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Evolution

Habitat-dependent hybrid parentage and differential introgression between neighboringly sympatric *Daphnia* species

(cyclic parthenogenesis/mtDNA/polymerase chain reaction/allozymes)

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ABSTRACT Allozyme, morphological, and PCR-generated mtDNA markers were used to analyze hybrid parentage and introgression between the neighboringly sympatric crustaceans *Daphnia galeata mendotae* and *Daphnia rosea*. mtDNA analysis of *D. galeata mendotae* from 15 lakes and of *D. rosea* from 8 lakes revealed species-specific fragment patterns resulting from *Taq* I and *Rsa* I digestion. No individuals of one parent species possessed the typical mtDNA of the other parent species, suggesting that mtDNA introgression is rare or nonexistent. Hybrids from 18 lakes possessed either the mtDNA patterns of *D. rosea* or of *D. galeata mendotae*, indicating that reciprocal hybridization occurs. The mtDNA genome of the dominant parent species in a lake was overrepresented in the hybrids, suggesting that hybridization most often involves females of the common species and males of the rare species. Such a pattern is consistent with the differing importance of density to the environmental induction of males and sexual eggs in *Daphnia*. For the assessment of nuclear gene flow, eight sympatric populations of each parental taxon and seven allopatric populations of *D. galeata* were analyzed for allozyme variation at nine polymorphic loci. Our results provided evidence for asymmetric interspecific gene flow involving alleles at six loci that are unlikely to be convergent or symplesiomorphic. This reticulate evolution accounted for much of the genetic divergence between European and North American populations of *D. galeata*.

Hybridization and introgression have long been proposed as important evolutionary processes in the crustacean genus *Daphnia* (e.g., see refs. 1–4). Early evidence of hybridization was limited to the frequently reported morphological convergence among coexisting species (1, 4, 5). Yet the convergence of morphological characters can also be due to environmentally induced responses and to stabilizing selection. The resolution of these competing hypotheses depends on identification of independent heritable markers for the putatively hybridizing taxa. Recent allozyme studies (e.g., see refs. 6–8) identified such markers and established that interspecific hybridization is even more prevalent than was initially proposed. Ironically, these studies have shown that hybridization is absent where it was suspected on morphological grounds (e.g., *Daphnia pulex* × *Daphnia middendorffiana* and *D. pulex* × *Daphnia rosea*) but present in other cases in which morphological variation was attributed to phenotypic plasticity (6, 8, 9).

The prevalence of hybrids between *Daphnia* species may have important evolutionary implications. For example, hybridization events could lead to speciation if hybrid clones were able to produce asexual resting eggs or were able to continually reproduce by parthenogenesis in a persistent environment (4). Alternatively, if the hybrids were incompletely isolated from their parent taxa, then opportunities for introgressive hybridization exist. Gene flow across species boundaries may confer adaptations, alter habitat preferences, or even lead to phylogenetic reticulation (10, 11).

Unfortunately, detailed characterizations of *Daphnia* hybrid systems are lacking. As a result, little is known about the extent of introgression or about the process of hybridization itself. Analysis of molecular markers, particularly cytoplasmic DNA, has consistently yielded important insights concerning hybridization and introgression in both plant and animal hybrid systems (12, 13). mtDNA is especially useful in determining the importance of introgression and hybrid parentage because the molecule is generally nonrecombinant and maternally inherited. Yet, because of the small size (0.8–3.0 mm) of *Daphnia*, molecular analysis of natural populations has been difficult (14). Application of the PCR eliminates this problem because analysis of small amounts of DNA is possible.

The present study uses allozyme and PCR-generated mtDNA markers to investigate hybridization between *D. rosea* and *Daphnia galeata mendotae*. These species are neighboringly sympatric (sensu Grant, ref. 11) throughout much of temperate North America. That is, the two species often exist within dispersal range of one another in adjacent but ecologically different habitats. *D. rosea* lives in permanent ponds and small lakes while *D. galeata mendotae* resides in larger lakes and reservoirs (4, 8). Despite extensive geographic and temporal sampling, this ecological segregation is strong enough that authentic coexistence of sympatric *D. rosea* and *D. galeata mendotae* has not been reported (refs. 4, 8, and 15–17; D.J.T. and P.D.N.H., unpublished data). Allozyme analysis of this species pair in the Indiana Lake District revealed that interspecific hybrids are common (45%) and are more numerous than the parent taxon in some lakes (8). Because hybrids ordinarily coexist with only one of their parent species, hybridization probably involves rare *D. rosea* in *D. galeata mendotae*-dominated lakes and rare *D. galeata mendotae* in *D. rosea*-dominated lakes.

Past cytoplasmic DNA investigations of hybridization have shown that nonrandomness is commonly observed with respect to parentage, symmetry of introgression, and cytonuclear disequilibrium (12). Moreover, evidence exists that nonrandomness of both introgression and directionality is compounded when one of the hybridizing taxa is rare (18, 19). Another pattern emerging from plant and animal hybridization studies is that cytoplasmic DNA is apparently more susceptible to interspecific gene flow than is nuclear DNA (13, 20). Thus, when evidence of nuclear introgression is found, as has been proposed for the *galeata–rosea* complex (8), cytoplasmic introgression also usually occurs. Given this background, nonrandom hybrid parentage and mtDNA introgression might be expected in the *galeata–rosea* complex.

The present study seeks to (*i*) identify mtDNA markers for *D. rosea* and *D. galeata mendotae*, (*ii*) test the randomness of parental crosses that lead to successful hybridization, and

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(*iii*) assess the relative importance and degree of mtDNA and nuclear gene flow across species boundaries.

MATERIALS AND METHODS

mtDNA Analysis. A total of 295 individuals composed of 150 *D. galeata mendotae* from 24 lakes, 49 *D. rosea* from 8 lakes, and 96 F_1 hybrids from 18 lakes was analyzed for mtDNA variation. The samples were taken in May and June 1992, from four U.S. states (Indiana, Michigan, New York, and Washington) and two Canadian provinces (Ontario and New Brunswick; Table 1). Fifteen hybrid populations were from Indiana, two were from Michigan, and one was from Ontario (Table 2). Morphs (based on helmet shape) within lakes were characterized as *D. galeata mendotae, D. rosea*, or hybrids by their allozyme patterns at two apparently unlinked diagnostic loci (8). Prior to DNA extraction, individuals were assigned to a parent taxon or hybrid on the basis of helmet morphology. Taylor and Hebert (8) have shown that helmet shape characters allow accurate taxonomic assignment in most of the lakes in the present study. Because we have used only two diagnostic allozyme loci and backcrosses cannot be distinguished morphologically from F_1 hybrids, our classification of F_1 hybrids probably includes some backcrosses. Nevertheless, the influence of backcrosses on our interpretation is likely to be negligible because recombinant genotypes constituted <3% of hybrids. If 25% of backcrosses had hybrid genotypes, as expected with two diagnostic loci, then <4% of the F_1 hybrid class in the present study were actually backcrosses.

For DNA extractions, single live adult *Daphnia* were homogenized in 2× hexadecyltrimethylammonium bromide (CTAB) buffer in a 1.5-ml microcentrifuge tube and incubated for 10 min at 50°C. Single organic extractions of chloroform, phenol, and chloroform again were then conducted. The total DNA was then ethanol precipitated and resuspended in 50 μl of 1 mM Tris/EDTA.

Oligonucleotide primers used for amplification were homologous to portions of the 12S and 16S gene regions. The sequence of the 12S primer (5′-ATCGTGCCAGCCGTCGCGGTTA-3′) was based on a conserved region of the *D. pulex* 12S gene. The second primer (5′-CCGGTCTGAACTCAGATCA-3′) is a universal primer for the 16S gene.

The PCR mixtures contained the following reagents: 36 μl of distilled H_2O; 2 mM $MgCl_2$; 5 μl of 10× *Taq* polymerase buffer (500 mM KCl/100 mM Tris·HCl, pH 9.0/1% Triton X-100); 2.5 μl of a 10 mM mixture of dATP, dGTP, dCTP, and TTP; 2 μl of a 10 μM solution of each primer; 0.3 μl of *Taq* polymerase; and 5 μl of DNA template. The thermal cycles consisted of 1 cycle of 1 min at 94°C; 38 cycles of 1 min at 94°C, 1 min at 56°C, and 2 min at 72°C; and 1 cycle of 7 min at 72°C.

Table 1. Frequency of mtDNA haplotypes (based on *Rsa* I and *Taq* I digestion) in *D. galeata mendotae* and *D. rosea*

Taxon	mtDNA haplotype				Region	No. of lakes
	AA	BB	BC	BD		
D. galeata mendotae	—	36	29	—	IN	9
	—	7	—	—	MI	2
	—	—	15	—	NB	2
	—	7	—	—	NY	2
	—	8	25	23	ON	9
D. rosea	37	—	—	—	IN	6
	11	—	—	—	ON	1
	1	—	—	—	WA	1

See Table 3 for an explanation of haplotype letter designations. Number of individuals for each heplotype is shown.

Table 2. Frequency of mtDNA fragment patterns (based on *Rsa* I and *Taq* I digestion) in *D. galeata mendotae* × *D. rosea* hybrids

Dominant parent species	Lake	mtDNA haplotype				*D. galeata* haplotype frequency
		AA	BB	BC	BE	
D. rosea	Adams, IN	1				0.00
	Big, IN	5				0.00
	Big Crooked, IN	4				0.00
	Blue, IN	2	1			0.33
	Little Crooked, IN	9				0.00
	Loon, IN	1				0.00
	Lower Long, IN	4	2			0.33
	Smalley, IN	4			1	0.20
	Waubee, IN	10				0.00
D. galeata mendotae	Big Chapman, IN	3	1	1		0.40
	Eagle, MI		1			1.00
	Fine, MI		6			1.00
	Kuhn, IN	4			1	0.20
	St. George, ON			11		1.00
	Sylvan, IN	6	4			0.40
	Waldron, IN	1	6			0.86
	Wawasee, IN		2			1.00
	Webster, IN		4		1	1.00

Restriction enzyme analysis used 5–8 μl of the PCR product. The following 13 enzymes were screened for their ability to cut the amplification product: *Alu* I, *Ava* II, *Cfo* I, *Dde* I, *Dra* I, *Hae* III, *Hin*fI, *Hpa* II, *Msp* I, *Nci* I, *Rsa* I, *Sau*96I, and *Taq* I. Digestion products were then separated electrophoretically in 2% agarose gels and visualized with ethidium bromide and UV light.

Assessment of Nuclear Gene Flow. Allozyme electrophoresis followed the cellulose acetate gel methods of Hebert and Beaton (21). The nine polymorphic loci (see ref. 8) scored in the present study were aminoaspartate transferase (AAT; two loci; EC 2.6.1.1), aldehyde oxidase (AO; EC 1.2.3.1), glucose phosphoisomerase (GPI; EC 5.3.1.9), phosphoglucomutase (PGM; EC 5.4.2.2), lactate dehydrogenase (LDH; EC 1.1.1.27), fumarate dehydrogenase (FUM; EC 4.2.1.2), proline dipeptidase (PEP-D; EC 3.4.13.9), and dipeptidase (PEP-A; EC 3.4.13.11). Alleles were given ascending letter designations based on relative anodal migration—"a" was the most distant allele from the anode.

The simplest method of resolving the competing hypotheses of introgressive hybridization, convergence, and shared ancestral characters is to compare allopatric and sympatric parental populations (12). Independent genetic markers that are shared between hybridizing taxa in areas of sympatry but are reduced in frequency in allopatric populations within a taxon are most likely to be introgressed. Allopatric *D. rosea* has been reported from the Canadian high Arctic (22), whereas allopatric *D. galeata mendotae* has been reported from central Mexico (23). Unfortunately, our examination of populations from Mexico and from 300 lakes and ponds in the Canadian Arctic has led us to conclude that the allopatric reports were misidentifications (D.J.T. and P.D.N.H., unpublished data). As an allopatric reference group for North American *D. galeata mendotae*, we have used seven populations (Nanpanton Reservoir in England; Bodensee in Germany; and Hadí, Římov, Slapy, Velký Pálenec, and Žabinec Reservoirs in the Czech Republic) of the Eurasian subspecies *Daphnia galeata galeata*.

Metric multidimensional scaling of Rogers' genetic distance was used to assess genetic intermediacy of introgressed populations and to assess geographic variation in the extent of introgression (24). To minimize possible regional biases in interspecific nuclear gene flow, eight pairs of *D. galeata mendotae* and *D. rosea* populations were analyzed from five widespread sites in eastern North America. Populations were

from the following sites: Center Lake, Indiana; James Lake, Indiana; Baseline Lake, Michigan; Guelph Lake, Ontario; Belwood Lake, Ontario; Lake St. George, Ontario; Pinehaven Lake, New York; and Lake Morey, Vermont, for *D. galeata mendotae*; Hammond Lake, Indiana; Bear Lake, Indiana; Three Lakes Two, Michigan; Miller Lake, Ontario; Guelph unnamed pond, Ontario; Haynes Lake, Ontario; Round Pond, New York; Lake Mitchell, Vermont, for *D. rosea*. Interspecific hybridization occurs in or near each of the sampling sites (D.J.T., unpublished data) but F_1 hybrid genotypes (Ao^{ab}, $Aat\text{-}m^{ab}$) were excluded from the analysis.

RESULTS

mtDNA Variation. The PCR-amplified product was ≈1800 bp for all 295 individuals. Initial screening revealed no apparent restriction sites in *D. galeata mendotae* or *D. rosea* for *Cfo* I, *Ava* II, *Msp* I, *Nci* I, *Sau*96I, and *Hpa* II. Restriction sites were, however, present when the amplification product was digested with each of *Alu* I, *Dde* I, *Dra* I, *Hae* III, *Hin*fI, *Rsa* I, and *Taq* I and all of these enzymes except *Hae* III yielded interpretable restriction site differences between the parent taxa. Restriction enzyme profiles were subsequently obtained using *Rsa* I and *Taq* I for each specimen.

Digestion of the amplification products with *Rsa* I resulted in two fragment patterns differing by one restriction site (Table 3). All 49 *D. rosea* from three different sites possessed *Rsa* I pattern A, whereas all 150 *D. galeata mendotae* exhibited pattern B (Table 1). Five fragment patterns resulted from *Taq* I digestion and apparently differed by one to four restriction sites (Table 3). All 49 *D. rosea* possessed pattern A, whereas *D. galeata mendotae* possessed patterns B, C, and D (Table 1).

With the exception of *Taq* I pattern D, which occurred only in *D. galeata mendotae* populations north of the analyzed hybrid populations, all the fragment patterns found in *D. rosea* and in *D. galeata mendotae* were detected in individuals of hybrid origin (Table 2). The rare *Taq* I pattern E was only detected in three hybrid individuals from each of three neighboring lakes of the Tippecanoe River system. In each case, *Taq* I pattern E was associated with the *Rsa* I pattern B typical of *D. galeata mendotae*. Apart from pattern E, there was absolute linkage between the parental patterns of *Rsa* I and *Taq* I in hybrids.

Mitochondrial haplotypes were determined for a total of 96 hybrid isolates with 52 of these hybrids from lakes dominated by *D. galeata mendotae* and the balance from lakes dominated by *D. rosea*. Parentage of the hybrids was markedly nonrandom with respect to the parent species that dominated the lake (Table 2). In *D. galeata mendotae*-dominated lakes, hybrids possessed more *D. galeata mendotae* mtDNA patterns than expected by random mating (38 *D. galeata mendotae* versus 14 *D. rosea*; *G* test of goodness-of-fit, $G = 5.92$; $P < 0.025$). In contrast, the hybrids in *D. rosea*-dominated lakes possessed more *D. rosea* mtDNA patterns than expected by random mating (40 *D. rosea* versus 4 *D. galeata mendotae*; $G = 19.02$; $P < 0.001$). We reduced the influence of nonindependent hybridization events (due to hybrid clone mates) by comparing relative frequencies of *D. galeata mendotae* haplotypes from the two lake classes (Table 2). Although preliminary allozyme results indicated that each hybrid population possessed a minimum of three to five clones, using lake comparisons reduces the probability of hybrid nonindependence because each lake tends to possess unique hybrid clonal arrays (ref. 8; D.J.T., unpublished data). Still, the same parentage pattern emerged as hybrids from *D. galeata mendotae*-dominated lakes possessed a greater proportion of *D. galeata mendotae* mothers than were expected by chance (Wilcoxon signed-ranks test; $T = 4$; $P < 0.05$), and the hybrids from *D. rosea*-dominated lakes possessed more *D. rosea* mothers than were expected by chance (Wilcoxon signed-ranks test; $T = 0$; $P < 0.05$).

Nuclear Gene Flow Analysis. Allele frequency summaries for populations of *D. galeata galeata*, *D. galeata mendotae*, and *D. rosea* are given in Table 4. *D. galeata mendotae* and *D. rosea* share three alleles ($Aat\text{-}m^a$, $Aat\text{-}s^a$, $Pep\text{-}D^c$) that are absent and another allele (Gpi^c) that is greatly reduced in frequency in the allopatric reference populations of *D. galeata*. These alleles are inferred to have crossed species boundaries from *D. rosea* to *D. galeata mendotae*. Three of these alien alleles ($Aat\text{-}s^a$, $Pep\text{-}D^c$, and Gpi^c) have become

Table 3. Approximate fragment sizes and pattern designations of PCR-amplified *D. galeata mendotae*, *D. rosea*, and hybrid mtDNA segments digested with *Rsa* I and *Taq* I

Rsa I patterns		*Taq* I patterns				
A	B	A	B	C	D	E
1070	565	820	610	610	610	820
530	530	310	370	370	370	665
195	505	295	310	235	295	310
	195	195	295	210	235	
		175	210	160	210	
				135	75	
				75		

Table 4. Mean allele frequencies (AF) at nine polymorphic loci for eight populations each of *D. galeata mendotae* and *D. rosea* and seven populations of *D. galeata galeata*

		D. galeata galeata		*D. galeata mendotae*		*D. rosea*	
Locus	Allele	AF	*n*	AF	*n*	AF	*n*
Aat-m	*a*		240	0.023	368	1.000	405
	b	1.000		0.977			
Aat-s	*a*		262	0.579	546	1.000	527
	b	1.000		0.421			
Ao	*a*		262		429	0.819	432
	b					0.022	
	c	1.000		1.000		0.159	
Fum	*a*		239	0.054	402		353
	b	0.998		0.946		0.991	
	c					0.009	
	d	0.002					
Gpi	*a*	0.016	262		657		520
	b	0.906		0.062			
	c	0.061		0.913		1.000	
	d			0.025			
	e	0.017					
Ldh	*a*		234		429	0.028	515
	b	1.000		1.000		0.972	
Pep-A	*a*	0.017	259		484		457
	b	0.925		0.083		0.953	
	c			0.038			
	d	0.058		0.879		0.047	
Pep-D	*a*		231	0.786	594		480
	b	0.101					
	c			0.214		1.000	
	d	0.686					
	e	0.213					
Pgm	*a*		242	0.007	569		392
	b	0.124					
	c	0.342		0.629			
	d	0.527		0.361		0.659	
	e	0.007		0.003		0.323	
	f					0.018	

Frequencies of proposed introgressed alleles are underlined.

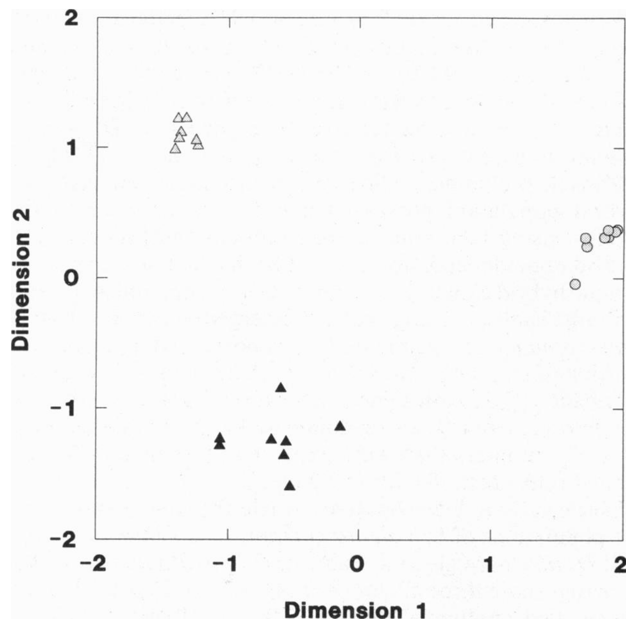

FIG. 1. Two-dimensional scaling of Rogers' genetic distances among seven populations of *D. galeata galeata* (stippled triangles), eight populations of *D. galeata mendotae* (solid triangles), and eight populations of *D. rosea* (stippled circles). The squared correlation increases from R^2 = 0.715 for a one-dimensional solution to R^2 = 0.977 for a two-dimensional solution.

more frequent than native alleles in some populations and even replaced the native alleles in four populations: $Aat\text{-}s^a$ in Lake St. George and Pinehaven Lake, and Gpi^c in Center Lake and James Lake. Limited gene flow may also have occurred in the other direction as the uncommon alleles at Ao^c and $Pep\text{-}A^d$ in *D. rosea* are the common alleles in *D. galeata mendotae*. Multidimensional scaling of genetic distances revealed the extent of introgression among populations (Fig. 1). In dimension one, all eight *D. galeata mendotae* populations grouped closer to *D. galeata galeata* than to *D. rosea* but were genetically intermediate to *D. rosea* and *D. galeata galeata*. No geographic component to the introgression was apparent. Overall, the allelic arrays and multidimensional scaling are consistent with widespread asymmetric bidirectional nuclear introgression.

DISCUSSION

The finding that hybrids can possess either the mtDNA genome of *D. galeata mendotae* or the mtDNA genome of *D. rosea* indicates that successful reciprocal hybridization occurs. Yet the direction of this cross is nonrandom, as the maternal genome of the dominant parent is overrepresented in hybrids. We believe the most plausible explanation for this pattern is linked to the induction and phenology of sex in *Daphnia*. Males are first produced parthenogenetically and then, independently, sexual eggs are formed in females. Generally, these responses are induced or correlated to periods of high density (spring and/or fall) for a given species (25). *D. rosea* × *D. galeata mendotae* hybridization must occur when one parent is rare and the other is common because both species are never codominant. So how is sex induced in the rare species?

Sex determination in *Daphnia* is environmental and the induction of males is mediated by a chemical (probably a metabolite) that results from crowding (26, 27). Moreover, Hobæk and Larsson (27) have shown that this chemical is not species specific and can induce male production even among distantly related species. Interestingly, the interspecific chemical density cues do not induce sexual egg formation, which is apparently initiated by more stringent or different environmental cues. If this model of sexual induction applies to *D. galeata mendotae* and *D. rosea*, then sex in the rare species should be biased toward males and, as a consequence, the mtDNA genome of the rare species should be underrepresented in hybrids. This mechanism of hybrid formation is generally consistent with our data but some lakes (especially those dominated by *D. galeata mendotae*) do contain hybrids from both reciprocal crosses. Such a pattern may be due to hybrid immigration or to the influence of environmental cues other than density for sexual induction. For example, decreasing photoperiod may be the prime sexual cue for *D. rosea* (and its relatives) because sexual phases are not as strongly associated with population density as they are in *D. galeata* (28–32).

Our study also provides insight concerning the evolutionary implications of introgression between *D. galeata mendotae* and *D. rosea*. As these species exhibit widespread neighboring sympatry and frequently coexist with large numbers of hybrids, the potential for interspecific gene flow is great. Nevertheless, our results indicate that nuclear gene flow has occurred in the absence of accompanying mtDNA gene flow. It is possible that sampling of the parental genomes was sufficient to detect nuclear gene transfer but insufficient to detect mtDNA transfer. The model of Avise and Saunders (18) shows that, for a given backcross introgressant, the mean number of mtDNA markers is half of the number of nuclear markers. In addition, because the effective population size of mtDNA is a quarter of the population size of nuclear genes, a rare introgressed mtDNA genome is more likely to be eliminated by fixation than are alien nuclear genes.

Even so, several lines of evidence suggest that mtDNA gene flow is negligible between *D. galeata mendotae* and *D. rosea*. First, our sample sizes were large with respect to both number of parents and number of populations analyzed in an attempt to offset the effects of haplotype fixation. Second, in the absence of selection, Takahata and Slatkin (33) have shown that very low levels of introgression are enough for alien mtDNA to become established and eventually replace the native genome. Third, empirical evidence suggests that cytoplasmic DNA is generally more susceptible than nuclear DNA to introgression in both animals (12) and plants (13). Finally, hybrid females with sexual eggs may be unimportant in backcrossing as they are very rare compared to hybrid males (D.J.T., unpublished observation). Carvalho and Wolf (34) also noted that hybrids between *D. galeata* and *Daphnia hyalina* (a relative of *D. rosea*) frequently produced empty ephippia. Consequently, the lack of accompanying mtDNA introgression may be due to a rarity of fertile sexual female hybrids. If so, then our results reveal that biased reciprocal backcrosses may influence the penetrance of species boundaries by cytoplasmic DNA in organisms that are not prone to Haldane's rule (i.e., species in which sex is environmentally determined).

Of course, it is also possible that we have not detected mtDNA gene flow because there is no gene flow of any sort. Yet the available evidence is inconsistent with ancestral or convergent alleles as an explanation of the shared alleles. First, morphological and allozyme results agree that *D. galeata mendotae* and *D. rosea* are not sister taxa (4, 35–37). The sister taxa are *D. galeata galeata* for *D. galeata mendotae*, and *Daphnia thorata* or *D. hyalina* for *D. rosea*. Our results show that of the possible introgressed alleles, only $Pep\text{-}A^b$ in *D. galeata mendotae* can be considered a shared ancestral allele with *D. galeata galeata*. Although we did not have allopatric reference populations for *D. rosea*, the proposed alien alleles in this species ($Pep\text{-}A^d$ and Ao^c) are probably not shared ancestral alleles because two closely related species (*D. thorata* and *D. hyalina*) are fixed for the

common *D. rosea* alleles at these loci (D.J.T., unpublished data). Second, *D. galeata mendotae* is allozymically (ref. 8; D.J.T., unpublished data) and morphologically (17) the most polymorphic sexual species of the subgenus *Daphnia* in North America. Much of the allozyme variation is due to alleles whose presence can be explained by introgression of alleles that are shared with *D. rosea*. The simultaneous convergence at six of the nine polymorphic loci in *D. galeata mendotae* and *D. rosea* is improbable. An additional observation that neither convergence nor shared ancestry predicts is that *D. rosea* from pond populations have no *D. galeata mendotae* alleles at the marker loci, whereas *D. rosea* in nearby lakes (Haynes, Sunfish, Miller, Round) do possess *D. galeata mendotae* alleles. This observation, however, is consistent with introgression because *D. galeata mendotae* never occurs in ponds (4).

Although the low number of backcrosses suggests that introgression is limited, our results show that alien alleles may be amplified by processes other than direct infusion and eventually replace native alleles. Such a pattern may be due to a combination of long-term introgression, selection, and founder events. We conclude that *D. rosea* × *D. galeata mendotae* hybrids are not sterile but have indeed served as a conduit for introducing new alleles into the parental gene pools. The asymmetric leakage has provoked the convergence of North American populations of *D. galeata* toward *D. rosea* and accounts for a large portion of its genetic divergence from conspecific European populations. This reticulate evolution has set the stage for speciation and may also contribute to the extraordinary morphological variation found in *D. galeata mendotae*.

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