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Does local adaptation to resources explain genetic differentiation among *Daphnia* populations?

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Abstract

Substantial genetic differentiation is frequently observed among populations of cyclically parthenogenetic zooplankton despite their high dispersal capabilities and potential for gene flow. Local adaptation has been invoked to explain population genetic differentiation despite high dispersal, but several neutral models that account for basic life history features also predict high genetic differentiation. Here, we study genetic differentiation among four populations of *Daphnia pulex* in east central Illinois. As with other studies of *Daphnia*, we demonstrate substantial population genetic differentiation despite close geographic proximity (<50 km; mean $\theta = 0.22$). However, we explicitly tested and failed to find evidence for, the hypothesis that local adaptation to food resources occurs in these populations. Recognizing that local adaptation can occur in traits unrelated to resources, we estimated contemporary migration rates (m) and tested for admixture to evaluate the hypothesis that observed genetic differentiation is consistent with local adaptation to other untested ecological factors. Using Bayesian assignment methods, we detected migrants in three of the four study populations including substantial evidence for successful reproduction by immigrants in one pond, allowing us to reject the hypothesis that local adaptation limits gene flow for at least this population. Thus, we suggest that local adaptation does not explain genetic differentiation among these *Daphnia* populations and that other factors related to extinction/colonization dynamics, a long approach to equilibrium F_{ST} or substantial genetic drift due to a low number of individuals hatching from the egg bank each season may explain genetic differentiation.

Keywords: dispersal, founder effects, local adaptation, microsatellites, monopolization hypothesis, reciprocal transplant

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Introduction

Evolutionary biologists have long understood that natural selection, mutation, migration and genetic drift influence population structure, but understanding their relative importance in any system remains a large challenge. For organisms with high dispersal capacity, strong genetic differentiation among populations is paradoxical, as dispersal should facilitate gene flow and reduce genetic differentiation that results from drift and selection. For example, high dispersal rates can break down both stochastic and adaptive diversification among populations and in extreme cases, lead to the homogenization of regional gene pools (e.g. Fuller *et al.* 1996; Roslin 2001; Gilbert-Horvath *et al.* 2006). Under lower dispersal, selection and drift are less quickly counteracted, resulting in increased population genetic differentiation (Ehrlich & Raven 1969; Slatkin 1985).

Zooplankton populations in the genus *Daphnia* commonly exhibit strong population genetic differentiation, even over small geographic scales (Boileau & Hebert 1988; Spitze 1993; Vanoverbeke & De Meester 1997; Morgan *et al.* 2001; Haag *et al.* 2006; Thielsch *et al.*

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2009). This observation is paradoxical in light of the wealth of ecological literature demonstrating high dispersal capacities for daphniids. For example, *Daphnia* rapidly colonize newly created habitats (Cohen & Shurin 2003; Louette & De Meester 2005; Johnson *et al.* 2008). Why then do cyclically parthenogenetic zooplankton such as *Daphnia* exhibit strong genetic differentiation?

Patterns of population differentiation are likely related to the life history strategy of daphniids. *Daphnia* typically reproduce by cyclic parthenogenesis, an alternation between clonal reproduction during the growing season and sexual reproduction when cued by their environment (but see Paland *et al.* 2005). Sexual reproduction results in the production of diapausing eggs that are encased in a desiccation-resistant ephippium and dispersal of ephippia appears to be the primary mode of dispersal (Hebert 1978). Consequently, habitats are effectively colonized through ephippial dispersal and hatching and subsequent clonal reproduction allows for rapid population growth from a few initial colonists. Given this life history, it is generally accepted that founder effects play an important role in initial genetic differentiation among populations (Boileau *et al.* 1992). At least for recently colonized habitats (<5 years), there is evidence that such founder events and priority effects occur and lead to population genetic differentiation (Haag *et al.* 2006; Louette *et al.* 2007). Despite the high dispersal capacity of daphniids, this genetic differentiation often does not erode quickly (as evidenced in high F_{ST} values). Hypotheses explaining this fall into two categories: adaptive and non-adaptive mechanisms. Non-adaptive mechanisms include a non-equilibrium model (drift-migration imbalance) (Boileau *et al.* 1992) and clonal selection and recurrent bottleneck models (Berg & Lascoux 2000). Alternatively, divergent selection (local adaptation) may maintain or increase equilibrium levels of differentiation (De Meester *et al.* 2002).

A primary non-equilibrium hypothesis explaining genetic differentiation among cyclic parthenogens was set forth by Boileau *et al.* (1992). This hypothesis posits that colonization by a few individuals and subsequent rapid population growth through clonal reproduction creates large genetic differences among populations through a founder effect. These initial founder effects persist for long periods of time, because while equilibrium F_{ST} depends on the number of migrants per generation ($N_e m$), the approach to that equilibrium depends only on the migration rate (m) which may be small. Thus, Boileau *et al.* (1992) suggested reaching equilibrium F_{ST} may take hundreds to thousands of generations and estimates of migration rates based on F_{ST} from younger populations were drastically underestimated. Hence, genetic differentiation in this 'Persistent Founder Effects' hypothesis results from a lack of equilibrium between migration and drift.

Berg & Lascoux (2000) modelled equilibrium genetic differentiation among cyclic parthenogen populations accounting for several major features of the life history. They found that high equilibrium F_{ST} could result from a combination of low dispersal during the asexual phase coupled with an erosion of effective population size during that time. Low dispersal during the asexual phase is likely for *Daphnia* as dispersal occurs primarily via ephippia (Hebert 1978). In addition, genetic diversity has been found to decrease during the growing season in several studies, presumably owing to clonal selection (Lynch 1984; Pfrender & Lynch 2000; De Meester *et al.* 2006). If clonal selection results in a relatively small effective size when sexual eggs are produced, then strong genetic differentiation may result from drift. Drift may be even more pronounced if the number of eggs hatching from sediments is low (e.g. Cáceres & Tessier 2003). An increase in the rate of clonal selfing, resulting from decreasing effective size during the growing season, could also lead to increased differentiation among populations, though the conditions under which this could occur make it unlikely to be important (Berg & Lascoux 2000). In addition, Berg & Lascoux (2000) demonstrated that recurrent bottlenecks due to frequent population crashes could lead to genetic differentiation among populations and increase the time to migration-drift equilibrium in a manner similar to Boileau *et al.* (1992).

In addition to the non-equilibrium and drift models above, population differentiation can result from natural selection. For example, neutral loci linked to genes under selection may differentiate strongly between populations (Lynch 1987; Vanoverbeke & De Meester 1997). In a more specific formulation, DeMeester *et al.* (2002) set forth the 'Monopolization Hypothesis' as an explanation for strong genetic differentiation among cyclic parthenogens. The Monopolization Hypothesis builds on Boileau *et al.*'s (1992) persistent founder effects model by positing that populations rapidly adapt to local environmental conditions in the few generations following colonization (De Meester *et al.* 2002): clonal selection in the local environment and annual bouts of sex lead to the rapid development of a large, locally adapted diapausing egg bank that restarts the population in subsequent years. Thus, locally-adapted clones limit gene flow from later-arriving colonists by outcompeting them for local resources (a priority effect), resulting in reduced gene flow and an equilibrium F_{ST} that is much higher than expected based on dispersal ability alone.

For local adaptation to be considered an important factor in genetic differentiation among populations,

some trait(s) must exhibit local adaptation and there must be evidence for reduced gene flow using methods that do not rely on calculations based on F_{ST} (since these methods rely on equilibrium conditions) (Boileau *et al.* 1992; Whitlock & McCauley 1999). Such a gene flow reduction must be substantial enough to alter equilibrium dynamics (either equilibrium F_{ST} or the rate of approach to equilibrium). One way to test for local adaptation is to employ reciprocal transplant experiments (Kawecki & Ebert 2004) and several methods are now available for identifying gene flow with molecular markers that do not rely on F_{ST} (Pritchard *et al.* 2000; Wilson & Rannala 2003). Although several studies have presented data consistent with local adaptation in cyclically parthenogenetic zooplankton (e.g. life history traits—Boersma *et al.* 1999; phototaxis—Cousyn *et al.* 2001; migration behaviour—Michels *et al.* 2007; resources—Vanni 1987; Tessier *et al.* 2000; Sarnelle & Wilson 2005) and there is a rich literature documenting significant genetic differentiation among populations (Boileau & Hebert 1988; Spitze 1993; Vanoverbeke & De Meester 1997; Morgan *et al.* 2001; Haag *et al.* 2006; Thielsch *et al.* 2009), few studies have combined an estimate of gene flow with an experimental demonstration of local adaptation (but see Declerck *et al.* 2001).

Here, we study genetic differentiation among four populations of *Daphnia pulex* in central Illinois (Allen 2010). We demonstrate significant and strong genetic differentiation among these ponds despite their close geographic proximity using five microsatellite loci. To explain this differentiation, we tested both adaptive and non-adaptive hypotheses. We first tested for local adaptation to resources (Monopolization Hypothesis). Although local adaptation may occur in any number of traits, adaptation to resources is a good starting point for research aimed at testing for local adaptation in daphniids because: (i) they are logistically feasible to study using reciprocal transplant experiments; (ii) ponds differ strongly in quality and quantity, factors known to elicit selective responses (Tessier & Consolatti 1991; Declerck *et al.* 2001; Sarnelle & Wilson 2005); and (iii) other studies suggest resource-related traits are very important for daphniids (Vanni & Lampert 1992; Tessier *et al.* 2000; Tessier & Woodruff 2002; Weider *et al.* 2005). Next, since local adaptation should lead to reduced gene flow, we used Bayesian clustering analyses to test for both dispersal and gene flow among differentiated populations. Such evidence should be present regardless of the trait being selected if Monopolization is occurring. Finally, we tested the non-adaptive hypothesis that clonal selection was present in the populations, one of Berg & Lascoux (2000)'s alternate mechanisms for population genetic divergence.

Materials and methods

Pond characteristics

We chose four shallow, fishless ponds containing cyclically parthenogenetic populations of *Daphnia pulex* located within 50 km of one another in east-central Illinois, USA. The ponds vary in physical, chemical and biological parameters (Table 1). To quantify the resources of each pond, we first measured nutrient and chlorophyll-*a* levels during mid-April 2008. Chlorophyll content was determined by filtering pond water through a 0.7 μm filter (Whatman GF/F), extracting the chlorophyll in ethanol and measuring the absorbance using a Turner Designs—700 fluorometer (Welschmeyer 1994). For particulate phosphorus, carbon, and nitrogen

Table 1 Biotic and abiotic characteristics of Illinois study ponds in April 2008. Variables: Size (m^2) as measured from aerial photos in ArcGIS Desktop. Maximum depth in m. Hydroperiod given in months: 12 + (pond fails to dry in wet years); sp (semipermanent, rarely dries). Resources: juvenile specific growth rate per day from *D. pulex-pulicaria* bioassay. Chlorophyll-*a* concentration (Chl-a) and total phosphorus (TP) in μg/L. C:P (C:N): seston carbon to phosphorus (nitrogen) ratio was calculated for each date and the average calculated. SE are given, where available

Pond	Location	Size (m^2)	Depth (m)	Hydroperiod	
BridgeS	40.1221 N, 87.7367 W	2230	1.5	7–12+	
Busey	40.1287 N, 88.2131 W	12150	1.0	8–12+	
Dump	40.2428 N, 87.7795 W	270	1.5	Sp	
Top	40.2420 N, 87.7824 W	290	0.5	3–6	
	Resources (d^{-1})	Chl-a (μg/L)	TP (μg/L)	C:P	C:N
BridgeS	0.504	4.60 (0.48)	203.4	69.6	8.9
Busey	0.269 (0.04)	1.60 (0.24)	21.0	128.9 (75.7)	8.2 (0.6)
Dump	0.234 (0.05)	0.75 (0.22)	48.4	65.1 (7.2)	7.4 (0.6)
Top	0.386	0.49 (0.06)	30.1	104.6 (89.6)	9.0 (1.8)

(PP/PC/PN), water was filtered through 75 μm mesh to extract large invertebrates and then through precombusted GF/F filters. Phosphorus filters were frozen and nitrogen filters were stored in a dessicator until analysis. Pond water for total phosphorus (TP) was frozen prior to analysis. Total and particulate phosphorus were extracted by the molybdate-ascorbic acid method (APHA 1980) and analysed using a Unico Spectrophotometer 2800. We measured particulate carbon and nitrogen on a Carlo Erba NCS2500 elemental analyzer, with acetanilide used for standards. We used particulate carbon to nitrogen (C:N) and phosphorus (C:P) ratios to examine differences in resource quality among the ponds (Sterner *et al.* 1997; Hall *et al.* 2004). We also used a juvenile specific growth rate bioassay (see description below) to quantify a composite measure of resources on each of the water types. The relative growth rate was used as an indicator of resource richness (Lampert & Trubetskova 1996; Desmarais & Tessier 1999; Tessier & Woodruff 2002).

Local adaptation experiment

To test for local adaptation to resources, clones from each population were reciprocally grown in water from each pond in a laboratory common garden. Juvenile specific growth rates (Lampert & Trubetskova 1996; Desmarais & Tessier 1999) of each clone on each water source served as the response variable. Resource richness was quantified using the bioassay described above. We predicted that if clones are locally adapted to resources, then native clones should have higher average growth rates than foreign clones on each water source (Kawecki & Ebert 2004).

To ensure that all experimental animals were unique genotypes, we hatched *Daphnia* from diapausing eggs collected from sediments during fall 2007. In January 2008, the sediment was incubated in filtered lake water in the laboratory under early spring-like conditions. Containers were checked twice per week for 2 months for hatching. All hatched *Daphnia* were transferred to 150 mL beakers. Eight clonal lines from each pond were selected randomly for use in the experiment. Clones were split into multiple sublines and grown in low density culture for at least two generations to reduce maternal effects (Lynch 1985). Clones were kept at 20 °C and fed a satiating amount of *Ankistrodesmus falcatus* daily.

To measure the relative fitness of each clone on each resource, we measured the 4 day somatic growth rate of each clone. This juvenile specific growth rate (JGR) is a standard fitness measure for daphniid species and been shown to be a reliable indicator of total lifetime reproductive output (Lampert & Trubetskova 1996; Tessier & Woodruff 2002). Using a 4×4 factorial design (population × water type), we grew eight *Daphnia* clones per population, with two replicates per clone in each water type. We blocked the experiment over six consecutive days with two water types per clone started on each day. Neonates from a single mother's third or greater clutch were gathered within 18 h of birth. To estimate initial weight, five sisters from each clone were harvested, dried at 60 °C and weighed on a UMX2 microbalance (Mettler Toledo). Five additional sisters from each clone were placed in a 200 mL beaker with each water resource and grown for 4 days. Water was filtered through 70 μm mesh to remove invertebrates and changed daily. On day 3, densities in each beaker were reduced to two individuals. On day 5, all individuals were harvested, dried at 60 °C and weighed. JGR was calculated for each clone as the log of the average final weight minus the log of the initial weight divided by the number of days grown.

We followed the methods of Kawecki & Ebert (2004) to test for adaptation to local resources. We used a two-way ANOVA with water source (S) and clone source (population—P) as fixed factors. Clones were nested within population as a random factor. A planned contrast was used to test for local adaptation: clones grown on their local resources vs. foreign clones grown on those same resources. The analysis was run using the Type 3 least squares approach with a Satterthwaite degrees of freedom correction. Due to insufficient reproduction of clonal mothers and death of some experimental animals during the experiment, we were not able to replicate fully the 4×4 factorial design. As such, for each clone by resource growth estimate we used the average of available sublines as our experimental unit. Clones for which we had growth estimates in their own water and at least one foreign water source were included (BridgeS: seven clones, Busey: seven clones, Dump: five clones, Top: five clones; Appendix 1). Running the analysis as a fully balanced design by eliminating clones with missing cells did not qualitatively change results.

The entire data set did not provide estimates for intraclonal variation. Hence, we tested for clonal plasticity or adaptation to resources using only those clones which had multiple estimates in at least two of the four habitat types (15 clones). We tested for plasticity to resource richness using a slope homogeneity test, using our bioassay of resource availability as a covariate crossed with clone (a random effect). Heterogeneous slopes among the clones would suggest a $G \times E$ response indicative of a genetic effect on clonal plasticity. We then performed ANCOVA to test for genetic variability among clones using this same covariate. As there were only 15 clones, we did not test for an effect of host population on the growth response. All analyses were run in SAS 9.1 (SAS Institute).

Genetic analysis

We used five polymorphic microsatellite markers to examine genetic diversity within and differentiation among our populations. The markers were chosen from Colbourne *et al.*'s (2004) library of *D. pulex* microsatellite markers (Dp26, Dp156, Dp244, Dp300 and Dp335). We included *Daphnia* clones hatched from sediments and individuals from early April plankton samples for our estimates of the genetic composition of each spring population. Forty-two to 45 individuals were genotyped from each population. We extracted DNA using the Qiagen DNeasy-96 tissue kit. Polychromase chain reaction (PCR) reactions used 3 μL of genomic DNA, 1 μL of 10 × PCR buffer (Invitrogen), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each primer, 1 U Taq DNA polymerase and water to a final volume of 10 μL. We employed a 'touchdown' thermocycling protocol consisting of an initial denaturation step at 94 °C for 2 min followed by 10 cycles of: 94 °C for 30 s (denaturation), an initial annealing temperature of 58 °C for 30 s, decreasing by 1 °C for each cycle and an extension at 72 °C for 1 min; 30 subsequent cycles using the same denaturation and extension as above but an annealing temperature of 48 °C; and followed by a final extension step of 72 °C for 10 min (Cristescu *et al.* 2006). Microsatellite loci were diluted and multiplexed as unique combinations of allelic size range by fluorescent dye colours (6-FAM, HEX, VIC, PET; Applied Biosystems). Genotyping was performed on an ABI 3730xl Genetic Analyzer at the University of Illinois W.M. Keck Center for Comparative and Functional Genomics. We used the program GeneMapper 3.7 to examine and confirm allele designations for all loci in each individual. In addition, we re-genotyped approximately 5% of our individuals to calculate genotyping error rates (Pompanon *et al.* 2005). Our error rate was very low (<3%).

We calculated allele frequencies, number of alleles per locus (A), number of private alleles (PVT), unique multilocus genotypes (MLG) and expected heterozygosity (H_E) within each population as measures of genetic diversity. Tests for Hardy-Weinberg equilibrium (HWE), significant levels of F_{IS}, and linkage disequilibrium (LD) were used to examine the potential for clonal selection within populations and the assumptions of AMOVA models and F_{ST}. Multiple comparisons within populations were accounted for by sequential Bonferroni correction. To test for population divergence, the total genetic variation among and within each of the populations was partitioned using AMOVA (Excoffier *et al.* 1992) and estimates of F_{ST} analogs (θ: Weir & Cockerham 1984) for pairwise combinations of populations were calculated in Arlequin 3.1 (Excoffier *et al.* 2005). We also tested for isolation-by-distance (a signature of dispersal) by comparing estimates of F_{ST} to all pairwise distances among populations using a Mantel test.

To test the hypothesis that dispersing organisms failed to enter local gene pools (a prediction for local adaptation), we used the program BayesAss+ (Wilson & Rannala 2003) which assigned individuals to one of the four populations using Bayesian posterior probability distributions. We ran the program for 3×10^6 iterations with a 999 999 iteration burnin and δ values of 0.15. We repeated the analysis three times to verify the stability of parameters and assignments. The assignments allowed us to test for recent migration and successful gene flow (first generation sex with residents) and to calculate rates of recent migration among populations. We also compared these results to those obtained using the program Structure (Pritchard *et al.* 2000), which tests for population admixture using a different Bayesian algorithm (see Appendix 3).

We finally tested the hypothesis that clonal selection is present in the populations and hence, may contribute to among population differentation (Berg & Lascoux 2000). We compared the results of our genetic analyses from the entire dataset (egg bank and water column individuals) to those obtained using only individuals collected from the water column. We also constructed a dataset of the unique multilocus genotypes within each of the populations. Analyses using these data subsets attempted to control for clonal reproduction/selection within the water column (Halkett *et al.* 2005). We tested for reductions in the number of loci in linkage and Hardy-Weinberg disequilibrium.

Results

Pond resources

The ponds varied substantially in size, hydroperiod and trophic status (Table 1). Differences in resources were reflected by significant variation in food quantity as measured by chlorophyll-*a* concentration in the ponds ($F_{3,\ 8} = 42.03$, $P < 0.0001$). Food quality was similar among the ponds, as there were no significant differences in the C:N ratio ($F_{3,\ 3} = 0.40$, $P = 0.77$) or C:P ratio ($F_{3,\ 3} = 0.18$, $P = 0.90$), the latter likely due to large SEs. These general trends were reflected in our bioassay of resource use, as Bridge South Pond had the highest quantity of food and most effective conversion rate to growth, while Busey and Dump ponds had lower quantities of food and the lowest growth rates (Table 1). However, there was not a significant linear relationship between individual measures of quantity and quality (i.e. chlorophyll or C:P) and the composite resource richness bioassay (chlorophyll-*a*: $r = 0.75$, $P = 0.25$; C:P: $r = -0.26$, $P = 0.74$) suggesting both quantity and

quality affect richness. Generally, resource concentrations (and ratios) among the ponds were similar to those found in other ponds in the Midwest during this time of year (Cáceres *et al.* 2008).

Local adaptation

We found no evidence for local adaptation to resources in our reciprocal transplant experiment (Fig. 1). The mean JGR of clones grown in their home water did not significantly exceed that of foreign clones for any of the four ponds (contrast: $F_{4,\ 54} = 0.53$, $P = 0.71$, Table 2). Likewise, there were no systematic differences in mean JGR among or within the populations. However, there was a large effect of environment (S) on the mean growth of clones across the four habitats (Table 2). At the clonal level, we found no evidence for differences in phenotypic plasticity among the clones (slope homogeneity test: $F_{14,\ 89} = 0.67$, $P = 0.80$) or genetic variability in growth rate across a resource gradient (ANCOVA: $F_{14,\ 89} = 1.00$, $P = 0.46$). However, we found evidence for an effect of resource richness on growth (ANCOVA: $\beta_Q = 0.71$, $F_{1,\ 103} = 82.77$, $P < 0.0001$; Fig. 2). This suggests clones from these ponds have a strongly plastic response to resource richness, but little genetic differentiation in this response.

Microsatellite diversity

We genotyped 174 individuals from the four populations (available online: http://hdl.handle.net/10255/dryad.1664). All five microsatellite loci were polymorphic with five to 15 alleles per locus. Populations generally had high allelic richness (range: 4.0–7.4; Table 3), with at least three (maximum 11) alleles found for each locus in each population. Total genetic variation was high (mean $H_E = 0.62$) and many alleles were shared among populations. Those populations with fewer alleles often contained a subset of the alleles in the more diverse populations (as private alleles were only in the two allelicly diverse populations). This suggests a shared colonization history from a regional gene pool or ongoing dispersal.

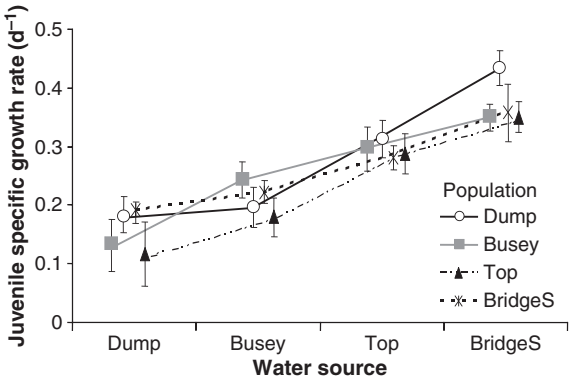

Fig. 1 Mean relative fitness within four *Daphnia* populations grown on four host resources. Relative fitness was measured as the 4 day somatic juvenile specific growth rate. Each set of points represents clones from a single source population. Host ponds are ordered from the lowest to highest relative resource abundance (from bioassay). There was a strong effect of water source, but no effect of population or clone on relative growth. Error bars represent SE and reflect among-clone variation.

Table 2 Analysis of variance (ANOVA) testing for the effects of water source (S) and population (P) on the relative fitness of *Daphnia*. The contrast tests the local vs. foreign interaction for local adaptation

Effect	d.f. effect	d.f. error	Mean Square	*F*	*P*
S	3	20.98	0.1992	33.45	<0.0001
P	3	54	0.0050	0.54	0.66
S × P	9	54	0.0055	0.92	0.51
Clone(P)	20	54	0.0094	1.57	0.10
Residual	54		0.0060		
Contrast					
Local vs. foreign	4	54		0.53	0.71

Population genetic differentiation and gene flow

Population genetic differentiation among all populations was significant. Overall, AMOVA indicated that 22% of the total variation was divided among populations, while only 3% was among individuals within populations (most was within individuals). All pairwise F_{ST} estimates were significant despite the geographic proximity of some of the populations [mean (SE) $F_{ST} = 0.22$ (0.05); Table 4]. These estimates hint at an isolation-by-distance pattern, but the relationship was not significant (Mantel: $r = 0.84$, $P = 0.17$), likely due to the low sample size (Appendix 2). Even Top and Dump ponds, which are separated by only 250 m showed moderate and significant differentiation ($F_{ST} = 0.12$). However, only two of the four populations met the assumptions of HWE and linkage equilibrium for *F*-statistics. To test the effect of these violations, we first removed identical multilocus genotypes, but this only slightly reduced disequilibrium for Busey and Dump ponds (Table 3). We next considered only genotypes collected from the water column (130 individuals). Here, Busey Pond came into complete linkage and HWE. This suggests the possibility of a Wahlund effect for the Busey population (the water column population being distinct from the egg bank). An AMOVA analysis considering the water column and hatched egg banks of individual ponds as separate subpopulations attributed <1% of the variance

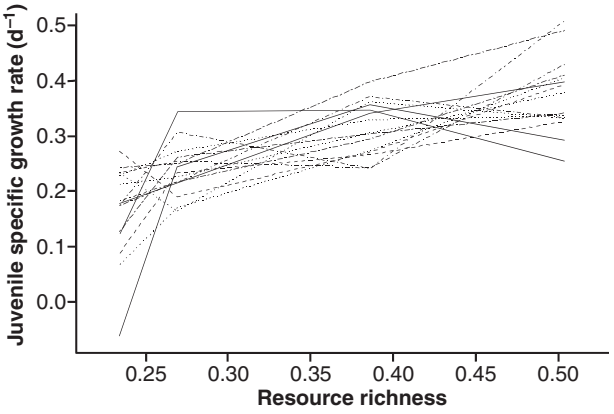

Fig. 2 Mean juvenile specific growth rate of *Daphnia* clones in four resource environments. Resource richness was calculated by the *D. pulex-pulicaria* resource bioassay (per day). Clones hatched from each of the four habitats were included in the analysis. There was a strong effect of resource richness, but no effect of clone or its interaction with environment. Resource richness from left to right: Dump Pond, Busey Pond, Top Pond and BridgeS Pond. For clarity, error bars have been removed.

among subpopulations and F_{ST} among subpopulations were <0.025, indicating a small effect. The analysis also revealed the possibility of clonal selection in Busey Pond. When only considering the water column individuals, F_{IS} was negative suggesting clonal replication led to a higher frequency of heterozygotes than expected by chance. Despite these caveats, when only individuals from the water column were considered, the F_{ST} among populations only increased slightly, reflecting the robustness of this measure to these violated assumptions [mean F_{ST} = 0.24 (0.06); Table 4].

Bayesian assignment analyses suggested contemporary among population migration rates were asymmetric, ranging from 0.003 to 0.212 (Table 4). A few individuals in Busey and Top ponds appeared to be recent immigrants, as well as many individuals from Dump Pond (Fig. 3; Table 4). Many of these Dump Pond individuals appeared to be immigrants from Top Pond or a pond with similar allele frequencies. The proportion of times individuals were assigned as first vs. second generation migrants to Dump Pond was 0.74, suggesting successful integration of migrants into the gene pool (Fig. 3). This is despite significant pairwise genetic differentiation among these populations (F_{ST} = 0.12). Similar conclusions were reached based on the Structure analysis, although higher rates of admixture were observed in the Bridge South population (Appendix 3). For example, the same individuals were identified as migrants in both analyses, and the admixture in Dump Pond was overwhelmingly from Top Pond (Appendix 3). Finally, when we ran the BayesAss+ analysis using only those individuals collected from the water column, movement rates and assignments were largely unchanged (data not shown).

Discussion

We found strong genetic differentiation among four populations of the cyclic parthenogen *Daphnia pulex* in east central Illinois, despite their geographic proximity (<50 km distant). This genetic differentiation was found whether or not we included identical multilocus genotypes or whether we analysed only those individuals from the water column or added those hatched from sediments. Such a pattern is widespread among populations of cyclic parthenogens (Spitze 1993; Vanoverbeke & De Meester 1997; Lynch *et al.* 1999; Morgan *et al.* 2001; Haag *et al.* 2006; Thielsch *et al.* 2009). Less clear

Table 3 Genetic diversity of *Daphnia pulex* populations using five polymorphic microsatellite loci. Analyses were run for the entire dataset (All) and a subset of the data where individuals were collected from the water column (WC). Variables: Number of genotyped individuals—hatched from eggs/total (*n*). Average number of alleles per locus (A). Number of private alleles/total alleles (PVT). Percent unique multilocus genotypes in the sample (MLG). Expected heterozygosity (H_E). Loci out of Hardy–Weinberg equilibrium (HWE) and locus pairs in linkage disequilbrium (LD) using all data/only unique multilocus genotypes. Diversity estimates are mean (±SE) by locus. The totals are averages (SE) or counts across populations. *Value is significantly different from zero

Pond	Dataset	*n*	A	PVT	MLG	H_E	HWE	LD	F_{IS}
BridgeS	All	15/44	7.4 (1.5)	6/37	43/44 = 97.7	0.731 (0.054)	0/0	0/0	0.01
	WC	29	6.8 (1.4)	5/34	29/29 = 100.0	0.741 (0.048)	0	0	−0.02
Busey	All	15/43	4.0 (0.5)	0/20	34/42 = 81.0	0.437 (0.092)	5/2	7/7	0.03
	WC	28	2.6 (0.6)	1/13	24/28 = 85.7	0.400 (0.110)	0	0	−0.18*
Dump	All	7/45	6.8 (1.2)	4/34	35/43 = 81.4	0.721 (0.051)	4/4	10/7	0.00
	WC	38	6.0 (0.8)	4/30	29/36 = 80.6	0.714 (0.052)	4	10	0.01
Top	All	7/42	4.6 (0.5)	0/23	42/42 = 100.0	0.574 (0.099)	0/0	0/0	0.13*
	WC	35	4.2 (0.5)	0/21	35/35 = 100.0	0.564 (0.098)	0	0	0.12*
Total	All	44/174	5.7 (0.8)	10/46	152/171 = 88.9	0.616 (0.070)	NA	NA	0.04
	WC	130	4.9 (0.5)	10/43	117/128 = 90.4	0.605 (0.035)			

Table 4 Recent pairwise population genetic divergence (F_{ST}) and migration rates. F_{ST} were calculated from all genotyped individuals ($F_{ST\text{-}A}$) or just individuals from the water column ($F_{ST\text{-}WC}$). A global test for population genetic differentiation revealed significant pairwise differentiation ($F_{ST\text{-}A}$ = 0.225). All pairwise comparisons were significant at the $P < 0.0001$ level using a permutation test. Contemporary non-migration and unidirectional migration rates were calculated in the BayesAss+ program. Non-migration rates represent the proportion of individuals that originated in the source population (i) each generation

				BayesAss+ rates		
Population pair		Observed data		Non-migration	migration	
i	j	$F_{ST\text{-}A}$	$F_{ST\text{-}WC}$	i → i	i → j	j → i
BridgeS	Busey	0.267	0.282	0.9904	0.0151	0.0030
BridgeS	Dump	0.064	0.070	—	0.0119	0.0031
BridgeS	Top	0.156	0.163	—	0.0069	0.0035
Busey	Dump	0.296	0.329	0.9773	0.0186	0.0038
Busey	Top	0.423	0.451	—	0.0033	0.0038
Dump	Top	0.119	0.128	0.7577	0.0038	0.2118
Top	—	—	—	0.9860	—	—

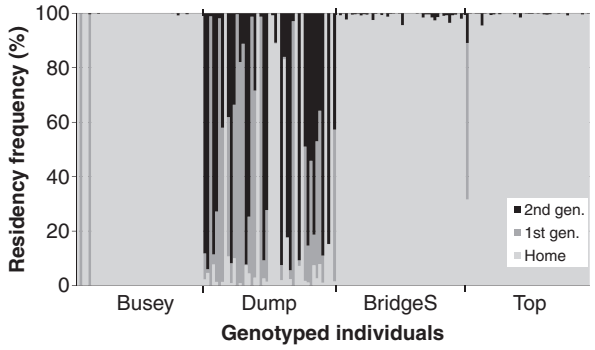

Fig. 3 Simulated assignment of individuals to populations using BayesAss+ based on their multilocus genotype. Bars represent the assignment frequency for individuals and are broken into a home fraction (assigned to the sampling location) and first or second generation (after sex) migrants. Individuals are sorted by sampled population.

is why such a pattern exists despite high dispersal capacity and gene flow potential. A variety of hypotheses—differing on the role of neutral and adaptive mechanisms—have been proposed. We used a combination of experiments and inference from our molecular data to evaluate the relative importance of local adaptation vs. the non-adaptive process of drift in structuring genetic variation. Overall, our data do not support the hypothesis that local adaptation to resources contributes to observed levels of differentiation among these populations. Although we cannot rule out the possibility that local adaptation to factors other than resources might explain genetic differentiation among population pairs where we found no immigrants or admixture, we show such adaptation is not strong enough to prevent gene flow in at least one population pair that exhibits substantial differentiation (F_{ST} of 0.12). Additionally, the genetic data do not support the hypothesis that clonal selection is a strong contributor to among pond differentiation in this case, as only one of the four populations showed evidence for clonal selection. Finally, while our contemporary genetic data do not provide an explicit test for Boileau *et al.*'s (1992) non-equilibrium model, evidence for historic and contemporary dispersal and the young age of the ponds suggest it is more likely than other examined alternatives.

No evidence for local adaptation to resources

We explicitly tested for a role of local adaptation to resources in genetic differentiation among populations, but found no evidence to support this hypothesis in our study. Instead, clones from each population exhibited plasticity to resource richness and had similar juvenile specific growth rate responses. Plasticity may result from the selection of broadly adapted, 'generalist' genotypes that are favoured over clones with specialized responses when individuals experience multiple environments over the course of their lifetime (either through spatial dispersal or where temporal environmental variability is a stronger selective force than spatial variability) (Reboud & Bell 1997; Kassen 2002). In both lakes and ponds, *Daphnia* can experience substantial variation in resource quality and quantity over the length of a growing season (e.g. Declerck *et al.* 2001; Cáceres *et al.* 2008). Several previous studies have also shown plasticity in a number of life history traits (e.g. size of offspring—Tessier & Consolatti 1991; resource allocation—Stelzer 2001).

We recognize that resource adaptation could manifest during a portion(s) of the life cycle not examined here. For example, while we measured responses to resources

during the asexual growth phase, selection for resources of differing quality may be required for allocation to somatic growth vs. reproduction (Wacker & Martin-Creuzburg 2007). As such, local adaptation to resources may occur among our populations for allocation during sexual reproduction. Similarly, resource selection may act most strongly on those individuals hatching from diapausing eggs early in the season and research suggests individuals hatched from dormant eggs may have different competitive abilities than those developing from parthenogenetic females (Arbaciauskas & Lampert 2003). While we cannot rule out local adaptation to resources during different portions of the life cycle, we argue that the large plastic response to resources among our populations provides strong evidence that local adaptation to resources is unlikely here, as trait plasticity should act to limit local adaptation (Kawecki & Ebert 2004).

Although not detected in our study populations, local adaptation to resources may be more important in other aquatic habitats. For example, Declerck *et al.* (2001) found that *Daphnia* clones from one lake experienced reduced survival relative to those from a second lake when grown on water from the second. One lake had consistently high food quantity levels (>100 μg/L chlorophyll-*a*), whereas the other had lower food quantity levels that varied by greater than an order of magnitude during the year [2–50 μg/L (approximately) chlorophyll-*a*]. This difference in the magnitude of and seasonal variation in food availability may cause strong selective differences among systems, but we have not observed such temporal patterns in our ponds (M. Allen, unpublished).

Finally, we recognize that local adaptation can occur in any number of traits that we did not measure. Prior work with *Daphnia* has demonstrated selection for chemical tolerance (Weider & Hebert 1987), behaviour (e.g. De Meester 1996; Cousyn *et al.* 2001; Michels *et al.* 2007), diapause timing (Hairston & Dillon 1990), neck tooth development (Parejko & Dodson 1991) and body size (Tessier *et al.* 1992; Boersma *et al.* 1999). Some of the above factors may be relevant in our study ponds. For example, body size distributions and peak ephippial production windows varied among our populations (M. Allen, unpublished). However, whether these differences reflect local adaptation vs. plasticity is unknown.

Extent of gene flow among populations

The Monopolization Hypothesis differs from other explanations for genetic differentiation in cyclic parthenogens in its emphasis on restricted gene flow resulting from selection against immigrant genotypes in locally-adapted populations. Although it was not possible to experimentally test for local adaptation to all factors that could potentially restrict gene flow from immigrant genotypes, we tested the hypothesis that gene flow was restricted among populations using our molecular data. Overall, we identified immigrant genotypes in three of the four populations using Bayesian approaches (BayesAss+ and Structure) (Pritchard *et al.* 2000; Wilson & Rannala 2003). However, the extent to which subsequent gene flow appears to occur following immigration differed among pairs of populations.

If genetic differentiation results from selection against immigrant genotypes in locally-adapted populations despite high dispersal rates, then genetic assignment methods should identify first generation immigrants with very few or no identification of admixed individuals from resident and immigrant genotypes. We found a pattern consistent with the above prediction in two populations: we identified two first generation immigrants from Bridge South in Busey Pond and one first generation immigrant from Bridge South in Top Pond with little evidence for admixture in either recipient population (Fig. 3; Appendix 3). Although patterns of immigration vs. gene flow in these two pairs of populations are consistent with the hypothesis of selection against immigrant genotypes, it is impossible to say unequivocally from the genetic data alone whether the lack of gene flow represents selection against immigrants *per se* vs. any number of other reasons that immigrants might fail to leave offspring (e.g. see clonal selection below). However, we did identify relatively high genetic admixture in Dump Pond, as only 78% of the individuals collected there were assigned as originating from Dump Pond using BayesAss+. The majority of immigrants into Dump Pond appear to originate from Top Pond, which is located only 250 m away (Table 4; Appendix 3). Surprisingly, however, we did not detect immigration in the opposite direction and the two populations did not exhibit the lowest pairwise F_{ST} (0.12); BridgeS and Dump ponds exhibited a lower pairwise F_{ST} (0.07). These results indicate that gene flow into Dump Pond from Top Pond is not high enough to prevent genetic differentiation, but are contrary to the hypothesis that selection against immigrants prevents gene flow.

Neutral processes provide alternate explanations for substantial genetic differentiation among pairs of populations such as Dump and Top. First, Berg & Lascoux (2000) hypothesized that frequent genetic bottlenecks or extinction/colonization dynamics would delay the time to migration-drift equilibrium and might lead to persistently high levels of genetic differentiation. While some *Daphnia* metapopulations exhibit frequent crashes, egg bank removal and recolonization (e.g. Pajunen & Pajunen 2003), this explanation is unlikely here as diapausing egg banks are well established in our populations and

reduce the severity of genetic bottlenecks due to population crashes.

Second, clonal selection could lead to genetic drift and high F_{ST} among populations if effective populations are reduced during a season and few individuals contribute to subsequent generations (Berg & Lascoux 2000). We found some support for the hypothesis that clonal selection occurs in the Busey and Dump populations, two populations with relatively long hydroperiods that may permit more asexual generations between bouts of sex. For both of these populations, nearly all loci deviated from HWE, many pairs of loci were significantly linked and the proportions of unique multilocus genotypes were much less than one. Additionally, the Busey population had a significantly negative F_{IS} value. However, we found no evidence for clonal selection in Top and Bridge South ponds, two ponds for which rates of gene flow were low. This suggests that although clonal selection may occur in some ponds across the metapopulation, it cannot explain the overall pattern of diversification observed among all populations.

We cannot rule out the hypothesis that observed genetic differentiation results from persistent founder effects (i.e. a lack of migration-drift equilibrium: Boileau *et al.* 1992). Two pieces of evidence align with this hypothesis. First, allele sharing among populations, some recent dispersal and a hint of an isolation-by-distance pattern in pairwise F_{ST} among ponds suggest dispersal has occurred historically and is ongoing to some extent. Low dispersal rates among most populations and large population sizes (resulting from thousands of eggs hatched annually) would theoretically require hundreds to thousands of generations for migration-drift equilibrium to be reached (Boileau *et al.* 1992). Second, few generations have passed since these populations were founded. Top and Busey ponds, for example, are fewer than 200 generations old, as Top Pond was formed in an old railroad bed and Busey Pond was formed by stream channelization. Consequently, a lack of drift-migration equilibrium among systems is a more likely contributor to divergence among our populations than local adaptation, clonal selection or recent bottlenecks.

Conclusion

Several features of *Daphnia* life history and ecology may lead to large F_{ST} among populations despite their high dispersal capacity. Specifically, colonization by a few individuals with subsequent rapid asexual reproduction should create genetic differentiation among populations via founder effects. Such founder effects, in the face of very large population sizes, will ultimately take a long time to reach an equilibrium F_{ST} that is lower than the original genetic differentiation from founding (Boileau *et al.* 1992). Furthermore, extinction/colonization dynamics and demographic/genetic bottlenecks, which may be experienced by *Daphnia* populations, will delay the time to reach equilibrium F_{ST}. Local adaptation may also lead to the maintenance of high equilibrium F_{ST} among populations. However, the degree to which local adaptation needs to be invoked to explain observed levels of genetic differentiation has rarely been considered.

Here, we have demonstrated that genetic differentiation among four geographically proximate, but genetically distinct, *Daphnia pulex* populations does not result from local adaptation to resource richness. While local adaptation to other factors may be important in some pairs of populations, we also show that restricted gene flow—a prediction for the maintenance of genetic differentiation via local adaptation—does not likely explain the substantial genetic differentiation observed between at least one pair of our populations. We therefore suggest that factors other than local adaptation operate to maintain genetic differentiation in these populations. Moreover, although a temporal record of genetic change within and among populations may provide insight into the relative roles of local adaptation vs. other factors, we stress that the use of molecular data alone is not likely to effectively elucidate the roles of local adaptation vs. other factors (e.g. deviation from migration-drift equilibrium, high rates of drift due to clonal selection, limited hatching from the egg bank at the beginning of the growing season, etc.). We therefore suggest that future studies of genetic differentiation in cyclic parthenogens combine experimental tests for local adaptation with genetic studies.

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This paper is part of M Allen's PhD dissertation which examined the ecological and evolutionary effects of dispersal on freshwater zooplankton. R Thum has broad interests in evolutionary ecology and particularly in the application of molecular markers to ecological questions. C Cáceres' interests in evolutionary ecology are currently focused on metacommunity dynamics and host parasite interactions.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Design matrix of local adaptation experiment. Cell contain the number of clones for each water by population source combination. Note that clones were nested within populations for the statistical analysis. While the target sample size was 8 for each treatment, some clonal mothers did not produce enough neonates during the experimental setup to be included in the experiment. Only two replicates were lost due to experimental deaths (one from Busey (Pop.) × Top (Res.) and one from Dump × Dump).

Appendix S2 Test for isolation by distance in genetic differentiation among populations. The correlation between genetic and geographic distance was high, but not significant (Mantel $r = 0.84$, $P = 0.17$), likely due to the low number of populations in the sample ($n = 4$).

Appendix S3 Individual assignments among four clusters using the program Structure. Each bar represents one individual and is broken into four colors whose heights correspond to the proportion of times the individual was assigned to a particular cluster. Individuals are grouped by their pond of origin for clarity. The program was run for 100 000 burn in replications and 500 000 MCMC replications to calculate cluster assignments.

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