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Mortality dynamics of *Daphnia* in contrasting habitats and their role in ecological divergence

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SUMMARY

1. The *Daphnia pulex-pulicaria* species complex has been proposed as an example of rapid ecological speciation, associated with divergence along the gradient of waterbody size from temporary ponds to deep, stratified lakes. However, this divergence is incomplete, and thus represents an opportunity to study ecological divergence as it is occurring.
2. Dynamics of twelve populations of *Daphnia* in the *pulex-pulicaria* species complex were monitored over 1 year. Six temporary pond populations and six permanent lake populations were compared to evaluate demographic differences that may contribute to ecological divergence in this complex.
3. Pond populations experienced greater changes in density, which were reflected in more extreme growth rates, higher birth rates and higher mortality rates than those of lake populations.
4. Mitochondrial DNA was isolated from up to three clones from each population, the D-loop of the control region was sequenced, and a phylogenetic tree was constructed. This tree revealed two strongly supported clades. The clades were not congruent with habitat type and nominal status, indicating that interhabitat gene flow occurs easily and that the nominal taxa are incompletely diverged.
5. Published reports of genetic life history differences in the *D. pulex-pulicaria* complex are consistent with the demographic differences reported here. This suggests that ecological differences between the habitats are selectively maintaining trait differences despite the possibility for genetic exchange. Thus, these taxa may be at the inception of ecological speciation.

Keywords: birth rate, death rate, demography, ecological speciation, phylogeny

Introduction

Populations separated in space will confront different selection pressures and, if the differences are maintained for a sufficient period of time, evolutionary divergence will occur as populations adapt to local conditions. This may ultimately lead to ecological speciation (Mayr, 1963). The role of adaptive change in speciation is a central issue in ecology and evolutionary biology because it offers a possible explanation for the origin and limits to biodiversity. Nonetheless, relatively few instances of natural divergence have been exploited by researchers because evolutionary divergence is transient and detailed reconstruction of past evolutionary change continues to be difficult. The best understood examples, such as Darwin's Finches (Grant & Grant 2002) and threespine sticklebacks (McPhail, 1994; McKinnon & Rundle, 2002), involve the recent tips of evolutionary branches, where observations can be made at the boundary of ecological and evolutionary timescales.

Recently, the freshwater crustaceans *Daphnia pulex* and *D. pulicaria* have been proposed as an example of rapid ecologically-based evolutionary speciation based on the association between population genetic structure and habitat types (Pfrender, Spitze & Leh-

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man, 2000) and differences in mating cues (Deng, 1997). These taxa offer a potentially powerful situation for examining ecological divergence because both taxa are widely distributed in North America and their populations exist across a habitat gradient that is geographically interspersed. *Daphnia pulex* typically resides in temporary ponds, whereas *D. pulicaria* is mainly found in deep, stratified lakes. The gradient of waterbody size creates fundamental changes in many components of community structure and therefore is likely to lead to many differences in the nature of selection at different parts of the gradient (Wellborn, Skelly & Werner, 1996). Populations of both nominal taxa can be found in close proximity across a large part of North America and thus their divergence is unlikely to be driven by regional geographic separation. Although these taxa have been given different names, they are identifiable only on the basis of allozymes (Hebert, 1995) that segregate on a gradient of waterbody size, and it is not clear that each are a genetically coherent taxon. Numerous molecular genetic studies place *D. pulex* and *D. pulicaria* at the outer tips of evolutionary branches (Lehman *et al.*, 1995; Colbourne & Hebert, 1996; Crease *et al.*, 1997; Colbourne *et al.*, 1998), with these taxa having the most recent divergence time of any pair of North American *Daphnia* species. Furthermore, there is considerable evidence of successful hybridisation between these two taxa (Crease, Stanton & Hebert, 1989; Crease, Spitze & Lynch, 1990; Hebert & Finston, 2001).

As a taxon spreads to new habitats, it is unlikely that its opportunity and ability to acquire resources or the optimal allocation of those resources will remain constant. An important first step to understanding ecological divergence is to determine how components of population dynamics differ in distinct habitats. This is particularly warranted when adaptive divergence depends on broadly integrative phenotypic traits, such as life history or resource use efficiency. In such cases, it may not be possible to identify key structural features or interactions that are driving divergence. Because alternate habitats are likely to differ in many important niche axes, examination of population demography can offer insight into aggregate selective challenges from the populations' perspectives.

A fundamental difference in selective challenges is the extrinsic mortality that different habitats impose on populations. Populations should respond evolutionarily to minimise the effects of the mortality agents they experience, but any such response will be tempered by competing demands, biological constraints and the availability of genetic variation. Different mortality levels mean that populations should ultimately diverge to different optimal life histories (Williams, 1957; Emlen, 1970; Partridge & Harvey, 1988; Reznick *et al.*, 1996; Rodd & Reznick, 1997). That is, the allocation decisions individuals make between investment in reproduction and survival, and between current and future performance, should differ among populations. As life histories are widely thought to be characterised by trade-offs, habitats that favour different life history strategies may promote evolutionary divergence.

The legacy of ecological research on *Daphnia* has presented us with many studies of *Daphnia* mortality rates (e.g., Hall, 1964; Tappa, 1965; Dodson, 1972; Threlkeld, 1979; Lynch, 1983; Hu & Tessier, 1995). However, no studies have been conducted to allow comparison of populations in the throes of evolutionary divergence. Evaluating past research in an evolutionary context is further complicated by recent genetic advances in *Daphnia* phylogenetics, which has revealed a large amount of unsuspected evolutionary separation (Taylor & Hebert, 1992; Colbourne & Hebert, 1996; Taylor, Hebert & Colbourne, 1996; Hebert & Finston, 2001; Omilian & Taylor, 2001) and precipitated substantial revision of the genus. As a consequence, for some classic ecological studies of *Daphnia* mortality, and in particular for species complexes that involve recent and cryptic divergence, it is unclear where to place the studied populations in our current understanding of *Daphnia* evolution.

In this study, I combine ecological and genetic data to illuminate important aspects of *Daphnia* spp. as a model system for investigations of ecological divergence. In particular, I quantify the population dynamics and components of demography that broadly characterise the selective pressures on the life cycle that populations face in alternate habitats. I seek to capitalise on *Daphnia's* power as a model system to draw together three types of information bearing on ecological divergence. First, I make a detailed comparison of the population growth rates, birth rates and death rates of *Daphnia* populations in both temporary ponds and permanent lakes. This represents a broad picture of how the selective environments differ between these habitats. Secondly, I use mtDNA sequence information to show that genetic exchange occurs between the habitat types. Finally, I draw on published life history information on these and other populations to give insight into how habitat differences may be promoting evolutionary divergence.

Methods

Choice of study populations

All of the study populations are located near the W. K. Kellogg Biological Station (KBS) in SW Michigan, U.S.A. Populations of the *Daphnia pulex-pulicaria* species complex can be found in this area across most of the gradient of waterbody size ranging from temporary ponds to deep, stratified lakes. To maintain a manageable scale for the experiment, yet allow for sufficient replication, I compared the extremes of this gradient, which are the typical habitats of the nominal taxa. In the spring of 1997, I surveyed 21 pond populations of *Daphnia* morphologically identified to the *pulex-pulicaria* complex and characterised the lactate dehydrogenase (LDH) allozyme (considered diagnostic for *D. pulex*, *D. pulicaria* and their hybrids) for at least 23 individuals per population. All populations that contained only the *D. pulex* allele were in temporary ponds; permanent ponds and a few temporary ponds had some or all individuals that were LDH heterozygotes. From these, six logistically convenient populations that had no LDH heterozygotes were chosen for the study. Natural lake populations in the KBS area have been the subject of considerable study, and always contain only the *D. pulicaria* LDH allele; for the lakes in this study (Lawrence, Pine, Warner, Hamilton, Three Lakes II, and Lake Sixteen), I verified this with at least 10 individuals per population. Throughout this paper, I refer to populations with only the *pulex* LDH allele as *D. pulex* and those with only the *pulicaria* allele as *D. pulicaria*, although these taxa are not entirely genetically distinct.

Field demography

In order to estimate extrinsic death rates, demographic parameters were measured in six replicate populations each of *D. pulicaria* and *D. pulex* from October 1997 through September 1998. The general procedure was to measure population densities, egg-ratio, egg age distribution and temperature to estimate instantaneous population growth (r), birth (b) and death (d) rates, in a manner similar to that of Tessier, Young & Leibold (1992). Egg age distribution was determined based on developmental stages described in Threlkeld (1979) and pictured in Esslová (1959). By incorporating information about the egg age distribution, many assumptions in calculating birth rates from clutch size and temperature are eliminated and a more accurate estimate of b can be obtained (Bottrell *et al.*, 1976; Threlkeld, 1979; Rigler & Downing, 1984). Once r and b have been estimated, d is taken from the equation $r = b - d$.

Habitat differences dictate different sampling protocols for lakes and ponds. In the six lakes, samples were taken with vertical hauls of an 80 μm mesh, 11.5 cm diameter Birge plankton net (Wildco, Buffalo, NY, U.S.A.) On each sampling date, three replicate samples were taken. Each sample consisted of three vertical hauls made at different locations (>10 m apart) in the deepest area of the lake and then pooled. No attempt was made to precisely duplicate locations on different dates other than returning to the deepest area of the lake. Lakes were sampled to obtain population densities throughout the year, and estimates of birth rate during focal periods (late October, late March, late May and early September) representative of the major changes in *D. pulicaria* dynamics in these populations (Threlkeld, 1979; Hu & Tessier, 1995). For each focal period, samples were taken on three dates separated by the egg development time indicated by the water's average temperature. Birth rate was also estimated in mid-December (under ice-cover) in four populations.

In the six ponds, three replicate spatially pooled samples were taken with a 3-L container. For each sample, the container was plunged into the water at 10–20 locations, moving through the pond without backtracking into disturbed areas. At each location, three samples of water were collected, passing each through a separate 80 μm mesh screen. At successive locations, both the depth from which the water was taken and the order in which the screens were used were varied. This procedure generated three samples composed of 30–60 L of water taken from the same locations, but minimised the effects of spatial structure in *Daphnia* distribution or behavioural response to disturbance. In some instances, water levels were too low to take three complete samples and fewer or smaller samples were taken. Ponds were monitored for animals starting when they first had water in them, which varied from November to late February. Once *D. pulex* appeared, samples were taken continuously, spaced by the egg development time, until ponds dried.

Samples were either preserved with cold sugar-formalin (Prepas, 1978) immediately or placed on ice for preservation in the lab. On dates when birth rate was estimated, egg data on at least 100 randomly chosen adult females (if available) from one sample was recorded within 36 h of preservation. Numbers of adult females, juvenile females and males were counted later. Usually a 5 or 10% subsample was counted, but if animals were rare, the entire sample was counted.

There can be substantial vertical structure to the distribution of *D. pulicaria* in lakes, and so it is inappropriate to report densities based on water volume. Here, densities are reported based on the surface area sampled (312 cm^2), assuming that horizontal structure is low and its effect was minimised by the sampling design. In contrast, densities in ponds are based on volume. Although this precludes absolute comparisons of the population size, the dynamics of population growth are the relevant comparison for this study.

DNA sequencing and tree construction

In order to better understand the relationships among the replicate populations, a tree based on a mitochondrial DNA sequences was constructed, generally following the protocol described in Lehman *et al.* (1995). When possible, up to three clones per population were included in the study. All of these clones were scored at the LDH locus and determined to be homozygous consistent with the populations' designation as *D. pulex* or *D. pulicaria*. DNA was extracted with chelating resin (Walsh, Metzger & Higuchi, 1991) and the rapidly-evolving D-loop of the control region was PCR amplified with the primers specified in Lehman *et al.* (1995). This produced DNA fragments that were roughly 730 bases long. Fragments were then purified with a Gene Clean® kit (Bio 101; Montreal, Canada) and bi-directionally sequenced at Michigan State University's automated sequencing facility. The sequences were then checked by eye and then aligned in ClustalW for analysis. The sequences have been deposited into GenBank under accession numbers AY531267–AY531299. Molecular genetic similarity among populations was evaluated with a gene tree based on 680 bp of sequence using PAUP* 4.0 (Swofford, 2000).

Results

Field demography

Populations were much more dynamic in temporary ponds than in permanent lakes (Fig. 1). *Daphnia pulex* populations expanded and contracted by over four orders of magnitude in <5 months, whereas *D. pulicaria* populations fluctuated at most by three orders of

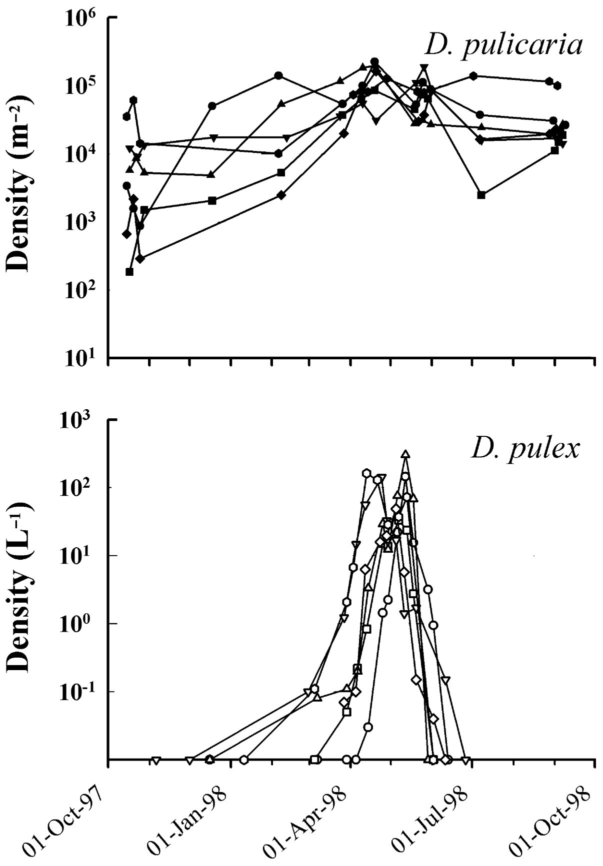

Fig. 1 Population dynamics of *Daphnia pulicaria* and *D. pulex* over 1 year. Density is reported on a log-scale, but at different magnitudes for the two species. The baseline of the lower panel represents densities below the pond detection limit of 10^{-2}. For *D. pulicaria*, circles = Warner Lake, squares = Pine Lake, up-triangles = Lawrence Lake, down-triangles = Hamilton Lake, diamonds = Three Lakes II and hexagons = Lake Sixteen. For *D. pulex*, circles = Wisdom Pond, squares = Otislake Pond III, up-triangles = POVI, down-triangles = Roughwood Pond, diamonds = Woodfrog Pond, and hexagons = Bittern Pond.

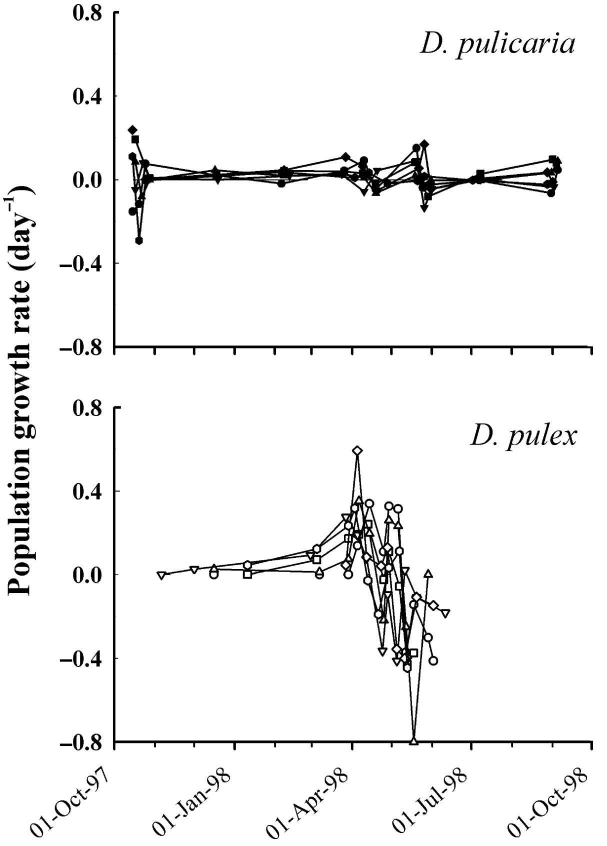

Fig. 2 Population growth rates of *Daphnia pulicaria* and *D. pulex* over 1 year. Symbols as in Fig. 1.

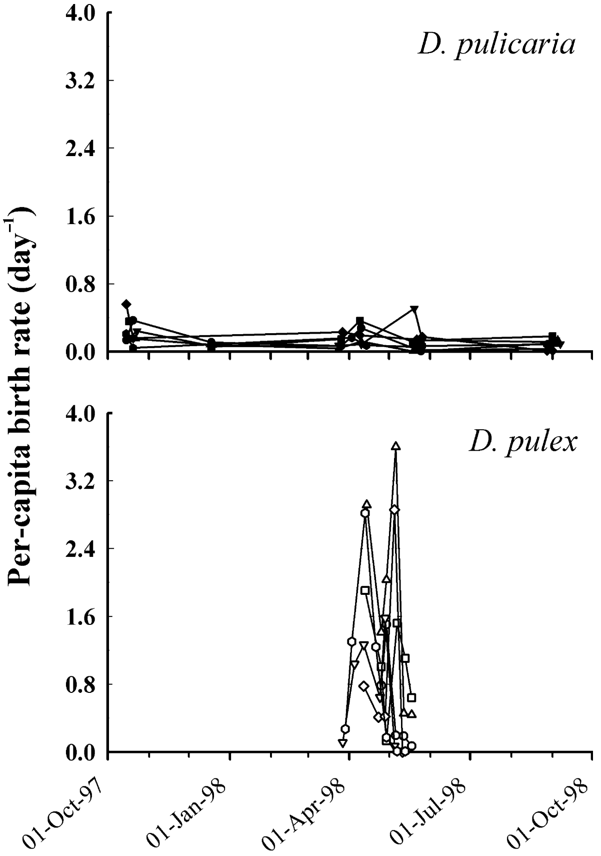

Fig. 3 Birth rates of *Daphnia pulicaria* and *D. pulex* over 1 year. Symbols as in Fig. 1.

magnitude over a year. This difference is also clear in the coefficients of variation for population density. For *D. pulex* the average CV was 179% whereas for *D. pulicaria* it was 103% ($t = -4.276$, d.f. = 5, $P = 0.008$). At the beginning and end of the season, pond densities were so low that no animals were collected in the samples. Though this may truly reflect the absence of *Daphnia*, these are plotted in Fig. 1 as the lower bound of detectability (0.01 L^{-1}) rather than zero. The stability of *D. pulicaria* is reflected in population growth rates that are generally near zero. *Daphnia pulex*, in contrast, shows extreme growth rates, flipping from +0.3 to −0.4 in the span of a month (Fig. 2). Birth rates in *D. pulicaria* were relatively low, with spring and fall peaks remaining below 0.5 neonates per adult female per day in most cases. In *D. pulex*, most populations had a birth rate peak in excess of 2.5 neonates per female per day (Fig. 3).

The differences in population growth and birth rates between the habitats resulted in strong differences in death rates. Death rate in *D. pulicaria* was both lower and more stable than in *D. pulex* (Fig. 4). The *D. pulex* populations also appear to harbour more among-population variation in death rate patterns. This can partly be attributed to asynchrony among the ponds in their emergence of *Daphnia* from the egg bank, and consequent differences in community timing. Despite this variation, four of the six pond populations reached daily death rates in excess of 2.7, whereas none of the lake populations ever exceeded a rate of 0.5.

In the *D. pulicaria* populations I assumed that hatching rate from the egg bank was negligible relative to overall population dynamics. This assumption is reasonable because these populations have a perennial phenology, and are therefore predicted to invest little in diapause. Recent evidence supports this assumption (Caceres & Tessier, 2003). Furthermore, *D. pulicaria* produced diapausing eggs only in mid-late May, and even then only 1–27% of adult females were carrying diapause eggs. *Daphnia pulex*, in contrast, must invest in diapause to persist through the dry

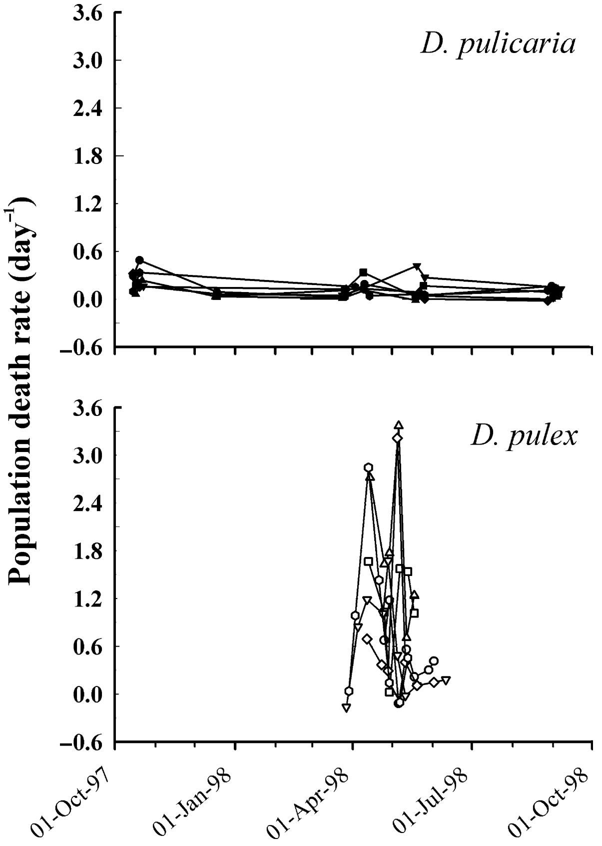

Fig. 4 Death rates of *Daphnia pulicaria* and *D. pulex* over 1 year. Symbols as in Fig. 1.

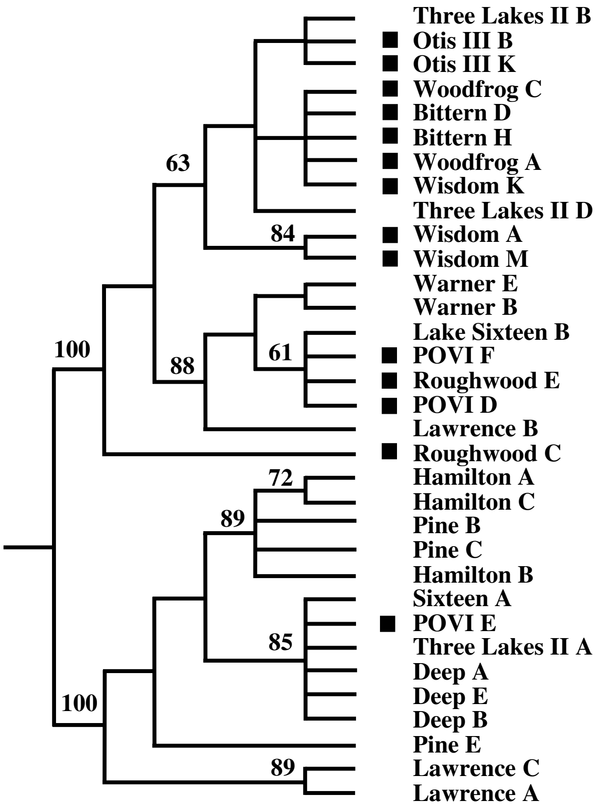

Fig. 5 Strict consensus of 13 best maximum likelihood trees based on 680 bp of control region D-loop mtDNA. Each terminal branch is a specific clone, identified by a letter and its source population. Labels preceded by a black square are LDH homozygous *D. pulex* from temporary ponds, otherwise clones are LDH homozygous *D. pulicaria* from lakes. Numbers adjacent to nodes indicate those with ≥60% bootstrap support, based on 500 replicates of the topologically congruent neighbour-joining tree.

period and the proportion of females carrying diapausing eggs peaked at 40–83%. In temporary ponds, genotypes that hatch late suffer a demographic cost relative to genotypes that hatch early, which should lead to the evolution of a narrow hatching window. I assumed that hatching was essentially complete by the time population densities were sufficiently large to estimate birth rate, and should therefore minimally affect the death rate estimates. However, failure of this assumption means that *D. pulex* death rates, and consequently the difference between species, were underestimated as emergence from the egg bank is not included in the estimates of birth rate.

All methods of tree construction (distance, maximum parsimony and maximum likelihood) strongly supported a division of the populations into two clades, one containing primarily *D. pulicaria* genotypes and one including a mixture of *D. pulex* and *D. pulicaria* (Fig. 5). These two clades were separated by 4.0% sequence divergence, whereas sequence divergence within each clade was only 1.1% (the mainly *D. pulicaria* clade) and 2.3% (the mixed clade). This indicates that genetic history and habitat do not covary. To test this further, a constrained maximum likelihood tree was constructed to force monophyly of the pond-derived clones. The orignial maximum likelihood analysis produced 13 best trees that differed slightly in the arrangement of the branch tips, but had equal likelihood scores. The constrained tree (keeping all clones from ponds a monophyletic group) yielded seven best trees that were markedly different from the original 13. Using a procedure that accounts for multiple comparisons, parametric bootstrapping showed that the best constrained trees were significantly worse than the best unconstrained maximum likelihood trees (likelihood-ratio = 98.68; $P < 0.0001$; Shimodaira & Hasegawa, 1999).

Discussion

Adaptive divergence in generalist herbivores may depend on the net effects of many different selective pressures that distinguish alternative habitats, rather than one or a few easily-identifiable selective agents. In *Daphnia*, the picture is further complicated by their remarkable morphological, behavioural and life historical phenotypic plasticity (Spitze, 1992; Stibor, 1992; Boersma, Spaak & De Meester, 1998; Dudycha, 2003). In this paper, I have shown that the mortality rates of *D. pulex*, a resident of temporary ponds, and *D. pulicaria*, a resident of permanent lakes, differ substantially in both their level and stability. These differences are aggregates of many different sources of mortality, including vertebrate and invertebrate predation, starvation, desiccation, pathogens and possibly intrinsic causes. Even without direct mechanistic understanding of the mortality differences, it is obvious that the two habitats should select for overall differences in life history.

Although it is not surprising that the mortality patterns differ between the habitats, the magnitude of the difference is quite large. In addition to occupying a much shorter window of time, the seasonal cycle of *D. pulex* population density ranges about two orders of magnitude further than *D. pulicaria*, indicating that extrinsic mortality is a much stronger force in the evolution of *D. pulex*. Because the habitat quality of temporary ponds degrades rapidly and completely, it is unsurprising to observe a sharp increase in mortality rates as spring progresses. In most cases this increase was followed by a sharp decrease in mortality rates, with the population dwindling and several weeks during which *Daphnia* densities were very low but the pond still had standing water. Populations were never eliminated en masse at the time of drying. Therefore, the drying of ponds cannot be the direct cause of increased mortality rates, and either density-dependent factors or other aspects of seasonal succession are probably responsible. Most of the mortality is likely to have some sort of extrinsic cause. Intrinsic mortality rates (measured in the lab at 20° and high resources) do not significantly differ from zero until *D. pulex* are over 1-month old, and over 2-months for *D. pulicaria* (Dudycha & Tessier, 1999). In the field, where temperatures are somewhat colder, substantial intrinsic mortality is likely to be delayed even further. The field mortality rates are sufficiently high, even in lakes, to imply that most individuals die before they are 1-month old. Determining the extrinsic mechanisms creating the mortality rate differences will require experiments that jointly evaluate the probable candidates, such as predation, resource scarcity, pathogens or abiotic stressors.

Common-garden experiments have revealed several distinguishing life history features between *D. pulex* and *D. pulicaria* (Dudycha & Tessier, 1999; Dudycha, 2003). *Daphnia pulex* generally have substantially shorter longevities, grow faster and have greater early reproductive output than *D. pulicaria*. All are characteristics that are expected to evolve under high extrinsic mortality risk. Furthermore, *D. pulex* has a relatively fixed investment in survival, whereas *D. pulicaria* is fairly plastic with respect to food and temperature (Dudycha, 2003). This is counterintuitive because *D. pulex* experiences a much wider range of mortality risk levels, so it may have more to gain in terms of efficiently exploiting its habitat by altering its investment in survival. However, the seasonal state that a pond individual finds itself in is predictable on the basis of maternal environment or photoperiod. Lakes, in contrast, present an environment where both the timing of change and the rate of change vary. As a consequence, individuals there may cue in to aspects of the environment that directly influence population growth, such as temperature and resource availability.

The life history differences between these taxa involve tradeoffs that are important in the context of their population dynamics. Although net lifetime reproduction does not consistently differ between *D. pulex* and *D. pulicaria*, laboratory measures of population growth rate from life tables do (Dudycha & Tessier, 1999). This difference is because of the greater reproductive output early in life of *D. pulex*, whereas *D. pulicaria* expands and prolongs its reproduction over a greater period. Reproductive investment at this time is further distinguished because *D. pulex* tend to produce more dormant eggs than *D. pulicaria*, and this production occurs in early adult instars (J. L. Dudycha, E. M. C. Morrin & C. A. Hassel, unpublished data).

The selective pressures promoting divergence will, however, be countered by gene flow between the habitats. Although the gene tree recovered two widely divergent groups, these did not correspond to the habitat differences, with one clade in particular showing substantial inter-habitat mixing. This indicates that these taxa are incompletely diverged, not at the end of speciation. In terms of life history, this means that trait divergence is strong and repeatedly maintained (Deng, 1997; Dudycha & Tessier, 1999; Dudycha, 2001, 2003).

Daphnia pulex and *D. pulicaria* are two nominal taxa that are at some point in the middle of adaptive speciation. These data join earlier work on genetic structure (Pfrender *et al*., 2000; Morgan *et al*., 2001), life history (Deng, 1997; Dudycha & Tessier, 1999) and comparative ecology (Demott, 1999) to form a basis for expanded understanding of joint roles of genetics and ecology in speciation. Mortality dynamics differ substantially between habitat types and are likely to be a key component of factors creating the potential for ecological speciation. Additional factors not addressed in this study are also likely to have important roles in the divergence of these taxa, in particular selection on resource acquisition, fecundity selection, selection associated with different temporal scales of temperature change, and aspects of shifts among reproductive modes and investment in diapause. On the ecological side, future work should compare the population dynamics of pond and lake populations over many years to gain an understanding of relative levels of interannual versus interhabitat variation in mortality risk. Populations existing at the margins of typical habitats, such as permanent fishless ponds and shallow lakes, need to be examined to better understand the limitations to divergence that are created by gene flow and hybridisation. Laboratory experiments are needed to further investigate the degree of reproductive isolation between these taxa, and uncover the link between resource availability and reproduction. On the genetic side, nascent genomic tools and the impending complete genome sequence of *D. pulex* open the door to investigating the association between environment- and taxon-specific gene expression and to determining the functional genetic basis of important tradeoffs. In conjunction, the potential for ecological, comparative, quantitative genetic and genomic research make *Daphnia* one of the best available systems for insight into adaptive speciation.

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