An AFLP-based test of clonality in widespread, putatively asexual populations of *Porphyra umbilicalis* (Rhodophyta) in the Northwest Atlantic with an in silico analysis for bacterial contamination

N. A. Blouin · S. H. Brawley

Abstract Asexual reproduction often predominates in populations of species experiencing range expansion. Locally selected genotypes can lead to the establishment of clonal populations that accumulate genetic diversity through time. Sexual reproduction has never been observed in extensive field and culture studies of the red alga *Porphyra umbilicalis* from Maine, USA, even though sexual reproduction predominates in this species in the eastern Atlantic (Europe). This suggests that Maine populations are indeed asexual and might consist of one or only a few genetic clones; we have tested this using AFLPs. Individuals were sampled at two sites in Maine (Cobscook Bay [$n = 25$], Schoodic Point [$n = 26$]) and compared to sexual individuals from England (Sidmouth [$n = 17$]). The AFLP analysis determined that individuals at two sites in Maine containing putative asexuals were not strictly clonal; however, two multilocus lineages were sampled more than once. Two genetic clones, one at each Maine site, were comprised of 6 individuals each; the 39 additional Maine individuals had distinctive AFLP genotypes. However, when the individuals from Maine were compared with a known sexual population from Sidmouth, England, much greater genetic diversity was found within the sexual population in England. Finally, we examine how preparation of field-collected material for AFLP investigations can affect the inclusion of non-target DNA and demonstrate an in silico approach for removing some cryptic contaminants from analysis.

N. A. Blouin · S. H. Brawley
School of Marine Sciences, University of Maine,
5735 Hitchner Hall, Orono, ME 04469, USA

N. A. Blouin (✉)
Department of Biological Sciences, University of Rhode Island,
120 Flagg Rd, CBLS287, Kingston, RI 02881, USA
e-mail: nblouin@mail.uri.edu

Introduction

Populations of species that are involved in range expansion often have small effective population sizes and can fix beneficial mutations through a combination of selection and genetic drift that often includes asexual reproduction (Hallatschek et al. 2007; Hallatschek and Nelson 2010; Song et al. 2011). Asexual reproduction can be an effective mechanism to establish a new population; however, it is not considered to confer adaptive advantage over longer evolutionary time scales because of asexuals presumed lack of genetic diversity (Otto 2009). Contrary to this idea, recent work with an asexual, clonal yeast that was dividing mitotically showed rapid local adaptation to salinity stress (Bell and Gonzalez 2011; Dhar et al. 2011). This adds to our understanding of persistent asexual reproduction in populations at the extremes of their ranges (e.g., in algae: Dixon 1965; Pearson and Murray 1997; Sosa and Lindstrom 1999; and references therein).

The intertidal red alga *Porphyra umbilicalis* has a typical sexual life history in the northeastern (NE) Atlantic (e.g., Brodie et al. 2008), but individuals in at least part of the northwestern (NW) Atlantic metapopulation appear to reproduce exclusively by asexual neutral spores released from blades (Blouin et al. 2007). The current described range for *P. umbilicalis* is from Long Island Sound to Greenland in the NW Atlantic and from Norway south to Portugal in the NE Atlantic (Sears 2002; Brodie et al. 2008; Teasdale et al. 2009). Influences on the NW Atlantic's intertidal marine biota include extirpation by glaciation and

recolonization by both natural processes and anthropogenic vectors (Maggs et al. 2008; Brawley et al. 2009; Muhlin and Brawley 2009; Adey and Hayek 2011). Given the lack of observed sexual reproduction in the NW Atlantic *P. umbilicalis* and recolonization of the NW Atlantic shores (from Europe or small NW Atlantic refugia) by *P. umbilicalis* after the last glacial maximum (LGM), the putative asexual population in Maine may be comprised of a group of clones. This is tested here with AFLPs (Vos et al. 1995).

In addition to investigating whether or not *P. umbilicalis* is clonal in Maine, we evaluate AFLP-relevant contamination of our field collections, provide an in silico approach to identifying additional potential contaminants, and discuss the importance of their removal when conducting an investigation with AFLP DNA fingerprinting. AFLPs are considered to be highly reproducible and robust against methodological artifacts, but can suffer from different types of scoring errors. Many of the nearly 7000 AFLP studies cited in the BIOSIS Citation Index make inferences concerning individuals or populations from wild-collected material. Some of these AFLP studies evaluated methodological or methylation errors (Donini et al. 1997; Bonin et al. 2004) or the proper analysis of AFLP data to avoid confounding results (Meudt and Clarke 2007; e.g., from heterozygotes, multiple alleles, and deviations from Hardy–Weinberg equilibrium; Bonin et al. 2004; Foll et al. 2010). Largely missing from the literature, aside from general warnings, is how to ensure removal of non-target DNA from AFLP DNA fingerprinting studies.

Materials and methods

Individual *P. umbilicalis* thalli were collected from three intertidal locations in the North Atlantic: Schoodic Point, ME (44° 20′ 20″ W 680 2′ 42″ ($n = 25$)); Cobscook Bay, ME (44° 52′ 54″ W 67° 7′ 53″ ($n = 25$)); and Sidmouth, England (50° 40′ 38″ W 3° 14′ 23″ ($n = 17$); Fig. 1). *Porphyra umbilicalis* haplotype P.um1 (UTEX #LB 2951, isolated from Schoodic Point in 2008 and being sequenced by The Joint Genome Institute, JGI) was included as a positive control against methodological artifacts in all of the AFLP reactions and was included in our analyses (adjusted sample size for Schoodic Point, $n = 26$). The Maine samples were examined for epiphytes and endophytes by brightfield microscopy immediately after collection. As a precaution against contaminating DNA, each individual was washed vigorously in soapy, deionized water for 1 min, and duplicate samples were frozen in liquid nitrogen (LN_2). To further determine whether our general sample preparation successfully removed contaminants, we performed AFLP analysis on another group of wild-collected plants ($n = 3$ individuals) from Schoodic as

Fig. 1 Collecting sites in the North Atlantic with a detailed map showing where in Maine and England collections were made

follows: The AFLP profiles for these individuals were generated in duplicate from equivalent longitudinal sections of each blade that were either (1) rubbed with paper towels (treatment: minimal cleaning), (2) cleaned in the manner described above (treatment: vigorous cleaning), or (3) cleaned and then subcultured in the antibiotic conditions described below for the English site (treatment: vigorous cleaning and antibiotics).

The Schoodic Point and Cobscook Bay collections of *P. umbilicalis* from $\sim 300 \times 30$ m sections of high-mid-intertidal zone at each site were collected using a transect line and a random numbers table. A 10-knotted string dropped onto the clumps so located allowed individuals to be collected randomly because knot position was determined with a random numbers' table. Sidmouth (UK) individuals were collected haphazardly at low tide from a single boulder (~ 1 m^3) on a predominantly sandy beach, blotted dry, and express-transported for our preparation. Sidmouth samples were examined for endophytes and epiphytes with light microscopy, vigorously washed in soapy water for 1 min, and separated into individual cultures for 1 week. All Sidmouth individuals were producing either male or female gametes. For culture, individual thalli were placed in 1 L Erlenmeyer flasks with 800 mL 1 μm-filtered, autoclaved seawater with ½ Provasoli's enrichment solution (WES; West and McBride 1999), 4 mg L^{-1} GeO$_2$, 10 mg/L penicillin G, and 2.5 mg L^{-1} dihydro-streptomycin sulfate ($1\times$ PenStrep; Guillard 2005). The cultures were gently bubbled (~ 1 L min^{-1}) at 60 μmol photons m^{-2} s^{-1}, 10 °C, 14:**10** (L:**D** in Percival I36LLVL incubators (Percival Scientific, Inc., Perry, IA)). All

individuals were determined to be free of endophytes or epiphytes by light microscopy after 1 week, and duplicate samples were frozen in LN_2.

DNA was extracted from cleaned, frozen tissue using a DNeasy Plant Mini Kit (Qiagen Inc. Gaithersburg, MD) following the manufacturer's instructions. The Small Plant Genome Mapping Kit (Applied Biosystems Carlsbad, CA) was used to generate the AFLP profiles for this investigation according to the manufacturer's instructions (after Vos et al. 1995). Briefly, 50 ng *P. umbilicalis* DNA was digested with *Eco*RI and *Mse*I endonucleases (New England Biolabs, Ipswich, MA). Ligation of adaptors for pre-selective amplification was carried out overnight at room temperature (T4 ligase, New England Biolabs). Preselective PCR amplification was carried out with no selective bases (*Eco*RI+ 0 and *Mse*I+ 0; Iitsuka et al. 2002; Yang et al. 2003; Niwa et al. 2004). Subsequently, selective PCR amplification of targets was accomplished with four fluorescently labeled *Eco*RI primer sets (*Eco*RI-AC/*Mse*I-CAA, *Eco*RI-AG/*Mse*I-CAA, *Eco*RI-AG/*Mse*I-CG, *Eco*RI-AC/*Mse*I-CT). Selective PCR products were analyzed on an ABI 3730 analyzer to create a multilocus genotype (MLG) from a presence–absence binary matrix using GeneMarker 1.8 software (SoftGenetics LLC State College, PA). Duplicate samples were extracted and amplified from every individual used throughout this investigation to ensure repeatability of AFLP scoring. When the presence or absence of any marker was inconclusive, additional preselective and selective PCRs were performed and compared to the original duplicates. Only those bands that occurred in duplicate runs were scored as present. Additionally, our laboratory has generated genome scaffolds assembled by JGI for 3 planctomycete contaminants known to survive antibiotic treatment in P.um1 cultures. We subjected these scaffolds to in silico AFLP analysis. Any bands from the *P. umbilicalis* individuals also found in the in silico AFLP analysis of the planctomycete scaffolds were removed from further analysis.

To investigate the genotypic diversity and the presence of clones within the Maine populations, we used the tools included within the GenoDive 2.0b22 software package (Meirmans and Van Tienderen 2004). Genetic distances were calculated using an infinite allele model (IAM), which assumes that a single mutation changes the state of any detected marker within an individual's MLG. The bimodal distribution of the pairwise distances and a comparison to the maximum pairwise distance was used to set an appropriate threshold for the assignment of a MLG to a single multilocus lineage (MLL, or "clone") for the Maine populations. We tested for the presence of clonal structure within the dataset by randomizing AFLP markers over individuals and comparing the observed diversity with the randomized set (Gomez and Carvalho 2000). If the

threshold were set too high, we would over-estimate the amount of variation in the MLG attributable to scoring error or somatic mutation and fail to reject the possibility of sexual reproduction within those individuals assigned to a single clone (i.e., sexual reproduction is suggested by $p_{sex} > 0.05$). Diversity associated with putative clonal asexuals compared to individuals known to be sexually reproductive was calculated with the following indices: number of genotypes, number of effective genotypes, and Simpson's diversity index. We also performed an analysis of molecular variance (AMOVA) to examine genetic diversity within and between sites. Finally, the genetic similarity between individuals was calculated using the *Primer v6* software (Primer-E Ltd., Ivybridge, UK).

Results

Sensitivity analysis for non-target DNA

A total of 566 polymorphic markers were scored for all individuals in this study. The in silico analysis of planctomycete scaffolds revealed that the presence of 10 AFLP bands in the *P. umbilicalis* preparations (1.8 % of *P. umbilicalis* bands) represent fragment sizes that may be contributed by known planctomycete contaminants of *P. umbilicalis*; these planctomycete bacteria remain associated with blades after vigorous washing. The putative contaminant markers were removed from further analysis, leaving a total of 556 markers. Verification of our cleaning protocol using a subset of 55 informative bands showed that vigorous cleaning was the most significant factor in reducing variation due to contaminants in AFLP profiles between preparation methods. Contamination from epiphytic bacteria and microalgae was still responsible for up to 15 % of the bands detected after minimal cleaning; however, another 1–2 bands/individual were removed in the vigorous cleaning and antibiotic treatment compared to vigorous cleaning alone (Fig. 2).

Similarity analyses

Markers in this study revealed 58 distinct MLLs (21, 20, and 17 for Schoodic, Cobscook, and Sidmouth, respectively), two MLLs, one at each Maine site, were comprised of 6 individuals each, whereas no clonal signature was found in the Sidmouth samples (Table 1). The frequency distribution of pairwise distances was weakly bimodal, suggesting the presence of non-sexual genomic rearrangements (Meirmans and Van Tienderen 2004; also see Lasso 2008 for discussion). The threshold for defining clonal lineages for the Maine collections was set to 2.7 % (Fig. 3). This threshold corresponds to the 2–4 % threshold
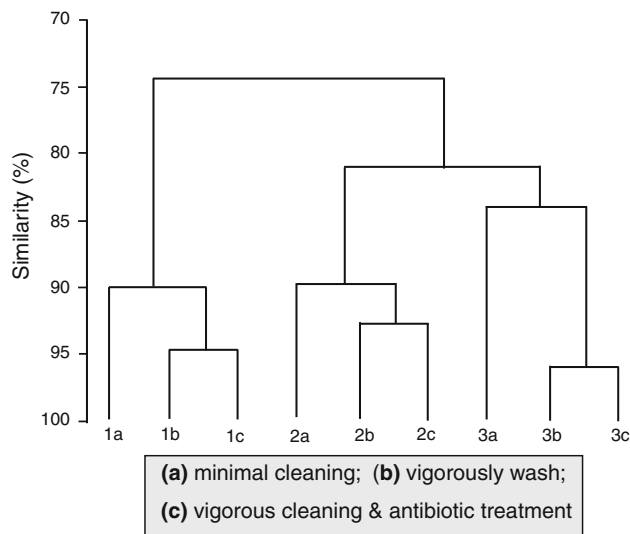
(a) minimal cleaning; **(b)** vigorously wash; **(c)** vigorous cleaning & antibiotic treatment

Fig. 2 Single linkage dendrogram generated from Bray–Curtis similarity coefficient showing that vigorous washing of field-collected samples greatly reduced contamination from bacteria and microalgae living on the surface of wild-collected *P. umbilicalis*

cited in previous studies (e.g., Arens et al. 1998; Winfield et al. 1998; Van der Hulst et al. 2003; Douhovnikoff and Dodd 2003; Lasso 2008). The probability that the 10 individuals comprising these two clones came from sexual recombination is not supported ($p_{sex} = 0.001$ for those clones at both Maine sites, Table 1). AMOVA results showed that the greatest amount of genetic diversity was found within sites rather than among sites (Table 2), and the Bray–Curtis genetic similarity analysis showed that the Sidmouth site exhibits the greatest within-site diversity as compared to the individuals from either of the two Maine sites (Fig. 4).

Discussion

AFLP analysis of P. umbilicalis. Although this study identified one clone at Schoodic that was sampled 6× and another clone at Cobscook that was sampled 6×, *P. umbilicalis* in Maine is neither a single clone nor a small

subset of clones. We are not able to determine robustly whether the remaining 39 MLLs from the Maine collections are asexual clones or were produced by sexual reproduction. However, the genetic similarity of all Maine individuals was high, and we have never observed sexual reproduction in the field in the northwest Atlantic, and that the closest source of sexual individuals is in Europe (Brodie and Irvine 2003; Blouin et al. 2007; Brodie et al. 2008). As expected, the (sexual) Sidmouth population displayed greater within-site variation when examined with AFLP DNA fingerprinting than the (putative asexual) Maine populations. The larger variation found within the Sidmouth site in comparison with the two Maine sites (Fig. 4) is further demonstrated by the scales over which the individuals in Maine and Sidmouth were collected. The Maine individuals were collected over several hundred meters of shoreline at each site whereas Sidmouth individuals were all from a single small boulder.

Teasdale and Klein (2010) recently reported that the genetic divergence found in *Porphyra* from both sides of the Atlantic is consistent with a recolonization of North America by a European population sometime after the LGM through drift or commerce (see Provan and Bennett 2008 for review). It should be noted that transatlantic introductions through shipping vectors (e.g., Brawley et al. 2009) would almost certainly have included sexual individuals given their prevalence in Europe (Brodie et al. 2008). Thus, the apparent lack of sexual differentiation in Maine *P. umbilicalis* remains notable. There is mounting evidence that many littoral animals and algae survived the last LGM in NW Atlantic refugia (Young et al. 2002; Govindarajan et al. 2005; Maggs et al. 2008; Muhlin and Brawley 2009; Coyer et al. 2011b) and that repeated cycles of glaciation have affected NW Atlantic intertidal algal genetic diversity more than in the NE Atlantic (Cunningham 2008; Olsen et al. 2010; Coyer et al. 2011a). This suggests that *P. umbilicalis* could have experienced a strong bottleneck effect from a previous glacial period and that this population might have survived the LGM in offshore refugia as a "trailing" edge population (Hampe and Petit 2005; Provan and Maggs 2012). Although asexual

Table 1 Descriptive statistics of genetic diversity parameters; *n*—sample size; *T*—threshold for multilocus genotype; MGD—maximum genetic distance in IAM; Clones MLL—number of Multilocus lineages detected at threshold; Clonal fraction—(*n*-Clones)/*n*; Div$_{pop}$—Simpson's diversity index; MLL = 1×—multilocus lineage sampled once; MLL >1×—multilocus lineage sampled more than once; p_{sex}—probability that sexual recombination is responsible for the repeated genotypes assigned to a MLL

Population	*n*	No. of markers	*T*	MGD	MLL	Clonal fraction	MLLs = 1x	MLLs >1×	Div$_{pop}$	p_{sex}
Schoodic	26	384	61	227	21	0.19	20	1	0.954	0.001
Cobscook	25	380	61	227	20	0.2	19	1	0.95	0.001
Sidmouth	17	316	*	120	17	*	17	0	1	*

* Sidmouth individuals are obligate sexuals

Fig. 3 Frequency distribution of genetic distances calculated by IAM for the Maine collection multilocus genotypes, the number of clones represented at each distance, and the threshold cutoff (*vertical line*) for assignment to the same multilocus lineage (MLL, clone). Genetic distances to the *left* of the threshold are assumed to be the result of non-sexual genomic rearrangements
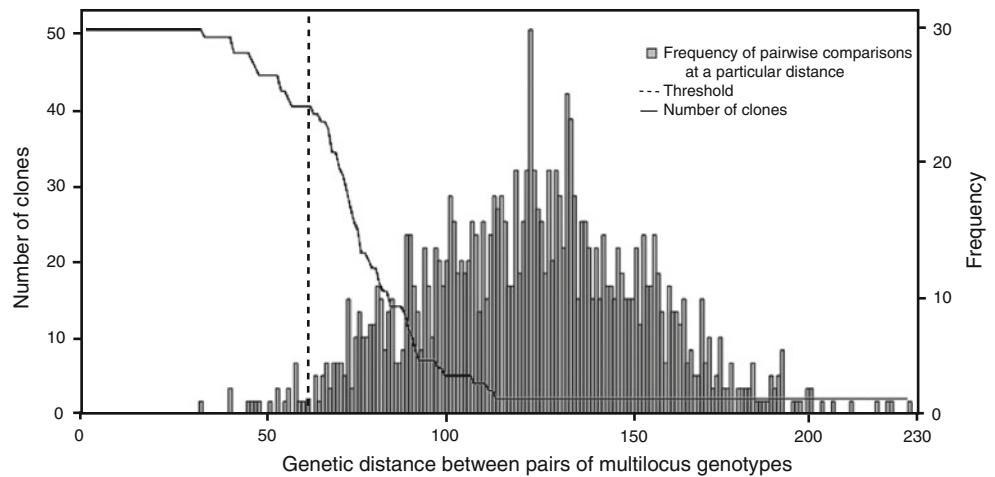

Table 2 Analysis of molecular variance (AMOVA) for *P. umbilicalis* individuals from Cobscook, Schoodic, and Sidmouth

Source of variation	df	MS	Variance components	Percentage of total variation	F_{st}	*p* value
Within populations	65	103.75	103.75	0.778	–	–
Among populations	2	764.44	29.62	0.222	0.222	0.001

There is no *p* value listed for within populations because this is effect a measure of inbreeding, which is assumed in populations with asexual reproduction
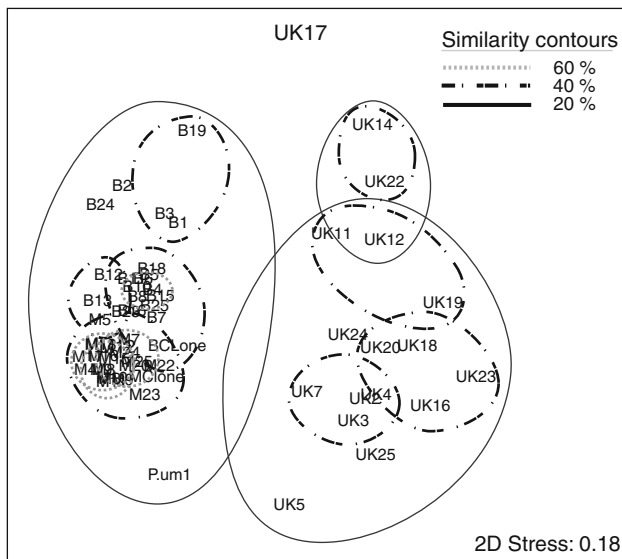

Fig. 4 Multidimensional scaling ordination plot derived using the Bray–Curtis coefficient showing similarity of *P. umbilicalis* using AFLPs for individuals in this study (UK = Sidmouth, B = Schoodic Point, M = Cobscook Bay)

reproduction could favor persistence of *P. umbilicalis* in the NW Atlantic, there are numerous related Bangiales (e.g., *B. birdiae*, *P. purpurea*) with transatlantic distributions that reproduce primarily by sexual reproduction throughout their distribution, including in the NW Atlantic (Sutherland et al. 2011). Thus, the apparent absence of sexual reproduction in *P. umbilicalis* in Maine remains

puzzling, and additional phylogeographic studies of *P. umbilicalis* and sister taxa may help solve the conundrum.

In addition to the potential for cryptic non-target DNA discussed below, genome rearrangements mediated by transposable elements (TEs) or viruses are known to be a major source of genomic variation for photosynthetic organisms (Slotkin and Martienssen 2007) and could account for the differences in AFLP profiles for putative asexuals (Maine collections) in our study. Further, theoretical and empirical assessments of the ability of asexual species to adapt to new environments find that rates of mitotic recombination may be adequate to allow asexual populations to adapt as quickly as sexual ones to new environments (Feschotte and Pritham 2007; Birky 2010). Finally, equivalent nucleotide substitution rates have been found in both sexual and asexual bdelloid lineages (Welch and Meselson 2001; Mandegar and Otto 2007). If similar rates of substitution or recombination exist in *P. umbilicalis*, this could explain the absence of sexual differentiation observed in extensive field and culture studies yet the high levels of AFLP-determined diversity demonstrated in this study.

Identification and removal of non-target DNA

Non-target DNA from bacteria and microalgae are potentially confounding agents in AFLP DNA fingerprinting; however, the potential for contamination is rarely addressed formally in AFLP studies (Bonin et al. 2004). Simply

wiping our field samples after collection removed some non-target DNA; however, vigorous washing in deionized water with detergent successfully removed nearly all of the non-target DNA as compared to vigorous washing followed by treatment with two antibiotics (penicillin and streptomycin). Caution should be used when applying our protocol to other organisms because our cleaning procedure was specifically designed to remove non-target DNA while not damaging the *P. umbilicalis*. *Porphyra umbilicalis* has a simple flat morphology, making it relatively easy to clean blades as described. Additionally, the epiphytic organisms that were removed in our vigorous cleaning treatment are adapted to living in full salinity seawater (32 PSU). The efficacy of our cleaning procedure was improved by the ability of deionized water to cause many organisms growing on the surface of the *P. umbilicalis* thalli to rupture, whereas *P. umbilicalis* is tolerant to these conditions.

Additional, non-target DNA sources may remain cryptic because they are not removed by the most rigorous cleaning protocols that still allow for successful extraction of target DNA. We were able to identify and remove potential planctomycete contamination because of our knowledge that planctomycete bacteria survive in unialgal *Porphyra* cultures that have been treated with penicillin and streptomycin, and because of the availability of their complete genomes. Planctomycete bacteria are resistant to β-lactams (penicillin G) and aminoglycosides (dihydrostreptomycin sulfate), and *P. umbilicalis* is killed by many antibiotics that eliminate epiphytic planctomycetes (Fuerst et al. 1997; Cayrou et al. 2010). In our approach, we have removed these bands from our analysis because they offer a potential source of contamination; further analysis is required (excision and sequencing of bands removed from analysis) to prove that these bands are planctomycete in origin. Although some investigators predict that whole genome analysis will replace molecular methods like the AFLP technique (e.g., Schlotterer 2004), it is just as likely that whole genome approaches will still be too expensive for many targets for the foreseeable future (Meudt and Clarke 2007; Gaggiotti 2010). Although most investigations do not have readily available reference genomes for known contaminants, recent advances in sequencing technology have resulted in a dramatic increase in the genetic data now available in public databases including whole genome sequences of many bacteria. Our in silico data successfully identified previously cryptic non-target DNA; however, we cannot rule out the presence of additional non-target DNA that was not removed by our cleaning procedure (e.g, bacteria living within the cell wall of *P. umbilicalis*). Even so, the application of an appropriate cleaning protocol and application of in silico AFLP analysis could identify potential contaminants from species known to be associated as parasites or commensals with the studied organism, and greatly improve the ability to interpret AFLP data from field-collected organisms.

References

Adey W, Hayek L (2011) Elucidating marine biogeography with macrophytes: quantitative analysis of the North Atlantic supports the thermogeographic model and demonstrates a distinct subarctic region in the northwestern Atlantic. Northeast Nat 18:1–128

Arens P, Coops H, Jansen J, Vosman B (1998) Molecular genetic analysis of black poplar (*Populus nigra* L.) along Dutch rivers. Mol Ecol 7:11–18

Bell G, Gonzalez A (2011) Adaptation and evolutionary rescue in metapopulations experiencing environmental deterioration. Science 332:1327–1330

Birky CW (2010) Positively negative evidence for asexuality. J Hered 101:S42–S45

Blouin N, Xiugeng F, Peng J, Yarish C, Brawley SH (2007) Seeding nets with neutral spores of the red alga *Porphyra umbilicalis* (L.) Kutzing for use in integrated multi-trophic aquaculture (IMTA). Aquaculture 270:77–91

Bonin A, Bellemain E, Eidesen PB, Pompanon F, Brochmann C, Taberlet P (2004) How to track and assess genotyping errors in population genetics studies. Mol Ecol 13:3261–3273

Brawley SH, Coyer JA, Blakeslee AMH, Hoarau G, Johnson LE, Byers JE, Stam WT, Olsen JL (2009) Historical invasions of the intertidal zone of Atlantic North America associated with distinctive patterns of trade and emigration. P Natl Acad Sci USA 106:8239–8244

Brodie J, Irvine LM (2003) Seaweeds of the British Isles. Vol. 1. Part 3B. Bangiophycidae. British Museum of Natural History, London

Brodie J, Irvine L, Neefus CD, Russell S (2008) *Ulva umbilicalis* L. and *Porphyra umbilicalis* Kutz. (Rhodophyta, Bangiaceae): a molecular and morphological redescription of the species, with a typification update. Taxon 57:1328–1331

Cayrou C, Raoult D, Drancourt M (2010) Broad-spectrum antibiotic resistance of planctomycetes organisms determined by Etest. J Antimicrob Chemoth 65:2119–2122

Coyer JA, Hoarau G, Costa JF, Hogerdijk B, Serrao EA, Billard E, Valero M, Pearson GA, Olsen JL (2011a) Evolution and diversification within the intertidal brown macroalgae *Fucus spiralis/F. vesiculosus* species complex in the North Atlantic. Mol Phylogenet Evol 58:283–296

Coyer JA, Hoarau G, Van Schaik J, Luijckx P, Olsen JL (2011b) Trans-Pacific and trans-Arctic pathways of the intertidal macroalga *Fucus distichus* L. reveal multiple glacial refugia and colonizations from the North Pacific to the North Atlantic. J Biogeogr 38:756–771

Cunningham CW (2008) How to use genetic data to distinguish between natural and human-mediated introduction of *Littorina littorea* to North America. Biol Invasions 10:1–6

Dhar R, Sagesser R, Weikert C, Yuan J, Wagner A (2011) Adaptation of *Saccharomyces cerevisiae* to saline stress through laboratory evolution. J Evol Biol 24:1135–1153

Dixon P (1965) Perennation, vegetative propagation and red algal life histories, with special reference to *Asparagopsis* and other Rhodophyta. Bot Goth 3:67–74

Donini P, Elias ML, Bougourd SM, Koebner RMD (1997) AFLP fingerprinting reveals pattern differences between template DNA extracted from different plant organs. Genome 40:521–526

Douhovnikoff V, Dodd RS (2003) Intra-clonal variation and a similarity threshold for identification of clones: application to *Salix exigua* using AFLP molecular markers. Theor Appl Genet 106:1307–1315

Feschotte C, Pritham EJ (2007) DNA transposons and the evolution of eukaryotic genomes. Annu Rev Genet 41:331–368

Foll M, Fischer MC, Heckel G, Excoffier L (2010) Estimating population structure from AFLP amplification intensity. Mol Ecol 19:4638–4647

Fuerst JA, Gwilliam HG, Lindsay M, Lichanska A, Belcher C, Vickers JE, Hugenholtz P (1997) Isolation and molecular identification of planctomycete bacteria from postlarvae of the giant tiger prawn, *Penaeus monodon*. Appl Environ Microb 63:254–262

Gaggiotti OE (2010) Bayesian statistical treatment of the fluorescence of AFLP bands leads to accurate genetic structure inference. Mol Ecol 19:4586–4588

Gomez A, Carvalho GR (2000) Sex, parthenogenesis and genetic structure of rotifers: microsatellite analysis of contemporary and resting egg bank populations. Mol Ecol 9:203–214

Govindarajan AF, Halanych KM, Cunningham CW (2005) Mitochondrial evolution and phylogeography in the hydrozoan *Obelia geniculata* (Cnidaria). Mar Biol 146:213–222

Guillard R (2005) Purification methods for microalgae. In: Andersen RA (ed) Phycological methods: algal culturing techniques. Elsevier Academic Press, Burlington, pp 117–132

Hallatschek O, Nelson DR (2010) Life at the front of an expanding population. Evolution 64:193–206

Hallatschek O, Hersen P, Ramanathan S, Nelson DR (2007) Genetic drift at expanding frontiers promotes gene segregation. P Natl Acad Sci USA 104:19926–19930

Hampe A, Petit RJ (2005) Conserving biodiversity under climate change: the rear edge matters. Ecol Lett 8:461–467

Iitsuka O, Nakamura K, Ozak IA, Okamoto N, Saga N (2002) Genetic information of three pure lines of *Porphyra yezoensis* (Bangiales, Rhodophyta) obtained by AFLP analysis. Fish Sci 68:1113–1117

Lasso E (2008) The importance of setting the right genetic distance threshold for identification of clones using amplified fragment length polymorphism: a case study with five species in the tropical plant genus *Piper*. Mol Ecol Resour 8:74–82

Maggs CA, Castilho R, Foltz D, Henzler C, Jolly MT, Kelly J, Olsen J, Perez KE, Stam W, Vainola R, Viard F, Wares J (2008) Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. Ecology 89:S108–S122

Mandegar MA, Otto SP (2007) Mitotic recombination counteracts the benefits of genetic segregation. P R Soc B 274:1301–1307

Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Mol Ecol Notes 4:792–794

Meudt HM, Clarke AC (2007) Almost forgotten or latest practice? AFLP applications, analyses and advances. Trends Plant Sci 12:106–117

Muhlin JF, Brawley SH (2009) Recent versus relic: discerning the genetic signature of *Fucus vesiculosus* (Heterokontophyta; Phaeophyceae) in the Northwestern Atlantic. J Phycol 45:828–837

Niwa K, Kikuchi N, Iwabuchi M, Aruga Y (2004) Morphological and AFLP variation of *Porphyra yezoensis* Uedo form *narawaensis* Miura (Bangiales, Rhodophyta). Phycol Res 52:180–190

Olsen JL, Zechman FW, Hoarau G, Coyer JA, Stam WT, Valero M, Aberg P (2010) The phylogeographic architecture of the fucoid seaweed *Ascophyllum nodosum*: an intertidal 'marine tree' and survivor of more than one glacial-interglacial cycle. J Biogeogr 37:842–856

Otto SP (2009) The evolutionary enigma of sex. Am Nat 174:S1–S14

Pearson EA, Murray SN (1997) Patterns of reproduction, genetic diversity, and genetic differentiation in California populations of the geniculate coralline alga *Lithothrix aspergillum* (Rhodophyta). J Phycol 33:753–763

Provan J, Bennett KD (2008) Phylogeographic insights into cryptic glacial refugia. Trends Ecol Evol 23:564–571

Provan J, Maggs CA (2012) Unique genetic variation at a species' rear edge is under threat from global climate change. P Roy Soc B-Biol Sci 279:39–47

Schlotterer C (2004) The evolution of molecular markers: just a matter of fashion? Nat Rev Genet 5:63–69

Sears J (2002) NEAS keys to the marine algae of the Northwestern Coast of North America. Northeastern Algal Society, Fall River

Slotkin RK, Martienssen R (2007) Transposable elements and the epigenetic regulation of the genome. Nat Rev Genet 8:272–285

Song YX, Scheu S, Drossel B (2011) Geographic parthenogenesis in a consumer-resource model for sexual reproduction. J Theor Biol 273:55–62

Sosa PA, Lindstrom SC (1999) Isozymes in macroalgae (seaweeds): genetic differentiation, genetic variability and applications in systematics. Eur J Phycol 34:427–442

Sutherland JE, Lindstrom SC, Nelson WA, Brodie J, Lynch MDJ, Hwang MS, Choi HG, Miyata M, Kikuchi N, Oliveira MC, Farr T, Neefus C, Mols-Mortensen A, Milstein D, Muller KM (2011) A New Look at an Ancient Order: generic Revision of the Bangiales (Rhodophyta). J Phycol 47:1131–1151

Teasdale BW, Klein AS (2010) Genetic variation and biogeographical boundaries within the red alga *Porphyra umbilicalis* (Bangiales, Rhodophyta). Bot Mar 53:417–431

Teasdale BW, West A, Klein AS, Mathieson AC (2009) Distribution and evolution of variable group-I introns in the small ribosomal subunit of North Atlantic *Porphyra* (Bangiales, Rhodophyta). Eur J Phycol 44:171–182

Van der Hulst RGM, Mes THM, Falque M, Stam P, Den Nijs JCM, Bachmann K (2003) Genetic structure of a population sample of apomictic dandelions. Heredity 90:326–335

Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP - a new technique for DNA-fingerprinting. Nucleic Acids Res 23:4407–4414

Welch DBM, Meselson MS (2001) Rates of nucleotide substitution in sexual and anciently asexual rotifers. P Natl Acad Sci USA 98:6720–6724

West JA, McBride DL (1999) Long-term and diurnal carpospore discharge patterns in the Ceramiaceae, Rhodomelaceae, and Delessariaceae (Rhodophyta). Hydrobiol 288(289):101–103

Winfield MO, Arnold GM, Cooper F, Le Ray M, White J, Karp A, Edwards KJ (1998) A study of genetic diversity in *Populus nigra* subsp. *Betulifolia* in the Upper Severn area of the UK using AFLP markers. Mol Ecol 7:3–10

Yang R, Liu B, Luo Q, Wang Y, Bao J (2003) Genetic variation of *Porphyra yezoensis* by using AFLP. Acta Oceanol Sin/Haiyang Xuebao 22:453–457

Young AM, Torres C, Mack JE, Cunningham CW (2002) Morphological and genetic evidence for vicariance and refugium in Atlantic and Gulf of Mexico populations of the hermit crab *Pagurus longicarpus*. Mar Biol 140:1059–1066