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Infraspecific mitochondrial sequence diversity in *Hydrobia ulvae* and *Hydrobia ventrosa* (Hydrobiidae: Rissooidea: Gastropoda): Do their different life histories affect biogeographic patterns and gene flow?

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Infraspecific relationships and population parameters are largely unknown in the ecologically significant mud snail genus *Hydrobia s.l.* We therefore studied infraspecific variation, population structure and gene flow in two *Hydrobia* species with different life history strategies: the marine, planktonic *H. ulvae* and the brackish-water, directly developing *H. ventrosa*. Based on sequencing data of a 638 bp fragment of the mtDNA gene for cytochrome oxidase I, we found considerable differences between the two species. *H. ulvae* shows high average pairwise nucleotide diversity, low population level differentiation (F_{ST}), and high average gene flow (Nm) between populations. Dispersal appears to accord with Wright's island model. In contrast, many populations of *H. ventrosa* have high population level differentiation and low gene flow. The average pairwise nucleotide diversity is relatively low; this species disperses according to Wright's isolation by distance model. Differences in dispersal modes and gene flow could be partly due to differences in type of early ontogeny and quantitative differences in passive dispersal. However, the fact that *H. ulvae* is a marine species with high tolerance to environmental stress and therefore less sensitive to migration barriers than *H. ventrosa* may better explain these differences. The extant lineages of *H. ulvae* and *H. ventrosa* most likely evolved in the northeastern Atlantic during the Pleistocene.

ADDITIONAL KEYWORDS:—mtDNA – cytochrome oxidase I – population genetics – evolutionary biology – dispersal – F_{ST} – Nm.

CONTENTS

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INTRODUCTION

The marine to brackish-water species of *Hydrobia s.l.* belong to one of the most intensely studied molluscan genera in the world. They have become a paradigm for diverse research topics involving parasitism, energetics, deposit feeding and nutrition, reproductive effort and food chain resources. However, our knowledge about infraspecific genetic variation is very limited. Studies of genetic diversity, biogeographic patterns and gene flow in these ecological very significant taxa are important for understanding their evolution. Infraspecific variation is the key to understanding patterns and processes of speciation in morphostatic species. Such studies also help testing of hypotheses concerning dispersal patterns (e.g. island or isolation by distance models) and can be used to estimate coalescence time (time since the appearance of the most recent common ancestor) and spatial origin of recent populations.

Studies of biogeographic patterns in *Hydrobia* species are difficult. *Hydrobia* taxa belong to morphostatic radiations, i.e. radiations with little niche differentiation and correspondingly low morphological and anatomical differentiation (Davis, 1994). Species lack robust morphological and anatomical characters which can be used for studies of infraspecific variation. However, species of *Hydrobia* are largely distinguishable by environmental factors (salinity, substratum, competition, parasitism).

Molecular methods are likely to provide a solution. There are few studies on *Hydrobia* using a molecular approach. In fact, the only studies dealing with infraspecific diversity are those by Davis *et al.* (1988, 1989) on the American *Hydrobia truncata* based on allozyme electrophoresis.

We used mtDNA sequencing data to compare genetic diversity, biogeographic patterns and gene flow in two *Hydrobia* species: *H. (Peringia) ulvae* (Pennant, 1777) and *H. (Ventrosia) ventrosa* (Montagu, 1803). Animal mitochondrial DNA has some characteristics that makes it interesting not only for phylogenetic studies in higher taxa but also for inter- and infraspecific comparisons: (1) mtDNA is usually uniparentally (maternally) inherited with no known recombination; thus each genotype is a haplotype; (2) the phylogenetic content of mtDNA gene trees reflects the past of a species and adds a historical perspective to population structure (Avise, 1992); (3) the rate of substitutions in mtDNA usually is higher than in single-copy nuclear DNA. This is essential for molecular work at the population level; (4) mtDNA genes are simple single copy genes, precluding problems with paralogy; (5) the mtDNA content in many tissues is rather high enabling DNA isolation from single *Hydrobia* specimens (often with dry weights under 1 mg).

We chose a fragment of the cytochrome c oxidase subunit I gene (COI) because

in the superfamily Rissooidea it does not show a significant degree of saturation below the superfamily level (Davis *et al.*, 1998). Thus, the data presented here for infraspecific variations can also be used for further studies at the species, generic and family levels. The substitution rate in COI is sufficiently high to provide a reasonable degree of divergence between and within populations necessary for the analysis of population structure and gene flow.

The two species used here are well suited for infraspecific studies. They have large distribution ranges: *H. ulvae* ranges from southern Spain (probably also the northwestern coast of Africa) throughout the North Sea and Baltic Sea to Spitsbergen, the Russian White Sea and Novaya Zemlya; *H. ventrosa* is found from the Mediterranean via the Iberian Peninsula, North Sea and Baltic Sea to Iceland and the White Sea. The populations usually occur at high densities in many intertidal ecological settings, and their life history and life strategies are well studied. The studies show that the biology of these frequently sympatric species differs in many regards—the reason why these two species have been chosen for the work presented here. The most striking difference involves their development. *Hydrobia ulvae* has planktonic, free swimming veliger larvae (planktonic development). In contrast, the nonplanktonic embryonic development of *H. ventrosa* takes place inside the egg capsule (direct development) (Pilkington, 1971; Falniowski, 1987).

Ecologically, the species are different as well. Whereas *H. ulvae* is a brackish-marine species tolerating higher salinity, *H. ventrosa* occurs predominantly in brackish waters with lower salinity (see Falniowski, 1987 for reviews). There also are major differences in their tolerance of water movement. *H. ulvae* frequently can be found in open waters, exposed to waves and with regular water exchange. It usually avoids stagnating habitats, such as backwaters, which are preferred by *H. ventrosa* (Cherrill & James, 1985; Falniowski, 1987). *H. ulvae* also seems to be much less sensitive to environmental stress than *H. ventrosa* and can, under some circumstances, replace *H. ventrosa* in its typical habitat (Fenchel & Kofoed, 1976; Falniowski, 1987).

In summary, *H. ulvae* is usually considered to be a more marine species with apparently high dispersal abilities and high tolerance to environmental stress, whereas *H. ventrosa* is a typical brackish-water species with limited dispersal abilities and usually restricted to isolated backwaters.

One would expect that *H. ulvae* might disperse according to the island model of Wright (1931) and *H. ventrosa* according to the isolation by distance model (Wright, 1943). The classical island model is characterized by thorough mixing of long-distance migrants from different source populations. The isolation by distance model is characterized by discrete populations where dispersal is usually restricted between neighbouring populations (Craddock *et al.*, 1997). Accordingly, we expected reduced geographic variation and population structure through increased gene flow in *H. ulvae*, and clear biogeographic patterns and genetically distinct populations with limited gene flow in *H. ventrosa.*

To examine these assumptions we determined nucleotide sequences for the mitochondrial COI gene from ten populations of each species. Sequence data were used to: (1) calculate infraspecific genetic variation; (2) analyse infraspecific relationships by phylogenetic networks; (3) test dispersal hypotheses (island model vs isolation by distance model); (4) calculate gene flow between populations; (5) estimate coalescence time and origin of the most recent common ancestor.

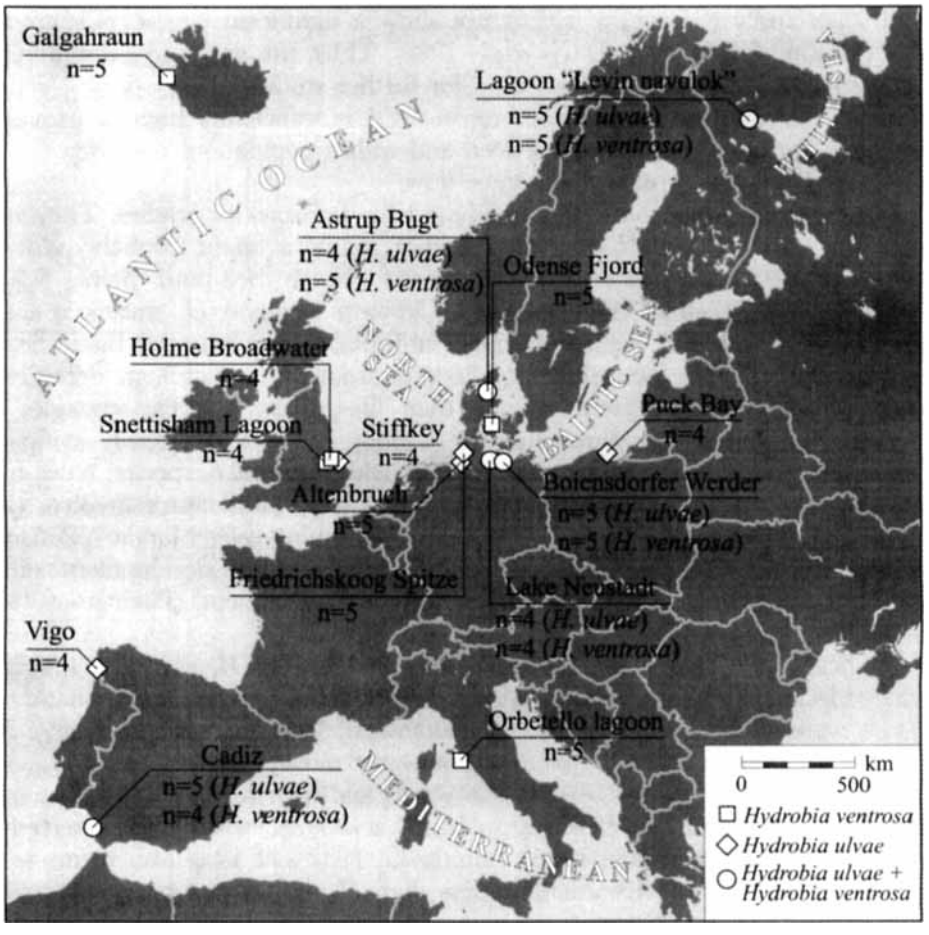

Figure 1. Localities sampled and number of individuals per population studied.

MATERIAL AND METHODS

Taxa studied

Localities sampled are distributed throughout the known distribution ranges (Fig. 1; Table 1). They include populations from the center of the distribution area (North Sea and Baltic Sea) as well as from the margins (southern Spain and the Russian White Sea for *H. ulvae*; the northern Mediterranean, west Iceland and the Russian White Sea for *H. ventrosa*). Four or five specimens respectively were sequenced from each population; a total of 45 individuals for *H. ulvae* and 46 for *H. ventrosa*. The specimens were brought to the USA alive and immediately prior to isolation of DNA quick-frozen at −80°C. *Cincinnatia winkleyi* (Pilsbry, 1912) (Hydrobiinae: Hydrobiidae) from Spurwink River, Maine, U.S.A. was used as outgroup (Individual #: 632; GenBank accession # AF118370).

TABLE 1. Localities and collection information for the specimens studied

Hydrobia ulvae			*Hydrobia ventrosa*		
Localities	Indiv. #	GenBank #	Localities	Indiv. #	GenBank #
North Atlantic, Spain, Bay of Cadiz, lagoon system 'La Imposible' (36°23.62′N, 6°8.25′W)	Ca 582	AF118279	Mediterranean Sea, Italy, Orbetello Lagoon (42°27′N, 11°13′E)	Or 663	AF118324
	Ca 814	AF118280		Or 665	AF118325
	Ca 815	AF118281		Or 666	AF118326
	Ca 1068	AF118282		Or 675	AF118327
	Ca 1069	AF118283		Or 676	AF118328
North Atlantic, Spain, Vigo, Bay O Grove (42°28.4′N, 8°51.3′W)	Vi 974	AF118284	North Atlantic, Spain, Bay of Cadiz, lagoon system 'La Imposible' (36°23.62′N, 6°8.25′W)	Ca 683	AF118329
	Vi 975	AF118285		Ca 684	AF118330
	Vi 979	AF118286		Ca 685	AF118331
	Vi 980	AF118287		Ca 1071	AF118332
North Sea, Great Britain, Stiffkey Saltmarsh (52°57.4′N, 0°55.8′E)	St 719	AF118288	North Sea, Great Britain, Snettisham Lagoon (52°51.8′N, 0°27.6′E)	Sn 715	AF118333
	St 720	AF118289		Sn 716	AF118334
	St 721	AF118290		Sn 717	AF118335
	St 722	AF118291		Sn 718	AF118336
North Sea, Germany, Altenbruch (53°51′N, 8°45′E)	Al 543	AF118292	North Sea, Great Britain, Holme Broadwater (52°58.6′N, 0°33.9′E)	Ho 711	AF118337
	Al 544	AF118293		Ho 712	AF118338
	Al 545	AF118294		Ho 713	AF118339
	Al 546	AF118295		Ho 714	AF118340
	Al 547	AF118296			
North Sea, Germany, Friedrichskoog Spitze (54°02′N, 8°50′E)	Fr 394	AF118297	North Atlantic, Iceland, Galgahraun, Alftanes (64°28′N, 22°10′W)	Ga 555	AF118341
	Fr 395	AF118298		Ga 561	AF118342
	Fr 399	AF118299		Ga 562	AF118343
	Fr 401	AF118300		Ga 563	AF118344
	Fr 402	AF118301		Ga 564	AF118345
White Sea, Russia, Lagoon 'Levin navolok' (66°32′N, 33°53′E)	Le 608	AF118302	White Sea, Russia, Lagoon 'Levin navolok' (66°19′N, 33°32′E)	Le 611	AF118346
	Le 609	AF118303		Le 612	AF118347
	Le 610	AF118304		Le 968	AF118348
	Le 1064	AF118305		Le 1066	AF118349
	Le 1065	AF118306		Le 1067	AF118350
Kattegat, Denmark, Astrup Bugt (56°41′N, 10°13′E)	As 971	AF118307	Kattegat, Denmark, Astrup Bugt (56°41′N, 10°13′E)	AS 851	AF118351
	As 972	AF118308		As 852	AF118352
	As 973	AF118309		As 853	AF118353
	As 1074	AF118310		As 1072	AF118354
				As 1073	AF118355
Baltic Sea, Germany, Neustadt in Holstein (54°06′N, 10°49′E)	Ne 920	AF118311	Kattegat, Denmark, Odense Fjord (55°30′N, 10°32′E)	Od 426	AF118356
	Ne 921	AF118312		Od 427	AF118357
	Ne 922	AF118313		Od 428	AF118358
	Ne 923	AF118314		Od 429	AF118359
				Od 431	AF118360
Baltic Sea, Germany, Boiensdorfer Werder (54°01.9′N, 11°31.4′E)	Bo 530	AF118315	Baltic Sea, Germany, Neustadt in Holstein (54°06′N, 10°49′E)	Ne 916	AF118361
	Bo 531	AF118316		Ne 917	AF118362
	Bo 532	AF118317		Ne 918	AF118363
	Bo 533	AF118318		Ne 919	AF118364
	Bo 534	AF118319			
Baltic Sea, Poland, Puck Bay, Kuznica (54°43′N, 18°37′E)	Pu 854	AF118320	Baltic Sea, Germany, Boiensdorfer Werder (54°01.9′N, 11°31.4′E)	Bo 373	AF118365
	Pu 857	AF118321		Bo 374	AF118366
	Pu 858	AF118322		Bo 375	AF118367
	Pu 859	AF118323		Bo 379	AF118368
				Bo 381	AF118369

DNA preparation

The methods of Spolsky *et al.* (1996) and Davis *et al.* (1998) were used for preparing DNA from individual snails.

DNA amplification

For PCR of a 658 bp long fragment of the COI gene the primer pair COF14 (forward: 5′ GGTCAACAAATCATAAAGATATTGG 3′) and COR722b (reverse: 5′ TAAACTTCAGGGTGACCAAAAAATYA 3′) was used. COF14 is identical to primer LCO1490 as described by Folmer *et al.* (1994). COR722b is a modification of Folmer *et al.* (1994) primer HCO2198 at position 24 (C→Y).

Cloned Pfu DNA polymerase (Stratagene) was used for the PCR. A total of 50 µl reaction mixture was prepared according to their protocol. PCR amplification was performed with the following profile: 90 sec at 95°C, 36 × (60 sec at 95°, 80 sec at 48°C, 70 sec incremented by 1 sec per cycle at 72°C), 300 sec at 74°C.

The quality of PCR product was determined by electrophoresis through a 1% agarose gel in TBE. For purification of DNA products we used Wizard PCR preps (Promega). Final DNA concentration was determined using a Hoefer TKO100 fluorometer.

Sequencing

Sequences were determined by automated cycle sequencing, using the LI-COR DNA sequencer Long ReadIR 4200 and Thermo Sequenase fluorescent labeled primer cycle sequencing kit with 7-deaza-dGTP (Amersham) according to their protocol. Cycling conditions consist of 30 cycles of 30 sec at 95°C, 15 sec at 51°C and 15 sec at 72°C for the forward primer and 30 cycles of 30 sec at 95°C, 15 sec at 56°C and 15 sec at 72°C for the reverse primer. 1.5 µl of stop solution were added after cycling. Before loading, the samples were heated 2 minutes at 95°C and loaded immediately on a 4% Long Ranger denaturing gel (FMC).

Data analyses

COI sequences for each individual were assembled and edited by eye using ESEE 3.0 s (Cabot & Beckenbach, 1989). In our sequences the first two to ten base pairs beyond each primer were often rich in ambiguities. We therefore uniformly excluded the first and last ten base pairs of each sequence, leaving a 638 base pair fragment for subsequent analyses.

A Kimura distance matrix was computed using DNADIST of PHYLIP version 3.57 (Felsenstein, 1989, 1993).

Population level differentiation (F_{ST}) and gene flow (Nm) were calculated (Hudson *et al.*, 1992) as follows:

$$F_{ST} = 1 - \frac{H_w}{H_b} \qquad Nm = \frac{1}{2}\frac{H_w}{H_b - H_w}$$

H_w is the mean number of differences between sequences from the same population, and H_b is the mean number of differences between sequences from different populations. Both equations assume Wright's island model with an infinite number of subpopulations of equal size, equal average diversity and no mutations.

The F_{ST} approach used here is substantially different from Wright's (1951) original exposition of the concept based on variance among gene frequencies. Accordingly, the results of both methods may be different and should and can not be compared.

F_{ST} and *Nm* values were determined using the software program DnaSP 2.52 (Rozas & Rozas, 1997). F_{ST} and *Nm* approaches are particularly sensitive to unequal population sizes and differential migration rates (Peter Beerli, pers. comm.). Therefore, all numbers given for F_{ST} and *Nm* are rough estimates only.

The geographic distance in ocean km between *Hydrobia* populations was determined as shortest waterway (straight line distances around major capes and islands) using the geographical information system Arc View 3.0a (ESRI Inc.) in connection with the digital map ArcWorld 1:3M (ESRI Inc.). The relationships between genetic distance or population level differentiation and geographic distance were tested by application of the Mantel test (ISOLDE procedure of the GENEPOP 3.1d software package; Raymond & Rousset, 1995).

The topologies of infraspecific relationships are here represented as networks using the proposal of Palumbi *et al.* (1997). According to these workers "in many cases sequences ... are hypothesized to reside at nodes of the phylogenetic trees". Thus, it is more informative to draw the stepwise evolutionary relationships among existing sequences as a network rather than a tree with many 'zero' branch lengths.

Infraspecific phylogenetic networks were obtained using the following procedures:

(1) haplotypes were determined by visual inspection of distance trees generated from Kimura distance matrices using the FITCH program of PHYLIP 3.57;
(2) maximum likelihood trees for each species were constructed from the haplotypes using DNAML of PHYLIP 3.57. *Cincinnatia winkleyi* # 632 and *Hydrobia ventrosa* # Sn 715 were used as outgroups for *H. ulvae* and *Cincinnatia winkleyi* # 632 and *Hydrobia ulvae* # St 719 as outgroups for *H. ventrosa*. Twenty-five repetitions, with randomized input order and optimization by global branch rearrangement, were run for each analysis;
(3) branches greater than zero were exported into the graphic package Corel Draw 7.0 to construct the phylogenetic networks;
(4) nucleotide substitutions between neighboring sequences were determined by comparing these sequences using ESEE 3.0s. Substitutions were enumerated along the branches of the network according to their positions in the aligned sequences.

RESULTS

Of the 45 sequences obtained for *Hydrobia ulvae*, 32 (71.1%) were different: one haplotype is shared by twelve individuals, three haplotypes are each shared by two individuals and 28 sequences are unique. A comparison of all sequences for *H. ulvae* shows that 69 (10.82%) of 638 sites are polymorphic.

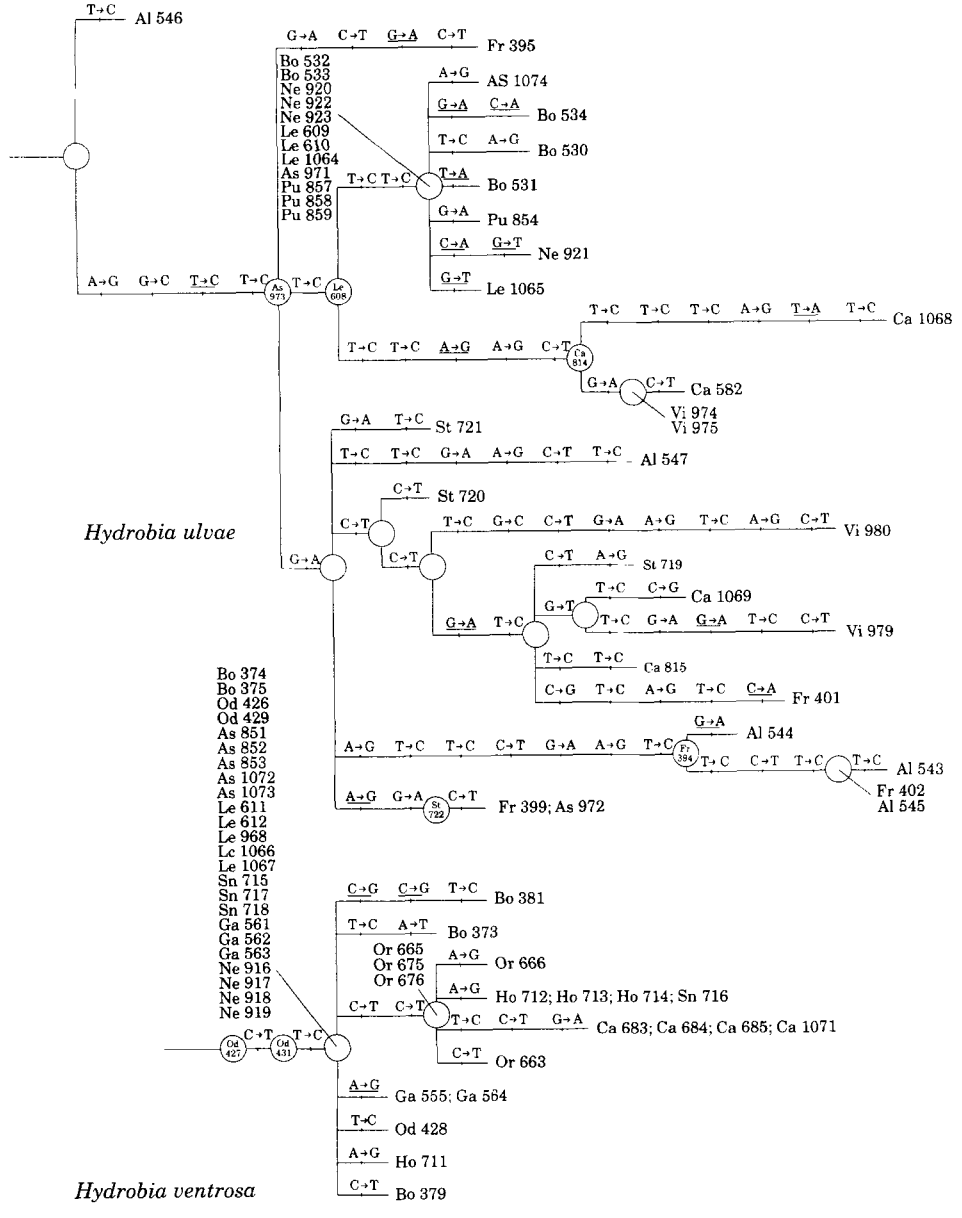

Figure 2. Phylogenetic networks for *H. ulvae* and *H. ventrosa* based on maximum likelihood trees. Substitutions are plotted along the branches. Non-silent substitutions are underlined. Ancestral sequences are shown in circles. Empty circles are hypothesized ancestral nodes not found in the data set presented here. For localities see Table 1. Network construction was adopted from procedures of Palumbi et al. (1997).

The relationships among specimens of *H. ulvae* (Fig. 2A) indicate: (1) few hypothetic ancestral sequences, sitting deep in the network, could be detected; (2) three of five sequences obtained from specimens from the Russian White Sea are identical with sequences from the Baltic Sea, although the geographic distances between the

populations ranges from 3518 to 4159 ocean km; (3) all sequences in the Baltic Sea either belong to the most common haplotype (shared by a total of 12 individuals), or originate from it.

The situation is quite different in *H. ventrosa*: from 46 sequences obtained, only 14 (30.4%) were different. One haplotype is shared by 24 individuals, two haplotypes are each shared by four individuals, one haplotype is shared by three individuals, one haplotype by two individuals, and nine haplotypes are unique (Fig. 2B). In the 46 sequences compared, 19 (2.98%) out of 638 sites are polymorphic. From the phylogenetic network the following points can be made: (1) all of the hypothetic ancestral sequences could be found; (2) the haplotype sitting in the center of the network, assumed to be the most recent common ancestor, is shared by 24 individuals from seven of ten populations, 20 more sequences originate from it; (3) all sequences obtained from specimens from or close to the Mediterranean form a distinct clade.

Regarding the quality of substitutions along the phylogenetic networks the differences between the two *Hydrobia* species are less significant. The ratio of transitions to transversions for the 32 haplotypes in *H. ulvae* is 6.4 and the ratio of silent to non-silent substitutions is 4.9. The 14 haplotypes of *H. ventrosa* show a ratio of 5.3 for transitions to transversions and 5.3 for silent to non-silent substitutions.

Average pairwise nucleotide differences in *H. ulvae* vary within populations between 0.08% and 2.04% with an average of 0.97%. The diversity between populations is slightly larger and ranges from 0.12% to 2.20% and averages 1.21%. Nucleotide differences in *H. ventrosa* are considerably smaller. The differences within populations vary from 0% to 0.38% with an average of 0.14%. Between populations these values range from 0% to 0.64% and average 0.28%.

Analysis of population level differentiation shows little F_{ST} values within *H. ulvae*: they average 0.25 and range from 0 to 0.60. Very small F_{ST} values are observed between the Baltic Sea populations, between the North Sea population and between the two Spanish populations. F_{ST} values are also very small between populations from the Baltic Sea and the White Sea. Average F_{ST} values in *H. ventrosa* are considerably higher than in *H. ulvae*. They range from 0 to 1 with an average of 0.41. Little population structure with corresponding low F_{ST} values is to be found between the Baltic Sea populations (including the Kattegat) and between the two populations from England.

Calculations of Nm values between populations with little differentiation is problematic. In these populations F_{ST} values are very low and the corresponding Nm values go to infinity. This is the case in 6 of 45 Nm values in *H. ulvae* and in 10 of 45 Nm values in *H. ventrosa*. Thus, we did not use these Nm values for further analyses. Instead, the data for populations from a particular geographic region with little genetic differentiation and corresponding low F_{ST} and high Nm values were combined (see Fig. 3). The genetic differentiation among the combined 'populations' is slightly higher than in the original populations and therefore Nm values can be calculated without problems. The results show substantial differences in gene flow between *H. ulvae* (Fig. 3A) and *H. ventrosa* (Fig. 3B). Thus, 6 out of 10 pairwise comparisons in *H. ulvae* show moderate ($1 \leq Nm \leq 5$) or high gene flow ($Nm > 5$). Low gene flow ($Nm > 1$) could only be observed between the White Sea population and the populations from the North Sea, between the White Sea population and the Spanish populations, between the Baltic and North Sea populations as well as between the populations from the North Sea and the Spanish populations.

In *H. ventrosa* 11 of 15 pairwise comparisons show low gene flow. Moderate gene

Figure 3. Gene flow (based on *Nm*) for *H. ulvae* (left) and *H. ventrosa* (right). Populations from a particular geographic region with little genetic differentiation were combined (circles within diagonally hatched ovals).

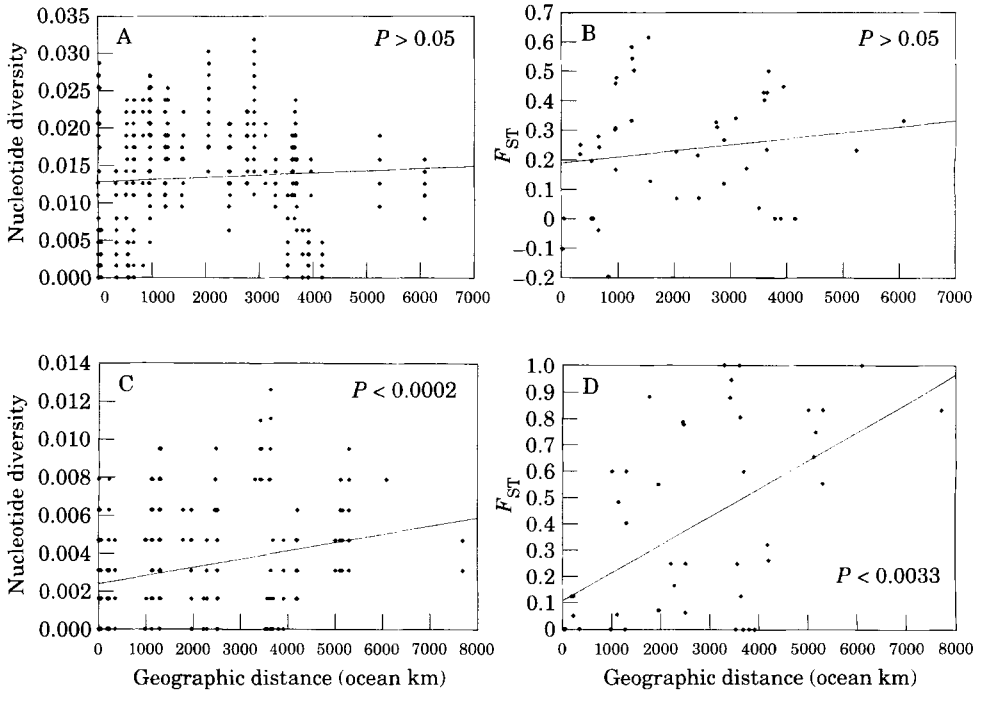

Figure 4. Relationships between F_{ST} and geographic distance (left) and average nucleotide diversity and geographic distance (right) for *H. ulvae* (A, B) and *H. ventrosa* (C, D).

flow occurs between the White Sea population and the Icelandic population, between the Icelandic population and populations from the Baltic Sea, and between English populations and the population from Italy. High gene flow could only be observed between the White Sea population and populations from the Baltic Sea.

To test whether the two *Hydrobia* species migrate according to the island model or to the isolation by distance model, the relationship between genetic diversity and geographic distance was studied. In the classical island model there is no significant relationship between gene flow and geographic distance. In an isolation by distance model the rate of gene flow decreases with geographical distance (Craddock *et al.*, 1997).

As the calculation of gene flow (based on *Nm*) was problematic for some of the populations (see above), we used F_{ST} values as an indirect measure of gene flow. Thus, assuming equal population sizes, we would expect a significant positive correlation between F_{ST} and geographic distance for the isolation by distance model whereas no significant relationship should be observed in the island model.

In *H. ulvae*, F_{ST} values increase slightly with geographic distance. However, this relationship is not significant ($P>0.05$). Thus we assume the island model for this species (Fig. 4A).

In *H. ventrosa*, there is a significant positive relationship between F_{ST} and geographic distance ($P<0.0033$), indicating migration according to the isolation by distance model (Fig. 4B).

As mentioned before, the F_{ST} approach used here assumes Wright's island model with populations of equal size and equal average diversity. These assumptions may

be violated in some (if not in all) cases introduced here. Thus, the relationship between F_{ST} and geographic distance may be particularly biased towards the island model.

We therefore also tested the relationship between genetic and geographic distance based on the simple average pairwise nucleotide diversity. This relationship is certainly not better suited to test for dispersal because mitochondrial nucleotide diversity does not reflect interactions within and between populations and it is population size dependent. Therefore it cannot replace the relationship of F_{ST} and geographic distance. However, as it is not biased towards the island model, it provides an additional evidence for one or the other dispersal hypothesis.

The results for the relationship between genetic and geographic distance are identical with the results obtained for the relationship between F_{ST} and geographic distance. There is no significant relationship between nucleotide diversity and geographic distance in *H. ulvae* ($P>0.05$) and a significant positive correlation in *H. ventrosa* ($P<0.002$) (Fig. 4).

DISCUSSION

Genetic differentiation

Our mtDNA data show distinct differences in genetic diversity between *H. ulvae* and *H. ventrosa* populations. Genetic variation in *H. ulvae* is higher than in *H. ventrosa*. The average pairwise nucleotide differences within a population of *H. ulvae* are more than seven times greater than in *H. ventrosa*; the average pairwise nucleotide differences between populations are more than four times greater. Similar relationships are to be found in a comparison of polymorphic sites and number of haplotypes.

These parameters have a strong impact on the topology of the phylogenetic networks. Thus, the network of *H. ulvae* has more clades and branches, and most of the branches are longer than in *H. ventrosa*. Another major difference between the species is that in *H. ventrosa*, all nodes have associated sequences. Moreover, these nodes are shared by more than one individual. Thus, the MRCA (most recent common ancestor, see Hoelzer *et al.*, 1998 for details) is shared by as many as 24 sequences (52.2% of all sequences obtained for *H. ventrosa*). In contrast, few sequences are located at nodes in *H. ulvae*, mainly in the clade formed by the populations from the White Sea, the Kattegat, and the Baltic Sea. Thus, the MRCA for the Baltic Sea is shared by 12 individuals. Palumbi *et al.* (1997) found similar results for different species of Pacific sea urchins. In some of the species, common nodes sitting deep within the phylogenetic structure could be found. In other species sequences at hypothesized ancestral nodes either were not present or were detected only for phylogenetically derived sub-populations.

The question is: Why did we find all of the sequences sitting at nodes deep within the structure of the phylogenetic network (which are, according to Palumbi *et al.*, 1997 old enough to have given rise to many derived mtDNA types) in *H. ventrosa* but not in *H. ulvae*? There are at least two possible explanations. The first could be a methodological problem. The genetic differentiation in *H. ulvae* is greater than in *H. ventrosa*. Thus, we found in 46 sequences of *H. ventrosa* only 14 haplotypes, whereas 32 different haplotypes in 45 sequences for *H. ulvae*. The limited number of individuals

studied here makes it impossible to detect all available haplotypes, especially in *H. ulvae*. The missing sequences could be due to a limited sample size. However, this alone would not explain the fact that we could find all the ancestral sequences in one clade of *H. ulvae* but not in the others. Another possible explanation would be differences in coalescence time and/or substitution rates. The possibly longer coalescence time and/or higher substitution rate in *H. ulvae* makes it likely that at least some of the ancestral sequences have mutated and therefore can no longer be found.

More intensive studies with more populations and individuals would be necessary to solve this enigma. The problem also raises the question of time and spatial origin of the radiation of *H. ulvae* and *H. ventrosa*.

Timing and spatial origin of infraspecific divergence

Estimates of the phylogenetic age of species are always difficult, particularly in taxa with morphostatic radiations where palaeontological data cannot (or only to a limited degree) be used to calibrate genetic distances. Moreover, to estimate the phylogenetic age of a species based on coalescence time to a common ancestor, the resulting sister taxon should still exist. This is the case in *H. ventrosa*. It has sister taxa (e.g. *H. truncata* and *H.* cf. *pontieuxini*) that can be used to estimate the coalescence time for this species. The situation is different in *H. ulvae*. It is considered by some workers to belong to its own genus and apparently does not have a closely related sister group. Thus, estimates of coalescent time based on genetic data are difficult.

Based on life history data, *H. ulvae* seems to be older than *H. ventrosa* as it has a planktonic development. According to Hansen (1983), who studied speciation in Tertiary neogastropods, there is an unidirectional trend from planktotrophy to nonplanktotrophy, and nonplanktotrophy is a derived character in many gastropod families.

Exact estimates of the phylogenetic age of the two *Hydrobia* species are difficult to make as there is no constant or universal molecular clock and nucleotide substitution rates in mtDNA differs among species and genes and may also be affected by geographical structure, population size and other factors (Hoelzer *et al.*, 1998). Given that the molecular clock rate is unknown for *Hydrobia*, but applying a molecular clock rate of 0.5–4% sequence differences per 1 Myr in mtDNA for invertebrates, the phylogenetic age of the recent *H. ulvae* clade can be estimated at about 0.62–5.0 Myr (2.5% sequence differences) and that of *H. ventrosa* at about 0.28–2.2 Myr (1.1% sequence differences). These crude estimates reveal two things. First, the radiation of the recent *H. ulvae* and *H. ventrosa* clades originate most likely in or shortly before the Pleistocene and second, the lineages of *H. ulvae* seem to be older than the lineages of *H. ventrosa*.

The theory that the infraspecific radiation originates in the Pleistocene is also supported by the biology and biogeography of the two species. Although both species are also to be found in subtropical waters, they are essentially temperate water species with a fairly high freezing tolerance (Hylleberg & Siegismund, 1987). The centre of their ranges is in or around the North Sea and they extend northwards as far as Iceland, Spitsbergen, Novaya Zemlya and the White Sea. Today, these northern areas have climatic conditions similar to the conditions during the Pleistocene in many parts of middle and western Europe.

TABLE 2. COI variation and population structure within *H. ulvae* and *H. ventrosa*

	Hydrobia ulvae	*Hydrobia ventrosa*
No. of populations	10	10
Total no. of individuals	45	46
No. of polymorphic sites	69 (10.82%)	19 (2.98%)
No. of haplotypes	32 (71.1%)	14 (30.4%)
Ratio transitions: transversions	6.42	5.33
Ratio silent: non-silent substitutions	4.93	5.33
Percent nucleotide differences within populations	0.974 ± 0.805 (0.078–2.038)	0.136 ± 0.142 (0–0.376)
Percent nucleotide differences between populations	1.209 ± 0.578 (0.118–2.203)	0.285 ± 0.177 (0–0.644)
Average F_{ST}	0.2519 ± 0.1842 (0–0.598)	0.4066 ± 0.3610 (0–1.0000)
Relationship between nucleotide diversity and geographic distance	$P>0.05$	$P<0.0002$
Relationship between F_{ST} and geographic distance	$P>0.05$	$P<0.0033$

Regarding the spatial origin of the recent *H. ulvae* and *H. ventrosa* lineages, there is some evidence from our mtDNA data that they originate in or around the North Sea—the same area that is still the centre of their current ranges. Thus, most of the ancestral sequences in the phylogenetic networks (Fig. 2) are from the North Sea, the Kattegat, and the western Baltic Sea. None of the ancestral sequences are from the Mediterranean or Spanish coasts. In fact, all sequences from these southern individuals reside on or close to the tip of the branches. They might therefore be derived and phylogenetically younger. From a biological perspective it is very unlikely that individuals from southern waters dispersed into cold water during a glacial period. It is much more likely that individuals from temperate areas retreated to relatively warm waters during the glacial advances. We therefore argue that populations of *H. ulvae* and *H. ventrosa* occurring during the Pleistocene in ice free shallow areas of the northeastern Atlantic (possibly the North Sea) are the ancestors of the recent populations.

Population level differentiation, gene flow, and distribution models

The phylogenetic content of mtDNA sequences, upon which our analysis of population structure and gene flow is based, reflects the past of a species. Therefore, the data presented here can be used to infer historical patterns of migration that might no longer occur.

We have, however, clearly to state that the average number of individuals per population studied in this paper is low (in the order of 4 to 5). Even with pooling populations we not always reach a number of 10 to 15 specimens which is often used for population genetics of conservative mitochondrial genes like COI. All our statements on gene flow and distribution patterns may lack accuracy, and the results are of preliminary nature.

As the average pairwise F_{ST} values are higher in *H. ventrosa* than in *H. ulvae* (Table 2), there must also be differences in dispersal patterns. Indeed, the relationships between F_{ST} values (or genetic distance) and geographic distance show that *H. ventrosa*

disperses according to the isolation by distance model and *H. ulvae* according to the island model (Fig. 4).

The low average population structure, high gene flow and dispersal according to the island model in the planktonic *H. ulvae*, and distinct population structure, low gene flow and dispersal according to the isolation by distance model in the nonplanktonic *H. ventrosa* are consistent with their biology. However, as shown by Todd *et al.* (1988) for the nudibranch mollusc *Adalaria proxima*, actual larval transport can be less than that which might be expected on the basis of larval culture data alone. This raises the questions of how relevant planktonic larval transport is for the dispersal of *H. ulvae*. Although the accounts in the literature are conflicting as to their mobility after hatching, it is generally agreed that, if populations of *H. ulvae* have a pelagic larvae stage at all, it is very short. Pilkington (1971) found that some populations showed a "complete suppression of any pelagic phase in the life history", and Chatfield (1972) stated that the veliger has a velum which lasts for only 3 days and is not very effective in movement. Because of these limitations the veligers of *H. ulvae* might not have the capacity for long distance dispersal. However, in *H. ulvae* as well as in *H. ventrosa* some passive dispersal modes have been observed: (1) both species show a typical floating behaviour (Anderson, 1971); (2) juvenile snails can disperse in rafting algal beds and (3) snails that are often preyed upon by migrating water birds and fish can frequently pass through the gut alive (Muus, 1967; Drake & Arias, 1995; Aarnio & Bonsdorff, 1997). All these factors indicate a potential for a long distance dispersal. However, only *H. ulvae* shows actual gene flow over large distances, whereas migration events in *H. ventrosa* are usually restricted to neighbouring areas (Fig. 3). These differences in dispersal modes and gene flow could be partly due to the more isolated habitats of *H. ventrosa* and to the different larval development and quantitative differences in passive dispersal. Migration barriers may be the most significant factor in the differential distribution of each species. Thus, after each 'random' migration event the snails must find a suitable habitat to survive. Here, *H. ulvae* has a clear advantage over *H. ventrosa*. As a marine to brackish-water species with high tolerance to environmental stress it is much more likely to survive dispersal than *H. ventrosa* which is restricted to isolated backwaters with low salinity (although the salinity is probably not the limiting factor for *H. ventrosa* but rather substratum, food, vegetation and restricted water movement that are correlated with many brackish-water habitats).

In spite of significant differences in gene flow between *H. ulvae* and *H. ventrosa*, patterns of gene flow between the populations from the White Sea and the Baltic Sea are similar for both species. Gene flow between these two areas is high, even though the geographic distance (in ocean km) between them is considerable. A possible explanation is that both areas are connected by a major migration route for water birds. The coastal areas of the White Sea are important breeding grounds for some bird species that prey upon mud snails, and the coastal areas of the Baltic Sea are the corresponding resting and/or wintering grounds. The snails might be carried in the gut of these birds during the semiannual migration events. As many habitats in the White Sea and Baltic Sea are similar regarding water movement, substrate, vegetation and salinity, these snails probably have a good chance to find suitable habitats when they pass through the gut alive.

Evolutionary scenario

Based on COI and life history data, the following scenario is probable for the evolution of the two species studied here: The marine, planktonic snail species *H. ulvae* failed further speciation. However, the recent lineages of that species, are characterized by a high degree of infraspecific variation. In contrast, the brackish, direct developing species *H. ventrosa* is characterized by a fairly high degree of speciation since the late Tertiary. The resulting morphostatic sister species evolved subsequently with little infraspecific variation (Wilke & Davis, unpublished data). This is not surprising, as nonplanktonic neogastropods in general show a higher rate of speciation than planktonic taxa (Hansen, 1983). However, this theory of contrasting modes of speciation within *Hydrobia s.l.*—little or no species diversity combined with high infraspecific variation and vice versa—needs further evaluation based on more taxa and different genes.

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