Molecular Dynamics of *Emiliania huxleyi* and Cooccurring Viruses during Two Separate Mesocosm Studies

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In this study we used denaturing gradient gel electrophoresis, sequencing analysis, and analytical flow cytometry to monitor the dynamics and genetic richness of *Emiliania huxleyi* isolates and cooccurring viruses during two mesocosm experiments in a Norwegian fjord in 2000 and 2003. We exploited variations in a gene encoding a protein with calcium-binding motifs (GPA) and in the major capsid protein (MCP) gene to assess allelic and genotypic richness within *E. huxleyi* and *E. huxleyi*-specific viruses (EhVs), respectively. To our knowledge, this is the first report that shows the effectiveness of the GPA gene for analysis of natural communities of *E. huxleyi*. Our results revealed the existence of a genetically rich, yet stable *E. huxleyi* and EhV community in the fjordic environment. Incredibly, the same virus and host genotypes dominated in separate studies conducted 3 years apart. Both *E. huxleyi*-dominated blooms contained the same six *E. huxleyi* alleles. In addition, despite the presence of at least six and four EhV genotypes at the start of the blooms in 2000 and 2003, respectively, the same two virus genotypes dominated the naturally occurring infections during the exponential and termination phases of the blooms in both years.

For more than 15 years we have known that viruses are abundant in the marine ecosystem and that they are capable of infecting the main primary producers (3, 30, 40). Viruses control microbial and phytoplankton community succession dynamics (7, 11, 19, 39, 43) and play important roles in nutrient dynamics (45) and biogeochemical (16, 25) cycling. Although these conclusions are now generally accepted, there are still many aspects of the ecological effects of marine viruses that remain unknown. Viruses are the most abundant (up to 10^7 to 10^8 particles ml^-1 (3) and genetically diverse biological entities in the sea (28, 37, 49), and this fact alone makes them incredibly difficult to study as a single entity. Therefore, it has become necessary to study specific groups of viruses to try to make sense of their propagation strategy and molecular dynamics.

To compensate for the limited realism of laboratory experiments and to avoid the difficulties of field work, transparent enclosures placed in a natural body of water (i.e., mesocosm experiments) have been employed in marine phytoplankton studies since the early 1960s (2, 23). The realism and reproducibility (13) of these systems make them well suited for studying effects of chemical, physical, and biological manipulations on natural plankton communities.

Coccolithophores are an abundant and widespread phytoplankton functional group. The bloom-forming coccolithophore *Emiliania huxleyi* (Lohmann) Hay and Mohler (Prymnesiophyceae) is the most abundant species of coccolithophores in the world’s oceans. Blooms of *E. huxleyi* are important for sediment formation (51), for being sinks of atmospheric carbon dioxide (20), and for the release of cloud-forming dimethyl sulfide into the atmosphere. In previous studies using mesocosm systems workers have investigated the effects of dissolved nutrient composition on the community dynamics of *E. huxleyi*-dominated systems and the role that viruses have in structuring different microbial components (4, 18, 48). It is clear from these studies that viruses are instrumental in the demise of *E. huxleyi* blooms and allow succession of different microalgae following rapid bacterial remineralization of organic matter (7, 19). As an *E. huxleyi* bloom crashes, there is a rapid increase in the number of large virus particles, which are easily discriminated by analytical flow cytometry (AFC), a technique that measures light scattering and fluorescence characteristics of individual particles (18, 46). These large virus particles can be isolated with relative ease by adding seawater samples to cultures of *E. huxleyi* (8, 47). Characterization of these large *E. huxleyi*-specific viruses (EhVs) has revealed that they belong to the *Phycodnaviridae* family of algal viruses, based on analysis of the DNA polymerase (pol) gene (34). The genome of one strain of EhV isolated from the English Channel was recently sequenced, which revealed a 407-bp genome (48a).

It has been recognized that there can be broad genotypic variation within populations of *E. huxleyi* (17, 24) and EhVs (33) during *E. huxleyi*-dominated blooms, although the available tools have never been used together to assess the virus-host molecular dynamics. It is necessary to develop appropriate tools before such an assessment can be made. Thus, for analysis of *E. huxleyi* Schroeder et al. (32) showed that a gene encoding a protein with calcium-binding motifs (designated GPA), thought to be involved in regulating coccolith morphology (10), could be used as a genetic marker to definitively...
resolve differences that could be attributed to different *E. huxleyi* genotypes within the A and B morphotypes kept in culture. *E. huxleyi* is currently separated into five morphotypes based mainly on coccolith morphology, physiological properties, and immunological properties of the polysaccharide associated with coccoliths. Morphotypes A and B are the best-characterized morphotypes. For EhV analysis Schroeder et al. (34) exploited variations in the major capsid protein (MCP) gene to assess the genetic diversity of EhVs. In another study Schroeder et al. (33) used the MCP marker to reveal the genetic richness of free-floating viruses during an *E. huxleyi* bloom in a mesocosm experiment in Norway in 2000. The DNA polymerase gene, which is more commonly used to resolve genetic variation among other algal viruses (9, 35), is not variable enough to differentiate EhVs. During the same study Jacquet et al. (18) employed AFC as a reliable tool to rapidly and accurately count the *E. huxleyi* and viruses in mesocosm samples.

In the current study we extended the research of Schroeder et al. (33) by using a combination of GPA and MCP molecular markers to monitor the dynamics and genetic richness of *E. huxleyi* isolates and cooccurring viruses during the same mesocosm experiment (in June 2000). Additionally, we analyzed samples from a second mesocosm experiment performed at the same site 3 years later (June 2003). There were two aims of this study: first, to assess the molecular dynamics of the host-virus system in situ; and second, to determine the genetic stability and variability of *E. huxleyi* isolates and cooccurring viruses over time (i.e., after a 3-year gap), particularly since *E. huxleyi* blooms occur annually in May through July in Norwegian coastal waters and fjords (4). In addition to the genotypic analysis, AFC counting was done, and the results were compared to those of Jacquet et al. (18).

### MATERIALS AND METHODS

**Experimental design.** Two mesocosm experiments designed to monitor the progression of a coccolithophore-induced bloom were carried out in Raunefjorden in western Norway at the Marine Biological Field Station at Espeland 20 km south of Bergen in June 2000 and June 2003. The experimental devices consisted of transparent polyethylene enclosures (11 m$^3$; 90% penetration of photosynthetically active radiation) purchased from ANI-TEX (Notodden, Norway) mounted on floating frames moored along the south side of a raft (for details, see reference 14). The experimental design and the method used for AFC analysis of *E. huxleyi* and its natural viral communities in enclosure 1 (nutrient enrichment with an N/P ratio of 15:1 [1.5 μM NaNO$_3$ and 0.1 μM KH$_2$PO$_4$]) for the 2000 mesocosm were the experimental design and method described by Jacquet et al. (18). In the 2003 mesocosm there were nine enclosures that were filled on 2 June with unfiltered seawater from a point adjacent to the raft at a depth of 2 m. The seawater in the enclosures was kept homogeneous by means of airlifts. The nine enclosures were divided into three treatment groups, allowing triplication of each treatment; nutrients were added daily at an N/P ratio of 15:1 to enclosure group A starting on 3 June, to group B starting on 4 June, and to group C starting on 5 June. An AFC analysis of the *E. huxleyi* and EhV communities in all the enclosures was performed daily as described by Martínez Martínez et al. (22).

**DNA isolation.** For total genomic DNA preparation, 1-liter seawater samples from the enclosures were filtered daily onto 0.45-μm-pore-size, 47-mm-diameter Supor-450 filters (PALL Corp.). The filters were transferred to 2-ml cryotubes, snap frozen in liquid nitrogen, and stored at −80°C until further processing. Genomic DNA was isolated using an adapted phenol-chloroform method previously described by Schroeder et al. (34). Filters were cut into small easily

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### TABLE 1. Primers used to assess host and viral diversity

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Reference</th>
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<td>GPA-F1</td>
<td>5′-GAG GAG GAG AAG CCG AGC CT-3′</td>
<td>32</td>
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<tr>
<td>GPA-F2</td>
<td>5′-CAG GCC TTC TTC GGG CTG GG-3′</td>
<td>This study</td>
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<td>GPA-R1</td>
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<td>5′-GGG GCC CTC TTC GGG CTG GG-3′</td>
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<td>MCP-R2</td>
<td>5′-GAC CTT TAG GCC AGG GAG-3′</td>
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</table>

* a The prefix GPA indicates primers used to assess host diversity, and the prefix MCP indicates primers used to assess viral diversity. F and R indicate forward and reverse primers, respectively.

### TABLE 2. *E. huxleyi* and EhV virus genotypes found in this study and GenBank accession numbers for sequence data

<table>
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<th>DGGE band</th>
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<th>GPA sequence</th>
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<th>MCP sequence</th>
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* a Fragments (284 to 287 bp) of the GPA gene encoding a protein with calcium-binding motifs.

* b Fragments (99 bp) of a gene encoding the putative major capsid protein.

* c GenBank accession numbers published previously. The same DGGE bands and corresponding sequences were also detected by Schroeder et al. (33).
dissolved pieces and placed in a 2-ml Eppendorf tube. Following addition of 800 μl of GTE buffer (50 mM glucose, 25 mM Tris-Cl [pH 8.0], 10 mM EDTA), 100 μl of lysozyme (10 mg/ml), and 100 μl of 0.5 M filter-sterilized EDTA, samples were incubated at room temperature for 1 to 2 h with gentle shaking. Sodium dodecyl sulfate (200 μl of a 10% stock solution) was added, and the mixture was incubated for 10 min at room temperature. DNA was then purified by phenol extraction as previously described (34). Finally, air-dried DNA pellets were resuspended in 50 μl Tris-EDTA buffer.

**PCR amplification and DGGE.** *E. huxleyi* genotypic richness was studied using a nested PCR and the total genomic DNA preparations. Three oligomers were designed for the GPA gene of *E. huxleyi* strain L (Table 1). Two-stage PCRs (first with primers GPA-F1 and GPA-R1 and then with primers GPA-F2 and GPA-
R1) were performed to amplify the variable region in the GPA gene that allows separation of the alleles into genotypes (32). The PCRs were performed using 100 ng of total genomic DNA for the first reaction and then a 2-μl subsample from the first PCR for the second reaction. The gels were stained with ethidium bromide, visualized with a UV transilluminator, and photographed with the Gel Doc 2000 system (Bio-Rad). Denaturing gradient gel electrophoresis (DGGE) of the second-stage PCR products was conducted as described by Schroeder et al. (33), with some minor adjustments. We used 30 to 50% linear denaturing gradient 8% polyacrylamide gels. PCR products were treated with mung bean nuclease (Promega) used according to the manufacturer’s recommendations in order to degrade single-stranded DNA ends. Single bands were excised, incubated in 50 μl of molecular-grade water (Sigma), reamplified, and verified by DGGE. PCR products were subsequently sequenced using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, United Kingdom) and an ABI 3100 capillary sequencer (Applied Biosystems, United Kingdom) according to the manufacturer’s recommendations. The data for each fragment (see Table 2 for GenBank accession numbers) were aligned together with the GPA sequences obtained for 15 E. huxleyi isolates kept in culture (32) using ClustalW (http://www.ebi.ac.uk/clustalw/).

The virus MCP gene was amplified in two stages using two pairs of MCP-specific oligomers (Table 1). The initial amplification was conducted using primers MCP-F1 and MCP-R1, and nested PCR of the resulting product was conducted with primers MCP-F2 and MCP-R2. DGGE of the second-stage nested PCR product and sequencing of excised bands were conducted as described by Schroeder et al. (33). MCP sequence data (see Table 2 for GenBank accession numbers) were aligned with corresponding MCP sequences of nine EhVs during the 2000 mesocosm experiment at Raunefjorden (34).

RESULTS

Flow cytometry analysis. AFC was used to monitor the total abundance of E. huxleyi and EhVs during the blooms in 2000 (18) (Fig. 1) and 2003 (Fig. 2). During the 2000 mesocosm study the concentration of E. huxleyi increased by 2 orders of magnitude, from 10^3 to 10^5 cells ml^-1, between the beginning of the experiment and the peak of the bloom. The concentration of E. huxleyi returned to the prebloom level (3 × 10^2 cells ml^-1) at the end of the study (Fig. 1A). An increase of ~2 orders of magnitude, from 4.7 × 10^3 to 3.5 × 10^7 virus particles ml^-1, was observed for the number of EhVs during the termination phase of the E. huxleyi bloom, revealing a classic lytic virus response to a susceptible host population (Fig. 1B).

During the 2003 mesocosm study the pattern observed for virus-host dynamics (in the nine enclosures) was similar to the 2000 mesocosm pattern (Fig. 2). For simplicity, only the AFC results obtained for samples from enclosure group A (mean values for the three enclosures in this group) are described below since they are representative of the results obtained for all the enclosures in the study.

The E. huxleyi concentration increased by 1 order of magnitude, from ~1.5 × 10^3 to ~4 × 10^4 cells ml^-1, over the 7 days prior to the bloom crash. Then the E. huxleyi concentration decreased rapidly and returned to approximately the same concentration as the concentration before the beginning of the bloom, 1.5 × 10^4 cells ml^-1 (Fig. 2A). The E. huxleyi-specific virus concentration also increased ~2 orders of magnitude, from 4.3 × 10^4 to 3 × 10^7 virus particles ml^-1, following the sudden demise of E. huxleyi (Fig. 2B).

E. huxleyi DGGE gels. DGGE analysis of the PCR products obtained using the GPA-specific primers did not reveal allelic changes in terms of the presence or absence of alleles in the E. huxleyi community during the bloom in the two mesocosm studies (Fig. 1A and 2A). A study of Schroeder et al. (32) revealed that while some E. huxleyi strains contain a single allele, other E. huxleyi strains contain two alleles. Therefore, the number of bands found in this study was just an indication of the number of different alleles present and did not show quantitatively the diversity of E. huxleyi strains. The gels contained at least six distinguishable bands throughout both study periods (Fig. 1A and 2A). All the alleles corresponded to E. huxleyi morphotype A, genotype CMM I (32) (Fig. 3). The authenticity of the DGGE bands was confirmed by excising 34 single bands (from gels from both study years), verifying their purity by PCR and a second DGGE (data not shown), and sequencing. The migration rates on DGGE gels and sequence alignment revealed that multiple bands obtained on an individual sampling day were different, while bands obtained on different days that migrated at the same rate had identical nucleotide sequences (Fig. 3). The alleles present in 2000 were identical to the alleles found in the 2003 mesocosm study and belonged to genotype CMM I (32) (Table 2).

Virus DGGE gels. The 2000 mesocosm DGGE gel had a diverse band pattern prior to the onset of the E. huxleyi bloom (Fig. 1B). At least six bands were distinguishable in the lag phase of the bloom profile (ehvOTU9a, ehvOTU3b, ehvOTU10c, ehvOTU5c, ehvOTU7f, and ehvOTU2h). There was a change in the bands present during the exponential phase of the bloom; ehvOTU7f and ehvOTU2h disappeared, and two new bands (ehvOTU1d and ehvOTU4g) became visible. During the termination phase of the bloom, ehvOTU3b, ehvOTU1d, and ehvOTU4g dominated; ehvOTU3b was detected during the entire study period.

The DGGE profile for the 2003 mesocosm (Fig. 2B) also seemed to indicate that there was a change in the abundance of virus groups, based on the appearance and disappearance of bands. In the 4 days prior to the onset of the bloom four bands were distinguishable (ehvOTU3b, ehvOTU5c, ehvOTU4g, and ehvOTU16j). During the exponential and termination phases of the bloom there was a change in the bands; the ehvOTU5c, ehvOTU4g, and ehvOTU16j bands disappeared and were replaced by ehvOTU11i and ehvOTU11d. Incredibly, the same two bands (ehvOTU3b and ehvOTU1d) dominated during the exponential and termination phases in both 2000 and 2003 mesocosms, and the same band shift from ehvOTU5c to ehvOTU1d was observed for the onset of the bloom in the 2 years.

Excision, sequencing, and alignment using ClustalW confirmed the diversity of the bands (Fig. 4) and revealed that single bands with different migration rates corresponded to different EhV genotypes. Four of the eight genotypes from the 2000 mesocosm (ehvOTU1d, ehvOTU3b, ehvOTU4g, and ehvOTU5c) were also detected in the 2003 mesocosm study. The rest of the genotypes were detected only during the 2000 study (ehvOTU2h, ehvOTU7f, ehvOTU9a, and ehvOTU10c) or the 2003 study (ehvOTU11i and ehvOTU16j) (Table 2). The sequence of the ehvOTU1d genotype was identical to the sequence obtained from the EhV-163 strain, which was isolated during the mesocosm experiment in 2000 (34). In addition, two of the genotypes detected only during the 2000 mesocosm study, ehvOTU9a and ehvOTU10c, had the same MCP sequence as the EhV-203 and EhV-207 isolates from the English Channel (34), respectively (Fig. 4).

DGGEs, conducted in triplicate, produced the same band
pattern for *E. huxleyi* and EhVs for the replicate enclosures (results not shown). The results presented here are representative of all the enclosures in the experiment.

**DISCUSSION**

In this study we examined and compared the succession and termination of two analogous induced *E. huxleyi* blooms separated by 3 years. Use of the molecular markers GPA and MCP (32–34) allowed us to resolve genetic variation among *E. huxleyi* isolates and EhVs, respectively. Our results revealed not only a genetically rich *E. huxleyi* and EhV community but also identical *E. huxleyi* genotypic compositions and the same shift in EhV genotypes during the exponential and termination phases in the 2000 and 2003 mesocosm studies. The AFC data gave an overview of the changes in the sizes of the *E. huxleyi* and EhV populations (Fig. 1 and 2). The concurrent rapid increase in the number of EhV particles as the *E. huxleyi* bloom declined indicates that lytic viral infection was the main cause of bloom termination. Virus-induced collapse of *E. huxleyi*
FIG. 3. Clustal alignment of partial E. huxleyi sequences obtained from excised DGGE bands from the two mesocosm studies (designated "ehuxOTUs") (Table 2) and from isolates kept in culture (32). The letters after ehuxOTU correspond to band letters in Fig. 1A and 2A. The box indicates the region in the sequences that allows differentiation between genotypes (CMMs). Conserved bases are indicated by dashes beneath the corresponding bases for L_original.
populations has been inferred from a number of studies both in the open ocean (5, 47) and in mesocosms (4, 7, 15). For the 2000 mesocosm experiment Jacquet et al. (18) reported that as viral particles accumulated, diel patterns of E. huxleyi physiological properties were lost, which is a characteristic of virally infected phytoplankton cells (6). In addition, during the 2003 mesocosm experiment E. huxleyi cell viability was determined using SYTOX staining, and high levels of compromised cells were detected during the decline of the bloom (C. Evans, unpublished data). The appearance of compromised cells was concurrent with a rapid increase in the size of the EhV group, providing further support for the idea of viral termination of the bloom. Although rates of grazing were not determined during this study, the lack of significant differences in bloom development and termination in another enclosure subjected to the same nutrient treatment plus additional zooplankton enrichment (data not shown) suggests that the contribution of grazing on E. huxleyi to the decline of a bloom is small.

The development of the same E. huxleyi community and the same succession of the dominant virus genotypes in both years, despite some differences in bloom dynamics (i.e., cell numbers and durations of the lag, exponential, and termination phases), is not likely to be a bias due to nutrient addition in the enclosures. Schroeder et al. (33) observed that during the 2000 mesocosm study the same viruses were responsible for the termination of the bloom in enclosures in which either P or N was depleted. Although these workers could not determine the genotypic composition of the E. huxleyi community in the enclosures, host range specificity indicated that the same E. huxleyi strains dominated despite the nutrient regimen (D. Schroeder, unpublished results). In addition, AFC analysis during the 2003 study revealed development of the same microbial community both inside the mesocosm bags and in the fjord (data not shown). Addition of nutrients to the seawater in the enclosures led to what other workers have described as an accelerated version of community succession commonly found in the environment (12, 13). Therefore, it seems reasonable to expect the same genotypic diversity in the enclosures and the adjacent fjord.

PCR and DGGE are techniques that are widely used for describing microbial community structure and diversity based on extracted DNA (27, 31, 36). However, these analyses provide only presence/absence data and not abundance data, mainly due to the qualitative nature of PCR and limitations in DGGE resolution. Truly quantitative information using molecular methods can be obtained only if DNA extraction efficiency and biases in the PCR step are under experimental control. This is not feasible when environmental samples containing an unknown amount of cells and virus particles, as well as inhibitors, are analyzed. Further investigations would benefit from the use of internal standards during the DNA extraction, PCR, and DGGE steps to allow at least a description of relative changes in abundance and diversity between samples, as described by Petersen and Dahllöf (29). Nonetheless, it is clear from the DGGE profiles produced prior to the onset of the bloom that a range of virus genotypes was present in the water column. It is likely that the viruses were remnants of previous bloom-lysis events. Detection of a diverse population of remnant viruses prior the onset of the bloom indicates that the viruses can remain in the water column long after their specific host(s) has “disappeared.” However, our detection methods do not necessarily mean that the viruses are still viable (50). Similarly, this would also explain why the EhV population does not crash immediately concurrent with the sudden demise of E. huxleyi. If a virus that is able to infect the dominant E. huxleyi genotype(s) is present during a bloom event, a very large part of the host assemblage could be infected and “removed.” This would allow other E. huxleyi ge-

FIG. 4. Clustal alignment showing E. huxleyi-specific virus genotype diversity (“ehvOTUs”) during the two mesocosm experiments based on amplified MCP fragments (Table 2). The letters after ehvOTU correspond to band letters in Fig. 1B and 2B. Sequences obtained from the known E. huxleyi-specific virus isolates used as DGGE standards are also included (34). Identical sequences are grouped together in boxes. Conserved bases are indicated by dashes beneath the corresponding bases for EhV-84.
notypes not susceptible to the now dominant viruses to occupy the niche and therefore would determine the succession of different host genotypes and the subsequent production of new viral genotypes in the fjord, as described by Thingstad (42) in the “kill the winner” model. Similar patterns of genetic succession were described for the Gulf of Aqaba in the Red Sea. Changes in the abundance and genetic diversity of the marine picophytoplankton Synechococcus during an annual cycle were determined by interactions with cooccurring cyanophages (26), proving that virus infection can play an important role in determining the succession of *Synechococcus* genotypes.

The fact that we observed “identical” events in 2000 and 2003 at the same time of the year indicates that there is a periodic annual succession of identical *E. huxleyi* genotypes, which in turn determines the viral genotypic succession. Our DGGE results did not reveal variation of the dominant *E. huxleyi* genotypes throughout each mesocosm experiment. This suggests that there is a genetically stable *E. huxleyi* population in the fjordic system examined and that the dominant strains have an efficient strategy for survival between blooms.

The stability of *E. huxleyi* populations in 2000 and 2003 was reflected by the identical population shifts of the dominant virus genotypes in the two studies (Fig. 1 and 2). This was despite the finding that there were two different genotypic virus profiles prior to the onset of the bloom. Metaphorically, viruses seemed to be jostling for position until the concentrations of the appropriate host *E. huxleyi* strains started to increase. Determining the ecological significance of high virus diversity in this prebloom situation is clearly a challenge for the future; it is likely that high diversity ensures that whatever host(s) is “successful” (i.e., goes on to form a bloom), there is always a virus with the ability to infect it. This raises the question of whether the diversity observed on a DGGE gel when the MCP marker is used can be translated into a phenotypic change in the virus (e.g., a change in the host that it infects). A recent study revealed that this is clearly the case when changes in the host range of a particular EhV does translate to diversity in the MCP sequence (1); however, there is no correlation between the sequence and the host(s) that the EhV infects. Therefore, we are not at a stage where we can determine the host that an EhV can infect from an environmental MCP sequence.

The fact that diverse populations of EhVs are present in the water column poses two questions. How do the same viruses persist and remain viable in the marine environment throughout the years even at times when the numbers of host cells are low? And what *E. huxleyi* strains do the viruses infect? For the first question, one hypothesis is that the viruses sink into deep water layers or sediments, where they can escape destruction by solar radiation (41, 44). Yet the ecological weight of algal virus reservoirs in sediments and deep waters and the importance of mixing in transferring viruses to surface water layers is currently subjects of speculation. Another hypothesis is that if *E. huxleyi* isolates do not completely disappear in a fjord, low virus production could be maintained year round. Mühling et al. (26) observed that the *Synechococcus* concentration always exceeded the density required for persistence of the phages that infect the bacteria (21, 38) in the Gulf of Aqaba. The second question could probably be answered after a much more comprehensive study comprising temporal and spatial sampling regimens to determine if more diverse *E. huxleyi* populations are indeed present. We know from culture studies that there is broad genotypic diversity of *E. huxleyi* (17, 24, 32). A recent study investigating *E. huxleyi*-EhV dynamics in the North Sea revealed a broad range of *E. huxleyi* genotypes, and genotypic shifts were observed in the Lagrangian study (J. Martínez Martínez, unpublished data).

Finally, it is noteworthy that ehvOTU9a and ehvOTU10c had MCP sequences identical to those of EhV strains EhV-203 and EhV-207, respectively (Fig. 4), both of which were isolated from the English Channel in 2001 (34). This is an indication of the similarity between these geographically distant EhV strains. Indeed, host range experiments have revealed that several virus isolates from the English Channel and the Norwegian fjord used this study could infect several cultured *E. huxleyi* strains originally obtained from very distant areas (1, 34, 47).

In summary, our study indicates for the first time the effectiveness of the GPA gene (32) as a molecular marker for differentiating *E. huxleyi* genotypes in the A and B morphotypes in natural communities. Our findings also provide new insights into the progression and structure, at a molecular level, of natural blooms of the globally important species *E. huxleyi*. We demonstrated that *E. huxleyi* blooms and the viruses that infect them are remarkably genetically consistent at the Norwegian fjordic study site, where the same *E. huxleyi* and EhV genotypes reoccur in annual cycles. Future challenges include determining the physicochemical, biological, and ecological parameters that influence such genetic stability.

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