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Daphnia galeata mendotae as a cryptic species complex with interspecific hybrids

Abstract—*Daphnia galeata mendotae* has been recognized as possessing the greatest morphological variation among North American *Daphnia* species. Prior work has suggested that this variation is a consequence of phenotypic plasticity. Using allozyme markers, we tested this hypothesis by examining the association between genotype and morphology in 22 populations of *D. galeata* from lakes in Indiana. Analyses indicated a genetic basis for the phenotypic variability, as two different species and their F_1 hybrids were detected. The parent species were *D. galeata mendotae* sensu stricto and a helmeted form of *Daphnia rosea*. F_1 hybrids were detected in 17 of 22 lakes and were dominant in 10. Backcross and F_2 individuals were rare, however, suggesting that introgression is limited by the existence of reproductive isolating mechanisms.

Despite its importance in freshwater habitats, the taxonomy of the crustacean genus *Daphnia* remains confused. Brooks (1957*a*) proposed that difficulties in species assignments resulted from extensive variation created by a combination of phenotypic plasticity, the coexistence of morphologically similar species, and interspecific hybridization. Genetic studies have now established that these three factors have interacted to create variation in both Australian (Hebert 1985) and European *Daphnia* species groups (Wolf and Mort 1986; Hebert et al. 1989*b*).

In North America, prior work has shown that *Daphnia galeata* Sars, 1864 *mendotae* Birge, 1918 comb. nov. Brooks, 1957 possesses a striking amount of phenotypic variation (Brooks 1957*b*; Jacobs 1961; Mort 1989). Indeed, Brandlova et al. (1972) suggested that *D. galeata mendotae* might represent a species group because its morphological variation was the greatest among North American *Daphnia* species. But studies of environmentally induced morphological changes (Jacobs 1961) and of allozyme variation (Stirling and McQueen 1987; Mort 1989) have failed to provide evidence for the species group proposal. In fact, all of these investigations have concluded that genetic sources of morphological variation in *D. galeata mendotae* are minor and that phenotypic plasticity is great.

However, such conclusions seem premature. The genetic studies were concerned mainly with allozyme-morphology associations within a single population and were therefore inadequate for assessing associations at the species level. Because multilake studies (e.g. Brandlova et al. 1972) have shown that phenotypic variation is much greater among lakes than within lakes, prior allozyme studies have examined only a small fraction of the total morphological variation

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in the species. Furthermore, the probability of detecting an association between genotype and phenotype increases with the number of allozyme marker loci used. Prior studies have used only two to four marker loci in their search for genotype–phenotype covariation.

Our purpose was to provide a more comprehensive evaluation of the sources of phenotypic variation in *D. galeata mendotae.* To do this, we examined the association between morphology and allozyme genotypes at 16 different loci among 22 populations of *D. galeata mendotae.* Samples of *Daphnia* were obtained from 24 glacial lakes in three watersheds (Tippecanoe, Wabash, and St. Joseph) and two drainage basins (Lake Michigan and Ohio River) in northeast Indiana.

Three morphs are prevalent in this region (Fig. 1). The first possesses an apically pointed helmet and a concave ventral head margin, while the second, Woltereck's (1932) *Daphnia longispina elongata indianae,* has a rounded helmet and a convex ventral head margin. Brooks (1957*b*) synonymized these two morphs with *D. galeata mendotae.* The third morph is intermediate in these characters and also agrees with the definition of *D. galeata mendotae.* The morphological relationships of males and juvenile females follow the same pattern as the adult females. Morph 1 males and juvenile females always have an apical spike, whereas morph 2 males and juvenile females are always apically rounded. Morph 3 has an intermediate phenotype, with males always, and juvenile females frequently, possessing an anterodorsal denticle. A more posterior and roseate neck-tooth develops in some males of morph 2. Another closely related species, *Daphnia rosea* Sars, 1862, has been identified from ponds in Indiana (pers. obs.) and is discriminated from *D. galeata* by its lack of a helmet (Brooks 1957*b*).

In all, 22 populations of *D. galeata* and 2 of *D. rosea* were analyzed. Samples were collected by duplicate vertical hauls of a 30-cm-diameter plankton net (200-μm mesh) near the point of maximum depth in the lake. *D. galeata* was sorted from other zooplankton and within 5 h of collection either frozen in liquid nitrogen, or preserved in 95% ethanol. Before electrophoresis, individuals were characterized as one of the three morphs. Cellulose acetate gel electrophoresis was conducted according to the methods of Hebert and Beaton (1989). The following 12 enzyme systems and 16 loci were scored: aldehyde oxidase (*Ao*), arginine phosphokinase (*Apk*), dipeptidase (*Pep-A, Pep-D*), fumarate dehydrogenase (*Fum*), glutamate-oxaloacetate transaminase (*Got-m, Got-s*), glyceraldehyde-3-phosphate dehydrogenase (*G3pdh*), isocitrate dehydrogenase (*Idh-1, Idh-2*), lactate dehydrogenase (*Ldh*), malate dehydrogenase (*Mdh-m, Mdh-s*), malic enzyme (*Me*), phosphoglucomutase (*Pgm*), and phosphoglucose isomerase (*Pgi*). Substrates for *Pep-A* and *Pep-D* were L-leucylglycine and L-phenylalanyl proline respectively. Relative allozyme mobilities were calculated as a percentage of the mobility of the most common *D. rosea* allele.

A locus was considered polymorphic when the most common allele had a frequency of <95%. Data from May 1990 were used for genetic analysis of most populations, but data from September 1989 and May 1990 were pooled for five populations of morph 2 (following a *G*-test of independence) because individual sample sizes for this morph were small in May. Three populations of morph 3, for which only seven loci were available, were excluded from the heterozygosity calculations.

Seven (*Apk, G3pdh, Idh-1, Idh-2, Mdh-m, Mdh-s, Me*) of the 16 loci were invariant among all individuals, but the other nine were useful in discriminating between morphs 1 and 2 (Table 1). Fixed allelic differences between these morphs occurred at two loci (*Ao, Got-m*), and large gene frequency differences occurred at four additional loci (*Got-s, Pep-A, Pep-D, Pgm*). Three other loci were partially diagnostic: morph 1 possessed alleles (Fum^a, and Pgi^b) that were not detected in morph 2, and morph 2 possessed an allele (Ldh^a) that was not detected in morph 1. The allozyme survey suggested that individuals of morph 2 were closely related to *D. rosea* as they had identical allelic arrays at all loci except *Ldh,* where morph 2 populations possessed a unique allele (Ldh^a).

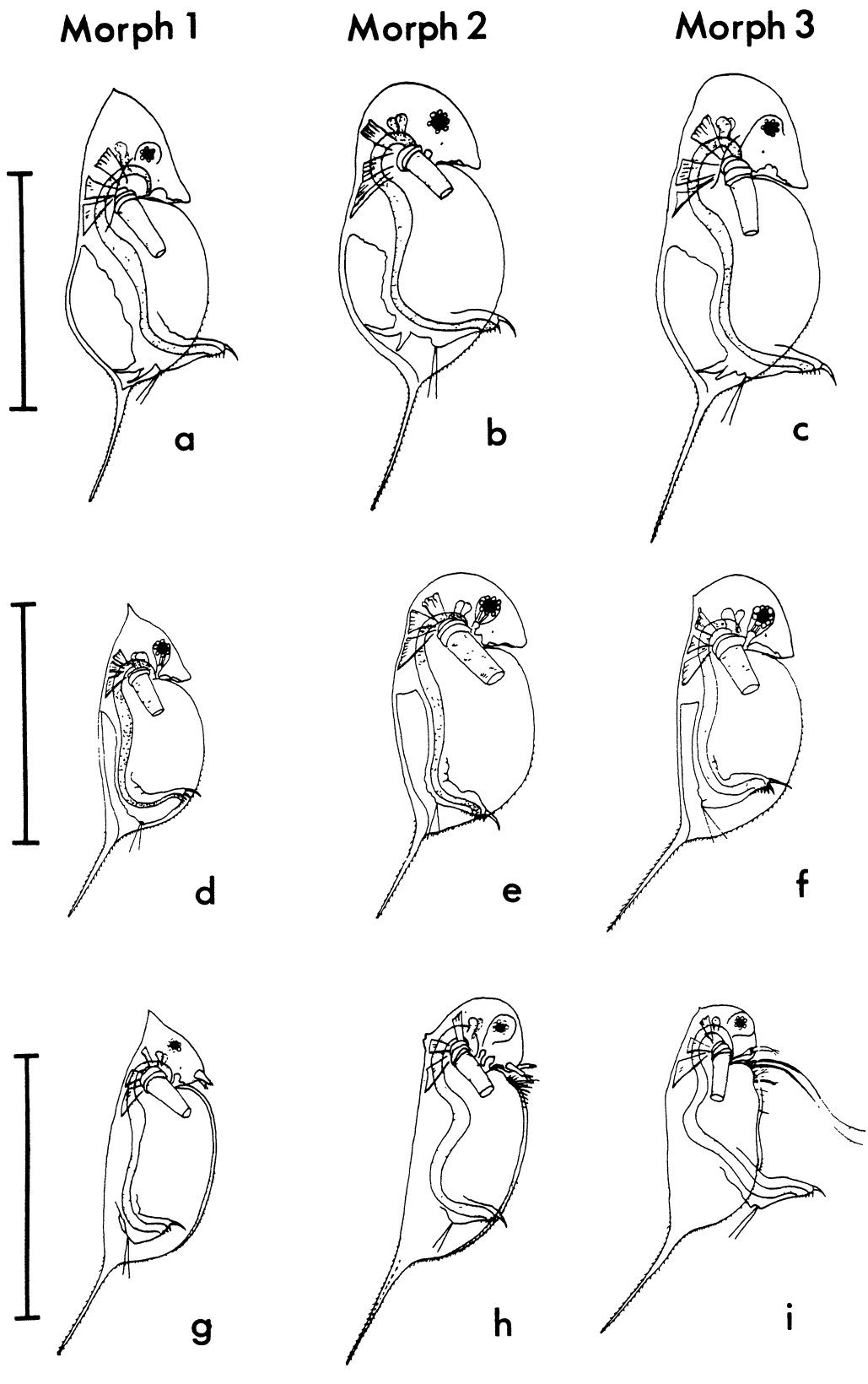
Morph 1
Morph 2
Morph 3
a
b
c
d
e
f
g
h
i

Table 1. Mean allele frequencies (AF) for morph 1, morph 2, and *Daphnia rosea,* and mean genotype frequencies (GF) for morph 3. Frequencies represent means of eight lakes for morph 1, nine for morph 2, and two for *D. rosea.*

Locus	Alleles (relative mobility)	Morph 1		Morph 2		*D. rosea*		Morph 3			
		AF	*N*	AF	*N*	AF	*N*	Observed genotypes	GF	*N*	No. of lakes
Ao	*a* (100)	—	418	1.00	575	1.00	325	*ab*	1.000	760	17
	b (142)	1.00		—		—					
Fum	*a* (88)	0.30	336	—	398	—	86	*ab*	0.282	436	17
	b (100)	0.70		1.00		1.00		*bb*	0.718		
Got-m	*a* (100)	—	269	1.00	225	1.00	66	*ab*	1.000	342	16
	b (127)	1.00		—		—					
Got-s	*a* (100)	0.20	321	1.00	522	1.00	147	*aa*	0.480	550	17
	b (114)	0.80		—		—		*ab*	0.520		
Ldh	*a* (68)	—	430	0.13	656	—	325	*ab*	0.008	772	17
	b (100)	1.00		0.87		1.00		*bb*	0.992		
Pep-A	*a* (100)	0.07	291	1.00	226	1.00	65	*aa*	0.156	317	14
	b (121)	0.93		—		—		*ab*	0.844		
Pep-D	*a* (81)	0.81	289	—	211	—	90	*ab*	0.812	292	16
	b (100)	0.19		1.00		1.00		*bb*	0.188		
Pgi	*b* (100)	0.96	249	1.00	557	1.00	218	*ab*	0.003	445	16
	c (107)	0.04		—		—		*bb*	0.995		
								bc	0.002		
Pgm	*a* (89)	0.60	296	—	621	—	255	*ab*	0.679	547	17
	b (100)	0.40		0.93		0.77		*ac*	0.100		
	c (109)	—		0.07		0.33		*bb*	0.219		
								bc	0.002		

Individuals of morph 3 were invariably heterozygous at *Ao* and *Got-m* (Table 1). These heterozygotes consisted of one allele typical of morph 1 and one typical of morph 2. At the remaining polymorphic loci, all but one allele (Pgi^a) in morph 3 individuals was detected in either morph 1 or in morph 2. This allele was, however, detected in morph 1 individuals from Michigan (D. Taylor unpubl.). The observed genotypes and their frequencies at all 16 loci were consistent with the hypothesis that morph 3 was an F_1 hybrid between the other two morphs.

Morph 2 was polymorphic at a lower proportion ($\bar{x}$ = 10.0%, range 0–12.5%) of the 16 loci than morph 1 ($\bar{x}$ = 21.9%, range 12.5–37.5%). Individuals of morph 3, however, showed extremely high levels of polymorphism ($\bar{x}$ = 38.4, range 25.0–50.0%). Differences in mean heterozygosity (H) over 16 loci mirrored those in polymorphism (Fig. 2). Populations of morph 3 (H = 0.337) had over 3 times greater heterozygosity than those of morph 1 (H = 0.101), and 14 times greater heterozygosity than those of morph 2 (H = 0.024). These differences provided a pattern of nonoverlapping heterozygosity estimates for the three morphs.

In May 1990, F_1 hybrids (i.e. morph 3) were detected in 17 of 22 lakes and were the dominant morphs in 10 (Fig. 3). Four lakes contained only hybrids, and on average, 44.5% of individuals collected in the 22 lakes were hybrids. Hybrids coexisted with either one or the other of the putative parent morphs in 12 lakes and with only a backcross/F_2 genotype in one lake. In three lakes (Blue, Kuhn, and Loon), hybrids were represented by single unique clones, while in seven others (Adams, Bear, Crooked, Lime, Lower Long, Wawasee, and Webster) hybrid populations consisted of two to five

←

Fig. 1. Morphs of *Daphnia galeata mendotae* from the Indiana lake district. Mature females: a—Center Lake, 3 October 1990; b—Smalley Lake, 2 October 1990; c—Tippecanoe Lake, 3 October 1990. Immature females: d—Silver Lake, 8 May 1990; e—Bear Lake, 6 May 1990; f—Bear Lake, 6 May 1990. Males: g—Center Lake, 3 October 1990; h—Old Lake, 3 October 1990; i—Blue Lake, 6 May 1990. (Scale bars—1 mm.)

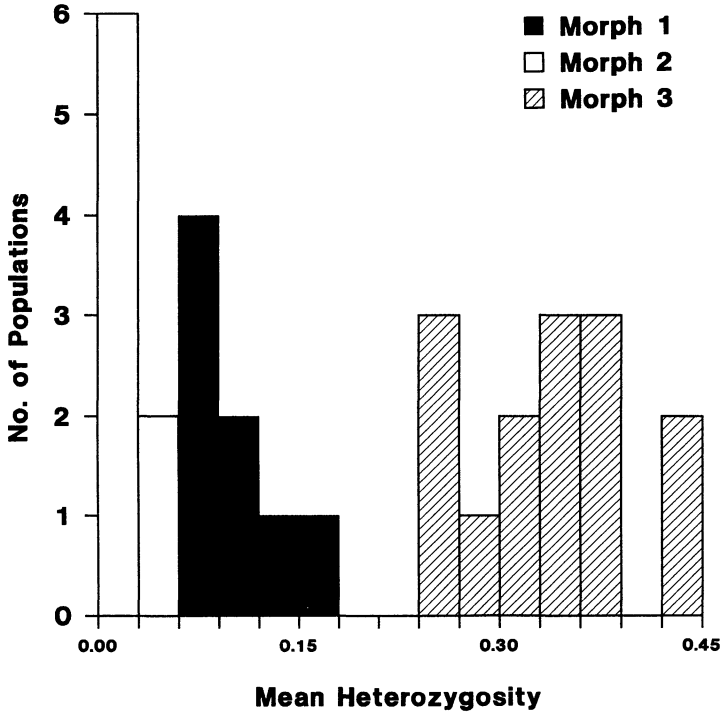

Fig. 2. Mean heterozygosity at 16 loci for three morphs of *Daphnia galeata* in 22 Indiana lakes.

clones each. The other seven hybrid populations were either clonally diverse (>5 clones) or sample sizes were too small to adequately assess clonal diversity (Little Crooked and Waubee). Putative backcrosses or F_2s, which were originally grouped with morph 3, were detected in four lakes. These individuals were identified by their possession of both recombinant hybrid genotypes and homozygous parental genotypes at diagnostic loci. At three sites, such genotypes included <10% of the population samples, but in one lake (Waubee), a single backcross clone constituted 91% of the complex. Among the remaining lakes, morph 1 dominated six lakes, while morph 2 dominated five.

Hybrids as well as both parent morphs were detected in each of the three watershed areas, but the morphs differed with respect to the size of lake in which they occurred. Morph 1 occurred in lakes of larger surface area [mean log(surface area in ha) = 4.90] than the lakes in which morph 2 occurred [mean log(surface area in ha) = 3.34], t = 3.01, df = 15, P = 0.009. Although the lake size distributions overlapped for the parent morphs, we never detected them coexisting. Instead, the lake size classes that overlapped between the parent morphs were dominated by the hybrid morph. The mean area of lakes occupied by hybrids [log(ha) = 4.32] was intermediate to those of the parent morphs, but differed significantly only from morph 2 (t = 2.07, df = 24, P = 0.05). *D. rosea* was detected in the two smallest lakes (Hammond and Allen) of the sample area. Even though lake depth (m) was correlated with surface area in the study area (r = 0.47, P = 0.005), no significant differences were found among depths of lakes in which different morphs occurred.

Allozyme analysis indicated that hybrids were less common in fall than in spring, being detected in only 9 of 15 lakes and comprising 18% of all individuals. The reduction in the relative abundance of hybrids was due to an increase in the abundance of morph 2 in small–medium-sized lakes. In larger lakes, however, hybrids actually increased in abundance relative to morph 1.

This study indicates that the phenotypic

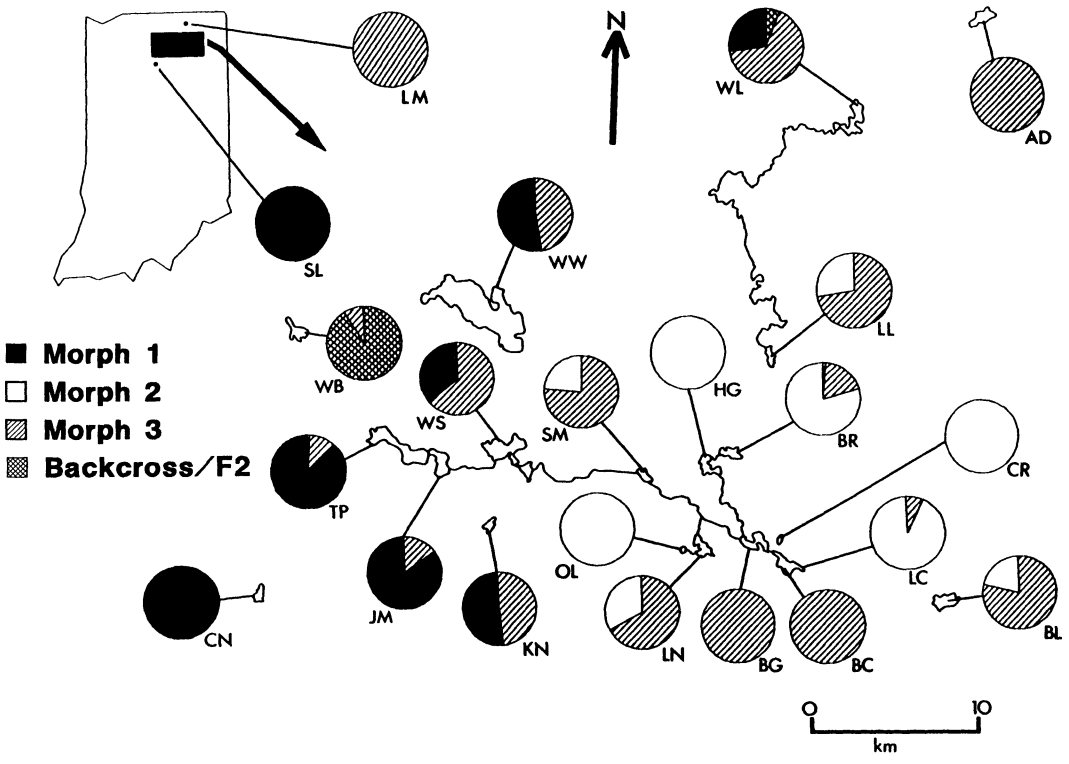

Fig. 3. Relative abundance of *Daphnia galeata* morphs (based on allozyme analyses) in May 1990 for 22 Indiana lakes. Sample sizes range from 35 to 168 with a mean of 79. Lake codes: AD—Adams; BC—Big Crooked; BG—Big; BL—Blue; BR—Bear; CN—Center; CR—Crane; HG—High; JM—James; KN—Kuhn; LC—Little Crooked; LL—Lower Long; LM—Lime; LN—Loon; OL—Old; SL—Silver; SM—Smalley; TP—Tippecanoe; WB—Waubee; WL—Waldron; WW—Wawasee; WS—Webster.

variability present among members of the *D. galeata* complex from Indiana has a strong genetic basis. It is clear that two species and their interspecific hybrids occur in these lakes. Our results suggest that only morph 1 individuals should be assigned to *D. galeata mendotae*. This was the only morph that possessed a tall, pointed helmet—a character that, according to Brooks (1957*b*), separates *D. galeata mendotae* from closely related species. The presence of fixed allozyme differences and a moderate genetic divergence (Nei's unbiased genetic distance = 0.35 for 16 loci) between *D. galeata mendotae* (i.e. morph 1) and morph 2 suggests that they are separate, closely related species. On the other hand, the near identical allozyme arrays and morphological similarities between morph 2 and *D. rosea* suggest that these two taxa are conspecific. Morph 2 shares with *D. rosea* the following characters: a slender tailspine, neckteeth, pinkish bodies, and a habitat preference for small lakes. Based on these similarities, we propose that morph 2 is a helmeted form of *D. rosea*.

Brooks (1957*b*) grouped morph 2 with *D. galeata mendotae* because of its morphological and ecological deviance from typical *D. rosea*. He described North American *D. rosea* as a western species that was most common in ponds and ordinarily lacked a cephalic crest. Morph 2's possession of a cephalic crest and its occupancy of lakes in the eastern half of North America provided Brooks (1957*b*) with the justification for its fusion with *D. galeata mendotae*. However, it is now well known that *D. rosea* occurs throughout most of North America (Brandlova et al. 1972). Moreover, Brooks (1957*b*) acknowledged that "robust" forms of *D. rosea* could develop crests (*see* the *robusta* and

dinotocephala synonyms of Kiser). More work is required to determine if morph 2 is a genetically divergent form of *D. rosea* or simply a phenotypic response to the unique conditions of Indiana marl lakes. These lakes are warmer (being at the southern extent of glaciation), more turbid (due to colloidal $CaCO_3$), and contain higher concentrations of calcium than other North American glacial lakes (Wetzel 1966).

Genetic evidence for interspecific hybridization between North American *Daphnia* has been obtained in only one case (*Daphnia pulex* × *pulicaria,* Hebert et al. 1989*a*). Morph 3, being intermediate with respect to *D. galeata* and *D. rosea* in genetic structure, morphology, and habitat occupancy, provides a second clear example of *Daphnia* hybridization in North America. Hybrids between members of the *D. galeata* complex and other sympatric *Daphnia* species (*ambigua, longiremis, pulicaria,* and *retrocurva*), could readily be recognized by fixed allozyme markers that exist for each of these species. However, as no heterozygotes were detected at these marker loci, we conclude that there is no hybridization between any of these species and members of the *D. galeata* group in Indiana (D. Taylor unpubl.).

The abundance of *Daphnia* hybrids from Indiana (at 44.5% in the spring) is the greatest yet recorded for lakes. F_1 hybrids constituted 21% of the *D. galeata* complex in Germany (Wolf and Mort 1986) and 1.3% of the complex in Czechoslovakia (Hebert et al. 1989*b*). Both of these studies did, however, find water bodies in which F_1 hybrids were temporarily dominant. Previous work has suggested that the hybrids present in many European lakes were the result of multiple hybridization events, as hybrid swarms were genotypically diverse (Hebert et al. 1989*b*). Moreover, Wolf and Carvalho (1989) found that F_1 hybrids hatched from ephippia collected from lake sediments. By contrast, the low number of multilocus genotypes detected within most Indiana hybrid populations suggests that high densities are often achieved by parthenogenetic replication of single hybrids. Given the parental variation, the evidence for asexual amplification of hybrids is particularly compelling in lakes where only one genotype was detected. Selection for certain hybrid clones may also have reduced diversity. Yet, as uniclonal samples were obtained at the probable peak of ephippial hatching (Wolf and Carvalho 1989), it is unlikely that selection accounts for the low diversity. In contrast to European lakes, in which cooccurrence of the parent species is common, the low frequency of hybridization events in Indiana lakes is almost certainly a consequence of the infrequent coexistence of both parent taxa.

A more challenging problem than the origins of hybrids is the maintenance of the entire hybrid complex. If hybrid forms were more fit than either parent taxa, then replacement of the parent species might occur on a regional scale. Such replacement did not occur in Indiana because of differing habitat preferences for the parent taxa and their hybrids. *D. rosea* was dominant in small lakes, hybrids were dominant in medium-sized lakes, and *D. galeata* was dominant in large lakes. These results support Lieder's (1983) suggestion that *Daphnia* hybrids have optimum habitats intermediate between those of the parents.

Their differing habitat preferences provide an ecological basis for persistence of the parent taxa, but hybridization might, if coupled with introgression, cause convergence of the parent forms. Although the present study detected both F_1 males and ephippial F_1 females, the number and abundance of backcross genotypes were low. There was, however, evidence for gene flow from *D. rosea* to *D. galeata* because uncommon alleles at three loci (*Pep-A*^{a}, *Pep-D*^{b}, *Got-s*^{a}) in *D. galeata* were the sole alleles present in *D. rosea.* Furthermore, the higher level of heterozygosity in *D. galeata* compared to *D. rosea* may, in part, be a product of this introgression.

It seems unlikely that hybridization in the *D. galeata* complex occurs only in Indiana. Several studies have figured animals from other parts of North America which bear a striking resemblance to the F_1 hybrids examined in this study (e.g. Woltereck 1932). These animals have often been interpreted as stages in a cyclomorphic succession. The present study reveals that hybrids are particularly cryptic when they coexist with *D.*

galeata. In such lakes, the temporal succession of hybrids and *D. galeata* creates a continuum of head shapes that is readily interpreted as cyclomorphosis. Clearly, the prevalence and geographic distribution of hybrids needs to be more thoroughly investigated.

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Cost of swimming by *Daphnia* during diel vertical migration

Abstract—*Daphnia hyalina* was grown under simulated conditions of diurnal vertical migration in vertical flow chambers, and its life-history parameters were evaluated. The amplitudes of the migrations were 3 and 60 m d^{-1}; food conditions were 0.1 and 1.0 mg C $liter^{-1}$. None of the investigated parameters (fecundity, individual growth rate, age of maturation) was significantly different between long- and short-distance migrating populations at high levels of food, whereas at low levels of food only the individual growth rate was higher in the short-distance migrating *Daphnia*. These results suggest that the energy spent to travel the vertical distance does not account for the metabolic costs of diurnal vertical migration.

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Several metabolic and demographic costs are associated with diel vertical migration (DVM) of zooplankton. Three components of these costs can be distinguished (*see* Lampert 1989): retarded metabolic rate due to migration through a vertical temperature