

## NOTES

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### Paleogenetic evidence for a past invasion of Onondaga Lake, New York, by exotic *Daphnia curvirostris* using mtDNA from dormant eggs

**Abstract**—Cladocerans possess traits such as resistant diapausing eggs and rapid parthenogenetic reproduction that make them efficient invaders of new habitats. Nearly all known invasions have been successful, perhaps because failed invasions are difficult to detect. It is possible, however, to identify past failed invasions, by studying the diapausing egg bank. *Daphnia* ephippia were found in the sediments of Onondaga Lake, New York that could neither be hatched nor identified using egg-case morphology. Instead, we used sequences of the 12S ribosomal ribonucleic acid (rRNA) gene of mitochondrial deoxyribonucleic acid (mtDNA) extracted from diapausing eggs to identify the unknown *Daphnia*. We compared these DNA sequences with those generated from morphologically identified *Daphnia* species collected in Onondaga Lake, and with published sequences for other North American *Daphnia* species. The invader was identified as *Daphnia curvirostris*, a Eurasian species that has been only reported once before from North America, in extreme northwestern Canada. The discovery of it in Onondaga Lake signifies a greater than 4,500-km range extension for this species. On the basis of the sediment ephippial data, *D. curvirostris* first appeared in the lake about 1952, reached maximum abundance during the period of peak pollution (1950s–1980s), and then essentially disappeared after 1983 when lake water quality improved. As with the finding of another exotic cladoceran, *D. exilis*, in Onondaga Lake (Hairston et al. 1999a), it is likely that chemical industry activities on the lakeshores were the original source of invading *D. curvirostris*, that pollution allowed this species to become established in the lake, and that the reduction in pollution ultimately led to its disappearance from the water column.

The dormant stages of zooplankton and phytoplankton provide a mechanism for persistence in varying environments (Hairston et al. 1996a; Hansson 1996). They represent a means to disperse among habitats and allow for temporal persistence over years with varying ecological conditions (Hairston and Cáceres 1996; Rengefors et al. 1998). These dormant propagules are also packages of biological information, like time capsules, that become buried in aquatic sediments, often in an ordered, stratigraphic manner. Paleolimnologists can exploit this fact to investigate historical changes in phenotypes (Hairston et al. 1996a, 1996b, 1999b; Kerfoot et al. 1999), genotypes (Weider et al. 1997), and community dynamics (Hairston et al. 1999a). We used diapausing eggs from sediment cores collected in Onondaga Lake, New York to reconstruct the dispersal of an exotic daphniid to this lake.

Three characteristics of cladoceran life history suggest that these animals should be excellent invaders of new habitats: They produce encased eggs that are resistant to physical stress and that can remain dormant for many years, they can reproduce asexually and thus multiply from a single individual, and they spend most of their lives in the plankton

where they are exposed to transport vectors. Resistant dormant eggs of cladocerans enable cladocerans to respond quickly to environmental disturbances. An egg that is transported to a new water body can remain dormant in the sediment until environmental conditions are favorable for hatching, survival, and reproduction. Numerous examples of recent cladoceran invasions attest to the efficacy of this colonization mechanism (e.g., Lehman 1987; Taylor and Hebert 1993; De Melo and Hebert 1994; Hairston et al. 1999a; Havel et al. 1995).

Although nearly all reported cladoceran invasions have involved studies of species or genotypes found in the plankton, it is also possible to detect past invasions by investigating evidence in lake sediments, even when the past invasion ultimately failed. One example is the invasion of Onondaga Lake, New York by *Daphnia exilis*, a species native to shallow saline pools of the southwestern U.S. and northeastern Mexico (Hairston et al. 1999a). The diapausing eggs of this species occurred in the sediments that were deposited during a period when industrial pollution raised the lakewater salinity. Both the eggs and the adults reared by hatching those eggs were identified to species on the basis of morphological characters.

While studying the response of the diapausing zooplankton egg bank to Onondaga's pollution history, we observed large numbers of ephippia from a second *Daphnia* species that did not match the morphology of any species known to have occurred in the lake. Like those of *D. exilis*, these ephippia were present exclusively in sediments deposited during the period of peak pollution (from the 1950s to 1980s). The morphology of these ephippia is intermediate between those of two species documented in the lake plankton, *D. pulicaria* and *D. galeata mendotae* (Auer et al. 1996). The ephippia of these native species were identified on the basis of drawings and photomicrographs in Brooks (1957) and Hebert (1995). Adults hatched from these ephippia keyed out as expected, and the mitochondrial deoxyribonucleic acid (mtDNA) sequences of the eggs in the ephippia were consistent with morphological identifications. The unknown ephippia are 800 to 900  $\mu\text{m}$  long (dorsal margin excluding the tail spine), comparable with *D. galeata mendotae* ephippia. Unlike *D. galeata mendotae*, however, which has a smooth dorsal margin, the unknown ephippia possess fine, short spines along their entire length. In addition, the unknown ephippia are almost as deep as they are wide, whereas those of *D. galeata mendotae* narrow toward the tail spine. *D. pulicaria*, on the other hand, has ephippia that are larger and more tapered than the unknowns, but *D. pulicaria* and the unknowns both are similar in that they both possess dorsal spines, although *D. pulicaria* spines are coarser (see Hebert 1995 for descriptions of *D. pulicaria* and *D. galeata mendotae* ephippia).

We identified the unknown *Daphnia* ephippia by taking advantage of the abundant data on the molecular phylogeny of *Daphnia* (Lehman et al. 1995; Colbourne and Hebert 1996). By sequencing the small subunit 12S ribosomal ribonucleic acid (rRNA) gene of mtDNA from both the unknown ephippia and from known species, we determined that the unknown ephippia do not belong to any *Daphnia* species currently present in Onondaga Lake or to any species reported from the lake in the past. Rather, the ephippia belong to *D. curvirostris*, a predominately European species that has only been reported in North America from the extreme northwestern portion of the Canadian Northwest Territories (Hebert and Loaring 1986).

**Study site**—Onondaga Lake is located in central New York State adjacent to the city of Syracuse. It has a surface area of 12 km<sup>2</sup> and a maximum depth of 19 m. The lake has two basins of 19- and 18-m depth separated by a “saddle” at 17 m. There has been a long history of industrial activity along the lakeshore dating from the 1880s. Maximum pollutant inputs occurred during the period 1926–1986 (Cominoli 1990; Effler and Harnett 1996). Throughout that time, chemical factories produced waste deposits of CaCO<sub>3</sub> and NaCl that resulted in lake salinities in excess of 3 g L<sup>-1</sup> and mercury inputs during 1946–1986 that reached a maximum in the late 1960s of 10 kg of Hg d<sup>-1</sup> (Effler and Harnett 1996). Following federal legal action in 1970, much of the direct input of pollutants was eliminated, though waste beds along the shore continue to drain into the lake, and nutrient loading from a wastewater treatment plant keeps algal biomass high, often in excess of 50 µg of chlorophyll *a* L<sup>-1</sup> during summer.

**Coring and core processing**—We obtained a 7-cm-diameter, 92-cm long piston core from the saddle location using self-contained underwater breathing apparatus on 13 May 1997. The core was wrapped immediately in aluminum foil, returned to the laboratory, stored at 4°C, and sliced within 2 d of collection. The core was sliced at 1-cm intervals between 0 and 20 cm, at 2-cm intervals between 20 and 40 cm and at 4-cm intervals between 40 and 92 cm. For each slice, the outer layer of sediment that had dragged along the wall of the core tube was separated from the inner sediments using a 5.9-cm-diameter cookie cutter. This outer layer was discarded. Sediment ages and sedimentation rates were determined by <sup>210</sup>Pb-dating (Hairston et al. 1999a).

To extract dormant eggs, sediments from each core slice were suspended in 1.6-µm-filtered Onondaga Lake water and washed through a 150-µm mesh. A stereodissecting microscope was used to search the retained material for *Daphnia* ephippia. Either the whole slice or two to three subsamples of each sediment slice were analyzed; a minimum of 12% of each core slice was analyzed. Each ephippium observed was opened, and the number of eggs was recorded. Isolated eggs that appeared viable were incubated in 1.6-µm-filtered lake water at 10°C and a 14 : 10 light : dark photoperiod. These conditions induced other cladoceran eggs in our samples to hatch, but none of the eggs from the ephippia of interest here hatched during our study.

**Paleogenetic analysis**—To identify the unknown *Daphnia* ephippia, we analyzed the 12s rRNA mtDNA gene and com-

pared our results with two previously reported *Daphnia* mtDNA phylogenies (Lehman et al. 1995; Colbourne and Hebert 1996). We compared the sequences obtained from the unknown ephippia with sequences for other *Daphnia* species known to occur presently in the plankton of Onondaga Lake or reported to have occurred in the lake in the past. These species include *D. ambigua*, *D. galeata mendotae*, *D. pulicaria*, *D. catawba*, and *D. exilis* (Makarewicz et al. 1995; Siegfried et al. 1996; Hairston et al. 1999a). *D. similis* was reported from the lake in 1969 by Waterman (1971), but Hebert and Finston (1993) divided this taxon into three sister species, and Hairston et al. (1999a) showed that what Waterman found was actually *D. exilis*. We compared sequences that we generated from ephippia or adults collected in Onondaga Lake, including the unknown species, *D. galeata mendotae*, and *D. pulicaria*, sequences reported in Genbank (*D. ambigua*, *D. curvirostris*, *D. galeata mendotae*, *D. pulicaria*, *D. pulex*), and sequences given to us (*D. catawba*, *D. exilis*, J. Colbourne pers. comm.). For *D. curvirostris*, we also generated a sequence from a laboratory culture that originated from the Czech Republic (W. Lampert pers. comm.).

An additional 7-cm-diameter piston core, 1.3 m long, was collected from the saddle site of Onondaga Lake on 7 April 1998. The core was obtained, stored, and processed as described above, except that slicing was done at 4-cm increments throughout. The unknown ephippia as well as *D. pulicaria* ephippia were taken directly from the sediments for molecular sequencing. *D. galeata mendotae* and *D. pulicaria* ephippia were collected from this core and cultured in the lab. Live animals from these cultures were collected for sequencing.

The mtDNA was extracted by placing adults or ephippia in either 30 µl or 10 µl of 5% Chelex 100 (Bio-Rad) in high-performance liquid chromatography water, respectively. Samples were incubated at 55°C for 3 h, vortexed, boiled at 100°C for 9 min, vortexed again, centrifuged at 16,000 g for 2 min, and held at 4°C for short-term storage or at -20°C for long-term storage. Fifty-microliter total volume polymerase chain reaction (PCR) amplifications were conducted containing 5.0 µl of 10× buffer (pH = 8.3; buffer contains 100mM Tris-HCl, 15 mM MgCl<sub>2</sub>, and 500 mM KCl per liter, as well as 10 mg of gelatin per ml), 1.5 µl of 25 mM MgCl<sub>2</sub>, 1.0 µl of deoxynucleotide triphosphate mix (2 mM; Boehringer-Mannheim), 10 µM (1.0 µl) of each primer (Biometra), 0.5 µl of dimethyl sulfoxide, 38.0 µl of ultrapure water, 0.5 µl of Taq polymerase (5 U µl<sup>-1</sup>; Boehringer-Mannheim), and 2.0 µl of template DNA in Chelex supernatant for each ephippium (or 1 µl of template DNA in Chelex supernatant for adults). A primer pair designed by Colbourne and Hebert from a conserved region of 12S mtDNA was used (5'-ATGCACTTTCCAGTACATCTAC-3'; 5'-AAATCGTGCCAGCCGTCGC-3'). The conditions for the PCR are described by Colbourne and Hebert (1996).

An initial screening of restriction fragment length polymorphisms (RFLPs) following the protocol of Taylor and Hebert (1993) using five restriction endonucleases (*Dpn*-II, *Dra*-I, *Hin*-fl, *Rsa*-I, and *Taq*-I) revealed that the unknown ephippia exhibited identical RFLPs to those of *D. curvirostris* (Dufresne 1995) for three (*Dra*-I, *Hin*-fl, *Taq*-I) of the five restriction enzymes. After this finding, we explored this unexpected result further using DNA sequencing.

Six DNA products were all sequenced manually, and two unknown ephippia and one known European *D. curvirostris*

were also sequenced using an automated sequencer. For manual sequencing, we used the dideoxynucleotide chain termination method with a Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham Pharmacia Biotech). For automated sequencing, PCR-amplified DNA was cleaned using a Wizard kit (Promega), and sequenced using an internal primer (5'-AATCCACCTTCCACCAACTT-3'). Sequences were obtained using a Perkin Elmer/Applied Biosystems DNA sequencer, model Prizm 377, using BIG Dye terminator chemistry and AmpliTaq-FS DNA polymerase. The sequences from each sample were then compared with sequences reported in the GenBank/EMBL database.

The sequences of five unknown ephippia, one known European *D. curvirostris* adult, one *D. galeata mendotae* adult from Onondaga Lake, and three *D. pulicaria* ephippia from Onondaga Lake were aligned using MegAlign along with the sequences reported in GenBank for *D. curvirostris*, *D. galeata*, *D. pulicaria*, *D. pulex*, *D. ambigua* and *D. similis* (accession numbers U13906, U34651, U13905, Z15015, AF064175, and U13911, respectively) and *D. catawba* and *D. exilis* sequences provided by J. Colbourne (pers. comm.). Sequences were aligned using the Clustal method and were adjusted by eye. Sequences approximately 110 base pairs (bp) long were imported into PAUP 4.0 (Swofford 1999); this segment is shorter than the 200–250-bp sequences that were generated during sequencing due to constraints imposed by alignment, because the portions of the 12S gene sequenced did not overlap perfectly. A heuristic search was used to find the most parsimonious trees using the sequence for *D. similis* reported in GenBank and the *D. exilis* sequence obtained from J. Colbourne as outgroups, because they belong to a distinct subgenus, *Ctenodaphnia*, within the genus *Daphnia*. A strict consensus of these most parsimonious trees was then computed.

**Results**—We found the unknown ephippia continuously between 15 and 40 cm sediment depth in Onondaga Lake with peak densities up to  $4.9 \times 10^4$  per square meter occurring between the late 1960s and the mid-1970s (Fig. 1). Low densities of these ephippia also occurred at two more recent sediment depths (approximately 1983 and 1990), and a minor peak was found between 30 and 34 cm (i.e., 1960 and 1965). No unknown ephippia were found below 40 cm (1952) or above 6 cm (1990).

In the RFLP analysis, 33 eggs were analyzed from sediment depths between 8 and 24 cm. In all cases, these eggs possessed identical RFLP patterns. Similarly, the five eggs from which 12S rRNA mtDNA genes were sequenced came from two different sediment slices (8–12 cm and 16–20 cm) and showed a minimum of 96% sequence similarity. These observations confirm that the morphological characters used to distinguish the unknown ephippia were consistently evaluated.

The 12s rRNA mtDNA genes of five of the unknown ephippia were, on average, 99% similar to the sequence reported in GenBank for *D. curvirostris* (accession number U13906) over sequences averaging 237 bp in length. Comparison of this mtDNA region between the unknown ephippia from Onondaga Lake and the known *D. curvirostris* adult from the Czech Republic also showed 99% similarity.

A heuristic search of the 17 12s rRNA mtDNA sequences generated in our study or obtained from other sources, using

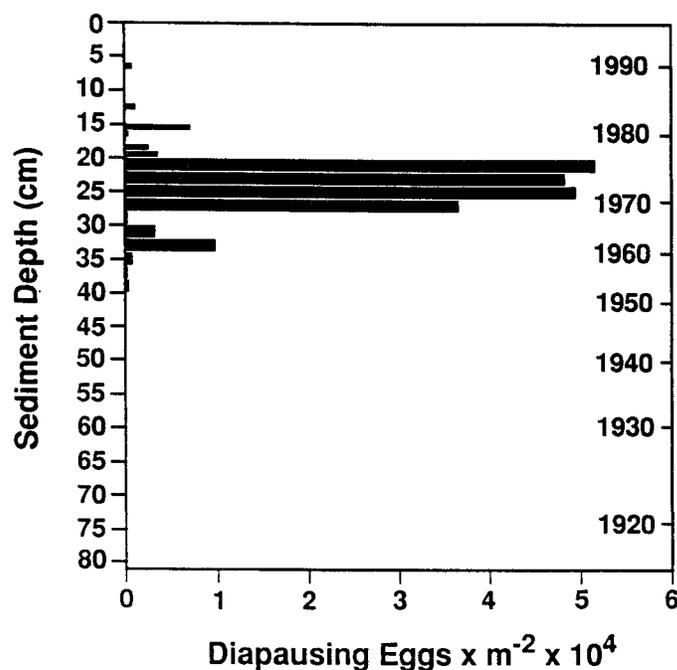


Fig. 1. Sediment distribution of the unknown ephippia in Onondaga Lake, New York, identified in this study as being *D. curvirostris*. Sediment dates from  $^{210}\text{Pb}$  analysis.

*D. similis* and *D. exilis* as outgroups, yielded five equally parsimonious trees (consistency index: 0.7300; retention index: 0.8784; one of these five trees is shown in Fig. 2). The only branch change between these trees lies in the resolution of *D. ambigua* and *D. catawba*. A strict consensus of the five trees only differed from the tree in Figure 2 in that *D. ambigua* and *D. catawba* are unresolved. In all five of the most parsimonious trees, the unknown ephippia found in Onondaga Lake cluster closely both with the *D. curvirostris* from the Czech Republic that we sequenced and with the *D. curvirostris* from the Canadian Northwest Territories reported in GenBank (Colbourne and Hebert 1996). Furthermore, within this consensus tree, the arrangement of all the taxa present in Onondaga Lake is consistent with the 12s rRNA mtDNA phylogenies previously reported by Lehman et al. (1995) and Colbourne and Hebert (1996).

**Ephippial comparison**—Live *D. curvirostris* adults from the population obtained from the Czech Republic were cultured in the lab in conditions that induced sexual reproduction and ephippial formation. The morphologies of the ephippia obtained from these cultures are the same size and shape, and the spines on the dorsal margin are the same as the unknown ephippia from Onondaga Lake (Fig. 3).

**Discussion**—Using the 12S rRNA gene of mtDNA, we identified an exotic cladoceran that invaded Onondaga Lake, New York during the 1950s as *D. curvirostris*. Sediment densities of the diapausing eggs of this species ranged between  $1 \times 10^4$  and  $4.9 \times 10^4$  eggs  $m^{-2}$  during the time of maximum abundance (1961–1981). This is comparable with the densities of diapausing eggs from other cladoceran species present in Onondaga Lake sediments at that and other time periods (Hairston

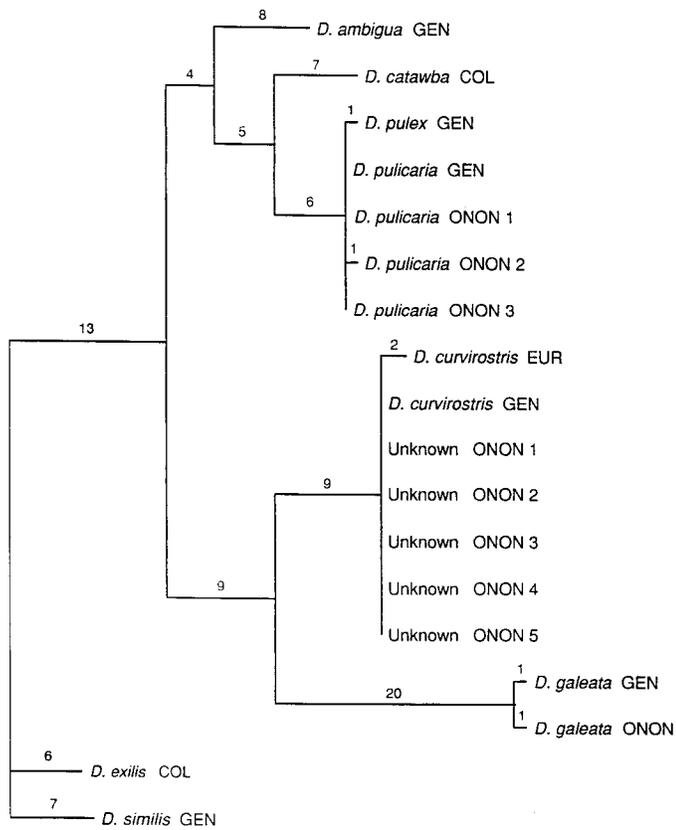


Fig. 2. Phylogram of one of five most parsimonious trees on the basis of 12S rRNA mtDNA gene sequences of *Daphnia* species occurring in Onondaga Lake, New York. Numbers shown are the branch lengths as computed in PAUP (Swofford 1999). COL sequences obtained from J. K. Colbourne (pers. comm.); EUR, sequence of adult from culture originating from Czech Republic; GEN, sequences obtained from GenBank; ONON, sequences from diapausing egg or adult from Onondaga Lake.

et al. 1999a, Hairston and Kearns unpubl. data), and suggests that this species was at one time a prominent member of the zooplankton community. It is thus surprising that *D. curvirostris* was not collected in studies carried out in Onondaga Lake in 1969, 1978, and 1979–1981 (Siegfried et al. 1996). Waterman (1971), however, reported finding *D. pulex* in the water column in 1969. We find no evidence of that species in the egg bank during this period, and it is possible that this represents a misidentification, because *D. curvirostris* would not have been present in a key to North American *Daphnia* at that time. Adult females of the two species have several key morphological features in common including similar adult body size and head shape, and a coarse middle pecten on the post-abdominal claw.

Even though none of the 1,204 viable-looking eggs we obtained from the Onondaga Lake sediments could be induced to hatch, we were able to extract and amplify diagnostic DNA sequences. The 12S rRNA mtDNA gene we used is a relatively conserved region that has proven useful in working out phylogenetic relationships among members of the Daphniidae (Lehman et al. 1995; Colbourne and Hebert 1996). This makes it possible to assign a species identity to our unknown eggs with reasonably high confidence even though definitive morphological characters are either un-

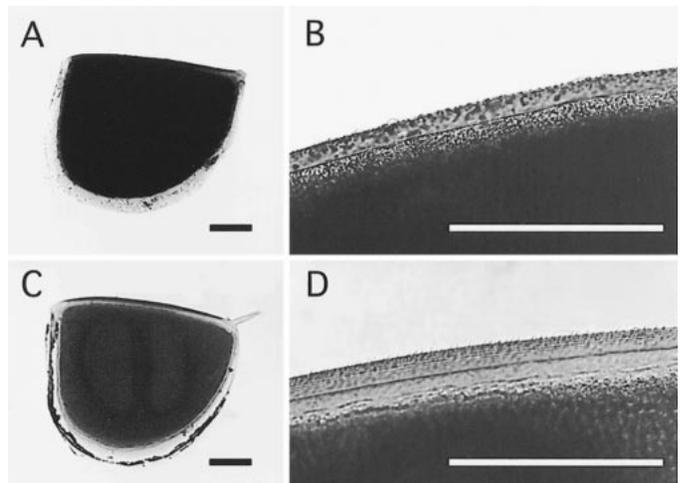


Fig. 3. Comparison of the morphologies of one of the unknown ephippia occurring in the sediments of Onondaga Lake with an ephippium obtained from a culture of *D. curvirostris* originating from the Czech Republic. Overall morphology of ephippia from (A) Onondaga Lake and (C) the Czech population. Note that the tail spine is broken off in (A) as were these spines for all of the unknown ephippia collected from Onondaga sediments. The breakage site is clearly visible in this photomicrograph. The dorsal margin of ephippia from (B) Onondaga Lake and (D) the Czech population showing small spines. These spines are somewhat obscured for ephippia obtained from Onondaga mud (B) by sediment debris adhering to the margin. All scale bars = 200  $\mu\text{m}$ .

available (i.e., none of the eggs hatched) or insufficiently distinctive (i.e., dependent solely on ephippial traits). The use of molecular characters from the sediment egg banks in lakes holds great potential broadly for investigations of ecological or evolutionary processes in zooplankton. Successful DNA extraction and amplification from diapausing eggs has been reported in the past by Schwenk (1993) for *Daphnia* and by Gómez and Carvalho (2000) for rotifers.

The detection of *D. curvirostris* in Onondaga Lake marks only the second time that this species has been found in North America. Hebert and Loaring (1986) first reported the species on this continent from the extreme northwest corner of the Canadian Northwest Territories, where it occurred exclusively in shallow freshwater ponds surrounded by frost wedge polygons formed in deep peat deposits. This is a very different habitat from Onondaga Lake, which is permanent, relatively deep, and, at the time of peak abundance of the species, had salinities exceeding 3‰ due to industrial pollution. It appears that the *D. curvirostris* population in Onondaga Lake was isolated both in time and space. Surveys of the regional zooplankton (e.g., Hall and Waterman 1967, 1968; Bloomfield 1978) and our examination of sediments from nearby Oneida Lake have not revealed its presence. Sediments from Onondaga Lake dating as far back as the late 1800s were studied, but *D. curvirostris* was found only in sediments deposited during the mid- to late 1900s, during peak industrial activity.

The natural range of *D. curvirostris* includes the British Isles across central Europe, south to central Africa, and across Asia to Japan (Green 1976; Hrbáček et al. 1978; Tanaka and Tominaga 1986), as well as northwestern Canada and probably Alaska (Hebert 1995). The species' occurrence

in a lake in central New York State is a marked range expansion of either greater than 4,500 km from the Northwest Territories or over 6,000 km from Europe. Although we have no direct evidence concerning the origin of the invading population, we surmise that it may have come from Europe as a part of the activity of the chemical industry on the shore of Onondaga Lake. For an extended period during the 1900s, this company was co-owned by industrialists with factories throughout the European range of *D. curvirostris*. We speculate that ehippial eggs were introduced in mud transported on equipment carried during one or more of the frequent exchanges of engineers between factories (Cominoli 1990).

Habitat disturbance makes ecosystems more susceptible to invasion by exotic species (Lodge 1993), so it is not surprising that Onondaga Lake, which is the most polluted body of water in the U.S. and Canada (Effler 1996; pers. comm.), has experienced multiple invasions. Recently, another exotic species, *D. exilis*, was found in the sediments of Onondaga Lake (Hairston et al. 1999a). Both this invasion and that of *D. curvirostris* occurred during the time when the lake was most heavily polluted, and when native *Daphnia* species had been eliminated (N. Hairston and C. Kearns unpubl. data). In both cases the invading species disappeared from the water column when the lake began to recover and return to what is presumed to be its natural state (Effler and Harnett 1996; Hairston et al. 1999a). Increased salinity probably facilitated the success of these exotic species in Onondaga Lake during the period of pollution. High salinity probably drove zooplanktivorous fish from the lake (Hairston et al. 1999a) and eliminated native species of *Daphnia* and other zooplankton (Auer et al. 1996).

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## Reconstructing the history of intercontinental dispersal in *Daphnia lumholtzi* by use of genetic markers

**Abstract**—After its appearance in 1989, the cladoceran *Daphnia lumholtzi* rapidly dispersed throughout the southern United States. In the current study, we used allozyme and mitochondrial deoxyribonucleic acid sequence data to infer the past dispersal of this species. Both genetic markers revealed the similarity among all U.S. populations and those from Uganda and Nepal but their divergence from Australian lineages. The extent of genetic divergence among populations, when coupled with estimates of rates of molecular evolution, suggests that the distribution of this species reflects a series of long-distance dispersal events over the last 4 million years.

Biogeographers attempt to explain the distributions of plants and animals from knowledge of ecology, systematics, and the geologic record. Species with disjunct intercontinental distributions are of particular interest because this pattern might arise either as a result of vicariance or through more recent dispersal from a center of origin (Brown and Lomolino 1998). Vicariance involves the appearance, over geologic time, of barriers that disrupt distributions that were originally continuous. For example, the division of Pangea (~200 million years ago [mya]) and the fragmentation of Gondwanaland (~65 mya) might explain the distribution of species now found on several continents. In contrast, the

center of origin hypothesis presumes the dispersal of modern lineages from a localized ancestral population. Conclusions about centers of origin are often controversial (Brown and Lomolino 1998) because dispersal corridors and mechanisms are necessary for this hypothesis to explain contemporary distributions, but these factors are usually difficult to investigate.

Freshwater cladoceran zooplankton provide an interesting opportunity to test biogeographic hypotheses. Fossil evidence indicates that the Cladocera originated in the Permian (280 mya) (Kerfoot and Lynch 1987), indicating that their origins predate the current position of the continents. Molecular data have additionally suggested that many of the species complexes in the cladoceran genus *Daphnia* are up to 100 million years old (Colbourne and Hebert 1996), suggesting the modern distributions of species might reflect the impact of vicariance events linked to the fragmentation of Gondwanaland. However, a much more recent exchange of lineages among continents, by dispersal, is also possible, given that cladocerans produce diapausing eggs, which are viable outside of water. In some cladoceran genera such as *Daphnia*, these eggs are held in an ephippial case that provides both additional protection and adherent structures (Benzie 1988; Dodson and Frey 1991; Hebert 1995), which