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Population Differentiation in *Daphnia* Alters Community Assembly in Experimental Ponds

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ABSTRACT: Most studies of community assembly ignore how genetic differentiation within species affects their colonization and extinction. However, genetic differentiation in ecologically relevant traits may be substantial enough to alter the colonization and extinction processes that drive community assembly. We measured significant molecular genetic and quantitative trait differentiation among three *Daphnia pulex* × *pulicaria* populations in southwestern Michigan ponds and investigated whether this differentiation could alter the assembly of pond zooplankton communities in experimental mesocosms. In this study, we monitored the invasion success of different *D. pulex* × *pulicaria* populations after their introduction into an established zooplankton community. We also monitored the invasion success of a diverse array of zooplankton species into different *D. pulex* × *pulicaria* populations. Zooplankton community composition depended on the *D. pulex* × *pulicaria* source population. *Daphnia pulex* × *pulicaria* from one population failed to invade zooplankton communities, while those from other populations successfully invaded similar communities. If population differentiation in other species plays a role in community assembly similar to that demonstrated in our study, assembly may be more sensitive to evolutionary processes than has been previously generally considered.

Keywords: invasion, population differentiation, hybridization, invasion resistance, *Daphnia pulex* × *pulicaria*, community assembly.

Introduction

Community composition is often the product of a process of community assembly. During assembly, communities are generated as a result of a series of successful colonization events. The colonists may subsequently cause the local extinction of other resident species (Law and Morton 1996). The eventual community composition that results from the assembly process depends on a number of factors, including the order of colonization and individual competitive differences between species (Drake 1990; Belyea and Lancaster 1999; Chase 2003). However, most theoretical and experimental studies of community assembly treat the species-specific traits that determine colonization success as evolutionarily fixed.

Ignoring intraspecific variation in these traits may be warranted if such variation is small or does not influence species composition. However, recent compelling studies indicate that the genetic variation harbored within species might influence the outcome of assembly. For example, host plant genotype has been shown to explain differences in resident herbivore community composition (Whitham et al. 2003; Johnson and Agrawal 2005; Wimp et al. 2005). Similarly, the genotype of resident species influenced the colonization success of other competing species in both experimental plant and zooplankton communities (Weltzin et al. 2003; De Meester et al. 2007; Crutsinger et al. 2008). Although these studies draw attention to the potential role of genetic differentiation in community ecological processes, they consider only genetic differences within resident species and not within colonizing species. They also do not characterize how resident populations differ from one another in ecologically important traits.

Genetic differences within species may also influence their colonization success in new habitats and therefore the outcome of community assembly. Few empirical studies address this possibility. A number of studies have shown that successful invasive species have diverged from native source populations in both morphological traits and molecular markers (Siemann and Rogers 2001; Lindholm et al. 2005; Yonekura et al. 2007; Dlugosch and Parker 2008; Beckmann et al. 2009). However, these studies did not determine whether this differentiation resulted from or caused colonization success. Crawford and Whitney (2010) recently established a link between increased genetic diversity in colonizing *Arabidopsis thaliana* populations and changes in fitness-related traits that may increase colonization success in a new habitat. However, their experiment did not consider the potential role that resident competitors could play in inhibiting colonization success. They also reported no difference in colonization success or failure among the various populations that would qualita-

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tively influence community composition. The link between precolonization genetic differentiation and invasion success remains to be established.

The goal of our study was to determine whether intraspecific genetic differentiation in one species, the freshwater crustacean *Daphnia pulex* × *pulicaria*, could alter the outcome of zooplankton community assembly. Because *Daphnia* often demonstrate substantial genetic variation among populations (Spitze 1993; Lynch et al. 1999; Morgan et al. 2001), we used a *D. pulex* × *pulicaria* source population to substitute for treatments with different genetic properties. We chose natural populations that likely receive colonists from the same regional species pool. We tested whether source population influenced two components of the assembly process: the ability to inhibit colonization by other species and the ability to successfully colonize habitats with established communities.

In southwestern Michigan ponds, obligately parthenogenetic *D. pulex* × *pulicaria* are often the dominant herbivorous grazer. Populations of this hybrid freshwater crustacean are genetically divergent from one another, and clones are able to disperse to other ponds in the region (Pantel et al. 2011). However, some ponds in this region also contain the cyclically parthenogenetic parental species, *D. pulex*. Aside from the mode of reproduction, *D. pulex* and their hybrid are morphologically and ecologically indistinguishable. Taxonomic assignment relies on a single diagnostic allozyme marker (Hebert et al. 1989). For simplicity's sake, we focused only on populations with no or a very low frequency of *D. pulex*. We quantified the molecular and quantitative trait differentiation among three *D. pulex* × *pulicaria* populations. We then placed the populations in two mesocosm experiments to determine whether source population influenced *D. pulex* × *pulicaria* colonization success and impact on the colonization success of other zooplankton species.

Material and Methods

Survey of Population Genetic Differentiation

Our mesocosm experiments required ponds with substantial genetic differences among resident *Daphnia pulex* × *pulicaria* populations. To determine the degree of molecular and quantitative trait differentiation among populations, we surveyed *Daphnia* from natural and artificial ponds within a 9-mi radius of W. K. Kellogg Biological Station (KBS; Kalamazoo County, MI) that were not connected to larger lakes or water bodies in the summer of 2006. In this study, we report only the molecular and quantitative genetic diversity of *D. pulex* × *pulicaria* populations from the three ponds included in the mesocosm experiments: Pond Lab 9 (PL9), KBS Fountain (KBSF), and Turner Pond (TP). We randomly selected these three ponds from the region because previous surveys indicated that they harbored *D. pulex* × *pulicaria* populations. Subsequent surveys found that *D. pulex* may reside in TP but at a very low frequency (J. H. Pantel, unpublished data). *Daphnia pulex* may also reside in the other two ponds at similar low frequencies, but they were not found.

In our analysis, we refer to the *D. pulex* × *pulicaria* assemblage at each pond as a population. We extracted DNA from a total of 38 individuals collected from the three populations. To extract DNA, we ground individual *D. pulex* × *pulicaria* in 1.6-mL microcentrifuge tubes with pestles in 500 μL of 65°C CTAB buffer solution (1.4 M NaCl, 0.2% 2-mercaptoethanol, 1 M Tris-HCl pH 8, 0.5 M ethylenediaminetetraacetic acid [EDTA] pH 8, 2% hexadecyltrimethylammonium bromide [CTAB], 1% polyvinylpyrrolidone [PVP]), vortexed the ground mixture, and incubated it for 60 min. We then added 500 μL of chloroform : isoamyl alcohol 24 : 1 to each sample, centrifuged the tubes for 15 min, and transferred the top layer of this mixture to new 1.6-mL microcentrifuge tubes. This new mixture was cleaned by adding 250 μL of cold isopropyl alcohol to the tube, incubating this sample at 4°C for 60 min, and then centrifuging the sample tubes for 15 min. The isopropyl alcohol was then poured out of the tube, leaving the DNA pellet. We cleaned the DNA pellet again by adding 1 mL of cold 80% ethanol, centrifuging this mixture for 5 min, and removing the ethanol with a pipette. Samples were then air-dried for 24 h to remove excess ethanol.

We genotyped each individual at seven microsatellite loci (Dp22, Dp27, Dp38, Dp102, Dp196, Dp433, and Dp461; Colbourne et al. 2004). The forward primers for each marker were labeled with a fluorescent dye (FAM and HEX; Integrated DNA Technologies). We performed multiplex polymerase chain reaction (PCR), using the Qiagen multiplex PCR kit. Each 8-μL PCR reaction contained 0.02 μg μL^{-1} DNA, 1 × Qiagen multiplex PCR master mix, and 0.2 μM of each primer (0.4 μM for Dp102). The PCR conditions consisted of an initial 15-min denaturing step at 95°C and then 25 cycles of 94°C for 30 s, annealing temperature (46.5°C for Dp433 + 27 + 102, 50.5°C for Dp461 + 196 + 78, and 45°C for Dp38 + 22) for 1.5 min, and 72°C for 1 min, followed by a final extension step at 60°C for 30 min. We added Rox-labeled size standards to each amplified sample. All fluorescently labeled fragments were detected with the Applied Biosystems 3130XL or 3730 Genetic Analyzer and interpreted for length, using GENEMARKER software (SoftGenetics, State College, PA). At each locus, we scored fragment lengths as alleles and sorted alleles into multilocus genotypes (MLGs; combination of alleles at multiple loci). Because *D. pulex* × *pulicaria* reproduce via obligate parthenogen-

esis, unique MLGs may represent clonal lineages. To summarize population genetic diversity, we calculated Simpson's index of diversity, using the equation $D = 1 - \sum [n_i(n_i - 1)/N(N - 1)]$, where n_i is the total number of individuals at a site with a given MLG, N is the total number of individuals sampled at that site, and D estimates the probability that two individuals chosen at random from the population belong to different MLGs (Simpson 1949). To determine whether populations were differentiated from one another, we used an analysis of molecular variance (AMOVA) in ARLEQUIN 3.1 (Excoffier et al. 2005) to calculate F_{ST}, the proportion of molecular genetic variation that was found among the three populations. ARLEQUIN tests the significance of F_{ST} by randomly permuting individuals among populations and comparing the observed F_{ST} value to the null distribution.

We also measured the distribution of two quantitative traits in each of the three *D. pulex* × *pulicaria* populations: juvenile growth rate (g; day^{-1}) and mass at birth (m_B; μg). Juvenile growth rate is the change in body mass each day from birth to sexual maturity:

$$g = \frac{\ln W_2 - \ln W_1}{t_2 - t_1},$$

where W_1 is the dry mass at birth (time t_1) and W_2 is the dry mass at sexual maturity (time t_2). Mass at birth is the dry mass of individual *D. pulex* × *pulicaria* neonates. At each of the three ponds, we collected 20 individual *D. pulex* × *pulicaria* clones and placed them in a common-garden experiment. All clones were placed in a Percival model I-60LL growth chamber set to a constant 20°C and a 14 : 10 L : D photoperiod. Clones lived in containers with 50 mL of filtered water from the KBS Reservoir, and this water was replaced every other day. On the days that the *D. pulex* × *pulicaria* clones received fresh water, they were fed 9.72 μg mL^{-1} of Shellfish Diet (Reed Mariculture), and on the other days, they were fed Shellfish Diet at a concentration of 4.86 μg mL^{-1}. To reduce the effect of maternal environment on trait values, we kept the clones in culture for two generations before measuring them.

We measured traits in two replicates of each of the 60 original clones, for a total of 120 experimental *D. pulex* × *pulicaria* lines. However, some of these experimental lines died at various stages of the common-garden experiment, allowing us to record measurements for only a subset of the 120 experimental lines. When an individual *D. pulex* × *pulicaria* from each line reproduced, we immediately preserved all but three members of the clutch in 90% ethanol. Six days later, we preserved the remaining three individuals in 90% ethanol. We dried the preserved individuals for 24 h in a Napco model 320 drying oven at 60°C and weighed them on a Sartorius SE2 microbalance to the nearest 0.1 μg.

We estimated juvenile growth rate (g) and mass at birth (m_B) in each population by averaging trait values for all clones sampled from the pond. Measurements for both replicates of each clone were averaged. To determine whether pond populations differed from one another in trait values, we formed a nested ANOVA model in JMP (ver. 4; SAS Institute, Cary, NC) with pond and clone nested within pond as the main factors. Data for all clones and their replicates were included. Pond and clone nested within pond were fitted as random factors, which allowed the model to estimate V_{GB}, the among-pond genetic variance, and V_{GW}, the within-pond genetic variance. In this model, the within-clone variance is included in the error term, and thus it is not considered in the among-pond genetic variance. To compare populations, we used the estimates that resulted from the ANOVA to calculate the proportion of trait variance found among populations, as opposed to within them: $Q_{ST} = V_{GB}/(V_{GB} + 2V_{GW})$ (Spitze 1993). We tested the significance of among-population variance by comparing the difference in the maximum likelihood value for the model with and without pond as a factor (Littel 1996). To determine which pairs of populations differed from one another in terms of g and m_B, we used R (ver. 2.8.1) to apply Holm's sequential Bonferroni correction to the results of pairwise t-tests among all populations. Degrees of freedom for these t-tests were estimated with the Welch approximation.

Mesocosm Experiments

During the summer of 2004, we placed *D. pulex* × *pulicaria* from three pond populations into two mesocosm experiments on the grounds of KBS. In the first experiment, we determined whether *D. pulex* × *pulicaria* populations differed in their ability to successfully colonize a new habitat. In the second experiment, we determined whether *D. pulex* × *pulicaria* populations differed in their ability to inhibit colonization of other zooplankton species.

Establishment Experiment

The purpose of this experiment was to determine whether *D. pulex* × *pulicaria* populations differed in their ability to successfully colonize a new habitat. We filled 10 300-L polyethylene tanks with 271 L of well water in July 2004 and waited for 3 days to allow the water to oxygenate. We then inoculated all of the tanks with a diverse array of planktonic algae and microbes by pooling water from 20 different ponds, filtering it though a 35-μm filter, and adding 150 mL of the water to each tank. Two days later, we provided tanks with target levels of nitrogen and phos-

phorus comparable to those in natural eutrophic ponds (150 μg L^{-1} phosphorus and 2,100 μg L^{-1} nitrogen) to serve as a resource base for phytoplankton. After the algal and microbial communities grew with nutrients for 8 d, we inoculated eight of the tanks with a diverse array of non-*Daphnia* zooplankton species. These zooplankton were collected at seven ponds, pooled, and split to form nearly identical inocula in the eight tanks. The other two tanks received only nutrients, algae, and microbes and were used as controls in the establishment experiment. The controls tested whether the presence of a zooplankton community influenced *D. pulex* × *pulicaria* growth.

We allowed the tank zooplankton communities to develop for 61 days before we tested the ability of different *D. pulex* × *pulicaria* populations to invade each of the communities. To test *D. pulex* × *pulicaria* invasion ability, we divided 80 L of water and biotic communities (algae and microbes, with zooplankton present or absent depending on the treatment) from each tank into four 20-L buckets. We used each bucket as an experimental unit to determine whether *D. pulex* × *pulicaria* populations differed in their ability to increase from a low density after introduction into a zooplankton community. The populations used in this experiment were the same as those in the genetic survey and inhabit natural or artificial ponds on the grounds of KBS or nearby Lux Arbor Reserve (Barry County, MI) that have existed for more than 30 years (M. A. Leibold, personal observation). Population PL9 inhabits Pond Lab 9, and population KBSF inhabits KBS Fountain. Both ponds are located on the grounds of KBS and are separated by ~1 km. Population TP inhabits Turner Pond, which is located on the grounds of Lux Arbor Reserve and is separated from both PL9 and KBSF by ~9 km. The mixed population was an equal mixture of populations PL9, KBSF, and TP that had been added together to form a genetically rich population for this experiment. For the establishment test, we collected *D. pulex* × *pulicaria* from PL9, KBSF, and TP in early September. We haphazardly isolated enough individual females to stock buckets at a density of 0.45 individuals L^{-1}. After allowing the *D. pulex* × *pulicaria* to grow for 15 d, we filtered and preserved the entire bucket zooplankton community in Lugol's solution for enumeration.

We defined establishment as the ability to increase from a low density. We calculated the intrinsic rate of population increase (r; day^{-1}) for each invading *D. pulex* × *pulicaria* population. Each replicate's zooplankton community originated from one of eight source tanks. To determine whether the source zooplankton community altered the invading *D. pulex* × *pulicaria*'s growth rate, we compared *D. pulex* × *pulicaria* growth rate among the eight source tanks, using a one-way ANOVA. To determine whether population (PL9, KBSF, TP, or mixed), zooplankton (present or absent), and their interaction influenced *D. pulex* × *pulicaria*'s growth rate, we used a two-way ANOVA with population, zooplankton, and population × zooplankton as factors. For post hoc comparisons, we applied Holm's sequential Bonferroni correction to the results of pairwise *t*-tests among all populations and estimated degrees of freedom for *t*-tests, using the Welch approximation. All statistical analyses were conducted in R (ver. 2.8.1).

Exclusion Experiment

The purpose of this experiment was to determine whether *D. pulex* × *pulicaria* populations differed in their ability to inhibit colonization of other zooplankton species. We established 20 cattle tanks at the same time as our eight stock zooplankton cattle tanks and prepared them in a similar fashion, except for zooplankton addition. To conduct the exclusion experiment, we inoculated tanks with only *D. pulex* × *pulicaria*. In 16 tanks, we replicated each of four treatments (different *D. pulex* × *pulicaria* populations: PL9, KBSF, TP, mixed) four times. The PL9, KBSF, and TP treatments were stocked with *D. pulex* × *pulicaria* females haphazardly isolated from each respective pond at an initial population density of 0.11 individuals L^{-1}. The mixed treatment received *D. pulex* × *pulicaria* from all three ponds at the same density as treatments from single ponds. To determine whether *D. pulex* × *pulicaria* influenced the growth of the colonizing zooplankton species, we set up four control tanks that received no *D. pulex* × *pulicaria*.

The 20 cattle tanks remained in isolation for 61 days before zooplankton colonization. We then collected and mixed water and zooplankton from all eight of our stock zooplankton cattle tanks (obtained from the establishment experiment described previously) and divided this mixture among all 20 experimental cattle tanks (see table 1 for colonizing zooplankton composition and density). After

Table 1: Densities of colonizing zooplankton species in the exclusion experiment

Species	Density
Simocephalus vetulus	.06 ± .08
Simocephalus serrulatus	.57 ± .04
Bosmina longirostris	.03 ± .02
Chydorus sphaericus	.56 ± .12
Ceriodaphnia reticulata	2.23 ± .18
Alona guttata	5.14 ± .46
Scapholeberis kingi	.07 ± .02
Macrothrix rosea	1.74 ± .19
Pleuroxus procurvus	1.38 ± .47
Cyclopoid copepod	1.27 ± .14

Note: Copepods were classified to order. Densities (individuals L^{-1}) are given as mean ± SE.

Table 2: Summary of molecular and quantitative genetic properties of three *Daphnia pulex* × *pulicaria* populations

Population	D	g (SE)	n	m_B (SE)	n
PL9	.71	.504 (.009)	19	2.4 (.1)	20
KBSF	0	.561 (.015)	17	2.5 (.2)	17
TP	.91	.548 (.022)	9	1.4 (.2)	9

Note: We measured two traits in a common-garden experiment, juvenile growth rate (g [day^{-1}]; change in body mass each day from birth to sexual maturity) and mass at birth (m_B [μg]). For the population at each of three ponds, we calculated Simpson's D (the probability that two individuals chosen at random from a population belong to different multilocus genotypes) and the mean and SE of both traits. n is the number of clones from that pond for which the trait was measured. PL9, Pond Lab 9; KBSF, Kellogg Biological Station Fountain; TP, Turner Pond.

allowing the zooplankton to establish for 15 days, we filtered the community present in 12 L of water from each tank and preserved them in Lugol's solution for enumeration. We defined exclusion as the ability to limit the establishment of colonizing zooplankton species. We calculated intrinsic rate of population increase (r; day^{-1}) for all colonizing zooplankton species. To determine whether the resident *D. pulex* × *pulicaria* population influenced the growth rate of colonizing zooplankton, we compared the r values of all 10 zooplankton species among treatments PL9, KBSF, TP, and mixed and the control with MANOVA.

Results

Population Genetic Differentiation

The three *Daphnia pulex* × *pulicaria* populations in our study differed in genetic diversity. The KBSF population contained only one MLG (table 2). The population from PL9 was much more diverse ($D = 0.71$), containing five MLGs. The TP population also had five MLGs present, but their distribution was much more even than the distribution in PL9 ($D = 0.91$). The KBSF and PL9 populations shared a single MLG, but all other MLGs in PL9 and TP were unique to those ponds. A significant portion of the molecular genetic variation was structured among populations ($F_{ST} = 0.26$, $P < .01$).

The three *D. pulex* × *pulicaria* populations also differed considerably in values of quantitative traits (table 2). *Daphnia pulex* × *pulicaria* clones from TP were significantly smaller at birth (m_B; TP: 1.4 ± 0.2 μg) than those from the other two sites (PL9: 2.4 ± 0.1 μg, $t_{11} = 4.28$, $P < .01$; KBSF: 2.5 ± 0.2 μg, $t_{21} = 3.81$, $P < .01$). Clones from PL9 and KBSF did not significantly differ from one another in terms of m_B ($t_{21} = 0.71$, $P = .48$), but they did differ from one another in juvenile growth rate (g; PL9: 0.504 ± 0.009 day^{-1}; KBSF: 0.561 ± 0.015 day^{-1}, $t_{27} = 3.29$, $P = .01$). The TP population had a juvenile growth rate that was intermediate (0.548 ± 0.022 day^{-1}) between PL9 ($t_{11} = -1.88$, $P = .18$) and KBSF ($t_{27} = 0.50$, $P = .62$). A large and significant proportion of genetic variance in juvenile growth rate was found among the three ponds, as opposed to within them ($Q_{ST} = 0.82$, $P = .01$). The Q_{ST} value for mass at birth ($Q_{ST} = 0.38$) was also significant ($P < .01$).

Establishment Experiment

Although each of eight replicates contained a resident zooplankton community that originated from a different source tank, source tank did not explain a significant amount of variation in invading *D. pulex* × *pulicaria* growth rate ($F_{7,24} = 1.14$, $P = .37$). The presence of a zooplankton community significantly decreased *D. pulex* × *pulicaria* growth (fig. 1; $F_{1,32} = 4.18$, $P = .05$). Source populations also differed significantly in their growth rates ($F_{3,32} = 4.08$, $P = .01$). Post hoc pairwise comparisons indicated that only treatments PL9 and TP differed significantly in growth rate ($t_{18} = 3.34$, $P = .02$). The interaction between zooplankton and population was not significant ($F_{3,32} = .35$, $P = .79$).

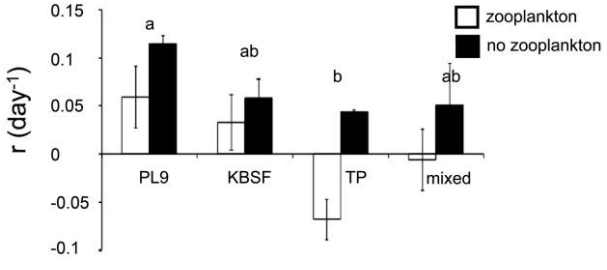

Figure 1: Growth rate (r; day^{-1}) of four different *Daphnia pulex* × *pulicaria* populations after introduction at low density into experimental mesocosms. Population PL9 is from Pond Lab 9, population KBSF is from KBS Fountain, and population TP is from Turner Pond. The mixed treatment is an equal mixture of populations PL9, KBSF, and TP that were added together for the purposes of this experiment, making up the genetically rich treatment. Open bars represent *D. pulex* × *pulicaria* that were introduced into experimental mesocosms that contained a diverse zooplankton community, and filled bars represent *D. pulex* × *pulicaria* that were introduced into experimental mesocosms that contained no other zooplankton species. The lowercase letters above the bars refer to the difference in mean r among treatments when a resident zooplankton community was present (*open bars*). Treatments with similar values of r share a letter. Treatments that do not share letters differed significantly from one another.

Exclusion Experiment

Zooplankton growth rate did not vary among the PL9, KBSF, TP, and mixed treatments and the control (Pillai's trace = 1.64, $F_{32,60} = 1.40$, $P = .19$).

Discussion

Because *Daphnia* are considered both evolutionary and ecological model organisms, numerous studies document both their strong genetic differentiation among populations (De Meester 1996; Boersma et al. 1998; Lynch et al. 1999; Aguilera et al. 2007) and their role in zooplankton community assembly (Louette and De Meester 2007; Steiner et al. 2007; Louette et al. 2008). Despite this breadth of knowledge, studies that link populations with genetic differences to altered outcomes of ecological processes are less common. *Daphnia pulex* × *pulicaria* from three ponds in southwestern Michigan harbored substantial molecular genetic and quantitative trait divergence among populations. The same three populations also significantly differed in terms of their success at invading a new habitat from a low density. The dependence of invasion success on source population indicates that the outcome of an ecological process, community assembly, is sensitive to the genetic differences found within participating species.

Although the lack of a significant interaction term indicates that source populations did not differ in their susceptibility to other zooplankton species, our results still indicate that genetic differences among populations altered zooplankton community assembly. All populations experienced positive growth rates (r) when they colonized in the absence of competitors, and the presence of a resident zooplankton community significantly decreased the growth rate of all populations. However, this influence was strong enough to cause only the population from TP to consistently experience a negative growth rate. The qualitative shift from a positive to a negative growth rate, which was observed for only one population and only in the presence of competitors, resulted in zooplankton community composition (with or without *D. pulex* × *pulicaria*) that makes sense only when intraspecific genetic differences are considered.

The quantitative trait assay indicates among-population divergence in traits that may influence establishment success in a new environment. Juvenile growth rate correlates strongly with and is often used as a proxy for population growth rate (Lampert and Trubetskova 1996; Tessier et al. 2000). The mean juvenile growth rate of TP was intermediate between the other two populations, yet TP had the lowest population growth rate in the establishment experiment. Therefore, it is unlikely that the TP population suffered a simple growth disadvantage relative to the other populations in the experiment. Instead, the competitive success of neonate *Daphnia* may be more critical in establishment success, putting the TP population at a disadvantage. Body size in *Daphnia* is linked to fitness, survival, and competition (Lampert and Trubetskova 1996; Milbrink et al. 2003; Vijverberg and Vos 2006; Iwabuchi and Urabe 2010). For example, variation in *Daphnia* body size influences both resource exploitation and susceptibility to predators (Gliwicz 1990; Tessier et al. 2000; DeMott et al. 2001; Riessen and Young 2005). The clonal variation in life-history traits that can result from food quality and stoichiometry also impacts intraspecific competition in *Daphnia* (Weider et al. 2005, 2008).

The *D. pulex* × *pulicaria* source population did not influence the colonization success of other zooplankton species in this study. However, there was no significant difference between the growth rates of colonizing zooplankton when *D. pulex* × *pulicaria* were present or absent. The second result is surprising, given that *Daphnia* is generally considered a keystone herbivore that is competitively dominant to other cladoceran zooplankton (Brooks and Dodson 1965; Hall et al. 1976; Leibold 1989; Ives et al. 1999). In other mesocosm experiments, the presence of *Daphnia* influenced the colonization success of other cladoceran zooplankton (Louette and De Meester 2007; Louette et al. 2008). Our experiment was designed to consider short-term colonization success and initial growth rate in a new habitat. It is possible that the competitive differences between *Daphnia* and other zooplankton species emerge at a longer time scale.

It is difficult to evaluate how resident *D. pulex* × *pulicaria* population differentiation influenced the colonization success of other zooplankton species, because *D. pulex* × *pulicaria* presence did not influence the growth rates of other species. However, we can conclude that source population did not qualitatively alter the ability of resident *D. pulex* × *pulicaria* to deter the invasion of other zooplankton species. This result contrasts with the small handful of studies that evaluate this possibility. The source population of resident *Daphnia magna* did influence the colonization of other cladoceran zooplankton in another mesocosm experiment (De Meester et al. 2007). Similarly, the genetic composition of resident *Arabidopsis thaliana* and *Solidago altissimia* populations influenced heterospecific plant colonization success (Weltzin et al. 2003; Crutsinger et al. 2008). The contrasting results may be attributed to the response variables measured in the other studies, which included changes in biomass and fitness-related traits in the invading species. We focused on population growth rates because we were more interested in qualitative changes in community composition (colonization success or failure) that result from genetic differentiation. Our goal was to determine whether intraspecific

genetic differentiation is important to consider when evaluating community composition. We can conclude that source population may be critical to understanding zooplankton composition patterns if its role in the assembly of communities is as strong as that observed in our study and that of De Meester et al. (2007).

Increased genetic diversity is often assumed to promote invasion success. Increases in additive variance in fitness promote more efficient responses to selection and increase the probability that the invading population will possess the traits necessary to survive in the new habitat (Fisher 1958; Frank and Slatkin 1992; Falconer and Mackay 1996; Vellend 2006). We included in our experiments a mixed population that was composed of an equal number of *D. pulex* × *pulicaria* from each of the three source populations. However, this population did not test the influence of genetic diversity on invasion success and invasion resistance because we did not separate the influence of genetic composition from genetic diversity. It is also possible that sampling error may have caused the mixed population to be less diverse than the TP or PL9 treatments. The mixed population colonized with growth rates that were intermediate between the populations that composed that treatment, supporting our assertion that the genetic composition of invading populations can influence invasion success.

Support for the hypothesis that increased genetic diversity influences invasion success is mixed and deserves further study. Comparisons of invasive and native population genetic diversity are common, but they cannot clearly distinguish between pre- and postinvasion diversification. For example, Poulin et al. (2005) surveyed populations of fountain grass (*Pennisetum setaceum*) that naturally varied in their invasion success. Although they found no relationship between genetic diversity and invasion success, it is possible that current levels of diversity do not reflect the levels of colonizing populations. Time since invasion certainly influences the observed level of genetic diversity (Dlugosch and Parker 2008). Crawford and Whitney (2010) directly linked levels of genetic diversity (number of ecotypes present in a colonizing population) with invasion success (an increase in fitness-related traits such as biomass and reproduction) in *Arabidopsis thaliana*. Increased genetic diversity led to nonadditive effects on invasion success. However, the experiment simulated colonization of open habitat with no competitors. Our study and that of Crawford and Whitney (2010) indicate that more direct empirical tests of the theoretical prediction that genetic diversity facilitates invasion success are needed. Our study also indicates that considering biotic interactions such as competition may be critical in determining the role of genetic differentiation in invasion success.

The ponds included in this survey are embedded in a larger regional group of ponds that contain both *D. pulex* and hybrid *D. pulex* × *pulicaria*. Because the taxa are morphologically indistinguishable and no true ecological distinctions have been quantified, taxonomic assignment relies on a single diagnostic allozyme (Hebert et al. 1989). Our allozyme surveys indicated that *D. pulex* × *pulicaria* inhabited the three ponds included in this study. However, an additional allozyme survey conducted 2 years after the mesocosm experiments indicated the presence of a single *D. pulex* in TP. It is possible that this *D. pulex* individual was a transient migrant, that *D. pulex* coexist with hybrids at an extremely low frequency in TP, or that *D. pulex* coexist in the other two ponds at low but undetected frequencies. Local coexistence is not uncommon. A survey of 145 ponds in the northeastern United States, including 68 ponds in Michigan, indicated that *D. pulex* co-occurred locally with hybrids in 14% of the ponds (Hebert and Finston 2001). Because of the low frequency of *D. pulex* in TP relative to the number of individual *Daphnia* used to stock our mesocosm experiments and estimate population trait means, it is unlikely that *D. pulex* were included in this study. If they were included, their low frequencies would have a negligible effect on our estimates of population growth. Naturally occurring *D. pulex* × *pulicaria* invariably reproduce by obligate parthenogenesis (Hebert et al. 1993; Hebert and Finston 2001), so genetic introgression between TP hybrids and potential resident *D. pulex* is also unlikely.

Substantial population differentiation is not unique to *Daphnia* or other zooplankton species. Thompson (1999, 2005) reviewed an extensive literature of the causes and consequences of such differentiation and stressed that differentiation influences species interactions in a spatial landscape. Differentiation may alter community assembly in a spatial setting via the "monopolization hypothesis" (De Meester et al. 2002). According to this hypothesis, a species that colonizes a habitat early might experience rapid adaptation to conditions at that habitat and would subsequently resist invasion by other genotypes of the same species. An extension of this hypothesis to communities predicts that different genotypes, which have undergone local adaptation and population differentiation, vary in their subsequent resistance to invasion by other species (Loeuille and Leibold 2008; Urban and De Meester 2009). This "community monopolization" hypothesis is important in suggesting how evolution may modify community assembly, but it focuses only on resistance to invasion. Our results indicate that population genetic differentiation can also alter invasion success, and they suggest that the community monopolization hypothesis must take a broader metacommunity view of community assembly (Leibold et al. 2004). Urban et al. (2008) suggest

that this interaction between microevolutionary and metacommunity dynamics likely influences multiple aspects of community assembly. Our results contribute to a growing body of evidence that this is the case.

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