Trait sorting in *Daphnia* colonising man-made lakes

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SUMMARY

1. We used a zooplankton metacommunity to ask how dispersal, genetic drift and selection act to determine the local and regional distributions of trait variation. Since the formation of the lakes 80 years ago, cladoceran species have sorted into local assemblages that cluster by lake depth. Given this species sorting, we ask whether intraspecific variation in an ecologically important phenotypic trait – body size – has sorted as well.

2. We quantified changes in body size through time by measuring ephippia from *D. pulicaria*, *D. dentifera* and *D. ambigua* recovered from sediment cores from two lakes. We then estimated mean body size of contemporary populations of two competing species, *Daphnia pulicaria* and *D. dentifera*, in a laboratory common garden experiment. Finally, we used microsatellite loci to characterise genetic diversity and gene flow among local sites in the metacommunity.

3. Body size was variable both within and among years for the three species of *Daphnia* examined using sediment cores. For two lakes where we examined body size distributions through time, we observed a significant shift in body size of the first species to arrive after colonisation by other *Daphnia* species, which suggests selection has occurred historically.

4. Despite heritable variation in body size in the laboratory, evidence for trait sorting was only found for *D. pulicaria*, which was larger in deeper lakes. Mean body size varied among lakes, but did not sort relative to depth for *D. dentifera*.

5. Microsatellite data indicated that neutral genetic diversity was low in the region; only 27% of the individuals assayed were unique multi-locus genotypes. We also found significant patterns of isolation by distance for both species. However, population structure was stronger in *D. dentifera* than in *D. pulicaria*. Hence, we conclude that a limited number of colonists have successfully invaded this metacommunity, and those genotypes arriving in this new region have experienced significant dispersal limitation among local sites.

6. Overall, while dispersal and selection have clearly led to the development of predictable community assemblages related to depth in this metacommunity, the distribution of phenotypic traits *within* species can differ substantially even between two trophically similar species. Our results highlight the complex roles of colonisation history, dispersal, selection and stochasticity in determining inter- and intra-specific patterns in metacommunities.

Keywords: *Daphnia*, dispersal, genetic drift, metacommunity, selection

Introduction

With its focus on dispersal, metacommunity theory is a promising conceptual framework for integrating processes that have traditionally been the focus of either community ecology or evolution (Holyoak, Leibold & Holt, 2005; Urban & Skelly, 2006; Loeuille & Leibold, 2008; Urban *et al.*, 2008). This theoretical framework predicts that in newly established habitats, species composition will be determined both by resident species adapting to

the novel environmental conditions and by species with suitable traits dispersing to and colonising the habitat (Urban & De Meester, 2009). If selection is spatially variable, then predictions can be made about how the traits of the community members should sort along an ecological gradient (Leibold & Norberg, 2004). But whether or not we observe trait sorting depends both on the magnitude of gene flow, the amount of heritable variation, and the intensity of selection (Hanski & Gaggiotti, 2004). At high rates of dispersal, populations may exhibit similar trait distributions regardless of selection because of ongoing immigration of less fit genotypes. In contrast, too little dispersal may limit the additive genetic variation on which selection may act and trait distributions may drift among populations (Slatkin, 1985; Leibold & Norberg, 2004). In short, to achieve the predicted patterns of phenotypic distribution, dispersal f must be sufficient to introduce genetic variation throughout the metacommunity, but it must not overwhelm local selection (Urban, 2011).

The interaction between dispersal, selection and genetic drift is likely to be influenced by colonisation order. Particularly for young communities, stochastic colonisation events and drift shape community structure, genetic composition and intra- and interspecific competition. As communities age and the richness and abundance of species increases, the effect of selection via intra- and interspecific interactions tends to increase, potentially influencing quantitative trait distributions (Mouquet *et al.*, 2003). Similarly, gene flow may alter neutral genetic distributions originally dominated by priority effects and drift, but evidence for these initial processes may remain evident in the metacommunity as spatial or temporal structure.

In *Daphnia*, body size is an ecologically important trait whose distribution can vary spatially and temporally. It is directly related to fitness, as larger animals tend to produce more eggs (Lampert & Sommer, 1997). A number of drivers are known to influence body size such as predation pressure, competition and resource availability (Goulden, Henry & Tessier, 1982; Lampert & Sommer, 1997). Larger animals can coexist with visual predators in lakes where a dark, deep-water refuge is available during the day; however, where predation pressure is strong (i.e. in lakes without a long-lasting refuge), smaller body sizes may be favoured (Tessier & Welser, 1991).

We took advantage of lake habitats constructed within the last century to ask whether trait sorting occurs in planktonic communities. Previously, we showed that cladoceran assemblages show evidence of species sorting over time along a depth gradient in these lakes (Allen,

Vandyke & Cáceres, 2011). Here, we focus on the three species (*D. pulicaria* Forbes, *D. dentifera* Taylor and Hebert and *D. ambigua* Scourfield) that have colonised all of our study lakes but now exhibit different abundances among lake types. In natural lakes, the co-existence of these competitors results from habitat segregation, body size differentiation and long-lived dormant eggs (Threlkeld, 1979; Leibold, 1991; Hu & Tessier, 1995; Cáceres, 1998). Of the two larger species, *D. pulicaria* (the largest) dominates deeper lakes (>12 m) in the spring, with a shift to summer dominance by the smaller *D. dentifera*. *Daphnia dentifera* is more common in lakes of moderate depth (7–9 m) (Allen *et al.*, 2011). Both species are extremely rare in lakes that are <7 m deep. The abundance of the smallest species, *D. ambigua*, also varies in each of the moderate depth and deep lakes.

Here, we test whether the ecological sorting observed at the community level has also occurred at the population level. We first examine the scope of historical body size variation using palaeoecological data from *Daphnia* species in two lakes and ask whether trait sorting has occurred through time. Although in natural lakes, mean body size of *D. pulicaria* is larger than *D. dentifera*, the distribution of body size of the two species overlaps substantially (Leibold, 1991, Cáceres & Tessier, unpublished). *Daphnia dentifera* body size also overlaps with *D. ambigua*. Given what is known about body size distributions in individual lakes, we tested the hypothesis that community composition influences body size by measuring the ephippial cases found in lake sediments (a proxy for body size) as the communities assembled. We predicted a shift in the magnitude and overlap of body size variation as the number of competitor species increased.

Next, we tested for the current patterns of sorting associated with the distribution of lake types (the factor affecting the community-level distributions). Specifically, we looked for evidence of intraspecific body size sorting along the depth gradient in the metacommunity as a whole. We predicted that body sizes of both *D. dentifera* and *D. pulicaria* would be larger in deep than in shallow lakes, because of the availability of a refuge, and tested this hypothesis with a common garden experiment using *Daphnia* from six lakes.

Historical and contemporary trait selection rely on sufficient dispersal to provide, but not overwhelm, genetic diversity. As a proxy for dispersal rate, both into and within the metacommunity, we used microsatellite markers to characterise the genetic structure of each population for each species. If dispersal is overwhelming selection, then we expect similar levels of genetic variation and low levels of genetic differentiation (as measured by F_{ST}) and

trait differentiation among lakes. If dispersal is low and selection is low in this metacommunity, then we expect high genetic differentiation and possibly high trait differentiation, but that trait differentiation would be randomly distributed because of drift. If selection is high relative to gene flow, but gene flow is not absent, we predict trait differentiation related to our environmental factor, but significant population genetic differentiation across the metapopulation and/or significant spatial or temporal isolation by distance at these neutral genetic markers.

Methods

We studied plankton communities in six lakes ranging from 7 to 16 m maximum depth that were created from a former strip mine in central Illinois. Ephippia extracted from sediment cores show that cladocerans colonised these lakes at various times between their filling (the 1920s or 1950s) and the present. These data suggest that community structure changed substantially over the decades, eventually sorting into groups associated with lake depth. A more detailed description of the study site (Kickapoo State Park, Vermilion Co, IL, U.S.A.) and associated cladoceran colonisation histories is available elsewhere (Allen *et al.*, 2011). To quantify changes in body size through time, and to test the hypothesis that changing assemblages caused selection on body size, we measured ephippia extracted from the sediment cores taken from High and Deep Lake. These two lakes had the most complete and continuous records of species invasion and establishment of the initial coloniser and were colonised by a second species at a point deep enough into the core to allow a before and after comparison of body size of the focal species. Other lakes did not meet these criteria for adequate comparisons. Ephippia were identified to species based on the shape and the presence/absence of spines along the dorsal margin. Body size was estimated by photographing each ephippium under a stereo-microscope (10–40×) and measuring its dorsal margin using S. Spot Advanced Software (Diagnostic Instruments, Sterling Heights, MI, U.S.A.). To examine body size variation through time, we plotted mean body size for each species in each core slice (note that we were not able to date individual core slices – see Allen *et al.*, 2011). We then tested for evidence of competition by calculating a shift in the median body size of the first species to colonise the lake (High Lake: *D. ambigua*; Deep Lake: *D. pulicaria*) using a Wilcox–Mann–Whitney *U*-test before and after the presence of the first daphniid competitor (a test for directional selection; data were not sufficient to subdivide further the core across subsequent daphniid invasion

events). For simplicity, we chose the deepest core slice where at least two competitor ephippia were found as the before/after point. Because we expected directional selection to cause a body size shift in the presence of competitors, we predicted a reduction in *D. ambigua* body size (the smallest species) in the presence of competitors and an increase in *D. pulicaria* body size (the largest species).

After testing for evidence of historical selection, we examined the current populations of *Daphnia* across all six lakes to test the hypothesis that trait sorting at the metapopulation level was related to lake depth (and hence predation or different community structures). To test for evidence of trait sorting in body size, we conducted a laboratory common garden assay to determine size at maturity. We collected individuals from multiple tows in the deepest sections of each lake using a full water column Wisconsin net sampler during May 2007, a time of the year when genetic diversity is expected to be at its peak (Lynch, 1984). From these collections, we established up to 25 female clonal lines (hereafter 'clones') from each of six *D. dentifera* and five *D. pulicaria* populations into laboratory culture. Clones reproduced asexually at a low density for three generations in environmental chambers set to a 10D: 14L cycle at 20°C and were fed 2 mg C L^{-1} day^{-1} of the green alga *Ankistrodesmus falcatus* (Corda) Ralfs to standardise maternal and grandmaternal effects (Lynch, 1985). After the third generation, clones were split into two sublines and neonates from the third clutch or later were used as experimental animals. For each subline, we collected three neonates (<18 h) that were placed into 200 mL GF/F filtered lake water and grown under experimental conditions. Experimental animals were monitored daily until the production of their first clutch of eggs, at which point the animal was measured. Females were photographed under the microscope and measured (top of the head to base of the tail spine). Sizes of all females that survived to maturity in each beaker were averaged, resulting in two estimates of size at maturity per clone (one from each subline). Because of incubator space and time constraints, the experiment could not be run for all clones simultaneously. Thus, the clones from each lake were split into groups of five. A group of five clones from each lake was considered one block of organisms (*D. dentifera*: five clones × two replicates/clone × six lakes = block; *D. pulicaria*: five clones × two replicates/clone × five lakes = block). A block of clones from each species was run simultaneously, and five blocks total were run sequentially during the late summer and autumn 2007. As some clones collected in spring did not survive through the autumn, and not all

clones matured in the experiment (i.e. dropped a clutch of eggs), data from 95 clones from five *D. pulicaria* populations and 117 clones from six *D. dentifera* populations were collected by the end of the fifth block.

To test for differences among populations in mean body size, we used mixed model ANOVAs for each species, with lakes as a fixed effect and clones nested within lakes as a random variable. The effect of experimental block was not significant in the initial analyses, so it was excluded. We used contrast statements to test whether average body size was larger in three deeper lakes relative to two shallower lakes. Broad sense heritabilities were also calculated from each population using the variance components of one-way ANOVAs fit with REML (Lynch & Walsh, 1998).

To provide the context for how historical levels of dispersal may have affected patterns of selection and drift on quantitative traits, we quantified current within and among lake molecular genetic variation. We genotyped 114 *D. dentifera* clones (out of 117) and 94 *D. pulicaria* clones (out of 95) that were included in the laboratory common garden. We extracted DNA using the Qiagen DNeasy-96 tissue kit and then genotyped polymorphic microsatellite loci using primers from Colbourne *et al.* (2004) and Fox (2004) (*D. dentifera*: Dgm105, Dgm106, Dgm107, Dgm113; *D. pulicaria*: Dp208, Dp231, Dp291, Dp304, Dp339). We then followed standard PCR amplification and analysis methods outlined in Allen, Thum & Cáceres (2010).

We used GeneMapper 3.7 to confirm allelic size variants at each locus for each individual. Allelic frequencies (A) and mean expected heterozygosities (H_E) were calculated as an estimate of the total genetic diversity within the lakes for each species, and intrapopulation F_{IS} was measured as a proxy for inbreeding or clonal selection. We then quantified among population divergence using AMOVA (Excoffier, Smouse & Quattro, 1992) and pairwise F_{ST}'s among all lakes (θ: Weir & Cockerham, 1984). Sequential Bonferroni correction (Rice, 1989) and permutation tests were used to assess the significance of pairwise F_{ST} values. Analyses were performed in ARLEQUIN v3.1 (Excoffier, Laval & Schneider, 2005).

To test the hypotheses that *Daphnia* within the metacommunity were limited spatially or temporally by dispersal, we used Mantel and partial Mantel tests to compare pairwise F_{ST} with distance matrices. Geographic distances were measured as the minimum straight line distance between lake pairs in ArcGIS Desktop (ESRI). To test for temporal structure within each species (i.e. lakes colonised at the same time have more similar genetic structures than lakes colonised at different times), we

calculated the difference between initial colonisation times between lake pairs using the colonisation years in Allen *et al.* (2011). Because data on initial colonisation year were not available for Long Lake, only five lakes were included in the temporal analysis and partial Mantel tests of distance versus time.

Results

Daphnia body size varied substantially through time in both Deep and High lakes (Figs 1 & 2). In Deep Lake, the median body size of *D. pulicaria* (the first colonist) increased following the introduction of additional *Daphnia* species ($W = 8472$, $P = 0.0005$). The shape of the body size distribution also changed from highly clumped about the mean [kurtosis (K) = 0.81; $\sigma^2 = 0.007$] to a more normal, dispersed distribution ($K = 0.43$; $\sigma^2 = 0.013$). In High Lake, the median body size of *D. ambigua* decreased following the introduction of *D. dentifera* and *D. pulicaria* ($W = 11\ 169$, $P < 0.0001$). The body size distribution became more leptokurtic ($K = -0.39$ to 0.89) and less variable ($\sigma^2 = 0.009–0.004$) as larger animals became less frequent.

For both *D. dentifera* and *D. pulicaria*, the common garden assay confirmed both that body size differed among populations for each species (Fig. 3; *D. dentifera*: $F_{5,111} = 5.35$, $P < 0.001$; *D. pulicaria*: $F_{4,90} = 5.34$, $P < 0.001$)
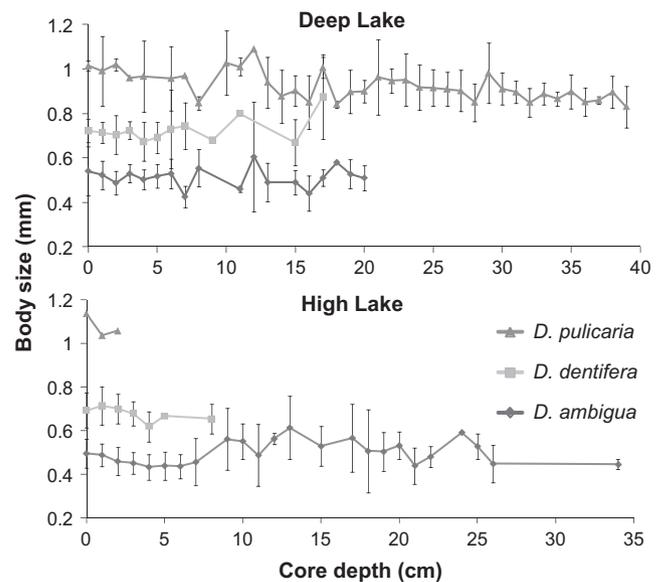

Fig. 1 Body size variation (mean ± SD) through time in two lakes. Body size was measured as the straight edge length of the ephippial spine from identifiable ephippia recovered from sediment core slices. The Deep Lake core represents 45 years of sedimentation, while the High Lake core represents 78 years of sedimentation.
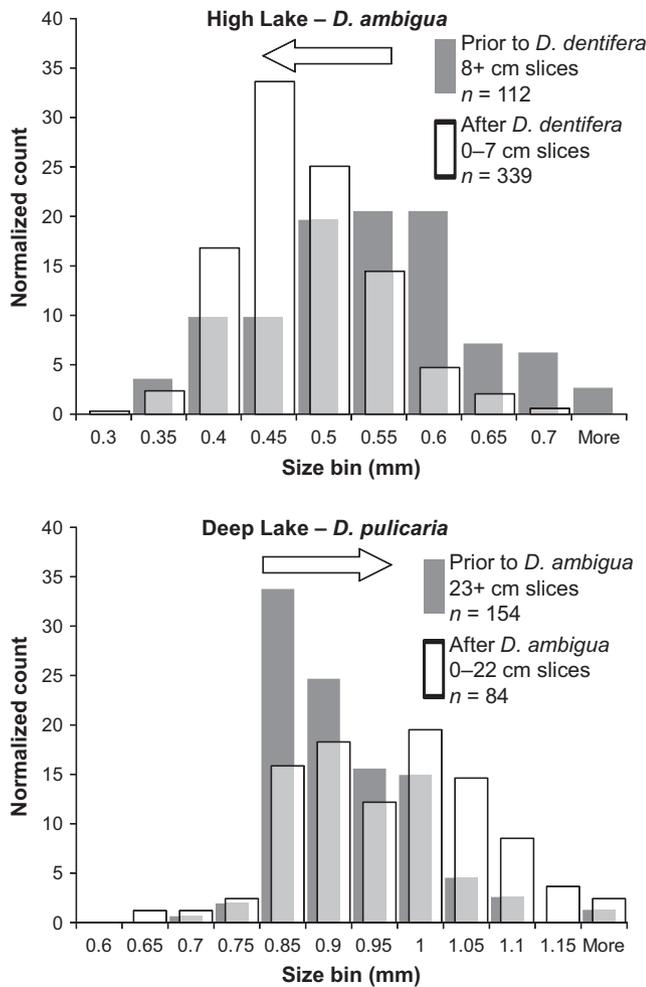
Fig. 2 Frequency histograms for size of initial colonising species before (solid bar) and after (open bar) subsequent colonisations. Counts were normalised for easier visual comparison of distributions. Median *Daphnia pulicaria* body size in Deep Lake increased after *D. ambigua* colonisation. Median *D. ambigua* body size in High Lake decreased after *D. dentifera* colonisation.

Fig. 3 Body size variation for two *Daphnia* species in Kickapoo State Park lakes. Boxes represent the interquartile range (IQR), the solid line is the median and whiskers extend to outliers within 1.5 IQR of the box. Body sizes were significantly different among lakes for both species. Solid bars represent total body size variation in the metacommunity, shaded bars moderate depth lakes, and open bars deep lakes. *Daphnia pulicaria* were not present in Inland Lake at the time of the study. Lakes: Sp, Sportsman's; Cl, Clear; De, Deep; Lo, Long; In, Inland; Hi, High.

and that this variation was heritable (*D. dentifera*: $H^2 = 0.54 \pm 0.06$ SE; *D. pulicaria*: 0.40 ± 0.12 SE). As predicted, *D. pulicaria* populations residing in deep lakes consisted of significantly larger animals than those found in moderate depth lakes (deep: 1.85 ± 0.01 mm; moderate: 1.75 ± 0.02; $F_{1,3} = 10.99$, $P = 0.045$). However, we observed no differences among lake types for *D. dentifera* (deep: 1.45 ± 0.01 mm; moderate: 1.43 ± 0.01; $F_{1,4} = 0.19$, $P = 0.68$).

Our molecular data suggest that this metacommunity was colonised by a limited number of individuals. The average number of alleles per locus (*A*) and expected heterozygosity (H_E) were low for both *Daphnia* species (Table 1); we found only 25 (*D. pulicaria*) and 29 (*D. dentifera*) unique multilocus genotypes of which five and nine
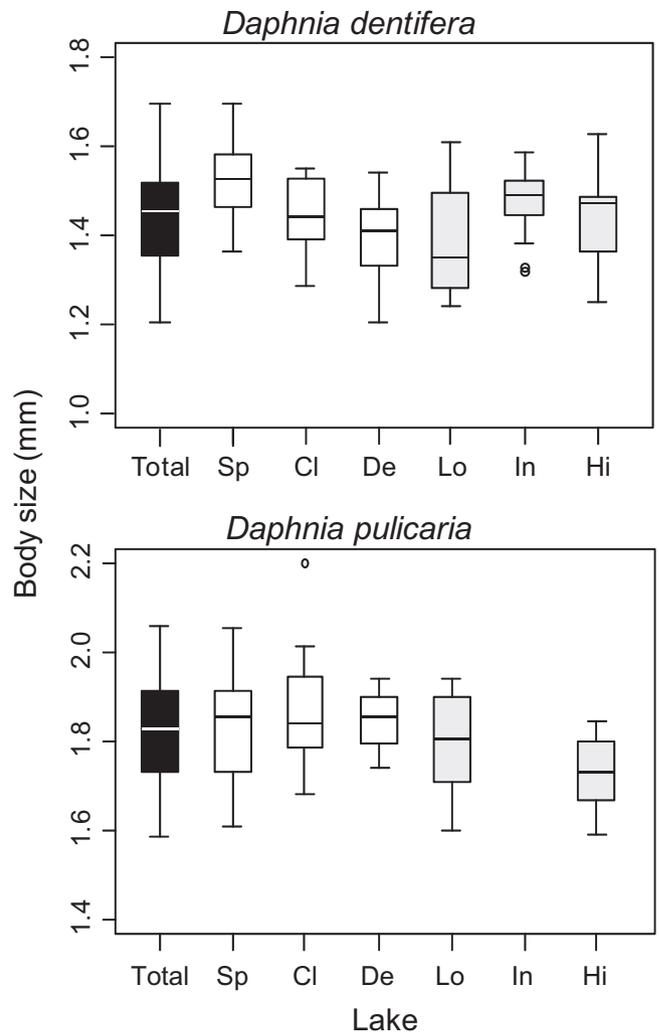
were shared among lakes, respectively. The probability that these multilocus genotypes were the same by chance (as opposed to representing the same genetic clone) was very low ($P < 0.01$, as calculated in MLGsim). Even though we used one fewer marker for *D. dentifera*, it tended to have higher mean genetic diversity than *D. pulicaria*. The exception was Deep Lake. However, this was the only lake in which *D. pulicaria* colonised before *D. dentifera*, and the species first colonising each lake had significantly higher expected heterozygosities than the later colonising species (parametric paired *t*-test: $t_4 = 3.13$, $P = 0.018$).

Table 1 Molecular genetic diversity of *Daphnia dentifera* and *D. pulicaria* in Kickapoo lakes

Lake	Depth class	Species	n	A	H_E	MLG	F_{IS}
Clear	Deep	*D. dentifera*	13	3.25	0.611 [0.10]	0.77	0.619***
Deep	Deep	*D. dentifera*	20	2.50	0.273 [0.26]	0.39	−0.260*
Sports	Deep	*D. dentifera*	21	2.75	0.396 [0.28]	0.35	0.518***
High	Moderate	*D. dentifera*	15	2.25	0.360 [0.25]	0.29	0.249**
Inland	Moderate	*D. dentifera*	23	2.75	0.304 [0.22]	0.26	0.285*
Long	Moderate	*D. dentifera*	22	3.00	0.595 [0.09]	0.63	0.181
Clear	Deep	*D. pulicaria*	22	1.80	0.421 [0.25]	0.43	−0.589***
Deep	Deep	*D. pulicaria*	15	2.00	0.374 [0.26]	0.31	−0.618***
Sports	Deep	*D. pulicaria*	20	2.00	0.398 [0.24]	0.25	−0.370***
High	Moderate	*D. pulicaria*	13	1.80	0.274 [0.34]	0.31	−0.547***
Long	Moderate	*D. pulicaria*	24	3.00	0.404 [0.29]	0.38	−0.456***

n, Number of individuals genotyped; A, average number of alleles per locus; H_E, mean [SD] expected heterozygosity across all loci; MLG, proportion of unique multilocus genotypes in sample; F_{IS}, population-specific inbreeding coefficient. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

We found significant heterozygosity excesses in all *D. pulicaria* populations, while *D. dentifera* populations were more often characterised by heterozygosity deficits (F_{IS}; Table 1). These deviations from the expected heterozygosity suggest non-random mating is occurring and are common when clonal selection is occurring in *Daphnia* populations (Lynch *et al.*, 1999; Morgan *et al.*, 2001). Despite generally low molecular diversity and sharing of multilocus genotypes among lakes, we observed significant genetic structure among populations of both species. Approximately 30 and 14% of the genetic variation was distributed among populations for *D. dentifera* and *D. pulicaria*, respectively, with moderate to high pairwise F_{ST} estimates for both species (Table 2).

Finally, our data support the hypotheses that spatial and temporal dispersal limitation influence the current microsatellite variation patterns. While neither distance (Mantel $r = 0.41$, $P = 0.06$) nor the difference between colonisation years ($r = 0.58$, $P = 0.08$) alone was significantly correlated with *D. dentifera* F_{ST}'s, the two variables interacted with one another, as partial Mantel correlations were significant and much larger (distance: $\rho = 0.69$, $P = 0.036$; time: $\rho = 0.77$, $P = 0.026$; Fig. 4). We found no interaction between spatial and temporal matrices for *D. pulicaria* F_{ST}'s, as there was no indication of a positive temporal relationship ($r = -0.56$, $P = 0.87$). However, there was clear evidence for a positive relationship between F_{ST} and distance among lakes ($r = 0.78$, $P = 0.004$; Fig. 4). Note that Long Lake was not included in analyses including colonisation time, as colonisation year data were unavailable.

Table 2 Pairwise F_{ST} for microsatellite differentiation among populations of (a) *Daphnia dentifera* and (b) *D. pulicaria* in Kickapoo lakes

(a) *Daphnia dentifera*

	Clear	Deep	Sports	High	Inland	Long
Clear		0.000	0.009	0.018	0.001	0.486
Deep	**0.172**		0.000	0.000	0.000	0.000
Sports	0.191	**0.537**		0.000	0.000	0.054
High	0.145	**0.431**	**0.167**		0.000	0.009
Inland	**0.349**	**0.675**	**0.223**	**0.425**		0.000
Long	0.004	**0.294**	0.072	0.090	**0.237**	

(b) *Daphnia pulicaria*

	Clear	Deep	Sport	High	Long
Clear		0.000	0.000	0.000	0.000
Deep	**0.170**		0.000	0.000	0.099
Sport	**0.079**	**0.214**		0.000	0.000
High	**0.172**	**0.130**	**0.180**		0.000
Long	**0.109**	−0.003	**0.160**	**0.102**	

F_{ST} values are below the diagonal, while permutation test significance values are above the diagonal. Lakes are sorted by depth class. Boldface F_{ST} values are significant after sequential Bonferroni corrections (a) $P < 0.003$ and (b) $P < 0.005$.
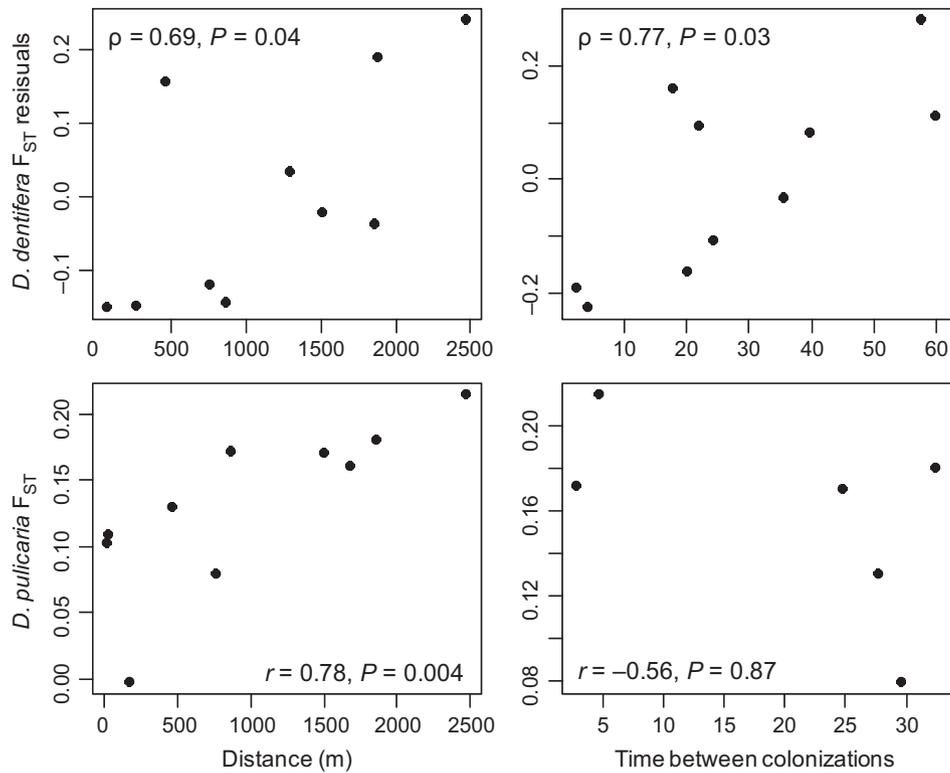
Fig. 4 Relationships between microsatellite differentiation and spatial and temporal distance among populations of *Daphnia dentifera* and *D. pulicaria*. Mantel correlations are inset on each panel, with *D. pulicaria* showing one-way tests and *D. dentifera* showing partial tests. Note the different axis scales between panels. Approximate colonisation years were not available for Long Lake. Hence, fewer data points are available for the time since colonisation analysis for *D. pulicaria* and the partial mantel tests for *D. dentifera*.

Discussion

For ecological trait sorting to occur in metacommunities, three main conditions are necessary: selection must be variable among populations, the trait of interest must exhibit sufficient heritable variation, and dispersal should be sufficient to maintain additive genetic variation across the metacommunity, but not high enough to erode it (Leibold & Norberg, 2004). We discuss each of these criteria in turn, providing evidence for shifting trait distributions through time, heritable variation and gene flow. Further, we discuss evidence for the hypothesis that community composition and predation contribute to selection on body size, noting other potential alternative explanations for the patterns we observed. We show that even for two ecologically similar species in a metacommunity, the balance of factors affecting an ecologically important trait (body size) can differ.

Physical and biological conditions are known to vary spatially and temporally in these lakes (Horner & Brummett, 1972; Cáceres, unpublished), probably leading to differences in selection intensity and direction among habitats. For example, our previous work

showed striking community composition changes through time that varied among lakes and led to different assemblages between deep lakes and shallow lakes (Allen *et al.*, 2011). Such assemblages were consistent with the premise that deeper lakes with an oxygenated hypolimnion maintain larger-bodied species (Tessier & Welser, 1991).

Substantial body size variation existed historically and remains within contemporary populations of *Daphnia* in this metacommunity. Historical populations of all three *Daphnia* species exhibited ephippia size variation throughout sediment cores. Additionally, field measurements taken in 2007 showed adult *D. pulicaria* ranging from 1.1 to 2.2 mm and *D. dentifera* ranging from 0.9 to 1.7 mm (Cáceres *et al.*, unpublished). Such body size variation is common within and among *Daphnia* populations. For example, Gliwicz & Boavida (1996) found that egg-bearing females of *D. pulicaria* from several lakes ranged in size from 0.7 to 2.5 mm; Hall *et al.* (2010) showed mean *D. dentifera* body size to range from 1.0 to 2.0 mm across 18 lakes. Most importantly, our laboratory experiments showed variation in contemporary populations was heritable and, assuming at least some fraction

of the heritable variation (in a broad sense) reflects additive variation (in a narrow sense), provides opportunities to respond to local selection pressures on body size.

Ecological trait sorting requires that dispersal is sufficient to maintain additive genetic variation across a metacommunity without being so high as to erode differences in traits predicted by local selection pressures (Leibold & Norberg, 2004). In our system, neutral genetic variation was low for each *Daphnia* species and, while dispersal occurred through time and space, it was limited. Several lines of evidence support this conclusion. First, differentiation estimates for each species were greater than zero between nearly all population pairs, a common finding in *Daphnia* metapopulation genetics (Vanoverbeke & De Meester, 1997; Morgan *et al.*, 2001; Thielsch *et al.*, 2009; Allen *et al.*, 2010). As these populations are young and of low diversity, they are likely to have been founded by few individuals over the course of several decades resulting in non-equilibrial dynamics (Boileau, Hebert & Schwartz, 1992). Second, for each species, only 27% of the individuals assayed were unique multilocus genotypes, with a number of these shared among lakes. Such estimates are extremely low in comparison with natural sexual populations, where up to 100% of individuals may be unique multilocus genotypes (Allen & Lynch, 2011). This pattern may result from low genetic diversity in concert with clonal selection, a pattern supported by F_{IS} estimates (Lynch *et al.*, 1999; Morgan *et al.*, 2001). However, evidence of shared multilocus genotypes among lakes provides support for ongoing dispersal. Third, spatial and temporal isolation by distance provide further support for limited dispersal.

Interestingly, our genetic results also suggest species-specific differences in colonisation history and genetic patterns. We found that whichever species colonised the lake first had higher genetic diversity. Five of the six lakes were colonised first by *D. dentifera* (despite both species being present in the metacommunity for decades), giving *D. dentifera* a longer time to build genetic diversity in the metapopulation. Consequently, *D. dentifera* had greater genetic diversity, higher unique multilocus genotype frequencies and higher F_{ST} estimates than *D. pulicaria*. Additionally, greater time between lake colonisations probably exaggerated the differences among the initial pool of colonisers in *D. dentifera* populations, contributing to the temporal isolation by distance observed. *Daphnia pulicaria* colonised lakes more recently and had shorter time between lake colonisations, which may explain lower genetic diversity and the lack of temporal isolation by distance in this species.

Given the inherent variability in, and history of, this system, we focussed on two potential selection pressures: community composition changes (i.e. the invasion of a conspecific) and basin shape (a proxy for predation intensity and the community composition differences associated with it). Our historical data support an effect of conspecific invasion on body size shifts in two lakes. In our contemporary populations, observing larger *D. pulicaria* in deeper lakes with a hypolimnetic refuge (relative to shallower lakes with more intense predation) is consistent with the hypothesis that selection on body size is related to basin depth. However, the mean body size of *D. dentifera*, while differing among lakes, was not related to basin depth. While the historical shift in body size patterns is striking, our palaeorecords limited us to only two species, each from a separate lake. More complete records from other nearby lakes would improve the power of the invasion analysis. Similarly, the power of our contemporary analysis was limited by the numbers of local populations and clones assessed within populations. Hence, generalising these results beyond this metacommunity must be treated with caution.

While both predation and competition are known to influence body size, even within a single season (Tessier, Young & Leibold, 1992), the influence of these selection pressures on body size is complex and likely to vary among habitats and through time (Wellborn, Skelly & Werner, 1996). Thus, predicting species effects can be difficult and observed effects may have many causes. For example, we expect a conspecific invasion to be a stronger selection pressure early in community assembly when few competitors exist. As communities mature, species may adapt to one another, species sorting may develop and other selection pressures may overwhelm the effect of such a conspecific. For example, previous work in these lakes has demonstrated adaptation of *D. ambigua* to the presence of *D. dentifera* using resurrected clones from early versus recent sediments (Steiner, Cáceres & Smith, 2007). Other work suggests in developed communities where fish predation is intense, smaller body size is generally anticipated (Machacek, 1995; Boersma, Spaak & De Meester, 1998; Lass & Bittner, 2002). If species interactions result in habitat segregation, bigger, hypolimnetic *D. pulicaria* could co-exist with smaller *D. dentifera* that occupy shallower water where fish predation is stronger (Tessier & Welser, 1991). Similarly, invertebrate predation (e.g. by *Chaoborus*) can cause both species and trait sorting in *Daphnia* species (Tollrian, 1993; Weber & Declerck, 1997; Sell, 2000; Garcia & Mittelbach, 2008). Additionally, interspecific competition probably differs among all lakes as community compositions and species

abundances vary historically and seasonally. Finally, other limnological conditions may influence species trait expression, including productivity, temperature regime and acidity (Lampert & Sommer, 1997). Unfortunately, while we know that physical and biological conditions have changed in these lakes since their creation (Horner & Brummett, 1972), sufficient historical data on fish stocking and abundance, lake productivity, temperature regime, etc., are not available to test these alternatives.

Taken together, our data support the hypothesis that metacommunity interactions influence trait sorting in these communities. Lakes vary historically and contemporarily, providing opportunities for variable selection on heritable variation. At the community level, an 'intermediate' level of dispersal has facilitated species colonisation and sorting in this metacommunity (Allen *et al.*, 2011). However, the effect of dispersal on intraspecific trait variation among sites within the metacommunity differs between the two focal species. For *D. pulicaria*, dispersal among sites has not been sufficient to prevent size differentiation among lakes of different depths, leading to our observed pattern that deeper lakes have larger *D. pulicaria*. In contrast, body size differences among sites are not related to depth for *D. dentifera*, and our results suggest that body size differences among *D. dentifera* populations either reflect genetic drift because of limited dispersal or a response to unknown factors. Thus, even for these trophically similar species, colonisation history, dispersal, selection and drift play complex, interactive roles in determining trait variation in metapopulations.

Acknowledgments

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