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Testing the ecological relevance of *Daphnia* species designations

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SUMMARY

1. Molecular approaches have increasingly revealed hidden genetic structure within ecologically important species, leading to the creation of sibling species whose ecological relevance is often unclear. A prime example is *Daphnia galeata mendotae*, which was split into *D. dentifera* and *D. mendotae* based on differences at two allozyme loci.
2. In a set of lake populations in Michigan USA, we test the geographical and temporal consistency of the genetic structure underlying this species split. We also test the morphological relevance of this molecular variation and its ecological significance in lakes. In essence, we ask: does recognition of these new species provide valuable information for plankton ecologists?
3. We found that *D. dentifera* and *D. mendotae* represent morphologically and ecologically distinct forms that are distributed among lakes in non-random fashion, which were remarkably stable over 6 years. Key differences between the species concern their body and head shape, vertical habitat use within lakes and distribution among lakes of different size. We hypothesise that these differences represent specialisation to habitats that differ in risk of invertebrate predation.
4. Reproductive barriers alone are insufficient to explain the pattern of genetic structure; in some lakes complete introgression is apparent. However, parent species and hybrids exhibit a stable co-existence in many lakes, which suggests that ecological specialisation reinforces divergence within this taxon.

Keywords: *Daphnia dentifera*, *Daphnia mendotae*, genetic structure, hybrid, lake size

Introduction

It is not uncommon for the taxonomy of a clade of organisms to be revised; existing species are split into two or more new species, based primarily on molecular differences (Knowlton, 1993; Gomez & Snell, 1996). To ecologists, this is sometimes viewed as an annoyance; do the split names convey ecological relevance or are they redundant in ecological function (Knowlton & Jackson, 1994)? In the plankton of lakes, the dominant grazers, *Daphnia*, have had a particularly fluid taxonomy (Brooks, 1957; Taylor & Hebert, 1992, 1994; Hebert, 1995; Hebert, Witt & Adamowicz, 2003). The reasons that *Daphnia* are prone to taxonomic confusion include their large amount of phenotypic plasticity (Brooks, 1957; Taylor & Hebert, 1992), scant exoskeletal fossil records (Frey, 1987) and relatively few phylogenetically informative morphological characters (Taylor, Hebert & Colbourne, 1996). As a result, *Daphnia* systematists have turned to molecular characters, such as allozymes and DNA, in an attempt to resolve this taxonomic confusion. While molecular data has clarified the genetic structure for several *Daphnia* complexes (e.g. *Daphnia galeata–cucullata–hyalina* complex; Schwenk, 1993), it potentially complicates the

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ecological picture by revealing new species diversity and hybridisation.

One such taxon is the North American *Daphnia longispina* group, which has been subjected to numerous taxonomic re-assignments (Colbourne & Hebert, 1996). Based on allozyme variation, Taylor & Hebert (1992) and Taylor *et al.* (1996) divided a subspecies within this group, *D. galeata mendotae* Brooks, into two new species, *D. dentifera* and *D. mendotae* and their hybrids. Taylor and Hebert reported that allelic differences at each of two allozyme loci [aldehyde oxidase (AO) and glutamate-oxaloacetate transaminase (GOT-m), now known as aspartate aminotransferase (mAAT)] corresponded to the extremes of morphological variation expressed among various lake populations. Allelic variation at a third allozyme (sAAT) also showed strong differentiation between morphs, but little variation within each morph. More importantly, they observed that individuals that were heterozygous for these allozymes had a morphology that was qualitatively intermediate to the two extreme morphs. As they observed these heterozygotes to co-occur with one or the other morph, but both morphs were never found in the same lake, they argued for species designation of the two morphs (Taylor & Hebert, 1992). Further, they presented evidence that the different morphs inhabited lakes with different surface area (Taylor & Hebert, 1992), suggesting ecological speciation.

Recognition of sibling species in what had previously been a polymorphic species was potentially of great ecological significance, as this is a widespread and often dominant grazer in the plankton of temperate lakes in North America. However, this original work was restricted to one set of lakes in northeastern Indiana, U.S.A., sampled in 1 year. Later work by Taylor & Hebert (1993) on a wider range of populations showed variation within morphs at both AO and mAAT. While these broader results were explained as the result of introgression, it effectively meant that species discrimination was problematic. The originally described morphological variation that correlated with the allelic variation was qualitative and based on lake-collected animals; it was not known to be genetically determined. Further, little evidence existed to determine whether these new species boundaries were ecologically relevant, or whether the pattern of genetic structure was stable across years. As a result, plankton ecologists have been slow to adopt the new taxonomy.

We examined 16 lake populations of this species complex in southwestern Michigan to see if the genetic structure was consistent with Taylor and Hebert's description of two species and hybrids. We also quantified morphological variation between the allozyme-defined species and hybrids for both field-collected and lab-raised clonal lines, and tested for additional ecological differences between these taxa. Based on Taylor and Hebert's earlier work, we specifically examined seasonal succession, vertical habitat segregation, lake size and co-occurrence with invertebrate predators. Finally, we re-sampled several lake populations 6 years apart to test the stability of their genetic structure.

Methods

Field sampling

To examine genetic structure within populations of the *D. dentifera–mendotae* complex, we collected samples from 15 lakes in southwestern Michigan, U.S.A. during 1995 (Table 1). In mid-June, 13 of the lakes (all except Eagle and Goguac) were sampled with single vertical hauls of a 30 cm diameter 80 μm mesh net. *Daphnia* within this complex were identified based on Brooks (1957) and mature individuals (11–22) were randomly selected and frozen at −80 °C for allozyme analysis. This broad survey was conducted to determine the regional diversity at the selected allozyme loci.

We conducted more extensive allozyme characterisation, in addition to morphological analysis, on eight of the populations (Table 1). These lakes were sampled one to four times during June and August, 1995. Mature individuals (mean = 94) from each population (or sub-population) were randomly selected and frozen at −80 °C for allozyme analysis. In addition, ten randomly selected individuals of each morph present in a lake were photographed for later morphological measurement. Eight lakes, seven of which had been sampled in 1995, were sampled in 2001 (Table 1). A single vertical tow was taken in each lake and 35–48 randomly selected adults were frozen at −80 °C for allozyme analysis.

Three populations (Goguac, Hall and Shaw) were selected, based on the morph that dominates the lake (*D. mendotae*, hybrids and *D. dentifera*, respectively),

Table 1 Summary of sampling scheme. All work was performed in 1995 except that eight lakes (designated by + in sampling year column) were sampled in 2001 to compare across years and the sediment study was carried out in 2001. All lakes except Whitford were initially sampled for AO as part of a broad lake survey prior to some lakes being picked for more extensive study. Individuals collected from Eagle and Goguac were subdivided by morphology into subpopulations for allozyme and morphological analysis

Lake	County	Sampling 1995/2001	Extensive allozymes	Morphology study	Invertebrate predator sampling	Sediment sampling
Hall	Barry	+/−	+	+	+	−
Head	Barry	+/−	−	−	−	−
Lower Crooked	Barry	+/−	−	−	−	−
McDonald	Barry	+/−	−	−	−	−
Shaw	Barry	+/−	+	+	+	−
Three Lakes 2	Kalamazoo	+/−	−	−	−	−
Three Lakes 3	Kalamazoo	+/−	−	−	−	−
Williams	Barry	+/−	−	−	−	−
Eagle	Kalamazoo	+/+	+	+	+	+
Fine	Barry	+/+	+	+	+	+
Goguac	Calhoun	+/+	+	+	+	+
Lawrence	Barry	+/+	+	+	+	+
Little Mill	Barry	+/+	+	+	+	−
Pine	Barry	+/+	+	+	+	−
Warner	Barry	+/+	−	−	−	−
Whitford	Barry	−/+	−	−	−	−

for further analysis. Animals were collected from net tows and five clonal lines were established into laboratory culture from each of the lake populations. One of the Goguac Lake clones was subsequently lost. Each clonal culture was split into replicate lines and reared at 20 °C under common environmental conditions for several generations (culture methods as in Tessier & Consolatti, 1991). Two 6-day-old animals from each of the replicate clonal cultures were photographed for morphological analysis.

During summer 1996, we examined diel vertical habitat use of *Daphnia* in Eagle Lake, which contained *D. mendotae* and apparent hybrids in high frequency. Samples were collected at two replicate locations within the lake at 0, 2, 4, 6, 8 and 10 m using a 15 L Schindler trap (Robert Richards, Tahoe Research Group, University of California, Davis, CA, U.S.A.) at mid-day and again at midnight. Temperature and oxygen depth profiles were also measured at noon. All *Daphnia* in each sample were counted and assigned to species (either *D. pulicaria*, *D. retrocurva*, *D. mendotae* or *D. mendotae* × *dentifera* hybrids) based on morphology using Brooks (1957) and Taylor & Hebert (1992). In addition, closing net tows were taken from 10–6 and 3–0 m at both locations during the nighttime sampling. We sampled 15–35 *D. dentifera–mendotae* complex adults from each of these four samples and froze the adults at −80 °C for allozyme analysis.

All of the study lakes contain bluegill sunfish (*Lepomis macrochirus*) as the primary fish planktivore (Tessier & Woodruff, 2002). However, we noticed substantial variation among lakes in density of invertebrate predators. Consequently, we sampled eight lakes (Table 1) during August 1995 at night using a vertical net (two tows of a 500 μm mesh, 41.5 cm diameter, 3 m length conical net). The density of large (3rd and 4th instar) *Chaoborus*, *Leptodora* and mites were quantified as potential predators of *Daphnia*.

Sediment analysis

In order to determine which morphs were present in the resting egg portion of the population, we collected sediment cores from four lakes. In many of these lakes, we are unable to detect *Daphnia* from this species complex during winter and early spring (Cáceres & Tessier, in press); therefore, the dormant eggs are likely to be important in re-founding the summer population. Three, 1-meter sediment cores were collected from each of four lakes (Table 1) in July 2001 using a Lauff Corer (George Lauff, Kellogg Biological Station, Michigan State University, Hickory Corners, MI, U.S.A.). Three additional cores were collected from both Eagle and Goguac in August 2001. The top 5–10 cm of each core was sliced in the field and stored in the dark at 4 °C. Ephippia were collected by passing sediments through a 150 μm sieve. As it is not possible to tell *D. dentifera*, *D. mendotae* and their hybrid ephippia apart and as AO is not expressed in resting eggs, it was necessary to hatch the animals in order to determine the species

of each resting egg. Resting eggs were incubated at 10 °C and 12 : 12 light : dark. Hatching was checked three to four times per week. Once hatching stopped, the resting eggs were transferred to a 20 °C, constant light environment. Hatching was once again checked three to four times per week. Hatchlings were raised 3–5 days, at which point their species identity was checked (morphologically). They were then stored at −80 °C for allozyme analysis.

Allozyme analysis

Allozymes were determined using cellulose acetate electrophoresis following methods in Hebert & Beaton (1989). For the animals collected in June 1995, two loci were scored: AO (EC 1.2.3.1) and phophoglucomutase (PGM, EC 2.7.5.1). AO was a locus that Taylor & Hebert (1992) originally considered diagnostic for the two species. Samples collected later in 1995 were characterised at these two loci, as well as at aspartate aminotransferase (sAAT, EC 2.6.1.1). We were unable to obtain reliable data for mAAT, which is the other locus Taylor & Hebert (1992) considered diagnostic, although both this and AO were later shown to exhibit strong introgression. Animals collected from Eagle Lake in 1996 were scored at AO and PGM. Animals collected in 2001 and animals hatched from the resting egg bank were simply scored at AO.

For the populations that were analysed at all three allozyme loci (i.e. AO, sAAT and PGM), a multi-dimensional scaling of their Euclidean distances was performed. We were able to capture >70% of the variation in a one-dimensional ordination, which we correlated to the morphological variation. The AO data from 1995, 2001 and the resting egg banks were used to test for agreement with Hardy–Weinberg expectations, after correcting for sample sizes (Lessios, 1992).

Morphological analysis

Animals were photographed for digital analysis using Image-Pro Plus software (Media Cybernetics, 1993). Coordinates were recorded for eight landmarks on the lateral view of the animal (the rostrum tip, the most anterior portion on the head, the point of helmet attachment to the body carapace, the centre of the eye, the point of tail spine attachment to the carapace, the tip of the tail spine, the point of greatest ventral head curvature and the point of greatest dorsal head curvature). The coordinates were standardised for differences in body size, using the distance between the rostrum tip and the point where the helmet attaches to the body as a baseline, scaled as 0,0 and 1,0 coordinates, respectively, according to the methods of Bookstein (1991). A principal components analysis (PCA) was performed on the covariance matrix of this standardised coordinate data.

Results

Daphnia in the *dentifera–mendotae* complex exhibited considerable molecular and morphological variation in our study lakes. We found 28 different multi-locus genotypes, (hereafter, clonal groups), among the nine lakes sampled in 1995, based on the AO, sAAT and PGM allozyme loci. Of these 28 genotypes, three comprised <1% of any population and so were ignored in further analysis. Individual lakes had between six and fifteen different clonal groups, with most lakes having seven or eight. A one-dimensional multi-dimensional scaling (MDS) explained 70.5% of the Euclidean distance variation. The differences among the lakes in this MDS axis were driven primarily by differences at the AO locus; the correlation between the MDS axis and the frequency of the AO slow allele was 0.9025 ($P < 0.0001$).

We found all three possible AO genotypes, suggesting the presence of *D. dentifera* (SS AO genotype), *D. mendotae* (FF AO genotype) and their hybrids (SF AO genotype), according to Taylor & Hebert (1992) (Table 2). Moreover, we found that lakes differed greatly in the relative frequencies of the three morphs. Many lakes were dominated by one parent and the hybrid, while the other parent species was rare or absent. However, more than half the lakes contained both parents and hybrids and in one lake, Little Mill, the ratio of *D. dentifera* : hybrid : *D. mendotae* was 1 : 2 : 1. For the 11 lakes sampled in 1995 for which deviations from Hardy–Weinberg Equilibrium (HWE) could be tested, six lakes showed significant deviations from HWE. In 2001, only one of eight lakes sampled showed significant deviations from HWE, although sample sizes were substantially smaller than in 1995.

We also found that the relative frequencies of the three AO genotypes in hatched resting eggs differed from those expected if the three morphs were randomly mating (Table 3). The egg banks of two of the four lakes had significantly fewer hybrid resting

Table 2 Frequencies of aldehyde oxidase (AO) genotypes in the study lake populations of *D. longispina* group animals. The sample size (pooled over all sampling dates within a year) and frequency of each of the three genotypes (SS, *D. dentifera*; SF, hybrids; FF, *D. mendotae*) are given, as well as the probability that the sample fits Hardy–Weinberg Equilibrium (HWE) at this locus. 'NS' is used to denote *P*-values >0.05 and a '–' indicates that the population was fixed for one genotype

Lake	Date	*n*	SS (%)	SF (%)	FF (%)	HWE *P*
Three Lakes 2	1995	11	100	0	0	–
Three Lakes 3	1995	66	74	15	11	<0.0001
Eagle	1995	66	12	64	24	0.0209
Eagle	2001	42	0	64	36	0.0027
Fine	1995	195	6	72	22	<0.0001
Fine	2001	40	43	38	20	NS
Goguac	1995	46	2	15	83	NS
Goguac	2001	48	0	10	90	NS
Hall	1995	190	21	78	1	<0.0001
Head	1995	22	100	0	0	–
Lawrence	1995	195	75	21	5	0.0069
Lawrence	2001	48	77	21	2	NS
Little Mill	1995	207	28	47	25	NS
Little Mill	2001	47	15	51	34	NS
Lower Crooked	1995	11	82	18	0	NS
McDonald	1995	11	100	0	0	–
Pine	1995	70	64	36	0	NS
Pine	2001	35	80	17	3	NS
Shaw	1995	79	92	5	3	<0.0001
Warner	1995	11	82	18	0	NS
Warner	2001	43	81	19	0	NS
Whitford	2001	37	65	32	3	NS
Williams	1995	11	100	0	0	–

Table 3 Frequencies of AO genotypes in animals hatched from ephippia collected from four lakes in southwestern Michigan. Table format as in Table 2

Lake	*n*	SS (%)	SF (%)	FF (%)	HWE *P*
Eagle	11	0	9	91	NS
Fine	12	17	0	83	<0.0001
Goguac	23	17	0	83	<0.0001
Lawrence	39	85	15	0	NS

eggs than expected given the frequencies of fast and slow AO alleles.

The relative frequencies of *D. dentifera*, *D. mendotae* and their hybrids in a given lake were stable across a period of 6 years (Fig. 1). The correlations between the proportion of a morph in 1995 and the proportion of that same morph in 2001 were all strong ($r = 0.888$, 0.793 and 0.988 for *D. dentifera*, hybrids and *D. mendotae*, respectively).

There was striking variation in the body shape of adult *Daphnia* collected from the study lakes and it was strongly related to genotype. The first PCA axis explained 56% of the variation in morphological landmark locations for field-collected animals. This axis described variation in the relative height and width of the head. Animals with high scores for PCA Factor 1 (i.e. heads large for their body size) tended to be homozygous for the fast AO allele (i.e. they would be considered *D. mendotae*), while those with negative loadings for Factor 1 tended to be homozygous for the slow AO allele (i.e. they would be deemed *D. dentifera*, Fig. 2). Hybrids were intermediate in head size, albeit

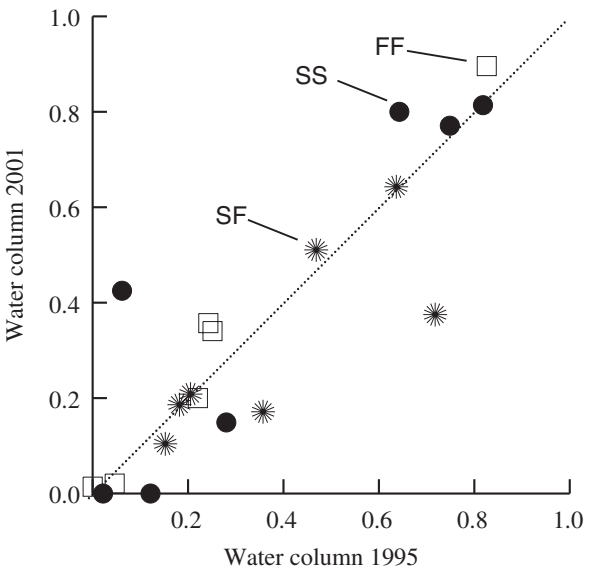

Fig. 1 Relative frequencies of each of the three AO genotypes (SS, *D. dentifera*; SF, hybrids; FF, *D. mendotae*) in seven lakes. The relative frequency of a morph in a lake in summer 1995 is plotted against its relative frequency in that lake in summer 2001. The dashed line represents a 1 : 1 correspondence.

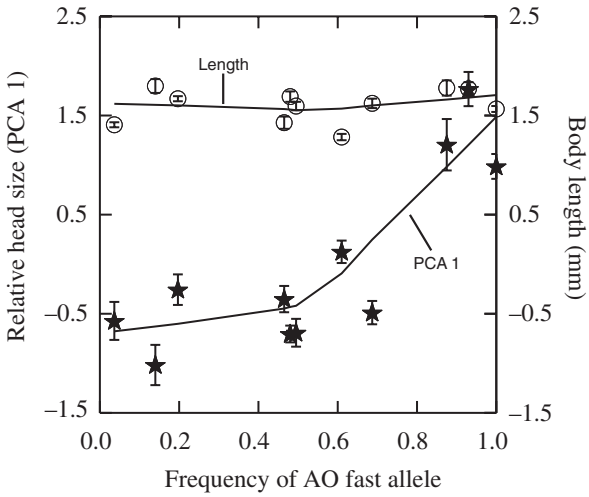

Fig. 2 Relationship between genetic variation in AO and morphology of field-collected animals. Mean body length reflects the specific adults chosen for morphological analysis and is not representative of population size structure. The first PCA axis of the standardised landmark coordinates describes the relative height and width of the head. Both data sets are fitted with locally weighted (LOWESS) smoothing lines. Values are means for the (sub)populations, from left to right: Shaw, Lawrence, Pine, Hall, Little Mill, Eagle 'Low Head', Fine, Eagle 'High Head', Goguac 'Round Head', Goguac 'Pointed Head' and Eagle 'Pointed Head'.

more similar to *D. dentifera*. Animal morphology (i.e. PCA axis 1) was also correlated ($r = -0.686$, $P = 0.020$) with the MDS axis of genotype distances. Only one population, Little Mill, did not fit this general relationship between allozymes and morphology. This lake contained individuals of all three AO morphs, yet all animals expressed the morphology of a hybrid.

Differences in morphology among the three AO morphs persisted for several generations in the laboratory, indicating that the differences have a genetic basis. A PCA on the landmark coordinate data collected from these lab-reared animals explained 40 and 32% of the total variation in the first two PCA axes, respectively. The first axis loaded primarily on the length of the carapace (excluding tail) and described the size of the body relative to the size of the head. The second axis loaded primarily on the location of the point of greatest dorsal curvature of the head and therefore described the shape of the head. The three taxa were significantly different when described using these two PCA axes (Pillai's $F_{4,22} = 10.5$, $P = 0.0001$; Fig. 3). In addition, the morphs differed significantly in body size ($F_{2,11} = 10.9$, $P = 0.0025$); *D. mendotae* were larger than *D. dentifera* and hybrids.

The three morphs showed temporal habitat specialisation (Fig. 4). Within a given lake, *D. dentifera* became more common and the hybrids less common, as the summer progressed. These trends resulted in significant regressions against sample day, even when corrected for multiple testing ($P = 0.0039$ for *D. dentifera* and $P = 0.0031$ for hybrids). The relative frequency of *D. mendotae* remained relatively constant.

Daphnia mendotae, *D. dentifera* and hybrids also differed in spatial habitat use. The proportion of *D. dentifera* in lakes decreased with both increasing lake surface area and depth (Fig. 5). The correlations between the frequency of *D. dentifera* and surface area ($r_p = -0.550$) and between *D. dentifera* and maximum depth ($r_p = -0.573$) were strong, however, the associations appear non-linear (Fig. 5). Vertical habitat segregation was also evident within a lake. *Daphnia mendotae* and hybrids, which co-exist in Eagle Lake, segregated vertically in the water column at night

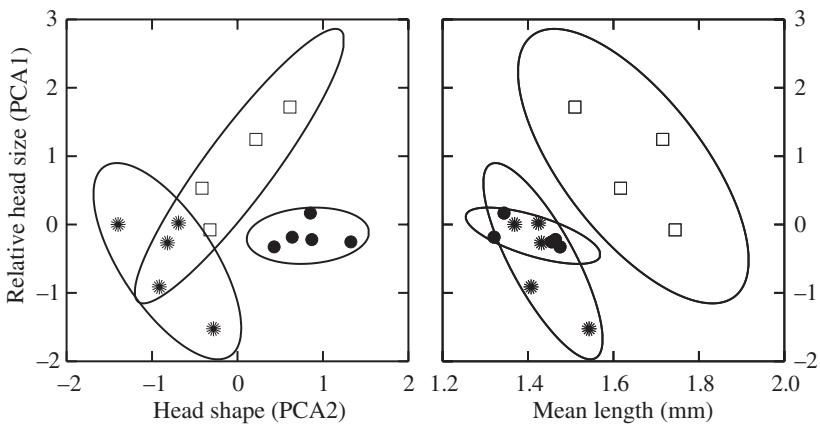

Fig. 3 Morphological differences among lab-raised *D. mendotae* (open squares), *D. dentifera* (filled circles) and hybrids (stars). Each point is the mean of a clone and the sample confidence ellipse ($P = 0.683$) for each species is also shown.

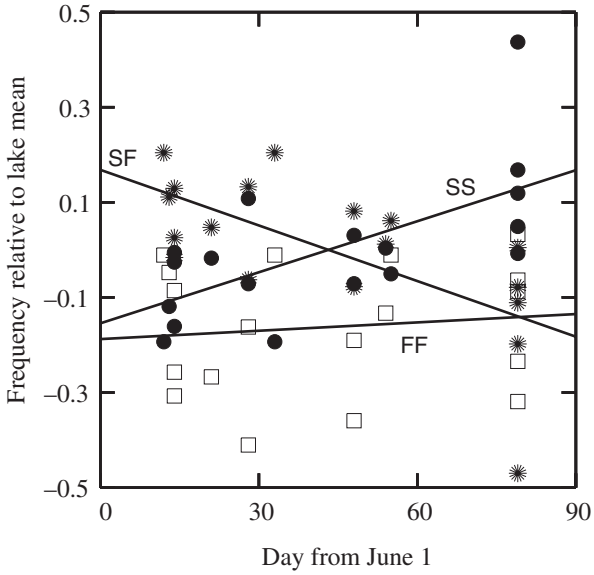

Fig. 4 Seasonal changes in relative abundances of morphs [*D. dentifera* (SS, filled circles), *D. mendotae* (FF, open squares) and hybrids (SF, stars)]. As lakes differed in mean frequencies of morphs, data is presented as residuals after removal of these mean lake differences. As there is no change in *D. mendotae* through the season, the changes in *D. dentifera* and hybrid frequencies are negatively correlated.

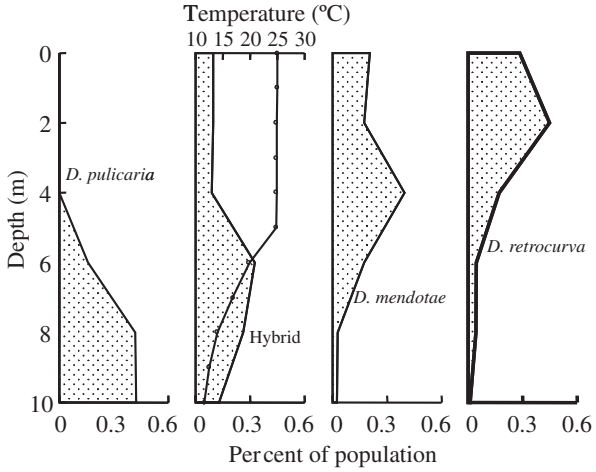

Fig. 6 Vertical habitat segregation among *D. pulicaria*, *D. retrocurva*, *D. mendotae* and *D. dentifera* × *D. mendotae* hybrids during summer in Eagle Lake at night. Water temperature is shown in the second panel. For clarity, only nighttime distributions are shown, as the daytime distributions differed only slightly.

(Fig. 6). Clonal composition, based on AO morph, was significantly different between the shallow (0–3 m) and deep (6–10 m) samples (exact $P < 0.01$ for both lake sites) and closely matched counts of individuals based on morphology. *Daphnia mendotae* preferred shallower waters than the hybrid morph.

Variation in the relative frequency of the three morphs among lakes was also associated with variation in the abundance of invertebrate predators (Fig. 7). Levels of invertebrate predation in lakes clearly fell into two groups: high (>118 invertebrate predators m^{-2}; Fine, Eagle and Goguac) and low (<45 invertebrate predators m^{-2}; Hall, Lawrence, Little Mill, Pine and Shaw). These two groups of lakes significantly differed in the frequency of the AO fast allele (pooled variance $t = 3.12$, $P = 0.021$); the fast AO homozygote (*D. mendotae*) was much more common in lakes with high invertebrate predation. Interestingly, these high invertebrate predator lakes were also the largest surface area lakes.

Discussion

The *D. dentifera–mendotae* complex in our lakes is comprised of distinct morphs that reflect genetic and ecological variation. These morphs form assemblages

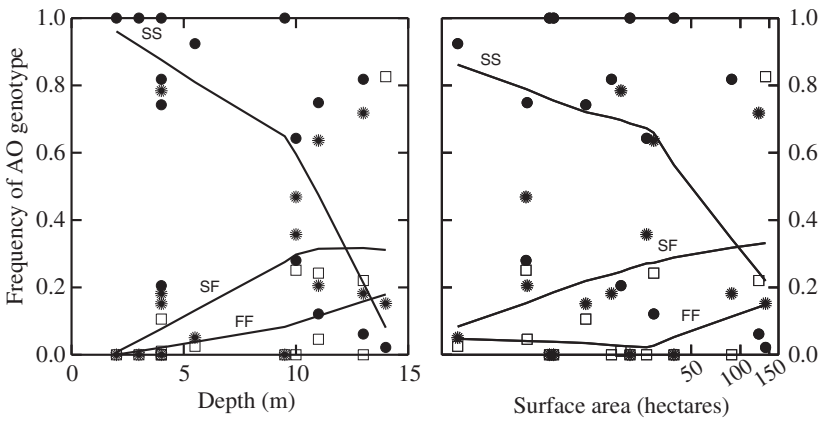

Fig. 5 Relationship between lake size and the frequency of AO genotypes (SS, *D. dentifera*; SF, hybrids; FF, *D. mendotae*). Locally weighted (LOWESS) smoothing lines are fit for each genotype.

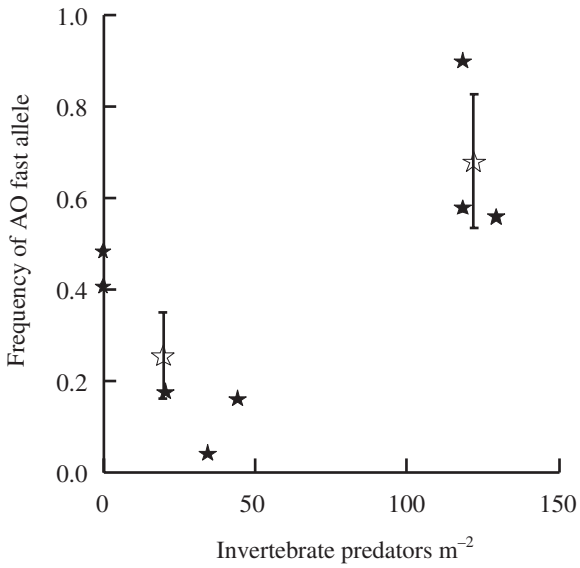

Fig. 7 Relationship between the number of invertebrate predators and the frequency of the AO fast allele in different lakes (depicted by closed stars). Lakes were split into two groups: low invertebrate predation (<45 invertebrate predators m^{-2}) and high invertebrate predation (>118 invertebrate predators m^{-2}) and the open stars represent average AO (± 1 SE) allele frequencies and average number of invertebrate predators for the two groups.

that are stable over multiple years, both within and among populations and that are distinct ecologically. This strong patterning of genetics and morphology supports Taylor and Hebert's conclusion that this complex is comprised of two entities that experience hybridisation and introgression. However, at least occasionally (e.g. Little Mill) introgression can be large, indicating that the genetic barriers between these entities are not complete. We suggest, therefore, that the force responsible for maintaining the distinction between these morphs in most lakes is ecological. Specifically, we speculate that the distinctness of these morphs reflects adaptive specialisation to habitats that differ in risk of invertebrate predation. Whether these morphs deserve species status or represent a polymorphism within one species is debatable, but our results indicate that the names clearly have value to ecologists.

Daphnia mendotae have much larger heads, relative to the size of their bodies, than *D. dentifera*; hybrids are intermediate in head size. In addition, *D. mendotae* are significantly larger in overall body size than *D. dentifera* and hybrids. Together, these traits indicate that *D. mendotae* would be better equipped than *D. dentifera* to avoid predation by the common invertebrates *Chaoborus* and *Leptodora*, whose efficiencies of prey capture decrease with increasing *Daphnia* body length (Zaret, 1980; Mort, 1986; Pijanowska, 1990; Branstrator, 1998). Within a lake, *D. mendotae* occurs higher in the water column than hybrids, which would make *D. mendotae* particularly vulnerable to predation by the common fish predator, bluegill sunfish. This risk is somewhat minimised by preferential investment in head growth, which is the most transparent part of the animal. However, *D. mendotae* was common only in the larger lakes, which also seemed to have relatively higher densities of the invertebrate predators.

We suggest that the trend of higher frequency of *D. mendotae* and of invertebrate predators in larger lakes reflects a shift in the relative risk of predation from fish to invertebrates. Bluegill sunfish are known to feed selectively on larger prey and especially favour *Chaoborus* and *Leptodora* over *Daphnia* (Branstrator & Holl, 2000). Further, bluegill sunfish, especially younger age classes, prefer to remain close to the littoral zone (Hall & Werner, 1977). Therefore, the open water of large surface area, deep lakes is likely safer in terms of fish predation compared with small lakes, but riskier in terms of invertebrate predation. Hence, the relationships we observed among lake size, invertebrate predators and frequency of *D. mendotae* may be reflecting an overall shift in food web structure and size selective predation on *Daphnia*. We know of no studies that explicitly examined this idea.

Invertebrate predators, such as *Chaoborus* spp. and *Leptodora kindtii*, also tend to be in the epilimnion at night and *Leptodora* prefers warmer water even during the day (Vijverberg, 1991; González & Tessier, 1997). Therefore, while the ecology of *D. mendotae* means it is exposed to more invertebrate predators, its morphology allows it to avoid predation by them. On the other hand, *D. dentifera* and hybrids are morphologically more susceptible to these invertebrate predators. Hence, vertical segregation of these morphs within single lakes may reflect the same ecological specialisation to predators that we see among lakes of different size. The maintenance of hybrids in a European daphniid species complex is also believed to depend upon variation in vulnerability to vertebrate and invertebrate predators (Spaak, 1995; Spaak, Vanoverbeke & Boersma, 2000).

While ecological specialisation to habitats that differ in predation regime may explain the selective value of the polymorphism, it does not explain the discrete nature of two parent species and a common hybrid. In most lakes, hybrids must not backcross successfully with either parent as multiple allozymes are so strongly linked to the morphological traits over a period of many years. However, an important unknown is the frequency of sex by each parent and especially the hybrids. In laboratory cultures we have seen no evidence that any of the three morphs is capable of producing asexual resting eggs. Hence, sex appears necessary for investment in dormancy. Our populations of *D. dentifera* do not overwinter in the water column and instead rely on resting eggs to re-establish the summer population (Cáceres & Tessier, in press). However, in the lakes dominated by *D. mendotae* (Eagle and Goguac) we have observed overwintering adults (M. Duffy and A. Tessier, personal observation). This suggests that there may be important distinctions between these species in their reliance on resting eggs to overwinter.

When we examined the relative frequencies of AO genotypes of animals hatched from the resting eggs, two of the four populations differed strongly from Hardy–Weinberg expectations. In both cases, hybrids were under-represented in hatchlings. A larger study is warranted, but these results suggest non-random mating or lower viability of hybrid resting eggs.

Against this backdrop of persistent genetic structuring that is linked to morphology and to seasonal and vertical habitat segregation, we find one population where the linkage appears to have broken down. The Little Mill Lake population engages in sexual reproduction during each fall, disappears from the water column and emerges from resting eggs during each spring. All individuals share a similar morphology and AO alleles are consistent in a 1 : 2 : 1 ratio. The breakdown in the correlation between morphology and the AO allele is not inconsistent with Taylor & Hebert (1993); they report an overall frequency of 0.16 for the AO fast allele in the *D. dentifera* morphs they sampled. Apparently, whatever the barriers that prevent a similar situation from occurring in the majority of lakes, they are not absolute.

The interaction of genetic and ecological constraints that promotes polymorphism in these and other *Daphnia* is largely unexplored. The application of molecular techniques that provide greater resolution of genetic structure than allozymes, e.g. microsatellites, aids in this work (Schwenk, Ender & Streit, 1995). However, an explicit focus on mechanisms such as mating system variation, assortative mating, clonal competition, selection and dormancy is also needed. Animals in the *D. dentifera–mendotae* complex show important ecological differences and form assemblages that are stable over multiple years. Whether one wishes to denote these as two species or not depends largely on what definition of species concept is being advocated and what mechanisms are stabilising the polymorphism in most lakes. However, the existence of morphological and behavioural differences that also relate to food web structure provide an ecological justification for the different names, *D. dentifera* and *D. mendotae*.

Taylor and Hebert's original studies revealed hidden genetic structure within *D. galeata mendotae*, an ecologically important species, leading them to split that subspecies into two species, *D. dentifera* and *D. mendotae*, and their hybrids. Our study demonstrates that this genetic structure is mirrored by important ecological differences and we speculate that the differences are associated with differences in susceptibility to invertebrate predation. Combining studies of the genetics and ecology of these taxa has enabled us to begin to understand the evolution of this group. Paleoecological studies have the potential to further this understanding. Therefore, the recognition of *D. dentifera* and *D. mendotae* is ecologically and evolutionary relevant and not just taxonomically reverent.

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