

5 EVOLUTIONARY ORIGINS OF PHENOTYPIC DIVERSITY IN *DAPHNIA*

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Freshwater zooplankton are extraordinarily favorable targets for evolutionary studies. Their population sizes are so enormous that sample sizes rarely constrain investigations, and their development is so rapid that multigenerational studies are frequently feasible. Many groups also employ unusual breeding systems that are thought to accelerate evolution (Lynch and Gabriel 1983). Their "insular" environments crystallize population boundaries and provide exceptional venues for disruptive selection.

Terrestrial archipelagoes – where the most celebrated examples of rapid adaptive evolution have been documented – provide no analog to the covariation in biological and physical factors that zooplankton encounter in moving from lakes to ponds. Lake zooplankton feed on phytoplankton, are consumed by visual vertebrate predators and occupy a medium favorable to their persistence for millennia. By contrast, pond species often feed on detritus, are exposed to tactile invertebrate predators and require resistant stages to survive recurrent but unpredictable bouts of desiccation. These ecological dichotomies between ponds and lakes would likely create divergent selection pressures similar to those that have provoked explosive radiations seen in many insular fauna (Freed et al. 1987; Kambyzellis et al. 1995; Shaw 1996).

Yet, despite the confluence of factors favoring rapid diversification, freshwater zooplankton are notorious for their morphological stasis (Frey 1987). Evolutionary theorists have attributed this lack of divergence to the homogenizing effects of gene flow, arising through the dispersal of their resistant stages (Mayr 1963). However, more recent allozyme and DNA studies have shown a surprising amount of local population differentiation (DeMelo and Hebert 1994; Gómez et al. 1995; Hebert 1995), challenging the notion that stasis is simply a consequence of gene flow, and highlighting the need for further research.

Among more than a hundred genera of freshwater zooplankton, none has attracted more attention than the genus *Daphnia* (Crustacea: Cladocera). Named after the virgin nymph Daphne of Greek mythology, these organisms first attracted interest because of their unusual mode of reproduction, involving both sexual and asexual propagation. Although most of the subtleties of their breeding system were resolved by the mid-19th century, *Daphnia* has maintained its scientific appeal because of its central importance in freshwater foodwebs and its amenability to laboratory culture. As a result of extensive research, we now have a deep understanding of morphological and ecological diversity in the genus. Its taxonomy has been less satisfactorily resolved, because hybridization and phenotypic plasticity have combined to defeat the best efforts of morphologists (Brooks 1957; Hebert 1978). However, the use of molecular markers to detect the genetic discontinuities associated with reproductive

isolation is now providing the stable taxonomic platform needed for phylogenetic studies (Hebert and Finston 1993, 1996; Taylor and Hebert 1994). *Daphnia* appears to entail nearly 200 species, most of which are restricted to single continents. Nonetheless, species on different continents are often closely allied, suggesting that sweepstakes dispersal has often extended the geographic ranges of individual taxa which then led to speciation. While it seems likely that most speciation occurs allopatrically (Hebert and Wilson 1994), there is evidence of more exotic processes. Some taxa have apparently arisen through reticulate speciation, fostered by the parthenogenetic amplification of F_1 hybrids and subsequent introgression (Taylor and Hebert 1993). A few polyploid lineages are known, but they are closely related to diploid taxa, and appear to have played a minor role in diversification (Dufresne and Hebert 1994). A number of closely related pairs of species seem to have arisen through sympatric speciation, linked to disruptive selection between habitat types (Taylor et al. 1996).

Despite 200 years of detailed morphological studies, daphniids are seen to possess relatively few morphological characters that are phylogenetically informative, suggesting that their morphological evolution has been constrained. In general, phenotypic stasis has been attributed to a number of genetic factors (e.g., pleiotropy, epistasis, or lack of variation), including ecological restriction (Morris et al. 1995) with an emphasis on the role of interactions with existing competitors in slowing diversification (Paul 1977; Valentine 1980; Conway Morris 1989). Information on the timing of both speciation and phenotypic change in *Daphnia* is of particular value, given the potential for their strong association with habitat shifts or competitive release.

Results from our most recent investigations into *Daphnia* phylogenesis were presented in earlier papers (Colbourne and Hebert 1996; Taylor et al. 1996). This paper examines the origins of morphological diversity in the North American members of the genus. We restrict our analysis to this region because ecological and taxonomic knowledge of *Daphnia* in other biogeographic areas is poor. Fortunately, the North American fauna is thought to include representatives of most major species complexes in the genus. Our work aims to describe the nature and patterning of morphological diversification within *Daphnia*, and to ascertain if this diversity has arisen at a relatively uniform tempo. The ecological conditions associated with character-state transformations are examined to determine the possible adaptive value of variation in several key traits (see Wiley et al. 1991). We begin by summarizing some salient biological features of the genus.

***Daphnia* in North America – a model system**

Species of *Daphnia* are common in all areas of the globe except the tropics. The intensive application of genetic methods has identified 34 species inhabiting North America (Hebert 1995). They have been assigned to one of three subgenera: *Ctenodaphnia*, *Daphnia* and *Hyalodaphnia*. Given the lack of morphological resolution among members of a subgenus, it is not surprising that 19 of these 34 species were unrecognized by earlier taxonomists (see Brooks 1957). Nevertheless, species do differ in body size, coloration, possession of predator-induced headshields and neckteeth,

morphology of the carapace and ephippia, and chromosome number (see below). Table 5.1 summarizes some of the key biological attributes of the North American fauna.

Members of the genus typically reproduce by cyclic parthenogenesis, with populations being founded from females that hatch from diapausing eggs. These females ordinarily produce broods of diploid eggs that develop solely into daughters, fostering high rates of reproduction and rapid population growth. However, under conditions that depress their metabolic rate, individuals switch to the production of both male and female offspring (see Hebert 1987). Although broods from individual females are usually single-sexed, the populational shift to a 1:1 sex ratio is remarkably precise. Following this shift in sex ratio, females begin to produce haploid sexual eggs that require fertilization in order to develop. These eggs – packaged in a protective structure termed an ephippium – resist desiccation and freezing, and are the means of long-distance dispersal. The unusual reproductive system of *Daphnia* is important, in that cyclic parthenogenesis may significantly accelerate phenotypic evolution (Lynch and Gabriel 1983; Lynch 1985).

There is only limited information on the evolutionary origins of the genus. Fossil ephippia of *Daphnia* and the closely related *Simocephalus* are known from the early Cretaceous (Fryer 1991a; Smirnov 1992). Biogeographic patterns provide further support for the ancient origin of the genus, as the southern continents are dominated by the subgenus *Ctenodaphnia*, while the faunas in the northern hemisphere consist largely of the subgenera *Daphnia* and *Hyalodaphnia* (Hebert 1978; Benzie 1987). The age of species lineages is ill defined, although Brooks (1957) suggested that many North American species had originated since the Pleistocene. Speciation mechanisms in the group are unclear, although Lynch (1985) proposed that speciation via founder effects was important and reflected the establishment of populations from single ephippia. Other research has suggested the importance of speciation linked to introgressive hybridization (Taylor et al. 1996) as well as geographic isolation (Hebert and Wilson 1994).

The origins of North American *Daphnia*

The uncertainties in the timing of speciation events and the origins of major sublineages within *Daphnia* motivated us to analyze DNA sequence variation within the entire North American fauna (Colbourne and Hebert 1996; Taylor et al. 1996). Sequence comparisons were made on a 503 bp region of the relatively slowly evolving mitochondrial 12S rRNA gene, which was selected for analysis because of the ancient origin of the genus. The arthropod mitochondrial DNA clock (Brower 1994) of 2.3% sequence divergence per million years was used for dating speciation events among closely related taxa. For divergence values in excess of 10%, a 12S rDNA clock was calculated according to Lynch and Jarrell (1993). The estimated asymptotic identity (I_{∞}) for our data was 0.281, while other parameters, including the nucleotide substitution rate of 0.489% per million years, were obtained from Lynch and Jarrell's (1993) investigation of mitochondrial DNA evolution. The resultant molecular phylogeny (Figure 5.1) shows that the North American fauna consists of three very distinct species assemblages, corresponding to the subgenera proposed by earlier workers. These subgenera show sufficient 12S divergence (>20%) to suggest that they

Table 5.1. Biological and ecological attributes of the 34 *Daphnia* species found in North America. Key to table: **Size** (range of core body length in mm); **breeding system** (C = cyclic parthenogenesis, O = obligate parthenogenesis); **range** (N = Nearctic, H = Holarctic, B = several biogeographic regions); **habitat** (E = ephemeral ponds, P = permanent ponds, L = lakes); **biome** (S = subtropical, W = warm temperate, C = cool temperate, B = boreal, A = arctic, where more than one biome is listed, they represent the distributional boundaries); **tolerance of water turbidity** (Y = yes, N = no); **presence of melanin in carapace** (Y = yes, N = no).

Taxon	Size	Breeding system	Range	Habitat	Biome	Water turbidity	Melanin carapace
Subgenus <i>Daphnia</i>							
<i>ambigua</i>	< 1.3	C	N	L	S,C	N	N
<i>latispina</i>	1.8–3.8	C	N	E	C	Y	N
<i>villosa</i>	1.4–2.3	C	N	E	C	N	N
<i>oregonensis</i>	1.6–3.7	C	N	E	C	Y	N
<i>catawba</i>	1.3–2.1	C	N	L	C,B	N	N
<i>minnehaha</i>	1.1–3.2	C	N	E	C,B	N	N
<i>parvula</i>	1.1–1.4	C	N	L	W,C	N	N
<i>retrocurva</i>	1.0–1.8	C	N	L	W,B	N	N
<i>cheraphila</i>	1.3–1.7	C	N	P,L	S,C	Y	N
<i>prolata</i>	1.5–2.0	C	N	E,P	S,C	Y	N
<i>obtusa</i>	1.1–2.2	C	H	E	W,C	N,Y	N
<i>neo-obtusa</i>	1.4–2.8	C	N	E	C	Y	N
<i>pileata</i>	1.6–2.8	C	N	E	S,W	Y	N
<i>middendorffiana</i>	1.9–2.7	O	H	E	A	N	Y
<i>pulicaria</i>	1.4–3.2	C,O	N	P,L	S,A	N	N
<i>pulex</i>	1.1–3.5	C,O	H	E	C,B	N	N
<i>arenata</i>	1.5–3.5	C	N	E	C	N	N
<i>melanica</i>	1.6–2.5	C	N	E	C	N	Y
<i>tenebrosa</i>	1.6–2.5	C,O	H	P,L	A	N	Y,N
Subgenus <i>Hyalodaphnia</i>							
<i>laevis</i>	1.1–1.8	C	B	P,L	S,C	N	N
<i>dubia</i>	1.1–1.9	C	N	L	C	N	N
<i>curvirostris</i>	1.3–1.8	C	H	E,P	A	N	N
<i>umbra</i>	1.1–2.7	C	N	P,L	A	N	Y
<i>thorata</i>	1.3–1.6	C	N	L	C	N	N
<i>dentifera</i>	0.9–2.2	C	N	P,L	C,B	N	N
<i>mendotae</i>	1.2–2.8	C	N	L	C,B	N	N
<i>longiremis</i>	0.6–2.4	C	H	L	C,A	N	N
Subgenus <i>Ctenodaphnia</i>							
<i>exilis</i>	1.8–4.5	C	N	E	W,C	Y	N
<i>similis</i>	2.0–4.5	C	H	E	C	Y	N
<i>salina</i>	2.0–3.5	C	N	E	C	N	N
<i>lumholtzi</i>	1.6–2.6	C	B	L	S,W	N	N
<i>magna</i>	2.0–5.0	C	B	E	C,B	N	N
<i>ephemeralis</i>	1.3–3.0	C	N	E	C	N	N
<i>brooksi</i> ^a	< 1.6	C	N	E	C	Y	N

^a The sole species excluded from our study; it is known from only nine individuals and may be an introduced species.

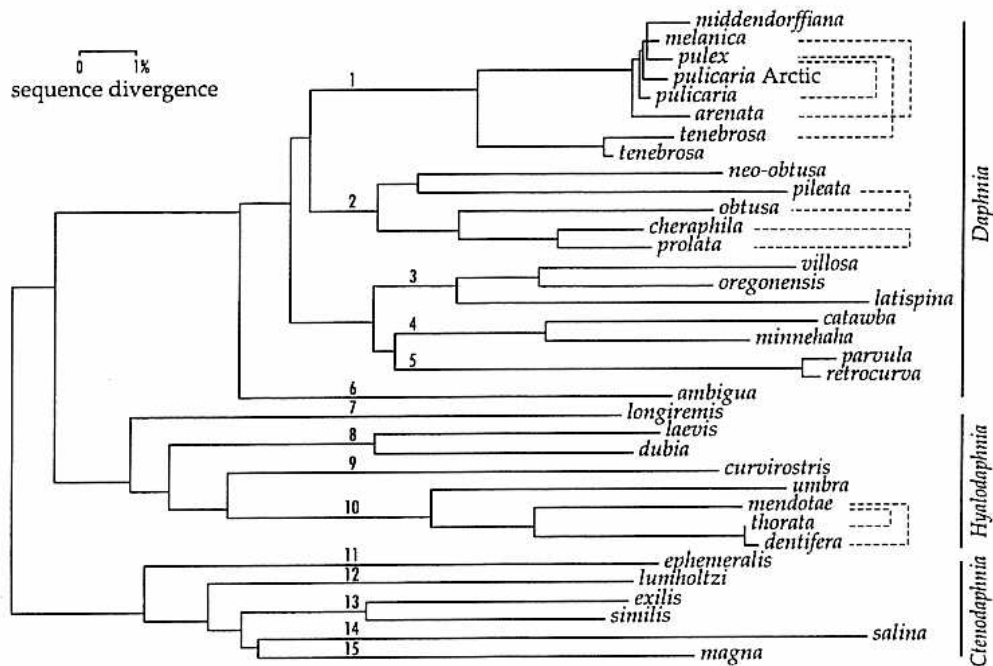


Figure 5.1. A neighbor-joining tree based on sequence variation in the mitochondrial 12S rRNA gene in *Daphnia*. Estimates of sequence divergence were corrected using the Kimura two-parameter model (Kimura 1980) and analyzed using MEGA 1.02 (Kumar et al. 1993). Dashed lines connect taxa known to hybridize. Numbers indicate species complexes within each of the three subgenera. The six complexes within subgenus *Daphnia* are: *pulex* (1), *obtusa* (2), *villosa* (3), *catawba* (4), *retrocurva* (5), *ambigua* (6). Complexes within subgenus *Hyalodaphnia* are: *longiremis* (7), *laevis* (8), *curvirostris* (9), *longispina* (10). *Ctenodaphnia* complexes are: *ephemeralis* (11), *lumholtzi* (12), *similis* (13), *atkinsoni* (14), *magna* (15).

originated during the Mesozoic. Although the subgenus *Ctenodaphnia* appears to have diverged first, all three subgenera apparently differentiated during a brief interval, complicating the delineation of ancestry. For example, the majority rule consensus of the seven shortest cladograms (which is also one of the seven most parsimonious trees; Figure 5.2) suggests that *Hyalodaphnia* could be polyphyletic. However, because only one extra step to the tree length is needed to place the (*laevis*, *dubia*) clade within *Hyalodaphnia* (dashed line in Figure 5.2), we used this latter tree for character-state optimizations using MacClade vers. 3.04 (Maddison and Maddison 1992). Additional details on the phylogenetic and evolutionary analyses are given in the figure legends and in Colbourne and Hebert (1996).

Each of the three subgenera includes a number of species complexes, with each complex defined as a set of species showing less than 14% sequence divergence in the 12S rRNA gene. This boundary condition was selected because it coincides with the maximum sequence divergence between species of *Daphnia* known to produce viable hybrids. The North American fauna includes five species complexes of *Ctenodaphnia*, four of *Hyalodaphnia*, and six within the subgenus *Daphnia* (Figure 5.1). Based on the expected rates of 12S rDNA evolution (see Lynch and Jarrell 1993), each of these

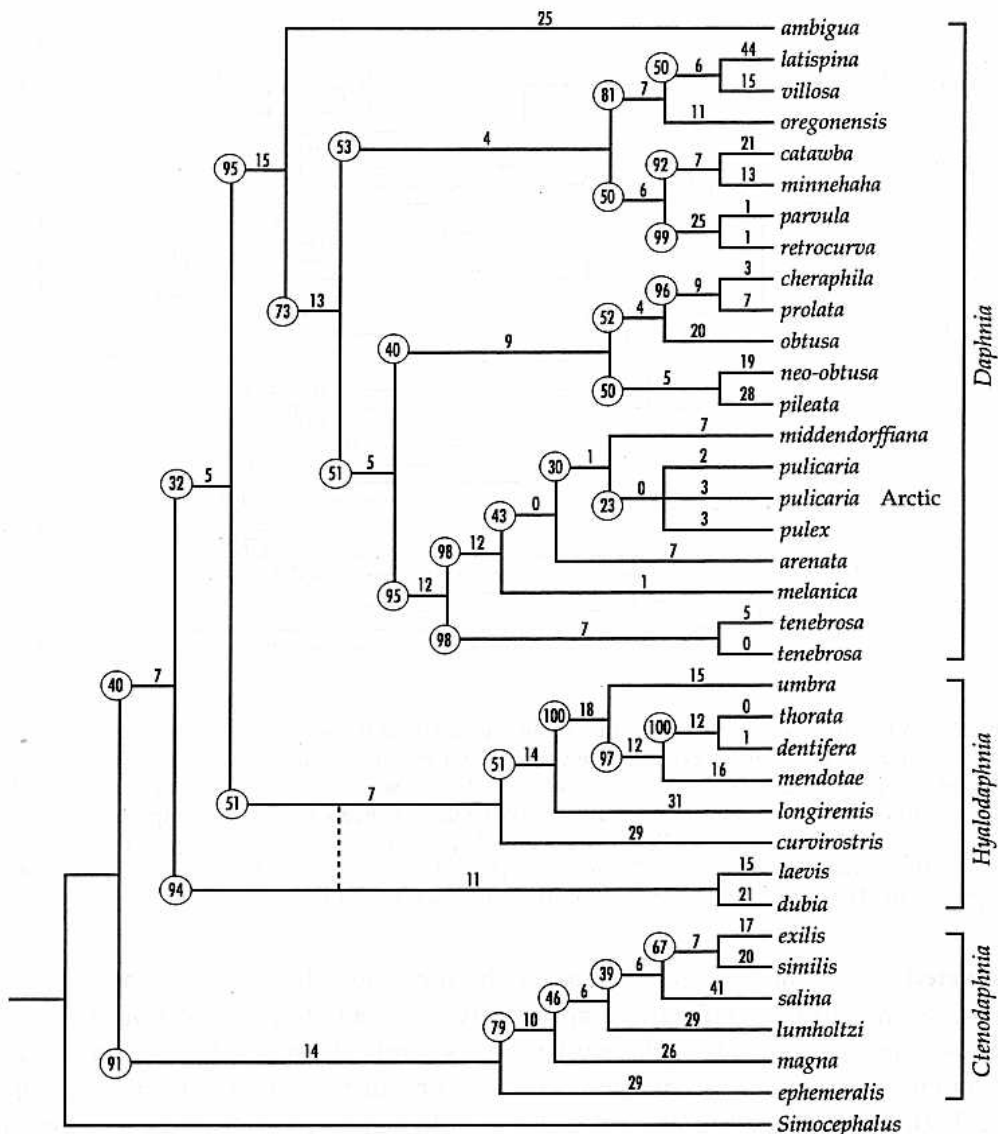


Figure 5.2. Fifty percent majority rule consensus and one of seven most parsimonious trees (length 1,186 steps, CI = 0.41, RI = 0.60) for *Daphnia*, based on 270 variable characters of the 12S rRNA gene. Transversions and transitions were weighted equally. Trees were obtained using a heuristic search in PAUP vers. 3.1.1 (Swofford 1993); taxa were added using the SIMPLE option with MULPARS and steepest descent options invoked; branch swapping used the tree bisection-reconstruction algorithm. No other equally short (or shorter) trees were found when taxa were added randomly in 25 replicate trials, with 10 trees being held at each step. Variation among the seven most parsimonious trees involved only the branches within the *pulex* complex. Four of the seven trees placed *D. melanica* at the basal node and placed *D. arenata* at the next higher branch. *D. middendorffiana* was the sister species to the unresolved (*pulex*, *pulicaria*) clade in five of the seven trees. The number of characters changing unambiguously (including all variable sites) is shown on each tree branch. Bootstrap percentages from 200 pseudo-replicates are shown in circles (calculated using Random Cladistics, vers. 2.1.0 [Siddall 1994]). Tree length using informative characters only was 1135 steps. A tree with one extra step suggesting the monophyly of *Hyalodaphnia* (dashed line) was chosen for character-state optimization analysis. Sequence alignments are available upon request from the authors.

complexes has persisted for at least 50 million years. It should be noted, however, that 11 of these 16 complexes contain only one or two species in North America. Four of the five remaining complexes (*pulex*, *obtusa*, *retrocurva*, *longispina*) show evidence of more active speciation in the last three million years. But even in these cases, diversification has not exceeded four species. Clearly, speciation rates have been constrained in North American *Daphnia*.

Phenotypic evolution

Despite their low rate of speciation, daphniids undoubtedly encountered diversifying selective pressures among different freshwater habitat types, many of which are sufficiently old for phylogenesis. For example, ca. 2,000 low-pH lakes have existed in north central Florida for at least several hundred thousand years (Hendry and Brezonik 1984). Similarly, some 30,000 lakes of playa origins have been present in the Texas/Oklahoma region since the Pleistocene (Osterkamp and Wood 1987). Water temperature, pH, and salinity, habitat permanency, and the intensity of exposure to ultraviolet radiation all vary on both local and regional scales. Aside from these physical variables, *Daphnia* populations also encounter local variation in predation regime (often linked most clearly to differences in habitat permanency). Lake populations are usually exposed to predation by fish, while those in ponds – where anoxia or complete freezing can kill fish, or where a lack of inlets or outlets may prevent fish dispersal – generally are exposed to predation by invertebrates only. To investigate the extent to which *Daphnia* have adapted to these physical and biological regimes, we analyze the ecological conditions associated with character-state transformations for six key phenotypic traits: cuticular melanization, headshield morphology, carapace setation, ephippial morphology, breeding system, and chromosome number.

CUTICULAR MELANIZATION AND ULTRAVIOLET RADIATION – Four North American species of *Daphnia* deposit melanin in their carapace (Figure 5.3). These pigmented daphniids predominate in the Arctic, but also occur in alpine and coastal sand-dune habitats. At these sites, *Daphnia* are found in shallow, clear-water ponds that are unshaded and lack dissolved humics, which absorb ultraviolet (UV) light in other aquatic habitats. Experimental studies have shown that the melanized carapaces of these daphniids intercept more than 90% of incident UV radiation, and transplantation experiments have shown that unpigmented *Daphnia* cannot survive the high UV exposure in clear-water ponds (Hebert and Emery 1990). Cuticular melanization clearly provides protection from UV radiation in these habitats (see Hobæk and Wolf 1991; Hebert 1995); in other contexts it is likely to be a net disadvantage because melanic carapaces render their bearers more conspicuous to potential predators.

Brooks (1957) hypothesized that melanization evolved only once in North American *Daphnia*, suggesting that the trait was shared among closely related species because of introgressive hybridization. However, character-state optimization of this trait onto the molecular phylogeny reveals that pigmentation in the genus has convergently evolved from a non-melanic state in members of two subgenera (Figure 5.4). Within *Hyalodaphnia*, one species (*D. umbra*) acquired the trait, while within *Daphnia*, three species of the *pulex* complex are melanic. These two subgenera show

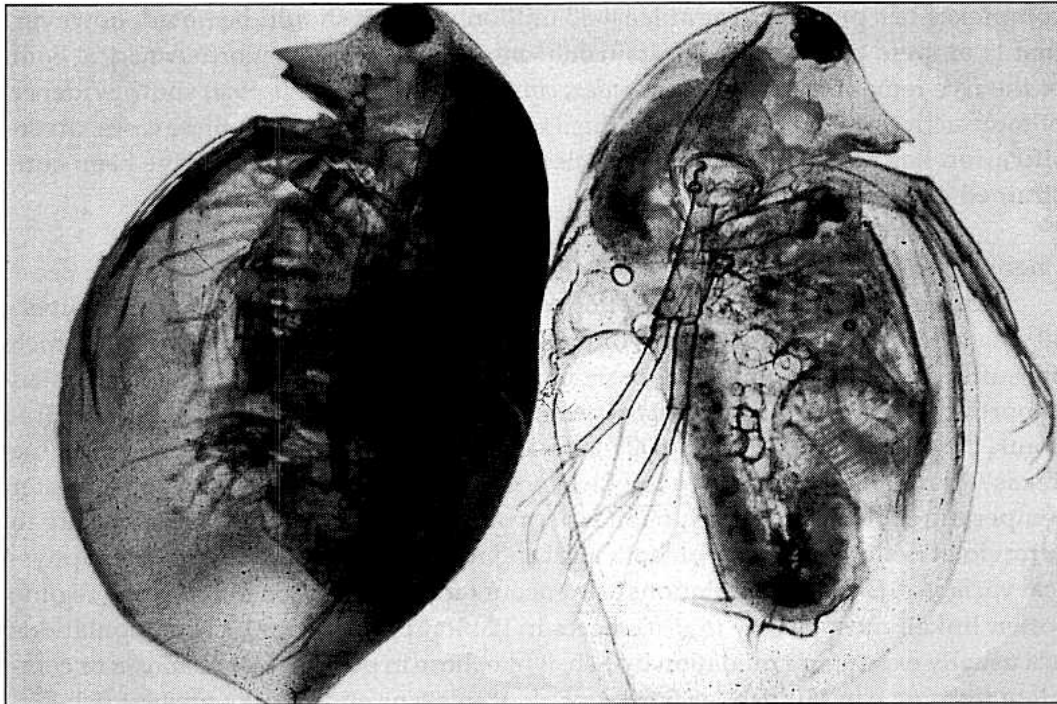


Figure 5.3. Melanic *Daphnia middendorffiana* (left) and non-melanic *D. pulex* from low-Arctic tundra ponds near Churchill, Manitoba, Canada.

approximately 24% sequence divergence at the 12S rRNA gene, representing over 180 million years of independent evolution. Hybridization is unknown between members of these two subgenera, making it apparent that melanization evolved on at least two occasions.

It is, however, much more difficult to ascertain the number of occasions on which melanization evolved in the *pulex* complex. Figure 5.4 suggests three origins of melanization, but this cladogram depicts only one of seven equally parsimonious patterns for the closely related members of the *pulex* complex, based on our 12S rDNA sequence data. In principle, the accumulation of additional sequence information at faster evolving genes could establish a single phylogeny and resolve this uncertainty. However, such efforts to reconstruct the evolutionary origins of melanization are complicated by the occurrence of hybridization among members of the *pulex* complex. It is possible that these three species share the character due to an introgressed gene that diffused rapidly among taxa, possibly because of the adaptive superiority of melanized individuals in clear-water habitats.

Nevertheless, biogeographical and historical evidence suggest that melanization has evolved recently (< 1 Mya) in the subgenus *Daphnia*. *Daphnia middendorffiana* and *D. tenebrosa*, although now endemic to the Arctic, are closely related to more southern pond species (Dufresne and Hebert 1994, 1995), suggesting that the *pulex* complex radiated by adapting to the huge number of northern ponds which were created following deglaciation. Indeed, these taxa appear to have originated during the Pleistocene. Melanization

in *Daphnia* may represent a key adaptation which arose following Pleistocene glaciations, and promoted access to vacant, shallow-water, high-radiation habitats.

HEADSHIELD MORPHOLOGY AND PREDATION – While adults of some species of *Daphnia* show little variation in head shape, others exhibit striking seasonal changes in morphology, termed cyclomorphosis (Figure 5.5; see Jacobs 1987). These changes can involve the production of either spikelike or bladelike extensions to the head (helmets) or thornlike spines in the neck region (neckteeth; Figure 5.5D). Although abiotic factors

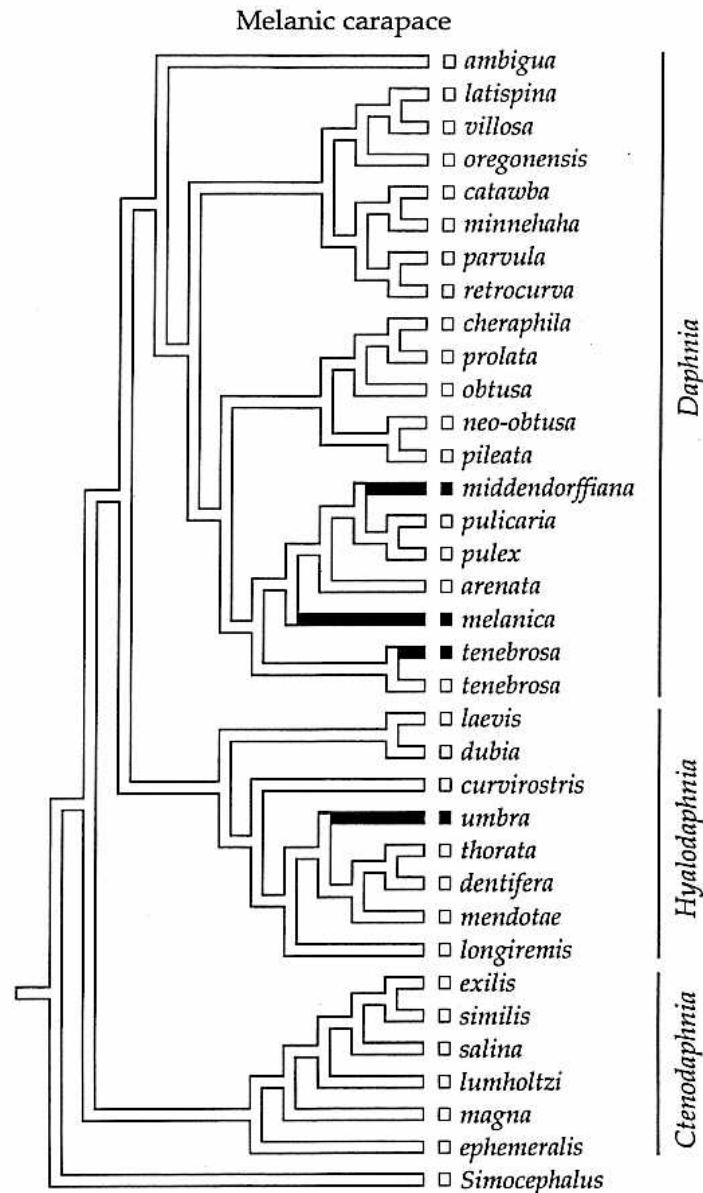


Figure 5.4. Character-state optimization of the presence/absence of cuticular melanization in *Daphnia*. Black shading denotes the presence of melanization.

modulate helmet development (Jacobs 1962; Havel and Dodson 1985), chemical signals from predators are largely responsible for prompting reconfigurations in head shape (Krueger and Dodson 1981; Hebert and Grewe 1985; Parejko and Dodson 1990). The predation regime encountered by *Daphnia* is very dependent on habitat occupancy; pond populations typically encounter only small invertebrate predators, while lake populations are exposed to predaceous fish as well. Studies have shown that even small shape changes provide substantial protection against invertebrate predators (Krueger and Dodson 1981; Havel and Dodson 1984; Tollrian 1995). Larger helmets provide defense against both invertebrate and vertebrate predators (Green 1967; O'Brien et al. 1979; Hanazato 1991), but in North America, they are generally only induced by invertebrate chemicals when both kinds of predators are present (S. Dodson, pers. comm.). This association likely reflects, in part, the effect of visual predation by fish in reducing

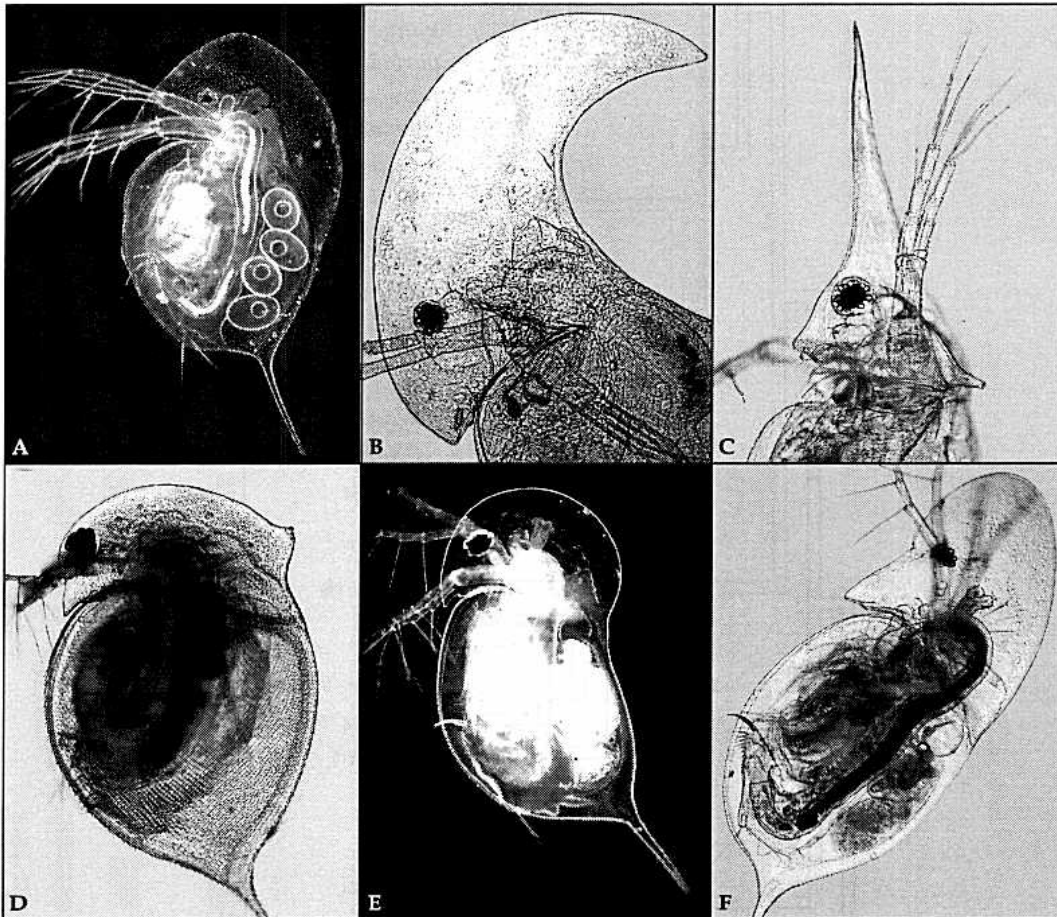


Figure 5.5. Predator-induced headshield morphology of selected adult female daphniids. (A) *Daphnia longiremis* from Eskimo Lakes 7, NWT. August 12, 1993. (B) *D. retrocurva* from Eagle Lake, Michigan. August 2, 1994. (C) *D. lumholtzi* from Grand Lake, Oklahoma. July 20, 1994. (D) *D. minnehaha* from a pond near Sault Ste. Marie, Ontario. May 29, 1991. (E) *D. pileata* from Santo Domingo, Mexico. February 28, 1992. (F) *D. mendotae* from Lake Mendota, Wisconsin. July 4, 1994.

the body size of lake daphniids, and indirectly enhancing their exposure to invertebrate predation (see Dodson 1988).

Figure 5.6 depicts the evolution of helmet and neckteeth formation on the phylogenetic tree for North American *Daphnia*. The plesiomorphic state exhibited by *Ctenodaphnia* appears to be a lack of both helmets and neckteeth. This observation coincides with their habitat, for all species excepting *D. lumholtzi* (Havel and Hebert 1993) are large-bodied animals restricted to ponds lacking vertebrate predators. The most parsimonious evolutionary pattern from accelerated transformation of the character-states (Figure 5.6A) indicates that on the two occasions where *Ctenodaphnia* dispersed into lakes, they evolved defensive helmets. *D. lumholtzi* produces huge spiked helmets when exposed to fish predation (Green 1967). Similarly, the *Ctenodaphnia* species ancestral to *Daphnia* and *Hyalodaphnia* (labeled I in Figure 5.6A), presumably a lacustrine species, also evolved a helmet, as indicated by the accelerated character-state optimization of both the trait and habitat (Figure 5.6B) onto the cladogram. Furthermore, helmet production persisted (with modifications) throughout the evolution of five lacustrine species of *Hyalodaphnia* and the oldest member of the subgenus *Daphnia*.

This association between habitat occupancy and helmet production is reinforced by other character-state changes. For example, one ancestral species of *Daphnia* (labeled II in Figure 5.6A) and three extant members of *Hyalodaphnia* lost the ability to produce helmets following their habitat shift from lakes back to ponds. On two other separate occasions, helmets originated in species of the subgenus *Daphnia*. *D. retrocurva* is one of these species which gained a helmet and fits the general pattern, as it is found only in lakes. However, *D. pileata*, the sole helmeted member of the *obtusa* complex, is restricted to ponds. This exceptional taxon produces a helmet very different in appearance from those of helmeted species in lakes (see Figure 5.5E), but similar to those developed by several Australian *Ctenodaphnia*. The latter species are also pond dwellers and in this case, helmet formation is known both to be induced by notonectids and to provide protection from these predators (Grant and Bayly 1981). In contrast to most other invertebrate predators encountered by *Daphnia*, which preferentially capture prey less than 1 mm in length (Krueger and Dodson 1981; Tollrian 1995), notonectids capture prey as large as 3 mm in length (Barry and Bayly 1985). Hence, helmet development is advantageous even in large-bodied daphniids exposed to notonectid predation (Grant and Bayly 1981; Barry and Bayly 1985). The fact that notonectid populations only reach high densities in warm regions explains the prevalence of helmeted pond daphniids in Australia, and the occurrence of this trait in *D. pileata*, which has one of the most southerly distributions of any North American species (Hebert 1995).

The association between the evolution of helmets and pond to lake habitat shifts is not as evident in optimizations generated by delayed transformation of the character-states onto the cladogram (Figure 5.7). Although Figure 5.7A indicates that helmet formation may have been derived by both *D. lumholtzi* and *D. ambigua* – coinciding with proposed habitat shifts by delayed transformation as well (Figure 5.7B) – the pattern is broken when inferring which (ancestral) taxa invaded lakes among the *Hyalodaphnia*. Nonetheless, this conflicting pattern does not diminish the strong

correlation between lake daphniids and defensive headshields. It does, however, suggest another equally parsimonious sequence for helmet evolution.

The ability to produce neckteeth has also arisen independently in both *Daphnia* and *Hyalodaphnia* (Figure 5.6), and occurs in both pond and lake taxa. In species able to produce this structure, neckteeth are induced by a chemical(s) released by larvae of the predatory dipteran *Chaoborus* (Krueger and Dodson 1981; Spitze 1992). The production of neckteeth by both lacustrine and pond species is not unexpected, as this predator occurs in both habitats. However, *Chaoborus* is absent from turbid water habitats and none of the *Daphnia* species from these settings (species belonging to the

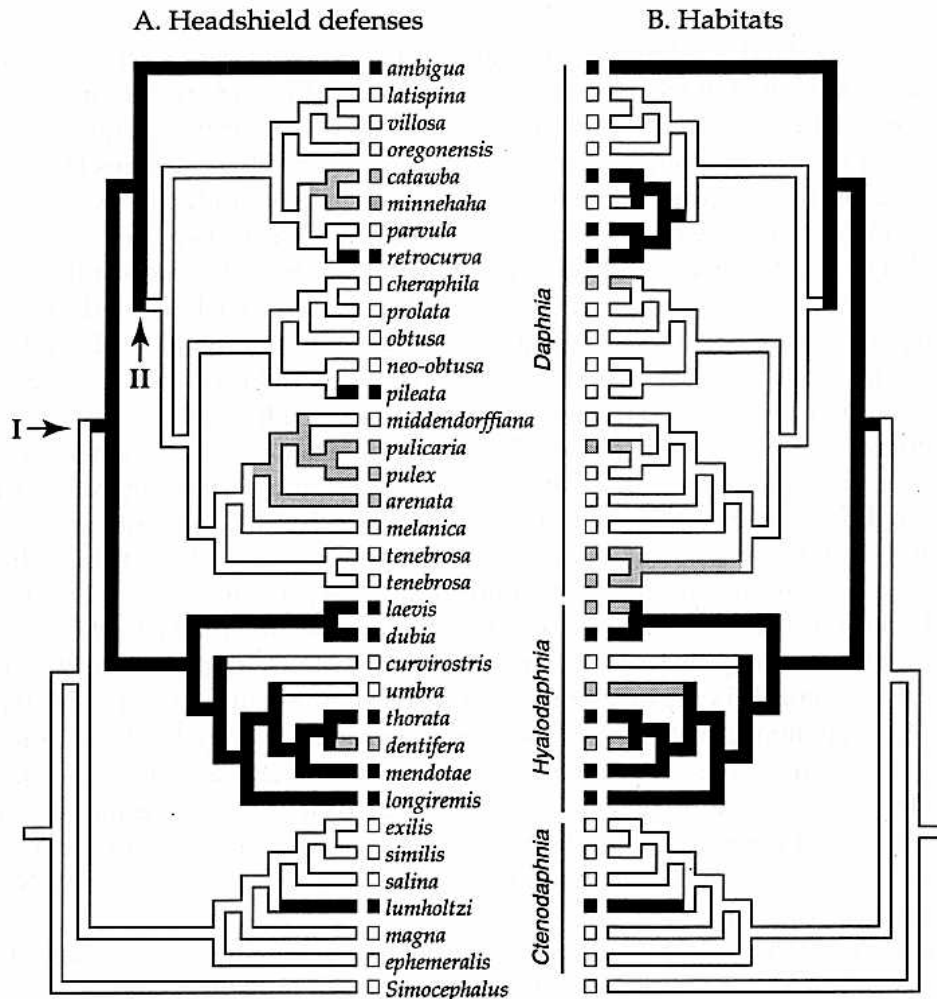


Figure 5.6. (A) Character-state optimization of helmet and neckteeth formation in the genus *Daphnia* assuming accelerated transformation. Black shading denotes the ability to produce helmets by adult daphniids, while gray shading denotes the inducible production of neckteeth. (B) Proposed habitat shifts associated with *Daphnia* speciation assuming accelerated transformation: ephemeral pond (white), permanent pool/small lake (gray), and larger lake habitats (black). Characters were not polarized prior to the transformations.

obtusa and *villosa* complexes) produces neckteeth, even when exposed to *Chaoborus* in the lab (Beaton 1995). In most taxa which produce them, neckteeth are evanescent, being retained only in the early juvenile instars when *Daphnia* is sensitive to this predator. However, the largest species of *Chaoborus* are restricted to ponds, and two species of *Daphnia* (*arenata*, *minnehaha*) which occur in these habitats have independently evolved the ability to sustain necktooth production even as adults.

This analysis demonstrates that the evolution of helmets is typically correlated with evolutionary shifts from pond to lake habitats, and in North America, apparently reflects an indirect response to vertebrate predation. Conversely, taxa invading pond

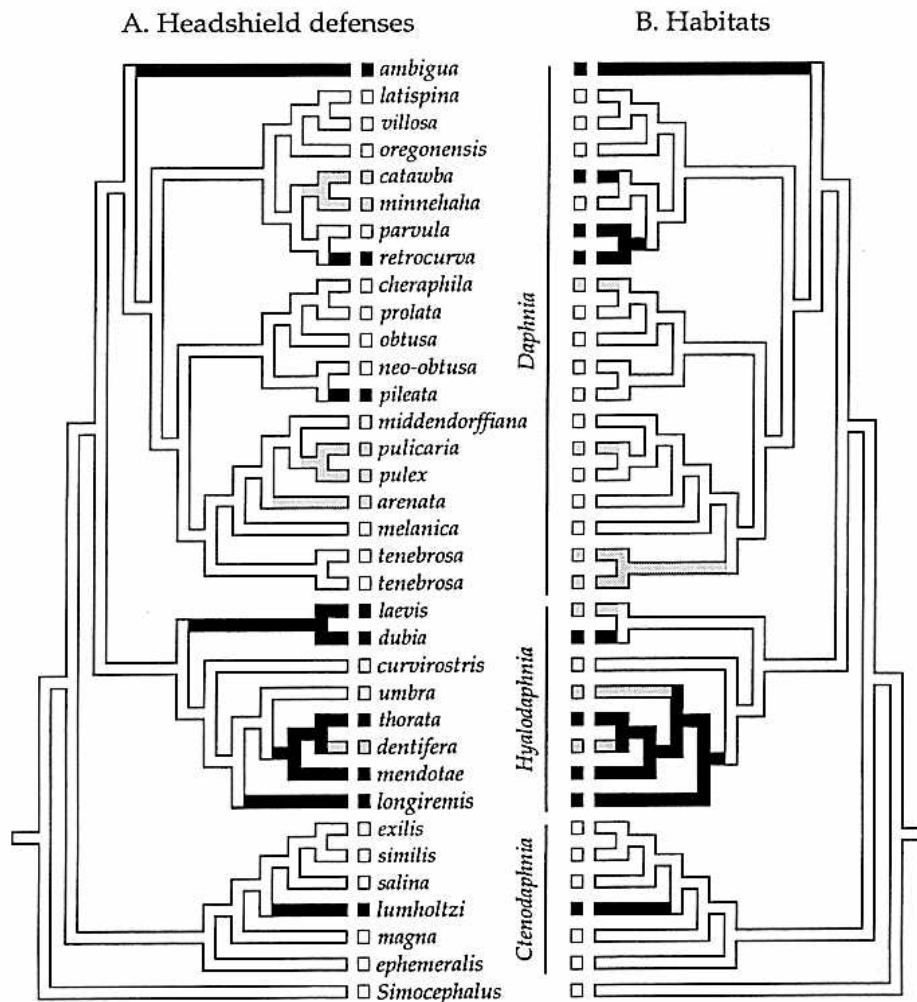


Figure 5.7. Alternative hypothesis for the sequence of evolutionary changes in inducible head morphologies of daphniids. (A) Character-state optimization of helmet and neckteeth formation in *Daphnia* assuming delayed transformation. Black shading denotes the ability to produce helmets by adult daphniids, while gray shading denotes the inducible production of neckteeth. (B) Proposed habitat shifts associated with *Daphnia* speciation assuming delayed transformation: ephemeral ponds (white), permanent pools/small lakes (gray), and larger lakes (black). Characters were not polarized prior to the transformations.

habitats with lower predation pressure lose helmets, presumably reflecting the cost of producing such structures (Black and Dodson 1990; Walls et al. 1991). In the case of neckteeth, species similarly gain the ability to produce and modulate neckteeth as they enter habitats with *Chaoborus*. The regularity with which daphniids modulate head shape, and the repetitious nature of these modifications suggest the maintenance of a simple proximate mechanism for such change (Beaton 1995). This conclusion differs from the past assumption of shared ancestry between helmeted forms, as shown for example by the subdivision of the *longispina* complex into helmeted and unhelmeted species assemblages before Taylor and Hebert (1994 and references herein).

CARAPACE SETATION AND SUSPENDED PARTICULATES – All *Daphnia* possess spines on the external margin of their ventral carapace, but only 13 species in North America have elongate plumose setae on their internal margin (Figure 5.8; Scourfield 1942; Hebert 1995; Hebert and Finston 1996). The carapace houses the filtering appendages which daphniids use to collect particulate food such as algae, and these plumose setae are believed to prevent debris from entering the carapace chamber (Fryer 1991b). The size of suspended debris encountered by a daphniid varies according to its habitat occupancy and feeding behavior. Species of *Ctenodaphnia*, although they are capable of foraging in the water column (Burns 1968), spend significant time recovering large particulate food from bottom sediments

(except *D. lumholtzi*; [Fryer 1991b]). Some members of the subgenus *Daphnia*, such as *D. obtusa*, employ a similar mode of foraging. The latter species uses, like *D. magna*, a specialized scraperlike medial spine on the second thoracic limb to resuspend ingestible materials (Fryer 1991b). By contrast, many other species of subgenus *Daphnia* and all *Hyalodaphnia* filter-feed in open water, which normally contains only smaller particles. It is interesting that daphniids that inhabit turbid habitats with high concentrations of colloidal clay invariably possess elongate setae (Hebert 1995).

Character-state optimization onto the phylogeny reveals that the presence of setae is the plesiomorphic state (Figure 5.9), coinciding with the benthic feeding behavior of most *Ctenodaphnia*. Although the ancestral *Ctenodaphnia* possess this trait, recent molecular phylogenetic

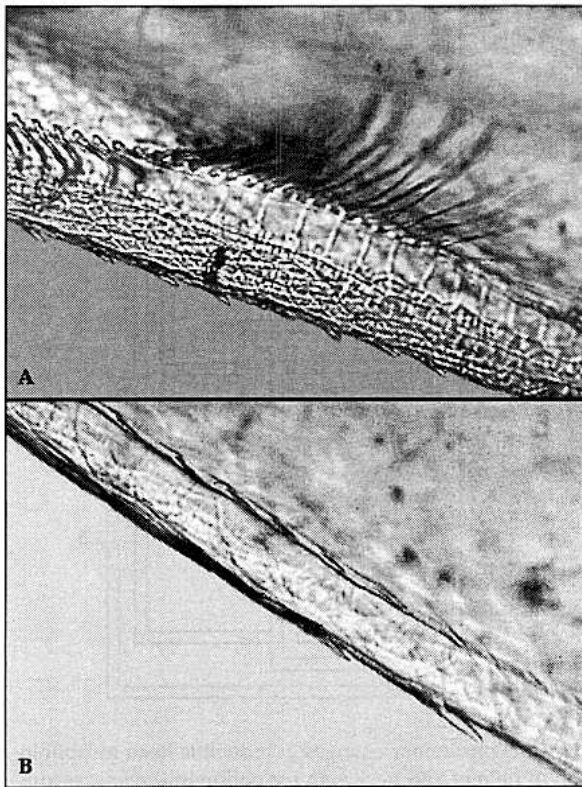


Figure 5.8. (A) Ventral margins of the carapace of *Daphnia* with internal plumose setae. (B) Same, but without plumose setae.

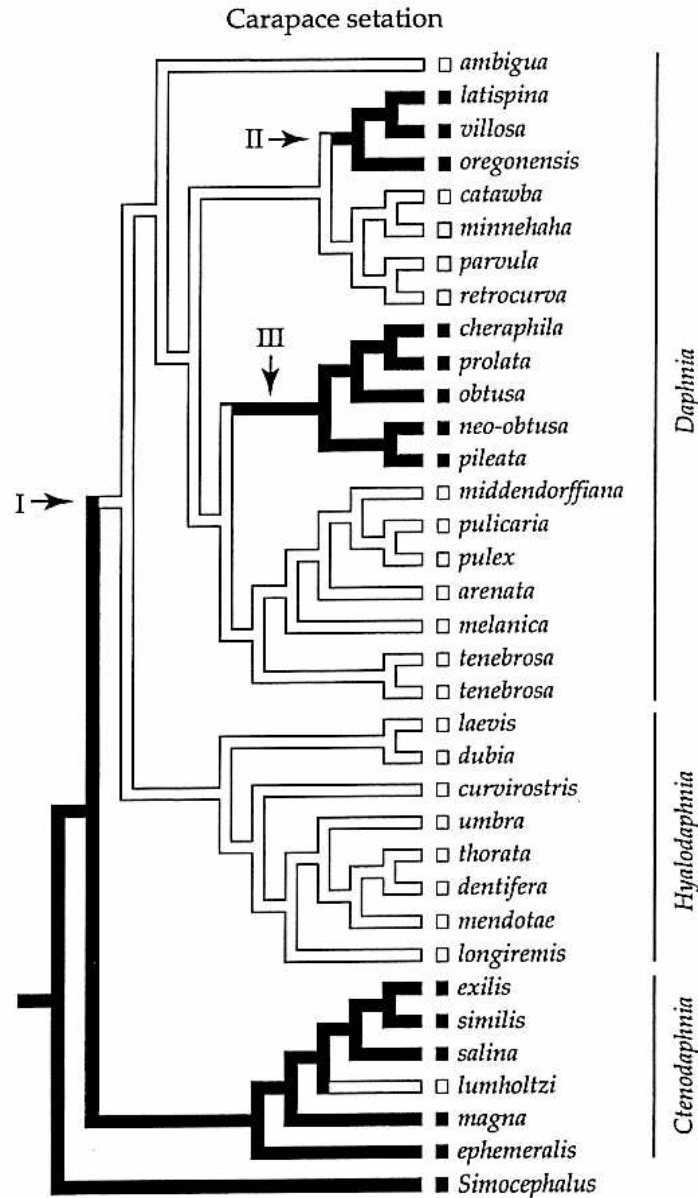


Figure 5.9. Character-state optimization of the presence/absence of plumose setae on the internal margin of the carapace in *Daphnia*. Black shading denotes the presence of plumose setae.

studies of branchiopod crustaceans (D. Taylor, in prep.) suggest that their benthic feeding behavior is derived. Moreover, the character was lost twice by *Daphnia* following habitat transitions from ponds to lakes. *D. lumholtzi* does not have these elongate setae (Fryer 1991b), nor did the species of *Ctenodaphnia* ancestral to the *Hyalodaphnia* and *Daphnia* clades (labeled I on the tree). Despite its absence in ancestral species, the character was regained twice in the subgenus *Daphnia*. The re-acquisition of this trait occurred in pond-dwelling lineages and was likely a consequence of a shift to a

detritus-based mode of feeding by members of the *villosa* and *obtusa* lineages (labeled II and III, respectively). However, the presence of this trait subsequently made it possible for these lineages to colonize a new environment – ponds with highly turbid waters – resulting in the origin of new species adapted to these environments.

EPHIPPIAL MORPHOLOGY – Ehippia display conspicuous morphological variation in the number/position of egg chambers, intensity of melanization, shape, and spinescence (Hebert 1995). All North American species have a two-egg ehippium, but at least one Australian species has a one-egg ehippium (Benzie 1988). The orientation of the egg chambers relative to the dorsal surface of the ehippium varies from horizontal to perpendicular, and is sufficiently invariant to discriminate the ehippial eggs of *Ctenodaphnia* from those of the other subgenera. The ehippial matrix varies in color from white to black, with the most melanized ehippia being produced by species showing melanization of the carapace. Ehippial shape varies from an elongate ellipse to subtrapezoidal, but quantification of shape variation is complex. As a result, we focus our analysis on the most easily scored trait – the presence/absence of spinescence on the dorsal margin of the ehippium (Figure 5.10).

Adult females of all species of *Daphnia* have dorsal spinescence on their carapace, but prior to ehippial production, 14 species suppress this trait and produce ehippia lacking spinescence. Our analysis indicates that absence of spines is the plesiomorphic state, as ehippia from both the outgroup (including other Cladocera such as *Daphniopsis* and *Ceriodaphnia*) and the oldest member of *Ctenodaphnia* are without spines. Figure 5.11 shows that ehippial spinescence originated twice in the genus, once in an ancestral *Ctenodaphnia*, and again in the ancestor to the subgenus *Daphnia* (labeled I on the tree). Subsequently, there have also been three independent losses of the trait, twice by ancestors to clades within the subgenus *Daphnia*, and once by a species in the *pulex* complex. As these lineages include both lake and pond species, there is no obvious association between the gain/loss of spines and habitat occupancy.

The adaptive significance of shifts in the spinescence patterns on ehippia is not clear. It has been argued that dorsal spines foster long-distance dispersal by fostering ehippial adhesion to birds and mammals or, contrarily, that they impede movement by serving as anchors (Frey 1982; Sergeev 1990; Fryer 1991b; Hebert and Wilson 1994). The information now available on the extent of gene frequency divergence among local populations of a number of *Daphnia* species may provide some resolution to this controversy. These findings (Colbourne et al., unpubl. data) suggest that ehippial spinescence is associated with pronounced gene frequency divergence among populations, supporting their potential role in anchoring eggs. Species of *Ctenodaphnia*, which possess the most spinescent ehippia, show much greater local differentiation in gene frequencies than species of *Hyalodaphnia*, which produce ehippia lacking spines.

BREEDING SYSTEMS – As noted earlier, most species of *Daphnia* reproduce by cyclical parthenogenesis, but four species have abandoned the sexual phase of the life cycle. These taxa produce apomictic ehippial eggs, in contrast to the sexual ehippial eggs produced by other species (Hebert 1981). It is interesting that three of these species (*pulex*, *pulicaria*, *tenebrosa*) include some populations which retain the ancestral

breeding system of cyclic parthenogenesis. The obligately parthenogenetic populations of each of these taxa show extraordinary clonal diversity, which seems to be the result of multiple transitions to obligate apomixis (Crease et al. 1989). Experimental studies have linked this shift in breeding system to the transmission of a sex-limited meiosis suppressor (Innes and Hebert 1988).

The *Daphnia* phylogeny indicates that obligate parthenogenesis has evolved in North America only in the *pulex* complex (Figure 5.12). Even though relationships are poorly resolved among members of this complex, it is clear from 12S rDNA sequence divergence data that this breeding system must have originated within the last million years or so. It is important to note that the derived mating system is only shared among species known to hybridize, or species which are themselves of hybrid origin (Hebert et al. 1989; Crease and Lynch 1991; Dufresne and Hebert 1994; Hebert and Finston 1996; P. Hebert, unpubl. data). This fact suggests that the trait may have originated only once in the North American fauna, and then diffused to other taxa by

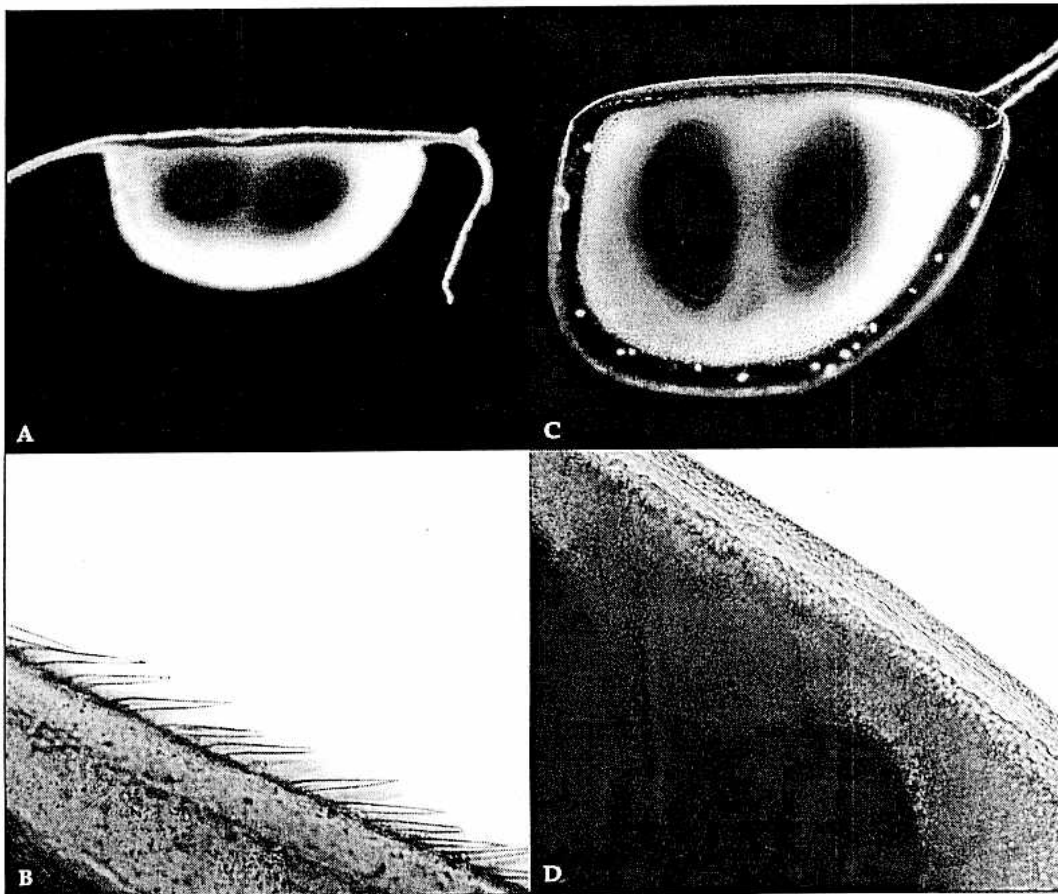


Figure 5.10. Examples of *Daphnia* ephippial morphology. (A) *D. magna* from Tuktoyaktuk, NWT. August 16, 1993. (B) Dorsal surface of the same ephippium possessing spinescence. (C) *D. laevis* from Rondeau Park, Ontario. June 9, 1992. (D) Dorsal surface of the same latter ephippium without spinescence.

introgressive hybridization. For this reason, it would be misleading to infer that the trait evolved in the common ancestor of the (*pulex*, (*pulicaria*, *middendorffiana*)) clade. Obligate apomixis may thus have had a very recent origin. The geographic distributions of breeding systems suggest that there may be selection for asexuality in a specific habitat, because all members of the *pulex* complex reproduce by obligate parthenogenesis in the Arctic. However, because these taxa are also polyploids, and

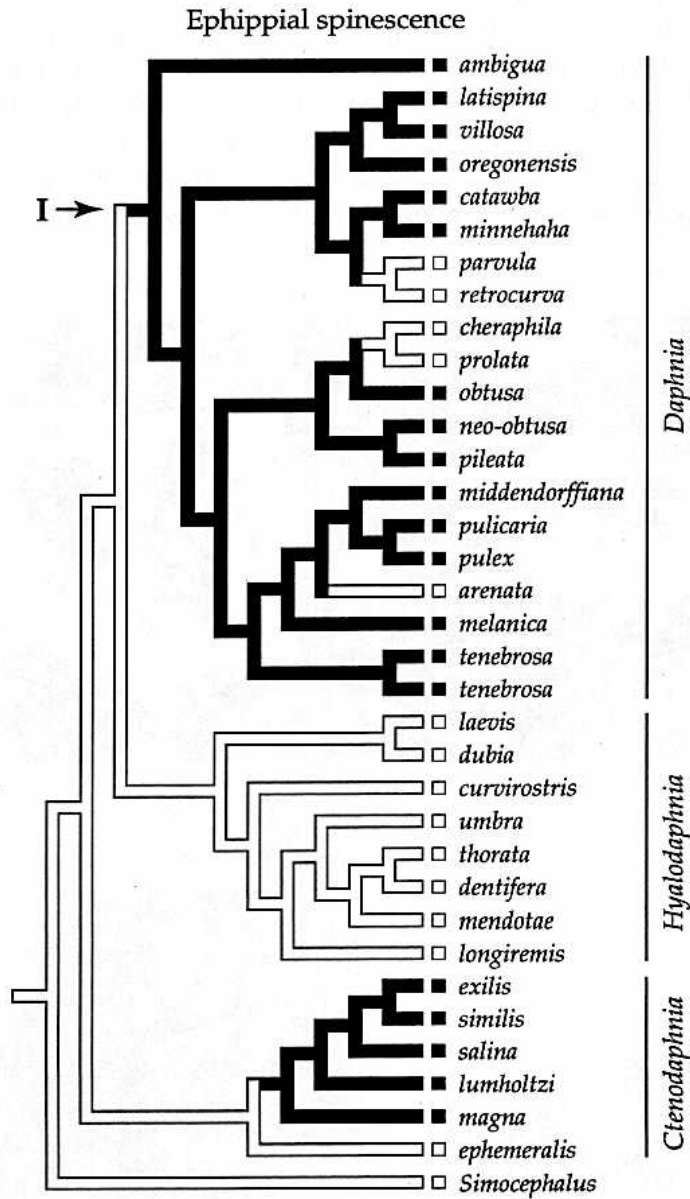


Figure 5.11. Character-state optimization of the presence/absence of ehippial spinescence in the genus *Daphnia* onto the cladogram of North American species. Black shading denotes the presence of ehippial spinescence.

because only diploids are present in temperate localities, the dominance of obligate apomicts at higher latitudes may simply be an indirect consequence of selection for greater ploidy levels (Beaton and Hebert 1988).

CHROMOSOMAL EVOLUTION – Species of *Daphnia* show relatively little variation in chromosome number (Zaffagnini and Trentini 1975; Trentini 1980). Current data indicate that all species of *Ctenodaphnia* and *Hyalodaphnia* possess 20 chromosomes, while all members of subgenus *Daphnia* have 24 (Beaton and Hebert 1994). There are poly-

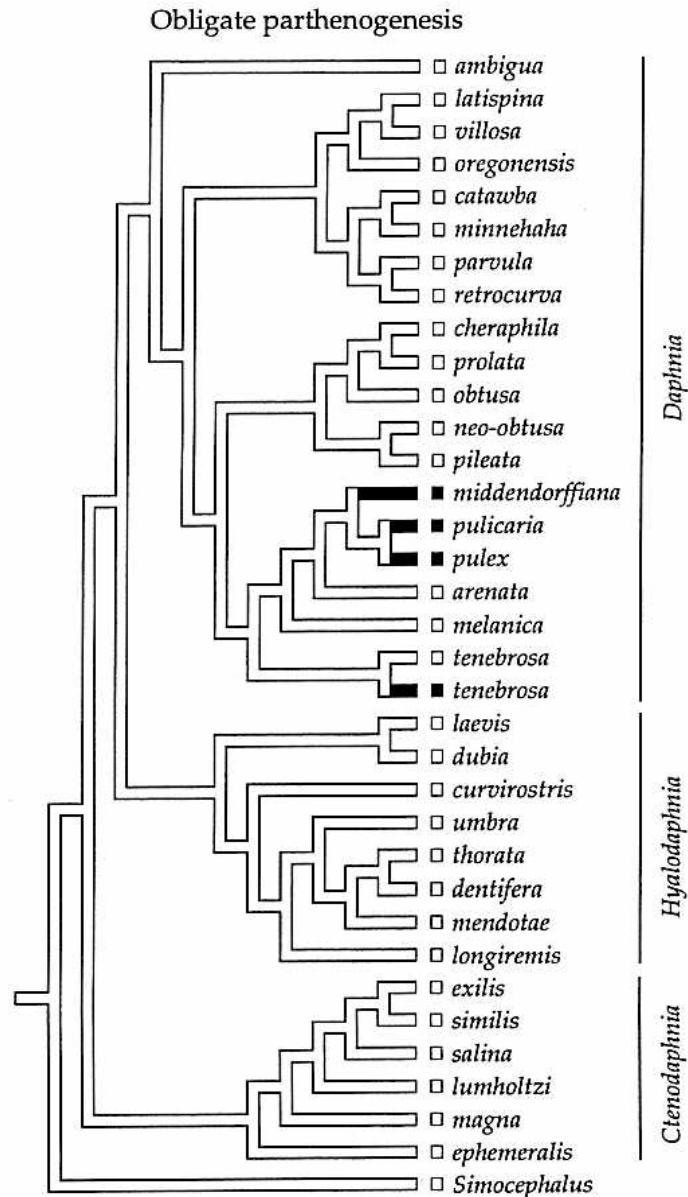


Figure 5.12. Shifts to obligate parthenogenesis in *Daphnia*.

ploid derivatives of several species in the latter group with higher chromosome numbers, but these polyploids are of recent origin and are incapable of sexual reproduction. Consequently, the sole shift in chromosome numbers within cyclically parthenogenetic lineages appears to have occurred early in the evolution of the genus (Figure 5.13). There is some information to suggest that even linkage relationships have been stable, because at least one pair of enzyme loci show similar map distances in species from two different subgenera (Hebert and Moran 1980; Innes 1989)!

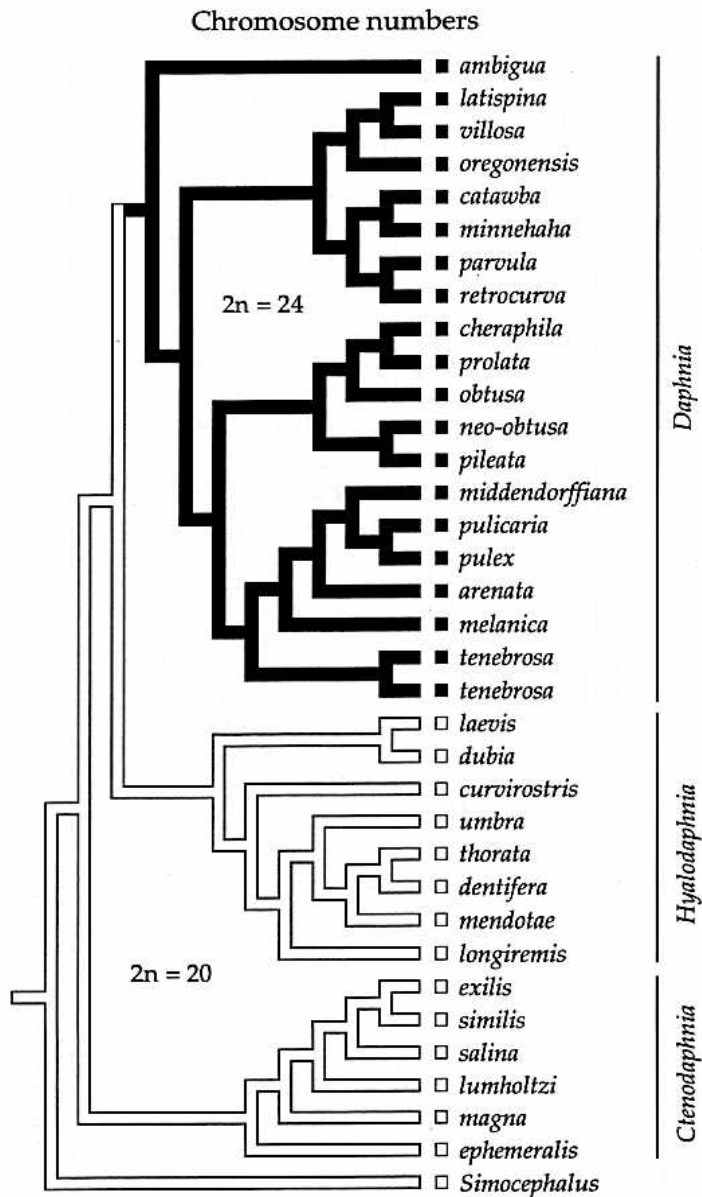


Figure 5.13. Character-state optimization of diploid chromosome numbers in *Daphnia*.

In addition to the stability in chromosome numbers and gene arrangements, genome sizes are relatively invariant within the genus (Beaton 1995). Variation in genome sizes among species of *Ctenodaphnia* and *Hyalodaphnia* is limited, with less than a 50% difference in mean genome size between the subgenera. More variation among species has been detected within subgenus *Daphnia*, with up to a four-fold variation in genome size. The largest genomes occur in obligately apomictic clones of the *pulex* complex that are recently derived polyploids (*middendorffiana*, *tenebrosa*).

There is only one exception to the small differences in genome size among taxa capable of sexual reproduction. Diploid clones of *D. tenebrosa* have a genome size which is nearly twice as large as that of other members of the *pulex* complex. Our 12S rDNA data suggest that this increase in genome size has occurred within the last million years. *Daphnia tenebrosa* is morphologically exceptional – it is not only the largest member of its subgenus, but it also produces the largest eggs of any species in the genus. Its increased body and egg size is likely a simple consequence of the usual genome size/cell size relationship (Cavalier-Smith 1985a,b; Beaton and Hebert, in prep.). These life history shifts are ecologically important, as they allow *D. tenebrosa* to co-occur with the predatory copepod *Heterocope septentrionalis*, while other small-bodied *Daphnia* are excluded (O'Brien et al. 1979; Hebert and Loaring 1980; Dodson 1984). Unfortunately, there is no understanding of the process which led to the rapid increase in its genome size, but it is clearly not a result of conventional polyploidy, as the species has the same chromosome number as other members of the subgenus *Daphnia*.

Conclusions

This study represents a preliminary effort to reconstruct the tempo and phenotypic pattern of evolution in North American *Daphnia*. Although the phylogeny employed to reconstruct character-state evolution is based on the analysis of sequence divergence in only one gene, our principal conclusions are likely to be robust, given the patterns of character-state variation within vs. between subgenera. For example, the multiple independent origins of cuticular melanization and helmets in two different subgenera – and the monophyletic nature of shifts in chromosome number and breeding systems corresponding to subgeneric or complex limits – are unlikely to be altered by more intensive analyses of sequence divergence at other loci. However, in other cases, such as the presence of melanization in several species of the *pulex* complex, our conclusions are less secure, reflecting in part the general problem of determining evolutionary trajectories where several closely related species share a distinctive trait. Such trait concordance has three possible explanations – its maintenance from a shared ancestor, its repeated independent acquisition, or its secondary acquisition as a consequence of introgressive hybridization. Unambiguous discrimination among these alternatives is only likely to result from the targeted analysis of sequence diversity in the gene(s) controlling the trait. At present, such analysis represents a substantial challenge, because there is usually no simple way to identify the gene(s) in question. Consensus phylogenies, based on the analysis of sequence diversity in a

number of genes, are certainly not a panacea for resolving such uncertainty in character-state transitions (also see Leroi et al. 1994), but such evidence is the only tool we now have to confront the vagaries of molecular evolution.

This study provides evidence that *Daphnia* has shown limited phenotypic innovation since the mid-Mesozoic. However, shortly after its origin, the genus showed a brief interval of intense diversification and phenotypic evolution, which led to the establishment of morphologically distinct forms that underlie the three modern subgenera. Following this initial radiation, rates of evolutionary diversification then slowed; pools trod by dinosaurs undoubtedly contained a daphniid fauna similar to extant forms. This result suggests that daphniids have not experienced global competitive release since the Mesozoic, implying that the zooplankton communities of inland waters were little impacted by the general faunal collapse at the Cretaceous boundary. Despite the lack of major evolutionary innovations, morphological diversification within the genus has not ceased. However, the pattern of diversification is one characterized by frequent character-state convergence. Placed in a lake setting, daphniids typically evolve helmets, and when placed with *Chaoborus*, they evolve neckteeth. A similar convergence involving cuticular melanization has occurred in response to intense ultraviolet exposure in two lineages of North American *Daphnia* as well as several Australian daphniids. As many other zooplankters sequester carotenoids gained from their food for apparently the same purpose, the repeated evolution of this adaptation by *Daphnia* suggests that underlying intrinsic factors (as well as the obvious extrinsic factor of UV radiation) favor its recurrent evolution. In this case, it is known that the genes coding for melanin formation antedate the evolution of a melanized carapace, for they are regularly expressed in both the eye and in the epidermal tissue surrounding egg chambers of the ephippium. The development of a melanic *Daphnia* may simply require the activation of these loci in other epidermal cells. Similarly, the loss of ephippial spinescence may only require the suppression of a trait normally present on the adult carapace. The gain of plumose setae may also not be revolutionary, given that all *Daphnia* males possess similar structures which they use to attach themselves to females when copulating. Some character-state transitions have occurred more rarely – for example, obligate apomixis has only evolved in four members of the *pulex* complex. This case may reflect character-state convergence due to introgressive hybridization rather than independent origin. Introgression may also account for the sharing of melanic carapaces by members of the same species complex.

The pattern of evolutionary diversification in the genus *Daphnia* appears similar to that of other groups of planktonic cladocerans, which also show limited species diversity and morphological variation (Frey 1987). However, the striking morphological diversity among different genera and families of cladocerans makes it clear that a varied body plan is compatible with success as a zooplankter (see Fryer 1991b). It seems likely that morphological divergence within each of these groups has been restrained by the long-term persistence of a stable community assemblage of different zooplankton groups. Further molecular phylogenetic work is now required to verify that the modern zooplankton fauna of inland waters derives from a single burst

of diversification in the early Mesozoic. It is interesting that no family of planktonic cladocerans shows as much taxonomic diversity as the Chydoridae (Frey 1987), specialists that occupy benthic and littoral habitats that are much more complex structurally than those occupied by pelagic forms. Molecular evolutionary studies are needed to determine whether chydorids show rates of morphological and genetic divergence that greatly exceed those of their planktonic counterparts. The verification of grossly different rates of differentiation linked to different kinds of habitat (as opposed to differences in competitive regime) would reinforce the situation-dependence of the evolutionary process.

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