Dormancy is an ecological strategy by which organisms avoid stressful environments, but it also can have genetic consequences. Many facultative parthenogens shift from asexual to sexual reproduction to enter dormancy. Hence, conditions that favour dormancy are predicted to select for more sex, which should increase clonal diversity. We examined lake populations of *Daphnia* that face different ecological risks to remaining active year-round, and quantified the extent to which they have differentiated in their investment in dormancy and sex. There was substantial genetic variation among populations and clones for sex induction and production of dormant eggs, and striking evidence of gender specialization. We also observed a positive association between the magnitudes of population-level investment in dormancy and of variance among clones in sex induction. These results document an ecological gradient in dormancy that is manifest as a genetic gradient in clonal variation for the propensity to engage in sex.

Keywords

Clonal gender specialization, diapause, facultative parthenogenesis, sex allocation.

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INTRODUCTION

Dormancy, dispersal and sex are often coupled in the life cycles of animals capable of producing both asexual and sexual eggs, i.e. facultative parthenogens (Bonner 1958; Bell 1982). For such individuals, the decision to invest in dormancy is also a decision to shift from clonal to sexual reproduction, as it is typically the sexually produced offspring that undergo dormancy and are readily dispersed. This association of sex (genetic recombination) and dispersal (in time or space) is understandable since both are means of dealing with variable environments (Burt 2000).

A consequence of a functional link between dormancy and sex is that ecological conditions that favour (or require) use of dormancy for population persistence should also enhance population genetic variance (Balloux *et al.* 2003). In contrast, environments that do not require dormancy may select for infrequent sex, which intensifies the process of clonal competition and selection, and reduces genetic diversity within populations (Lynch 1984). Hence, the ecology of dormancy should mediate the frequency of sex and determine population genetic variance and rates of evolution in facultative parthenogens (Wright 1931; Pálsson 2001). Studies of molecular variation in aphids, rotifers and cladocerans populations support a relationship between genetic variance and frequency of sex (Gomez & Carvalho 2000; Morgan *et al.* 2001; Vorburger *et al.* 2003a,b), but there is far less information on quantitative traits such as dormancy investment.

A crucial assumption underlying this expected relationship among ecology, genetics and evolution is that investment in sex (and dormancy) is a quantitative, heritable trait of individual genotypes. Unfortunately, most empirical work considers sex and dormancy to be discrete traits; one either does or does not reproduce sexually (i.e. obligate asexual), and the environment either does or does not require annual dormancy (Hebert *et al.* 1993; Delmotte *et al.* 2002). Consequently, much literature focuses either on environmental determinates of sex (Carvalho and Hughes 1983; Kleiven *et al.* 1992; LaMontagne and McCauley 2001), or on comparisons of sexual and obligate asexual forms (O'Connell & Eckert 2001; Vorburger *et al.* 2003a,b). It is so widely assumed that facultative parthenogens such as aphids, rotifers and cladocerans shift predictably to sex on some seasonal or annual cycle that they are commonly referred to as cyclical parthenogens. But not all habitats used by these organisms require dormancy and, consequently, there is no reason to assume that clones need invest equally in sex (Green & Noakes 1995; Rispe & Pierre 1998).

Several studies have looked for heritable differences in sex investment among clones of facultative parthenogens, especially in *Daphnia* and aphids. Clones of a single species have been found to differ in sex response to the environmental cues of photoperiod and crowding (Yampolsky 1992; Deng 1996; Innes & Singleton 2000). Further, it has been observed that when clones engage in sex, they can exhibit specialization in gender allocation (Innes & Dunbrack 1993; Rispe *et al.* 1999). The magnitude of this clonal variation within and among populations of aphids shows a geographic pattern driven by the need for dormancy in northern climates (Dedryver *et al.* 2001). Whether a similar relationship between sex and dormancy ecology occurs in other facultative parthenogens remains largely unexplored.

We have been studying dormancy in lake populations of *Daphnia pulicaria*, which are capable of persisting asexually as actively swimming individuals year-round. However, predators and competitors impose a bottleneck to population size during summer that varies in severity among lakes (Tessier & Welser 1991; Geedey *et al.* 1996; Cáceres & Tessier 2004, in press). Consequently, this species often produces dormant egg (i.e. engages in sex) during late spring. However, we have observed that natural populations of *D. pulicaria* display consistent differences in the magnitude of dormancy investment (Cáceres & Tessier 2004); populations that predictably experience benign summer conditions produce very few dormant eggs.

Here, we address whether this dormancy variation, observed in nature, represents heritable variation in the tendency to engage in sex. We also examine the nature of clonal specialization in sex and gender induction within each population and test whether populations characterized by greater overall sex investment also harbor more genetic variance. In short, we provide a first test that the ecology of dormancy can shape clonal variation for sex induction within and among populations of *Daphnia*.

METHODS

Populations

D. pulicaria is a common cladoceran (Crustacea : Branchipoda) that inhabits relatively deep lakes. Populations are most abundant in winter and spring when predation by fish is minimal; in summer the animals retreat to deeper, cold water, which provides refuge from competitors and fish predators (Leibold & Tessier 1997). However, lakes differ in the size and quality of refuge habitat (Tessier & Welser 1991). Lakes with a large, oxygenated refuge allow *D. pulicaria* populations to persist in the water column year round; in lakes with poor refuge opportunities, population abundance is greatly reduced in summer. Most of the year, *D. pulicaria* reproduce by parthenogenesis, but prior to summer (May and June) females may produce males (by parthenogenesis) and engage in sex. The sexual eggs are enclosed in a modification of the female's carapace known as an ephippium and delay hatching (i.e. are dormant) for months or years (Cáceres 1998), providing a means to escape poor conditions. In lakes where summer refuge opportunities are predictably good, *D. pulicaria* engage less in sex than in lakes possessing a small refuge (Cáceres & Tessier in press).

We examined nine populations of *D. pulicaria* living in glacial-kettle lakes in Kalamazoo Co. (Little Long, Whitford) and Barry Co. (Baker, Bassett, Big Long, Bristol, Little Mill, Pine, Warner), in southwestern Michigan, MI, USA. We chose these lakes to encompass a broad range of summer refuge and, consequently, of seasonal *D. pulicaria* dynamics (Cáceres & Tessier 2004, in press). *D. pulicaria* from each lake population have been examined using cellulose acetate electrophoresis (Geedey 1997; Tessier & Leibold 1997; Dudycha 1999; Tessier and Cáceres, unpubl. data), and found to be fixed homozygous for the slow allele of Ldh that is diagnostic for discerning *D. pulicaria* from its sibling species *D. pulex* (Hebert 1995).

All populations were sampled routinely (~weekly during the period of sex) from ice-out to June in 1999, 2000, 2001 (except that Big Long was not sampled in 1999) using, vertical net tows (80 μm mesh) from three deep-water locations. Samples were immediately preserved in ethanol and later counted for numbers of adult *D. pulicaria* carrying parthenogenetic vs. sexual eggs. For each sample date, we determined the fraction of clutches that were ephippial relative to the total number of clutches. In all populations this metric of sex activity exhibited a peak in May, but populations varied in magnitude and duration of sex. Hence, to compare populations we expressed annual ephippial production as the area under the curve of ephippial fraction vs. sampling day. A detailed presentation of seasonal and interannual population dynamics, and timing and magnitude of sex activity will be presented elsewhere (Cáceres & Tessier 2004, in press). Here, we simply summarize sex activity in each population as the average (among the 2–3 study years) of annual ephippial investment.

On one sampling date prior to the period of sexual activity in the populations, we isolated into clonal culture 15–20 adult female *D. pulicaria* from each lake population (six populations in 1999, three in 2000). Care was taken to minimize selection bias during this process; survival and propagation of all isolated individuals was greater than 95%. After cultures were well established, we split each clone into two lines (separate beakers) and placed all cultures into incubators set to inhibit sexual activity (10 °C, 6 : 18 *L* : *D* cycle). Cultures were fed high food and thinned weekly (following established culture methods, Tessier and

Consolatti 1991), and allowed to progress through >3 parthenogenetic generations prior to any experiments.

Acclimation

We used a two-generation acclimation designed to mimic the seasonal change from winter to spring. Animals were fed a high food level daily (>2 mg C L^{-1} *Ankistrodesmus falcatus*) and changed to fresh water every other day. Two to four neonates from each replicate line of each of the 146 clonal isolates were raised in high food at 15 °C, 8 : 16 *L* : *D* cycle in 110 mL water; these were the grandmothers for the experimental animals. No ephippial production was observed in this generation. After these animals matured (>2nd clutch adults), we collected two-four neonates from each line, and raised them in high food at 20 °C, 12 : 12 *L* : *D* cycle, in 110 mL water; these were the mothers for the experimental animals. About 10% of these animals produced either ephippia or males during this generation.

Experiments

When animals in the mother generation were >2nd clutch adults, we collected 12–14 female neonates from each replicate line and placed them into one of two different environments (i.e. six to seven neonates in each environment). Environment 1 was the same temperature and photoperiod as the mothers, but the culture water contained 20% filtered water from an extremely dense batch culture of *D. pulicaria* and *D. pulex*, which was routinely producing males and engaging in sex. Environment 2 was also characterized by 20 °C and 20% water from the crowded culture, but it had a longer photoperiod of 16 : 8 *L* : *D*. Hence, both environments provided cues of a high population density and background sexual activity, but differed in simulating early vs. late spring (hereafter referred to as spring vs. summer) periods, which bracket the timing of sex activity observed in the lakes. These two photoperiod conditions also bracket the relevant range of environmental control over ephippial production observed by Deng (1996) for this species of *Daphnia*.

These experimental animals were thinned as they grew such that by day 6 (nearing maturity) each beaker (110 mL water) had only four to five animals. Animals were fed (0.75 mg C L^{-1} until day six, 1.0 mg C L^{-1} thereafter), and changed to fresh water every other day; this simulated moderate food limitation typical of lake conditions. Animals were checked for reproductive status on the change days and categorized as either (1) carrying a clutch of parthenogenetic offspring, (2) being receptive to sex as evidenced by a carapace modified to form an ephippium, or (3) barren (no eggs, no ephippium: this was an infrequent and typically transient stage near molting). No female was ever observed to lay dormant eggs (i.e. deposit eggs in an ephippium), which confirmed that investment in dormancy requires sex since no mature males were present in the beakers. All parthenogenetic offspring were removed from each beaker on the change days, and counted by gender. We continued to record data from each beaker for 10 days past first clutch, i.e. until all animals had completed four to five clutches.

Analysis of sex investment

The data were used to create two metrics of sex investment, based on either male or ephippium investment. Male investment was calculated as the proportion of total number of parthenogenetic offspring that were male, produced by the four to five animals over all days of observation. Ephippium investment was calculated similarly as the proportion of the total number of adult clutches that were ephippial, ignoring barrens (including barrens had no qualitative effect on the analysis). Since there is no easy way to equate male and ephippial investment in terms of real time and energy costs, we simply summed these two investment metrics as a measure of the overall investment in sex. For statistical analyses, we rely on a single value (male, ephippial or summed investment) calculated for each replicate line of each clone. We stress, however, that each value is based on multiple individuals and >20 clutch-observations for each replicate line of each clone.

Since some clones in the mother's generation produced only ephippia and males, we were unable to set up all 146 clones for each environment; a total of 127 clones were established for Environment 1 and 136 for Environment 2. This created some imbalance in the experimental design. Consequently, for analysis of variance we used Proc Mixed (SAS 1999) and a mixed model specification in which environments (fixed effect) were crossed with population and clones nested within populations (both random effect). In all models, the error (residual) variance is based on clone replicates. We used the grouping option in Proc Mixed (Littell *et al.* 1996) to quantify and test for variance heterogeneity of clones among populations. We also performed some analyses based on a categorization of clones according to whether they invested exclusively in male or ephippium production. For these, we performed likelihood ratio tests using StatXact (Metha & Patel 1992), which determined *P*-values by randomization.

RESULTS

A surprisingly large number of clones were apparent gender specialists, engaging only in male or ephippial production (Fig. 1). Hence, we categorized each clone, in each environment, into four types of sex activity (no sex investment, male function only, sexual female function only, or both male and

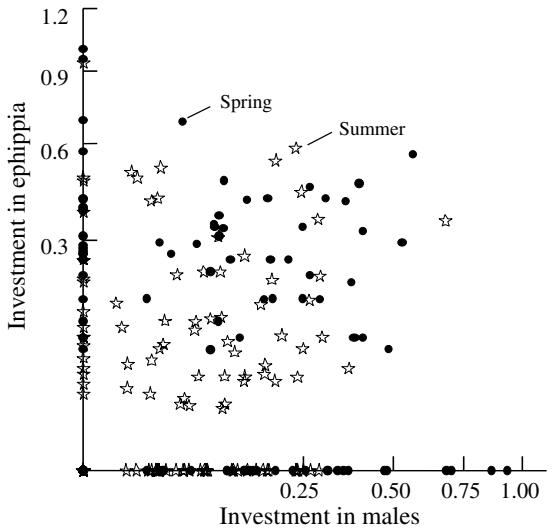

Figure 1 Plot of clone means for the proportion of parthenogenetic offspring that were male vs. the proportion of clutches that were ephippial (each metric runs from 0 to 1, but is plotted on a square root scale to better visualize the proportions close to 0). Each clone was measured separately in a spring (dots) and summer (stars) environment.

sexual female function). Comparison of the two environments revealed no difference in clone frequency distribution among the four categories (Likelihood ratio = 3.4, d.f. = 3, $P = 0.33$). However, the nine populations differed in frequency of clones in these four categories in both the spring environment (Likelihood ratio = 119.2, d.f. = 24, $P < 0.0001$) and the summer environment (Likelihood ratio = 49.9, d.f. = 24, $P = 0.005$). This suggests that populations are genetically differentiated with respect to gender allocation regardless of environment.

To explore population differentiation and specialization in sex activity more robustly, we categorized each clone based on their overall investment in sex in both environments combined (Table 1). For all clones, 18% never invested in sex, 31% were gender specific in their sex investment and 51% allocated to both male and sexual female function. The populations were highly differentiated in this categorization of sex (Likelihood ratio = 65.0, d.f. = 24, $P < 0.0001$). For example, in Baker Lake, 85% of the clones invested in both male and sexual female function, while this category is only 22% of the clones in Warner Lake. Gender specificity was overall quite high but varied greatly among populations. In Little Long and Whitford lakes, 56 and 31%, respectively, of the clones engaged only in male function regardless of environment and none were sexual female specialists. In contrast, in Bristol and Big Long lakes, 27 and 15% of the clones were sexual female specialists and no clones were male specialists.

Table 1 Distribution of *Daphnia pulicaria* clones among four categories of investment in sex for each of nine lake populations. Values in table represent percentage (rounded to whole numbers) of total clones from each population that never invested in sex, were male specialists, were sexual female specialists or invested in sex through both genders. Categorization of each clone reflects observations of numerous animals raised in both spring and summer environmental conditions

Population	No sex	Males only	Sexual female only	Both genders
Baker	10	5	0	85
Bassett	13	20	27	40
Big Long	15	0	15	70
Bristol	6	0	27	67
Little Long	6	56	0	38
Little Mill	13	13	19	55
Pine	36	29	6	29
Warner	45	22	11	22
Whitford	13	31	0	56
Total	18	20	11	51

Analyses of the quantitative metrics of sex investment revealed a similar partitioning of variance when performed on male and on ephippium investment separately, or on the combined (male + ephippium) metric (Table 2). Genetic variation at the level of populations and clones within populations (and their interactions with environment) explained approximately 60% of the total variation. The residual variation (based on replicate lines for each clone in each environment) suggests substantial maternal or micro-environmental influences. Much of the genetic variance was either within-population, clonal variation in response to environment (i.e. clone–environment interaction), or represented mean differentiation among populations.

Although explaining much less variance, populations also differed significantly in response to the environment (i.e. population–environment interaction). However, this variation in population responses was largely driven by the response of a single population, (Baker Lake; Fig. 2a). When Baker Lake is removed from the analysis the resulting variance partitioning is similar for clone and clone–environment interactions, but the population–environment interaction becomes nonexistent while mean population differentiation increases to 17% of the total variance ($P < 0.0001$). Hence, Baker Lake is the only population that changes significantly its overall investment in sex in response to the environment.

The largest genetic component of variance in all analyses was the clone–environment interaction (Table 2). Clones from all populations exhibited large and qualitatively distinct responses to environmental change (Fig. 2b). Some clones decreased sex investment from early to late spring conditions, while others increased investment. On average, these

Table 2 Analysis of variance for male, ephippium, or combined sex investment by clones of *Daphnia pulicaria* from nine populations raised in two environments. Models were run using SAS version 8, Proc Mixed with all terms deemed random except environment. Chi-square values represent the difference in the −2 REML log likelihood fits for models with and without that source of variance. Null probabilities for each random source are 1-tailed since variance is constrained to positive values (*$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$)

	Variance component	Percent of totals	Difference in −2 log likelihood
Male function			
Population	0.00697	23.7	112.9***
Population–Environment	0.00122	4.1	3.2*
Clone (Population)	0.00221	7.5	28.1***
Environment–Clone (Population)	0.00756	25.6	27.5***
Residual	0.01151	39.1	
Female function			
Population	0.00693	15.3	77.6***
Population–Environment	0.00377	8.3	10.2**
Clone (Population)	0.00445	9.8	29.5***
Environment–Clone (Population)	0.01314	29.1	36.6***
Residual	0.01695	37.5	
Male and female function			
Population	0.00223	12.1	68.1***
Population–Environment	0.00150	8.2	10.1**
Clone (Population)	0.00209	11.4	35.8***
Environment–Clone (Population)	0.00532	28.9	33.3***
Residual	0.00725	39.4	

responses cancel out to result in no significant population-environment interaction (except for Baker Lake), and no significant effect of environment on mean sex investment ($F = 3.1$, d.f. = 1,8.8, $P = 0.113$). However, some of this clonal differentiation in response to environment is associated with gender specialization. Notably, male specialist clones were especially active in their sex investment under spring conditions, but significantly reduced male production under summer conditions (Fig. 1, mean male production = 0.28 and 0.107 in spring and summer respectively, $t_{59} = 3.7$, $P < 0.001$).

In the analyses presented above, we treated clones as nested within populations. This amounts to an assumption of homogeneity of clonal variance (i.e. pooling clonal variance into a single term). However, plots, such as those shown in Fig. 2b, suggested appreciable differences among populations in the magnitude of clonal variation. To test whether variation among clones within populations might better be modelled as heterogeneous among populations, we compared the nested model to one in which clonal variance is fit separately for each population. Since clones clearly interact with environment, we ran the model comparison for each environment separately, obtaining similar results. In both environments, model fit is substantially improved when clonal variation is assumed unequal among lake populations (difference in −2 log Likelihood fit = 40.1 for spring, 23.9 for summer, d.f. = 8, $P < 0.0001$ and 0.002, respectively).

Since populations significantly differ in the magnitude of clonal variation in sex investment, we estimated this value separately for each lake population and environment. Interestingly, these estimates of within-population genetic variance exhibited a striking relationship to the mean level of sex investment for each population (Fig. 3, $r_p = 0.89$ $P = 0.0014$ spring environment, $r_p = 0.88$, $P = 0.0017$ summer environment). Only Baker Lake in the spring environment displayed a lower level of clonal variance than expected from its high mean sex investment.

Finally, we compared our laboratory estimates of population sex investment to observed investment in ephippial production in each lake (3-year average, Fig. 4). For the summer environment, sex investment was significantly correlated to the field observations ($r_p = 0.68$, $P = 0.045$). A weaker relationship in the spring environment is caused solely by one high value of laboratory sex (again, Baker Lake). If this population is ignored the relationship of lab and field sex investment also is significant for the spring environment ($r_p = 0.71$, $P = 0.05$). Hence, excepting one lake in one environment, our laboratory measures of population differentiation in sex investment match field observations of ephippial investment in these lake populations.

DISCUSSION

Dormancy is an ingenious and widespread adaptation to avoid perilous times, but it is not without risk. For

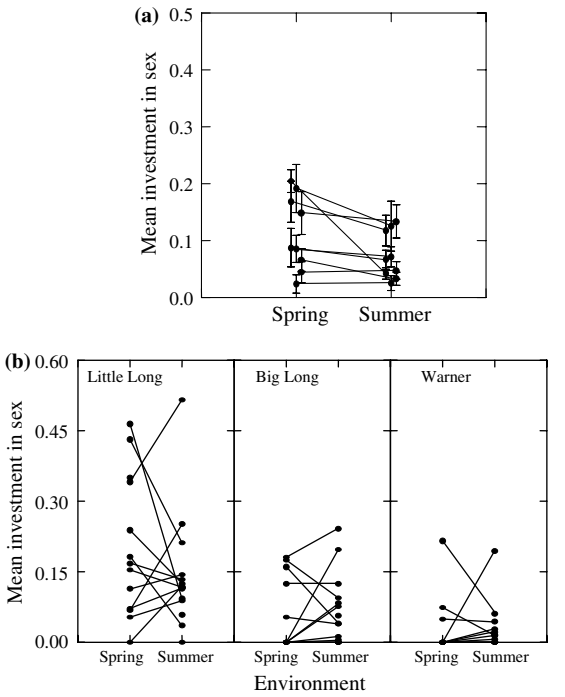

Figure 2 (a) Population means of clonal investment in combined male and ephippium production (mean ± 1 SE based on clone means) in each of two environments that mimicked spring and early summer conditions in the lakes. Lines connect the population means in the two environments. Note that one population, Baker Lake, shows extreme decrease in sex investment from spring to summer. (b) Clone–environment interactions in combined male and ephippium investment. Lines connect clone means in the two environments. Panels illustrate the population with the highest (Little Long), the lowest (Warner) and a population that exhibited intermediate (Big Long) mean sex investment.

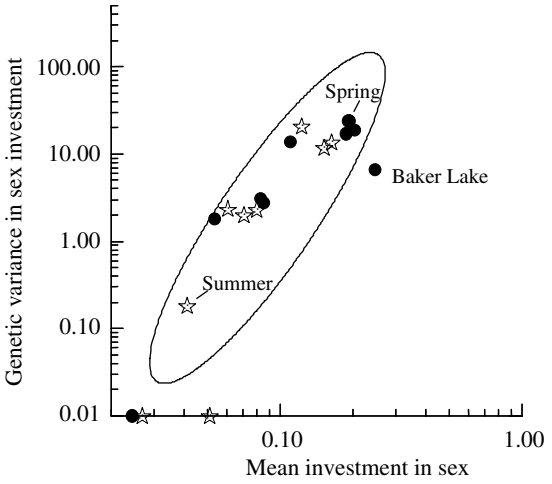

Figure 3 Relationship of genetic (clonal) mean and variance in sex investment measured in the laboratory for each population and environment. Oval indicates ±1 SE on bivariate relationship. Note that Baker Lake population is unusually high in sex induction but low in clonal variation.

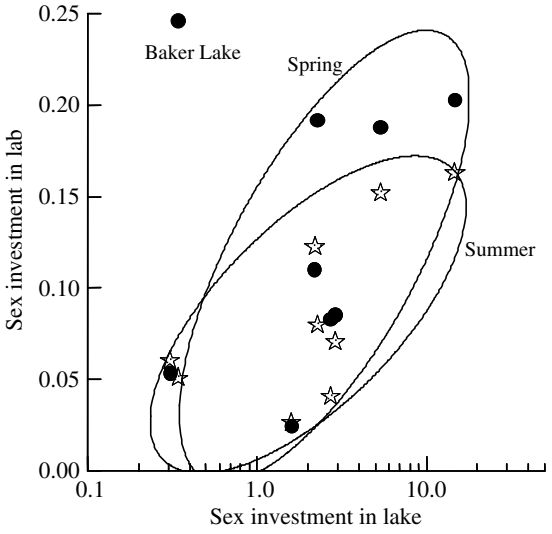

Figure 4 Comparison of mean of clonal sex induction measured in the laboratory for each population and environment related to sex activity observed in the lakes. Lake values for sex activity represent area under the curve of proportion ephippial females over all sampling dates. Ovals indicate ±1 SE on bivariate relationship estimated for each environment separately. Baker Lake clones were unusually high in sex investment in the spring lab environment.

zooplankton, the benefits of investing in a dormant egg bank are meaningless if you cannot make a withdrawal because your eggs are consumed by benthic predators (Cáceres & Hairston 1998) or permanently buried by sediment (Cáceres & Tessier 2003). Therefore, an optimal investment strategy should balance the risks of dormancy with those of remaining active. Our results demonstrate that populations of *D. pulicaria*, which face different risks to remaining in the water column year-round (Cáceres & Tessier 2004; in press), have differentiated genetically in their investment in dormancy. Since dormancy requires sex, in essence, we show sex induction to have a genetic basis that is subject to selection. Further, we demonstrate the expected positive association between the magnitude of population-level investment in dormancy and clonal variance for sex induction. Populations observed to invest more in production of dormant eggs in the lakes (and the lab) contained a greater variance among clones in the trait of sex induction (male and ephippial production). Hence, an ecological gradient in dormancy is manifest as a genetic gradient in sex induction and clonal variance.

Elsewhere (Cáceres & Tessier 2004; in press) we show that investment in dormancy by natural populations of *D. pulicaria* is related to the risk of remaining active in the water column through summer. *D. pulicaria* prefers cool temperatures and high quality food typical of autumn, winter and spring (Threlkeld 1979; Leibold 1991). Summer represents a stressful period of low resource quality, high predation risk and abundant competitors. However, not all lakes are equally stressful in summer; it depends on the lake depth and the availability of a deep-water refuge (Tessier & Welser 1991). In lakes where summer stress is low, populations display very little investment in dormancy and clones persist across years (Geedey 1997). Our results indicate that clones with a genetic predisposition for low incidence of sex dominate such lakes and, consequently, variance for sex induction is very low. We suspect that clonal richness is also reduced in these populations, compared to populations with high overall sex activity, as a consequence of extended periods (multiple years) of clonal competition. Testing this prediction will require a high-resolution study of molecular variation.

The notion that sex occurs periodically (cyclically) in facultative parthenogens can be traced to Weismann (1876–1879), who felt that the shift from asexual to sexual reproduction was controlled by an internal cycle or clock. Banta (1915) convincingly refuted this concept of an intrinsic cycle to sex, but unfortunately ingrained in the literature the belief that sex is environmentally determined. The modern textbook view of cyclical parthenogenesis is largely a substitution of seasonal and ecological cues (Stross 1968; Kleiven *et al.* 1992) for Weismann's internal sex drive. However, this legacy is undeserved; Banta (1939) documented clonal variation in the tendency to produce males and engage in ephippial production. He recognized that sex in *Daphnia* should be viewed as an interaction of genotype and environment. Our results and those of others (Korpelainen 1986; Yampolsky 1992; Deng 1996; Innes & Singleton 2000) confirm this perspective. Hence, clones use similar environmental cues to synchronize the timing of sex, but the magnitude of their sex induction is genetically variable among clones and populations. This suggests that selection on sex induction to cues is a means by which *Daphnia* populations adapt to an annual cycle of ecological change.

While the production of dormant eggs is obviously an ecological strategy, it is not without genetic consequences. Banta (1939) presents striking evidence of mutation accumulation in long-lived clones and inbreeding depression among their sexual, selfed offspring. These results have been broadly confirmed (e.g. DeMeester 1993; Deng & Lynch 1997; Haag *et al.* 2002) and suggest that inbreeding depression is an additional cost associated with a switch to sexual reproduction. Our finding of widespread gender specialization among clones within populations suggests an effective means by which *Daphnia* reduce this risk of inbreeding depression. It also suggests an explanation for the large component of variance expressed as a clone–environment interaction. Female *Daphnia* can switch from asexual to sexual egg production from one molt to the next, literally in a few days. However, fertilization of sexual eggs requires males that are produced as asexual offspring, and may take 2 weeks to mature under field conditions. Not surprisingly, male production typically precedes ephippial formation in natural *Daphnia* populations. We observed that clones specialized in male function were most active in male production when raised under spring conditions and significantly reduced sex investment under summer conditions. Given that an individual's median lifespan may exceed 2 months (Dudycha & Tessier 1999), which is longer than the typical length of sexual activity in spring (Cáceres & Tessier 2004), this seems a reasonable strategy. Other clones, engaged in sexual female function, increased sex investment from spring to summer, which also seems reasonable if mature males would normally be increasing in frequency over this time span. Together this pattern of sequenced gender specialization contributes to a pattern of clone–environment interaction that characterized nearly half of the total genetic variance we observed.

Several theoretical treatments (Wright 1931; Green & Noakes 1995) conclude that facultative asexuality is an evolutionary effective means of reproduction, so long as sex is frequent enough that selection does not eliminate clonal diversity. This theory suggests that while sex (and dormancy investment) should be reduced in permanent environments where the risk of remaining active is low, it should not be eliminated. However, some of our lake populations display quite low sex activity in the field and contained a high proportion of clones that were never observed to engage in sex in the laboratory. While frequent sex is clearly unnecessary when the risks to continued asexual reproduction are low, extended asexual reproduction and clonal competition leads to loss of clonal diversity (Lynch 1984; Tessier *et al.* 1992; Gomez & Carvalho 2000) and accumulation of mutations. Clones that emerge as dominants in a lake population face a high likelihood of selfing and severe inbreeding depression if they invest in sex. Gender specialization is no help in the case of extreme dominance by a single clone. In the short-run of a few years, such populations may be selected for clones that avoid sex. In the long-term, this could lead to a mutational meltdown (Lynch *et al.* 1993). Testing the extent to which this process occurs in nature will require long-term studies of population genetic structure.

Ecological constraints and opportunities determine the necessity for sex and dormancy investment, and this should shape genetic structure within populations of facultative parthenogens. Our results support this link between ecology

and genetics. A relationship between dormancy and the genetic structure of populations also has been confirmed in aphid species that occur as either obligate or facultative parthenogens (Delmotte *et al.* 2002;Guillemaud *et al.* 2003). These facultative parthenogens behave as annuals, undergoing sex and dormancy at the end of each summer and maintaining high population genetic variance. In warmer climates where dormancy is not needed to overwinter, clones that reduce investment in sex, or even possess obligate asexuality, dominate and local genetic diversity is low. While obligate asexuality does not occur in our populations of *D. pulicaria*, it is tempting to view their quantitative variation in sex investment as spanning the full range from annuals to perpetual asexuals. The likely feedback of this variation in sex and genetic structure on the evolutionary ecology of these populations awaits further study.

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