J. Phycol. **43,** 675–685 (2007)

DOI: 10.1111/j.1529-8817.2007.00369.x

GENETIC STRUCTURE IN POPULATIONS OF *FUCUS VESICULOSUS* (PHAEOPHYCEAE) OVER SPATIAL SCALES FROM 10 M TO 800 KM[1]

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Recent studies showing consequences of species' genetic diversity on ecosystem performance raise the concern of how key ecosystem species are genetically structured. The bladder wrack *Fucus vesiculosus* L. is a dominant species of macroalga in the northern Atlantic, and it is particularly important as a habitat-forming species in the Baltic Sea. We examined the genetic structure of populations of *F. vesiculosus* with a hierarchical approach from a within-shore scale (10 m) to a between-seas scale (Baltic Sea–Skagerrak, 800 km). Analysis of five microsatellite loci showed that population differentiation was generally strong (average F_{ST} = 12%), being significant at all spatial scales investigated (10^1, 10^3, $10^{4–5}$, 10^6 m). Genetic differentiation between seas (Baltic Sea and Skagerrak) was substantial. Nevertheless, the effects of isolation by distance were stronger within seas than between seas. Notably, Baltic summer-reproducing populations showed a strong within-sea, between-area (70 km) genetic structure, while Baltic autumn-reproducing populations and Skagerrak summer-reproducing populations revealed most genetic diversity between samples within areas (<1 km). Despite such differences in overall structure, Baltic populations of summer- and autumn-reproducing morphs did not separate in a cluster analysis, indicating minor, if any, barriers to gene flow between them. Our results have important implications for management and conservation of *F. vesiculosus*, and we raise a number of concerns about how genetic variability should be preserved within this species.

Key index words: **Baltic Sea; gene flow; microsatellites; population genetic structure; seaweed**

Abbreviations: **CA, correspondence analysis; NJ, neighbor-joining**

The study of biodiversity conservation is currently focused on species diversity. Nevertheless, diversity within species (genetic diversity) is recognized by evolutionary biologists as similarly essential, being the core component of evolution and long-term population sustainability (Frankham et al. 2004). Additional support for the crucial importance of genetic diversity emerges from recent studies showing that increased genetic diversity in habitat-forming species can have important ramifications for population- and community-level processes (Gamfeldt et al. 2005, Reusch et al. 2005, Crutsinger et al. 2006). Thus, the goal of maintaining genetic diversity within species is not solely to maintain viable and evolving species but also to secure ecosystem services and resilience by protecting the genetic diversity of species that have key roles in the ecosystem.

Bladder wrack, *Fucus vesiculosus*, is a dominant species of the temperate North Atlantic rocky shores (Norton 1994), and in the atidal brackish-water Baltic Sea, it is ecologically the most important perennial, large brown seaweed, providing shelter and food for associated flora and fauna (Kautsky et al. 1992). In much of the Baltic Sea, this species completely dominates the shallow hard-bottom areas down to 8–10 m depth (Snoeijs 1999). Since the 1940s, there have been major declines in the distribution of *F. vesiculosus* in some areas (Kangas et al. 1982, Kautsky et al. 1986) and even local extinctions (Nilsson et al. 2004), both a likely consequence of increased anthropogenic stress, such as eutrophication, and possibly of the cascade effects of overfishing or other factors changing the fish community

[1]Received 7 July 2006. Accepted 8 March 2007.
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(Nilsson 2004). With *F. vesiculosus* being a key structuring species of the Baltic ecosystem and at the same time being threatened by anthropogenic and natural perturbations, it is alarming that we have almost no information on the population genetic structure of the species in this area. Population genetic data provide essential input for management as well as restoration and support assessments of the threat level to local populations. Moreover, such data may indicate important background information on earlier distributions and population sizes of the species (Palumbi 2003).

A factor likely to have the biggest impact on the genetic structure of populations of marine sessile and sedentary species, such as macroalgae and benthic invertebrate species, is propagule and larval dispersal. Indeed, a review of population genetic data of 333 animal species revealed a strong general trend of negative correlation between larval dispersal ability and genetic differentiation among populations (Bohonak 1999). The situation for marine macroalgae is no exception, although it may be complicated by more or less frequent long-distance dispersal of fertile fragments of the plant in addition to dispersal of spores and gametes (van den Hoek 1987). Nevertheless, there are studies of genetic differentiation in macroalgae that show evidence of genetic isolation-by-distance effects, indicating restricted dispersal in at least some species (Williams and Di Fiori 1996, Coyer et al. 1997, 2003).

All fucoid plants have short-lived eggs and sperm, and the fertilized eggs disperse poorly before settlement (Williams and Di Fiori 1996, Serrão et al. 1997, Dudgeon et al. 2001; however, see Reed et al. 1988 for exceptionally long dispersal during particular circumstances in a related species). Thus, we expected *F. vesiculosus* to have a strong spatial genetic structure showing clear isolation-by-distance effects on scales similar to the related species *Fucus serratus* L. (Coyer et al. 2003). We designed the present study with the primary objective of assessing the spatial genetic structure of *F. vesiculosus* within a region where this species is a key ecosystem component.

Fucus vesiculosus is morphologically and physiologically extremely variable across its geographic range, and this is thought to be due to large phenotypic plasticity inherent to the species (Sideman and Mathieson 1985, Russell 1987, Scott et al. 2001), but at least part of it is shown to be genetically determined (Kalvas and Kautsky 1993, Pearson et al. 2000). The morphological variation of *F. vesiculosus* has given rise to various taxonomic considerations, with earlier literature describing a range of varieties sometimes suggested as separate species (Waern 1952, Luther 1981, Kalvas and Kautsky 1993). The limitation of older studies has been the lack of genetic methods to test if morphological and ecological separation of varieties is accompanied by interrupted gene flows, but various DNA techniques have now eliminated these inconveniences. As a result, species status was recently confirmed for the two closely related species *Fucus spiralis* L. and *F. vesiculosus* (Engel et al. 2005). Similarly, using a combination of morphological characters and DNA markers, we were able to show that the dwarf, bushy, and narrow-fronded morph of *F. vesiculosus* that dominates the northern part of the Baltic Sea is genetically isolated from the common morph, warranting it species status (*Fucus radicans* L. Bergström et L. Kautsky; Bergström et al. 2005, Tatarenkov et al. 2005).

The present study does not include *F. radicans,* but in the southern parts of the Baltic Sea, *F. vesiculosus* shows another phenotypic division: most plants have their main reproductive period during May–June, while others reproduce mainly in September–October (Carlson 1991, Berger et al. 2001). Plants with the two periods of gamete release show additional differences in size and number of eggs released and different response to photoperiodicity treatments in the laboratory (Berger et al. 2001). Moreover, repeated observations of the reproductive season of individual plants over 3 years demonstrate that plants maintain the same reproductive strategy (Berger et al. 2001). These observations suggest a potential for an additional subdivision of the *F. vesiculosus* gene pool in the southern parts of the Baltic Sea, namely, by reproductive strategy. Indeed, a similar case of reproductive season subdivision was earlier reported for *Fucus distichus* L. (Sideman and Mathieson 1983), and in this species, morphological differences were maintained in progeny raised in common conditions, indicating some genetic differentiation between the two reproductive morphs. Genetic separation of the two reproductive morphs of *F. vesiculosus* would heavily impact their evolution and, more specifically, would have important repercussions on the Baltic Sea ecosystem. On the other hand, if they are not genetically distinct, it is important to measure the gene flow and to explain the mechanisms that ensure continued gene exchange. Therefore, a second aim of this study was to compare the genetic variation within and between Baltic populations of *F. vesiculosus* representing the two reproductive strategies to test the hypothesis that different reproductive seasons impact the genetic relationship of the reproductive morphs and, consequently, the genetic structure of the species.

Thus, we analyzed the genetic diversity in the Baltic Sea–Skagerrak distribution of *F. vesiculosus* using a hierarchical sampling scheme designed to estimate diversity at spatial scales from between seas (800 km) to within shores (10 m). Within the Baltic, we included both summer- and autumn-reproducing populations to assess any effect on the genetic structure imposed by the reproductive characteristics of populations. To separate genetic structure owing to

geographic isolation from the effects of local gene flow between autumn and summer morphs, we sampled reproductively different morphs in separate sites. We interpret our results from the perspective of conservation of *F. vesiculosus*, particularly, considering its management in the Baltic Sea area where it is a key habitat-forming species.

MATERIALS AND METHODS

Study species. The life cycle of *F. vesiculosus* is diplontic (Mable and Otto 1998). The species is dioecious, with male and female gametes produced by separate, unisexual individuals. The gametes, eggs and sperm, are released in the water where fertilization takes place and a negatively buoyant zygote is produced, which typically settles within meters of the parents (Serrão et al. 1997).

Sampling. A total of 24 samples (16 summer reproducing and eight autumn reproducing) were collected in June or September 2002 from populations of *F. vesiculosus* in the southern Baltic Sea and from the Swedish west coast (Fig. 1). Each sample consisted of 50 reproductive individuals, which were harvested from an area of 2 m^2 at a depth of 0.5–1 m. Our sampling design for the summer-reproducing populations included several hierarchical levels: samples were collected from different SEAS (Baltic Sea and Skagerrak) at distances of 635–795 km; within seas samples were from different AREAS, which were separated by 70 km in the Baltic Sea and by 75 km in the Skagerrak; within-area samples were from two different SITES 1 km apart; and within sites, two samples were taken 10 m apart (Fig. 1). Eight samples of the autumn-reproducing populations were collected from the mainland shore of the Baltic Sea only; the sampling scheme mirrored that of the summer-reproducing populations from Öland (an island separated from the mainland by Kalmar Sound, see Fig. 1). The study by Berger et al. (2001) showed that only summer-reproducing plants occur on Öland, whereas mainland shores across Kalmar Sound usually harbor mixed populations, typically predominated by autumn-reproducing plants. Sampling areas of autumn-reproducing plants in our study approximately correspond to sites #6 and #12 in Berger et al. (2001). Only individuals with fully developed receptacles were collected in all sites, enabling identification of summer- and autumn-reproducing individuals.

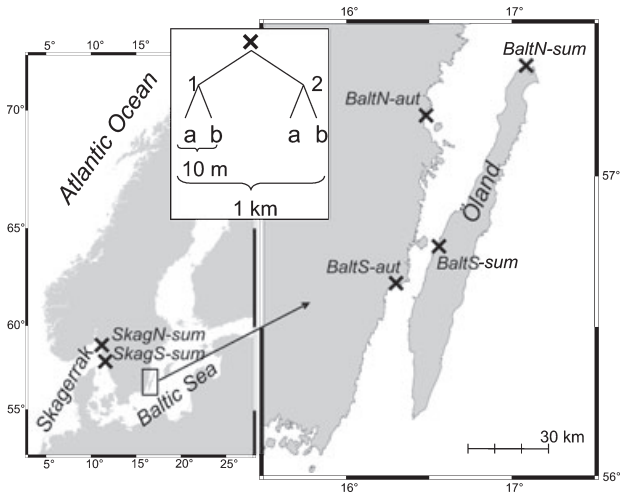

FIG. 1. Map of sampling areas in the Skagerrak and Baltic Seas with superimposed sampling design. AREAS are shown by crosses, with four samples in each AREA representing two SITES separated by 1 km of shoreline (see insets). Labels of the localities reflect both geographic position and time of reproduction: *BaltN-sum*, Baltic northern area of summer-reproducing *Fucus vesiculosus*; *BaltS-sum*, Baltic southern area of summer-reproducing *F. vesiculosus*; *BaltN-aut*, Baltic northern area of autumn-reproducing *F. vesiculosus*; *BaltS-aut*, Baltic southern area of autumn-reproducing *F. vesiculosus*; *SkagN-sum*, Skagerrak northern area of summer-reproducing *F. vesiculosus*; *SkagS-sum*, Skagerrak southern area of summer-reproducing *F. vesiculosus*.

Genetic analysis. The DNA was extracted from 10 mg of dried algal tissue using DNeasy Plant MiniKit (Qiagen, Valencia, CA, USA). Eluates of the first and second elutions were kept separately. Usually the second eluate (diluted 1:10 times with water) was used in the PCR reaction. When the first eluate was used occasionally for verification of genotypes, it was diluted 1:200 times. We genotyped the samples using five microsatellite loci (L20, L38, L58, L85, L94) developed by Engel et al. (2003). The PCR reactions were performed in 12 μL of solution containing 2 μL of template DNA, 0.6 units of Taq polymerase (MBI Fermentas, St. Leon-Rot, Germany), 1× PCR buffer (MBI Fermentas: 10 mM Tris-HCl [pH 8.0], 50 mM KCl, 0.08% Nonidet P40), 0.2 mM dNTP (Sigma, Manchester, UK), 2 mM $MgCl_2$, and 0.5 μM of each 5′ 5-Cy labeled forward primer and unlabeled reverse primer. Additionally, BSA was added to the PCR reaction mixture (final concentration 0.2 $\mu g \cdot \mu L^{-1}$) when amplifying loci L58, L85, and L94. Amplification conditions were as in Engel et al. (2003).

Six microliters of PCR product was loaded on to 8% acrylamide/bisacrylamide gels (ReproGel High Resolution from Amersham Pharmacia, Buckinghamshire, UK) and separated using ALFexpress II DNA Analyser (combined electrophoresis and laser fluorescent detection system from Amersham Pharmacia). Sizing of the PCR fragments was carried out using ALFwin Fragment Analyser software (ver. 1.00, Amersham Pharmacia) based on 50 bp DNA step ladder and two internal standards in each well. Additionally, two individuals with known genotypes were included on each gel to ensure consistency of scoring.

Statistical analyses. Departures from Hardy–Weinberg equilibrium (Rousset and Raymond 1995) were assessed separately for each locus using exact tests as implemented in GENEPOP (Raymond and Rousset 1995). Significance of F_{IS} across all loci was obtained by permutation procedure implemented in GENETIX ver. 4.04 (Belkhir et al. 2003). To avoid type I errors, we performed sequential corrections for multiple tests using Dunn–Sidak's multiplicative inequality for calculations of critical values of chi-square distribution (Sokal and Rohlf 1995). Dunn–Sidak's method is known to be slightly less conservative than the Bonferroni method. Tests for linkage disequilibrium between all pairs of loci were performed using the exact test in GENEPOP.

When evaluating the importance of the geographic subdivision on genetic differentiation, we used only summer-reproducing populations to not confound it with potential subdivision due to different reproductive periods. The package HIERFSTAT (Goudet 2005) was used to evaluate the effect of each hierarchical level; this package computes hierarchical *F*-statistics following Yang's (1998) algorithm and assesses the significance of differentiation by comparing the likelihood ratio *G*-statistic calculated for the observed data and for data sets obtained by randomization. The significance in randomization tests is calculated as the proportion of randomized data sets that resulted in statistics obtained through randomization that are greater than or equal to the observed statistic. With our sampling design, which included two seas with two areas each, it was meaningless to

use such an approach for estimating differentiation between seas and between areas because, on average, the proportion of randomized data sets that are identical to the observed data set (0.33 and 0.11, respectively) would be higher than the nominal level of significance (0.05; e.g., for the sea level there are three possible combinations of two groups with two items in each, and therefore, the combination identical to the observed data set would be generated in one out of three permutations [33.3%], which is higher than the 5% level of significance). Consequently, in the five-level hierarchy, we presented *F*-statistics without evaluating the significance of the two upper levels. In another analysis, we disregarded the area level, which allowed us to evaluate significance of differentiation between sites within seas ($F_{\text{Site,Sea}}$), and between seas ($F_{\text{Sea,Total}}$).

To compare the patterns of variation separately in the three major groups (i.e., Öland with summer-reproducing plants, continental shore facing Öland with autumn-reproducing plants, and Skagerrak with summer-reproducing plants), we applied a three-level hierarchical analysis (Weir 1996, implemented in the software GDA by Lewis and Zaykin 2001) with the following modification: In Weir's three-level hierarchical analysis, the correlation θ_S between two genes sampled from different individuals in the same subpopulation includes the sum of both the among-population variance component (σ^2_P) and the among-subpopulation variance component (σ^2_S) in the numerator. If all variance components are positive, then θ_S must be larger than θ_P, which includes only the among-population variance component σ^2_P in the numerator (denominators in θ_S and θ_P are identical). Thus, to evaluate variances separately, we subtracted θ_P from θ_S. The 95% confidence intervals for ($\theta_S - \theta_P$) were found by subtracting individual bootstrap replicate values of θ_S and θ_P, and by taking off the upper and lower 2.5% of the values from the distributions obtained. Confidence intervals around *F*-statistics for particular levels made possible their direct comparisons.

Cavalli-Sforza and Edwards' (1967) chord distances based on allele frequencies were used to construct a neighbor-joining (NJ) tree with PHYLIP (Felsenstein 1993). This distance was chosen because it performed best for reconstructing tree topologies based on microsatellite data in a simulation study by Takezaki and Nei (1996). Support for the tree nodes was found by bootstrapping loci 1000 times. Correspondence analysis (CA) was performed using the procedure implemented in GENETIX ver. 4.04 (Belkhir et al. 2003).

RESULTS

Genetic diversity. The genetic diversity within populations of *F. vesiculosus* was high (Table 1). The number of alleles ranged from four alleles at locus L85 to 15 alleles at locus L20. In total, there were 46 alleles across the five loci; of these, 35 alleles occurred across autumn-reproducing samples, 31 alleles were observed in the summer-reproducing populations in the Baltic Sea, and 40 alleles were in the summer-reproducing Skagerrak populations.

TABLE 1. Estimates of genetic variability at five polymorphic loci in *Fucus vesiculosus* populations.

				F_{IS}					
Population	*A*	H_E	H_O	All loci	L20	L38	L58	L85	L94
BaltN-sum-1a	4.2	0.57	0.56	0.03	0.02	0.00	−0.04	0.08	0.12
BaltN-sum-1b	4.6	0.60	0.58	0.03	−0.06	0.13	−0.07	−0.12	0.38*
BaltN-sum-2a	4	0.58	0.49	0.16***	0.32**	0.00	0.13	0.21	0.02
BaltN-sum-2b	4.6	0.59	0.58	0.01	0.25	−0.19	0.01	−0.07	0.06
BaltS-sum-1a	4.6	0.45	0.40	0.11*	0.22	0.19	−0.02	−0.14	0.11
BaltS-sum-1b	4.2	0.46	0.44	0.04	0.18	0.05	−0.12	−0.08	−0.06
BaltS-sum-2a	4.8	0.49	0.47	0.04	0.15	0.07	−0.15	0.10	−0.09
BaltS-sum-2b	4.6	0.44	0.45	−0.02	0.01	−0.10	−0.05	0.10	−0.01
SkagS-sum-1a	5.6	0.68	0.66	0.04	0.07	0.04*	−0.06	0.06	0.13
SkagS-sum-1b	4.4	0.64	0.54	0.16***	0.06	0.30	0.09	0.11	0.24
SkagS-sum-2a	5.2	0.67	0.63	0.06	0.14*	0.07	0.06	0.02	−0.04
SkagS-sum-2b	5.4	0.69	0.62	0.10**	0.22*	0.16	−0.15	0.11	0.15
SkagN-sum-1a	5.6	0.69	0.58	0.15***	0.43***	0.02	0.19	−0.04	0.07
SkagN-sum-1b	6	0.68	0.67	0.00	0.13	−0.06	−0.14	0.06	−0.02
SkagN-sum-2a	5.6	0.66	0.50	0.25***	0.52***	0.22	0.18	0.14	0.08
SkagN-sum-2b	5.2	0.64	0.52	0.18***	0.45***	−0.05	0.12	0.26**	0.05
BaltN-aut-1a	4.8	0.52	0.53	−0.02	−0.15	0.01	0.08	0.19	−0.14
BaltN-aut-1b	4.4	0.52	0.52	0.00	0.09	−0.15	−0.10	0.08	0.22
BaltN-aut-2a	5	0.53	0.47	0.10**	0.07	0.19	0.22*	−0.06	−0.04
BaltN-aut-2b	5.2	0.56	0.50	0.10*	0.01	0.21	0.18*	0.08	−0.01
BaltS-aut-1a	4.8	0.51	0.51	−0.01	0.04	0.08	−0.08	−0.13	−0.07
BaltS-aut-1b	4.6	0.46	0.44	0.05	0.04	0.17	−0.03	−0.04	−0.06
BaltS-aut-2a	5	0.52	0.50	0.04	0.11	0.15	−0.10	−0.07	−0.03
BaltS-aut-2b	4.8	0.51	0.49	0.05	0.04	0.10	0.07	−0.04	−0.03
Average over all samples	4.9	0.57	0.53	0.07	0.14	0.07	0.02	0.04	0.07
F_{IT}, all samples				0.16	0.19	0.14	0.12	0.12	0.23

A is the average number of alleles per locus. H_O and H_E are observed and unbiased expected heterozygosities, respectively. F_{IS} and F_{IT} are fixation indices within subpopulations and in the total population, respectively.

BaltN-sum, Baltic northern area of summer-reproducing *F. vesiculosus*; *BaltS-sum*, Baltic southern area of summer-reproducing *F. vesiculosus*; *BaltN-aut*, Baltic northern area of autumn-reproducing *F. vesiculosus*; *BaltS-aut*, Baltic southern area of autumn-reproducing *F. vesiculosus*; *SkagN-sum*, Skagerrak northern area of summer-reproducing *F. vesiculosus*; *SkagS-sum*, Skagerrak southern area of summer-reproducing *F. vesiculosus*.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Twelve alleles occurred in only one of the three groups (i.e., unique for that group), but these were all rare, and the majority of them were observed as heterozygotes.

The average number of alleles did not differ significantly among Skagerrak, Baltic summer, and Baltic autumn populations, although there was a tendency for a higher number of alleles in Skagerrak compared with Baltic summer populations ($0.05 < P < 0.1$; paired *t*-test). Mean expected heterozygosity (H_E) per sample in Skagerrak (0.668) was significantly higher than values observed in the Baltic Sea summer-reproducing populations (0.523) or in autumn-reproducing populations (0.516; two-tailed Student's *t*-test, $P < 0.001$ for both comparisons); the latter two values did not differ.

Out of 120 tests conducted, we observed departures from Hardy–Weinberg equilibrium in 11 comparisons, six of which were at L20 (Table 1). Three comparisons at L20 remained significant after correction for multiple testing (*SkagN-sum* samples 1a, 2a, and 2b); the departures were due to an excess of homozygotes. Based on F_{IS} over all loci, there were deficits of heterozygotes in nine samples. There was no evidence for genotypic linkage disequilibrium between any pair of loci. Of the 240 pair-wise comparisons, 15 were significant at the $0.01 < P < 0.05$ level, and none of these were significant after correction for multiple testing. Combined *P*-values for each locus pair across all populations were also nonsignificant.

Genetic differentiation and hierarchical F*-statistics analysis.* An important aim of this study was to evaluate the effect of geographic subdivision at various scales on the pattern of genetic variability. To avoid confounding effects due to different times of reproduction, only summer-reproducing populations were included. The overall result of the hierarchical *F*-statistics analysis clearly showed that *F. vesiculosus* was highly genetically structured over the investigated area and that differentiation at all spatial scales contributed to this pattern (Table 2), although the contribution of individual loci varied among particular levels. On average, 12% of the total variation was attributable to differentiation among samples over the whole region studied (F_{ST}). Significant differences were detected at the lowest hierarchical level (i.e., between samples within sites at distances of only 10 m apart); among five loci, only L94 did not display differentiation at this level, whereas the other four were highly significant ($P < 0.001$). Each of the upper levels added an even larger proportion of variation to the total differentiation (Table 2), with genetic differentiation between seas being the largest at 4.1%, even though we could not test the significance of $F_{\text{Areas,Sea}}$ and $F_{\text{Seas,Total}}$ with our design for a five-level hierarchy (see Materials and Methods). However, disregarding the level of areas, we showed that differentiation was significant at all levels in a four-level hierarchy (i.e., at scales of 10 m, 1–75 km, and 635–795 km), and in this analysis, the combined contribution of sites and areas ($F_{\text{Sites,Sea}}$) equaled the contribution of the sea level ($F_{\text{Seas,Total}}$; Table 3).

We also analyzed a five-level hierarchy using samples of summer- and autumn-reproductive individuals (not shown). *F*-statistics at each level remained remarkably similar to those obtained considering only summer-reproducing populations (compare the following values to Table 2, column ''All loci'': $F_{\text{Samples/Site}} = 0.026$, $F_{\text{Sites/Area}} = 0.038$, $F_{\text{Areas/Sea}} = 0.020$, $F_{\text{Seas/Total}} = 0.044$). It is noteworthy that

TABLE 2. Five-level hierarchical *F*-statistics analysis for 16 samples of summer morph.

Hierarchical level	All loci	L20	L38	L58	L85	L94
$F_{\text{Samples/Site}}$	0.023***	0.008**	0.023***	0.015***	0.067***	0.002 (n.s.)
$F_{\text{Sites/Area}}$	0.033*	0.020*	0.014*	0.020*	0.001 (n.s.)$^{\cdot}$	0.119*
$F_{\text{Areas/Sea}}$ (n.t.)	0.028$^{\cdot}$	0.033	0	0.094	0.035	0
$F_{\text{Seas/Total}}$ (n.t.)	0.041	0.032	0.116	0	0	0.079
F_{ST}	0.120***	0.090***	0.143***	0.120***	0.088***	0.166***

Program HIERFSTAT (Goudet 2005) was used to estimate *F*-statistics and their significance.

n.s., nonsignificant; n.t., not testable: this level is not possible to test by randomization with our sampling design. Values are given for evaluating relative contribution of this level.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 3. Four-level hierarchical *F*-statistics analysis for 16 samples of summer morph.

Hierarchical level	All loci	L20	L38	L58	L85	L94
$F_{\text{Samples/Site}}$	0.023***	0.008**	0.023***	0.015***	0.067***	$0.002^{\text{n.s.}}$
$F_{\text{Sites/Sea}}$	0.051***	0.042***	$0.010^{\text{n.s.}}$	0.083***	0.024*	0.102***
$F_{\text{Seas/Total}}$	0.051*	0.043*	0.114*	0.026*	0 (n.s.)$^{\cdot}$	0.070 (n.s.)
F_{ST}	0.120***	0.090***	0.143***	0.120***	0.088***	0.166***

Program HIERFSTAT (Goudet 2005) was used to estimate *F*-statistics and their significance. To be able to use permutation tests to estimate significance of differentiation at the SEA level, the AREA level was not considered in this analysis.

n.s., nonsignificant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

$F_{Areas/Sea}$, which in this analysis included comparisons of areas with both reproductive morphs in the Baltic Sea, did not increase, indicating no additional differentiation owing to differences in reproductive period.

Even though the above gene diversity analysis shows overall (general) trends of variation, situations in particular areas can be quite distinct. To determine whether the patterns of differentiation were similar in various regions on similar geographic scales, we conducted hierarchical *F*-statistics analyses separately for Skagerrak (summer reproducing), mainland populations in the Baltic Sea (autumn reproducing), and Öland populations in the Baltic Sea (summer reproducing). From bootstrapping across loci in GDA, we obtained confidence intervals around *F*-statistics for particular levels—thus making possible direct comparisons (Table 4). In the Baltic Sea, the pattern of differentiation among the mainland populations (autumn-reproducing plants) was somewhat distinct from that of the Öland populations (summer reproducing). Samples of summer-reproducing plants collected within one area were genetically similar, but genetic differences between areas were pronounced. This is in contrast to the pattern found in autumn-reproducing plants, where differentiation between samples within areas approached the level of differentiation between areas. In the Skagerrak, differentiation within areas actually surpassed differentiation between areas (Table 4). Altogether, there were no consistent differences in the pattern of variation between the two regions with summer-reproducing populations and the region with autumn-reproducing plants.

Isolation by distance. Population differentiation (F_{ST}) estimated between pairs of Baltic samples ranged from 0.000 to 0.146, with a mean value of 0.062. A plot of F_{ST} values against the geographic distances between populations revealed that genetic differentiation increased linearly with geographic separation over the scale studied, as predicted from an isolation-by-distance model (Fig. 2a). The Mantel test (10,000 permutations) was highly significant ($P < 10^{-5}$), and 56% of the variance in genetic differentiation was attributable to the variance in geographic distance among Baltic samples. In contrast, we observed no strong coupling between genetic and geographic distances among the Skagerrak samples. Although the Mantel test indicated a significant relationship ($P = 0.004$), only 13% of the

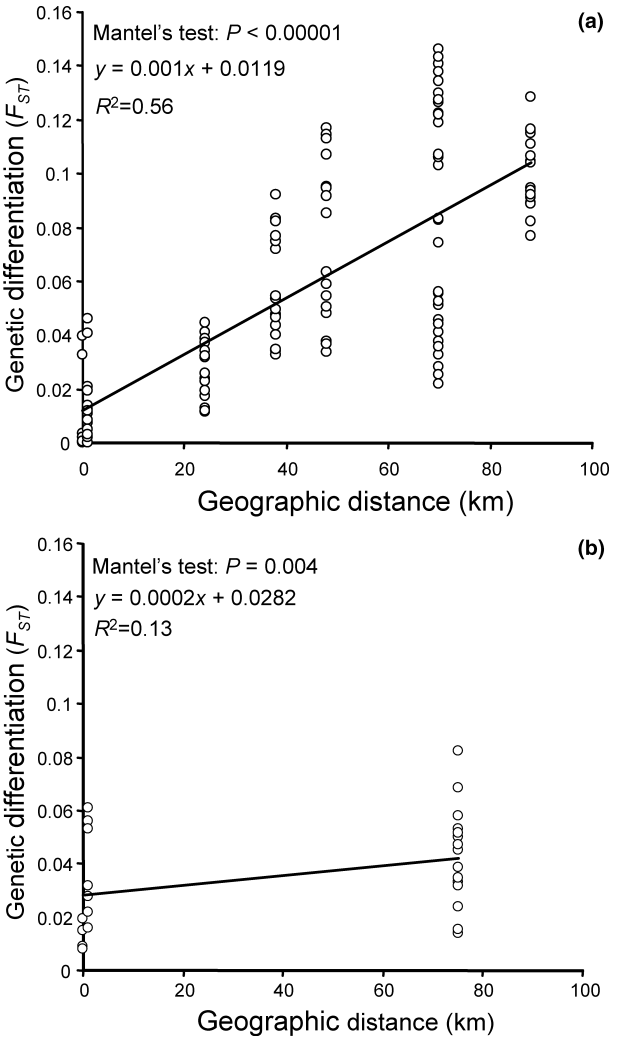

FIG. 2. Differentiation-by-distance relationship among the 16 *Fucus vesiculosus* samples from the Baltic Sea (a) and the eight samples from the Skagerrak (b). Pair-wise coefficients of genetic differentiation (F_{ST}) are plotted against geographic distances separating pairs of samples. Equation of linear regression, coefficient of genetic determination, and result of Mantel's test are shown directly on the figure.

TABLE 4. *F*-statistics from three-level hierarchical analysis according to Weir (1996), as implemented in the program GDA (Lewis and Zaykin 2001).

Hierarchical level	Populations	*F*-statistics	95% CI
Between areas (70 km)	Baltic summer reproducing	0.118	0.050–0.207
	Baltic autumn reproducing	0.025	0.008–0.048
	Skagerrak summer reproducing	0.012	0.004–0.019
Within areas (up to 1 km)	Baltic summer reproducing	0.003	0.000–0.006
	Baltic autumn reproducing	0.019	0.008–0.032
	Skagerrak summer reproducing	0.028	0.026–0.030

The 95% confidence intervals (CI) are based on 1000 bootstrap resamplings.

variance in genetic differentiation was explained by the geographic component (Fig. 2b).

Including samples of the two reproductive strategies in the Baltic analysis may partly explain the marked difference in genetic structure between Baltic and Skagerrak samples. To test this explanation, we calculated average pair-wise F_{ST} values between populations of the same reproductive strategy separated by 70 km of continuous shoreline in the Baltic Sea, and between populations of different reproductive strategies separated by 24–88 km of open sea across the sound separating Öland and the mainland (Fig. 1). If there was some impediment to gene flow between the summer- and autumn-reproducing plants of *F. vesiculosus*, we should expect increased genetic differentiation between them compared to that expected simply from isolation by distance. However, the average differences between populations were simply what were expected from the geographic separation between them (Fig. 3). In this comparison, as much as 90% of the differences between samples was determined by geographic distance, and it can also be noted that separation by open water did not impede gene flow more than separation over a similarly long distance of continuous shoreline. Slopes for regression lines for F_{ST} estimates versus geographic distance plots were almost identical for continuous populations ($y = 0.0013x$) and populations across open water ($y = 0.0014x$). Altogether, the conclusion from the F_{ST} estimates was that genetic differences were a function of geographic distances for comparisons within the Baltic Sea as well as between seas (Fig. 4).

Population clustering. There was a good correspondence between the clustering of samples and their geographic distribution (Fig. 5). Two clades representing the division between seas showed a high bootstrap support (98%) on an NJ tree. Within the Baltic Sea clade, samples from both southern areas clustered together (bootstrap support 76%), even though they represent distinct reproductive strategies. Moreover, all four samples of the summer-reproducing *BaltS-sum* formed a compact cluster with 80% bootstrap support. In the northern part of the Baltic Sea, the samples from the summer-reproducing populations grouped together according to the area in which they were collected (bootstrap 89%), whereas the autumn-reproducing samples *BaltN-aut* revealed a very loose pattern. In the Skagerrak, all samples clustered with a relatively high bootstrap support (57%–90%) according to the site from which they were collected, but only the southern samples *SkagS-sum* formed a group corresponding to area. Results of the correspondence analysis (Fig. 5) matched well with the NJ tree.

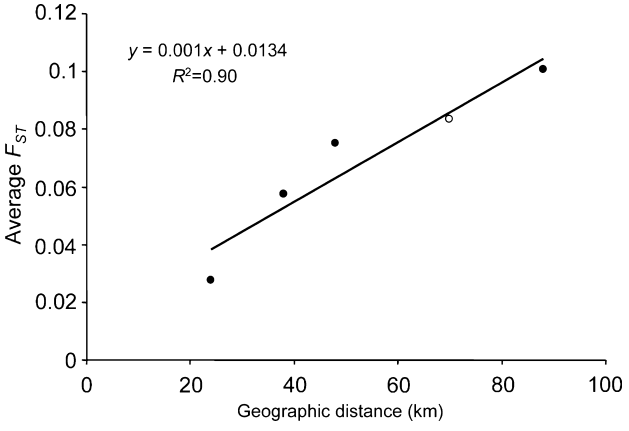

FIG. 3. The F_{ST} coefficients between areas in the Baltic Sea plotted against geographic distances separating areas (i.e., at 24 km distance or more). F_{ST} values are the average of all pair-wise comparisons between samples at a given distance. Closed circles, comparisons between summer- and autumn-reproducing samples of *Fucus vesiculosus*; open circle, comparison between samples of *F. vesiculosus* with the same strategy of reproduction—summer or autumn.

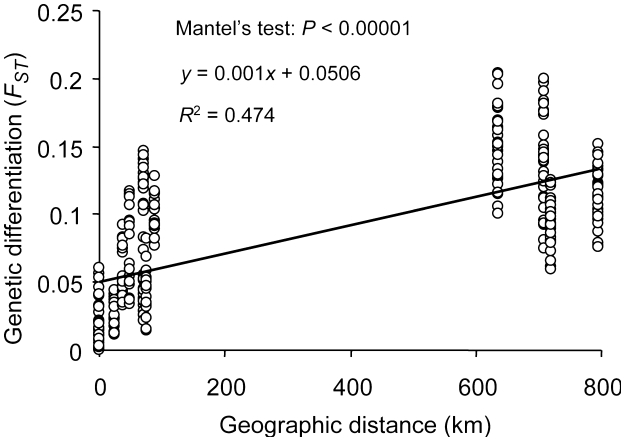

FIG. 4. Differentiation-by-distance relationship among all 24 *Fucus vesiculosus* samples. Pair-wise coefficients of genetic differentiation (F_{ST}) are plotted against geographic distances separating pairs of samples. Equation of linear regression, coefficient of genetic determination, and result of Mantel's test are shown directly on the figure.

DISCUSSION

There was significant genetic differentiation among *F. vesiculosus* samples at all hierarchical levels of geographic separation that we studied, from stands only 10 m apart to differentiation between seas at several hundred kilometers distance. On the other hand, our results did not support any substantial genetic subdivision of summer- and autumn-reproducing plants, a fact we discuss further below.

The overall strong geographic component of the genetic structure of *F. vesiculosus* is not surprising, given the fact that despite external fertilization, male and female gametes are short-lived and usually released in calm waters (Serrão et al. 1996a), which

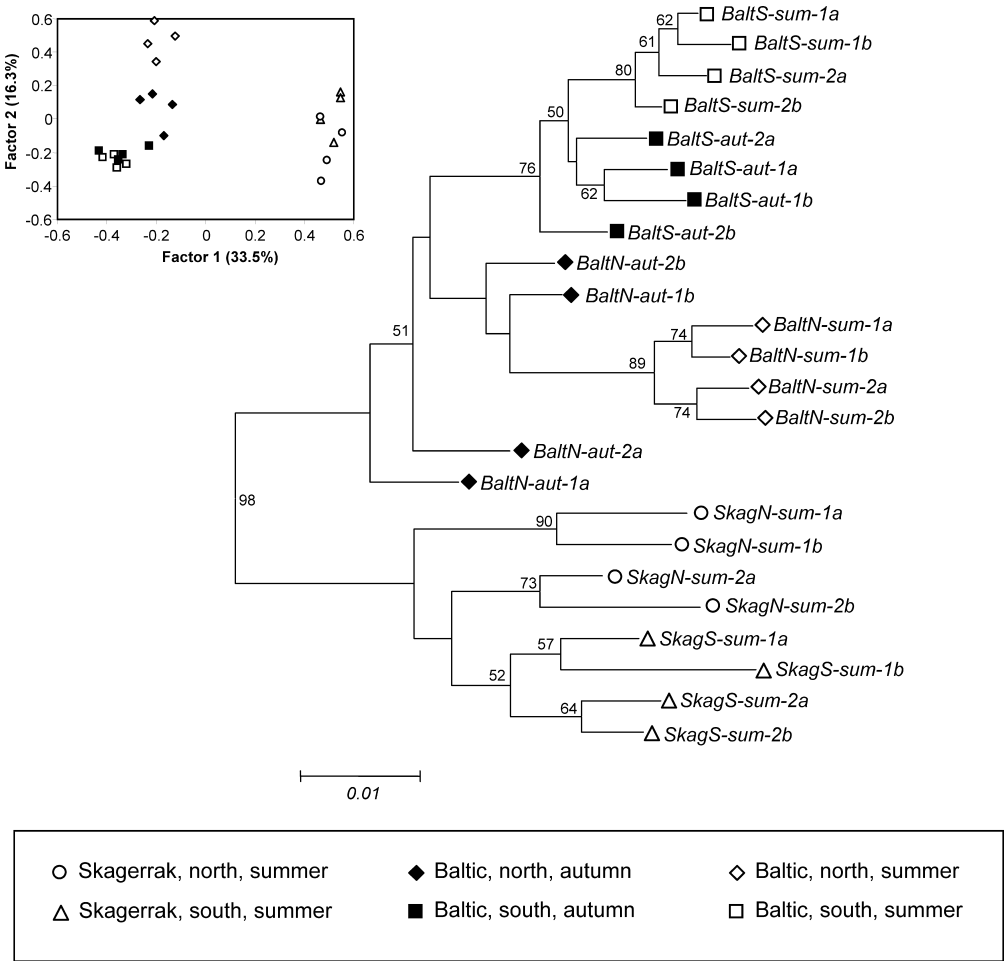

FIG. 5. Correspondence analysis (inset) and neighbor-joining tree [based on Cavalli-Sforza and Edwards (1967) distance] showing the genetic relationships among all 24 samples of *Fucus vesiculosus* from the Skagerrak and the Baltic Sea. Bootstrap support for the nodes was assessed from 1000 replicate samples. Only bootstrap values of at least 50% are shown. Factors 1 and 2 are the first and second principal factors of variability that explain the indicated percentages of total genetic variation.

promotes settlement of fertilized eggs within a few meters of the parent plant (Serrão et al. 1997). This pattern is similar to that observed in *F. serratus*, with genetic differentiation among populations becoming discernible at 0.2–1.3 km distance and being general at distances above 2 km between populations (Coyer et al. 2003). However, no spatial clustering of alleles was seen in *F. serratus* at the 1–100 m scale (Coyer et al. 2003), while in *F. vesiculosus*, we observed differentiation down to 10 m distance. Thus, genetic structuring in *F. vesiculosus* is perhaps even more fine-tuned compared with *F. serratus* at small geographic scales, despite the presence of flotation vesicles that potentially enhance dispersal abilities in the former while not in the latter. One possible explanation for this could be that dispersal over short distances is largely gamete or zygote driven, and that such dispersal may be more effective in *F. serratus*, which occupies a lower littoral zone as compared to *F. vesiculosus*. Another fucoid species, *Silvetia compressa* (J. Agardh) E. Serrão, T. O. Cho, S. M. Boo et Brawley, reveals genetic differentiation between groups of individuals at extremely small distances (1–6 m; Williams and Di Fiori 1996). Similarly, the kelp *Postelsia palmaeformis* Rupr. exhibits genetic differences at 1–25 m (Coyer et al. 1997), and the fucoid *Sargassum polyceratium* Mont. at somewhat longer distances (150–200 m; Engelen et al. 2001). On the other hand, the brown alga *Halidrys dioica* N. L. Gardner has the potential for longer dispersal and consequently shows low genetic differentiation at a distance of 4 km, but substantially more at 90 km (Lu and Williams 1994). Thus, brown macroalgae in general reveal small-scale patterns of genetic differentiation according to what is expected from their poor dispersing propagules, and *F. vesiculosus* is indeed no exception to this pattern.

In contrast to these observations, *F. spiralis*, which is very closely related to *F. vesiculosus* (Engel et al. 2005), showed overall low levels of genetic differentiation and no clear geographic component over

90 km of irregular coastline (Coleman and Brawley 2005). Moreover, surprisingly little genetic variation is observed in *F. spiralis*, both within and between samples, as compared to *F. vesiculosus*. The first of these observations is explained by a high degree of selfing in this hermaphroditic species (Billard et al. 2005, Engel et al. 2005), and the second may be a result of local extinction with subsequent recent recolonization from a common source owing to drifting algal rafts (Coleman and Brawley 2005).

A somewhat surprising finding of the present study was that distances of open water provided no additional barrier to gene flow. That is, we did not find any measurable effect on the level of genetic differentiation of geographic separation by water gap as compared to separation along a continuous coastline. Considering that the fertilized eggs in *F. vesiculosus* quickly sink, thus reducing opportunities for dispersal, it is expected that open gaps of water would increase genetic differentiation compared to continuous settlements over similar distances, as is documented in populations of intertidal snails with similarly poor dispersal (Johnson and Black 1996, Johannesson and Tatarenkov 1997). Our result suggests that the main genetic exchange over distances of tens of kilometers and more occurs via detached reproductively mature plants moved by currents or waves. Such exchange can be promoted by the presence of flotation vesicles in *F. vesiculosus*, potentially increasing traveling distance before the plant sinks; although, ultimately, the population structure is a result of the interplay of a number of biotic and abiotic factors, as evidenced by the absence of population structure in *F. spiralis* lacking air bladders (Coleman and Brawley 2005). Indeed, rafting is suggested as the main spreading mechanism in a dispersal model of *F. serratus* in marginal environments (Arrontes 2005). Likewise, it was concluded that rafting contributes substantially to gene flow between populations of *F. spiralis* along the U.S. East Coast (Coleman and Brawley 2005). Population history is suggested as an alternative to rafting to explain similarly inconsistent patterns of genetic differentiation observed in the green macroalga *Cladophoropsis membranacea* (Bang ex C. Agardh) Børgesen occupying sublittoral habitats around the Canary Islands. Here, owing to climate variation, populations are probably so recent that drift-dispersal equilibrium among populations of different islands might not yet have been reached, resulting in a structure wherein differentiation within islands (1–125 km) follows an isolation-by-distance model, while differentiation between islands (100–300 km) does not (van der Strate et al. 2003).

No isolation by distance was observed among *F. serratus* populations along the Swedish west coast, while such a pattern was obvious in other areas (Coyer et al. 2003). Notably, this result is similar to what we saw in *F. vesiculosus*. This similarity suggests that certain oceanographic features of this area (e.g., frequent storms or a complex system of currents) increase long-range dispersal in both species.

The genetic differentiation between autumn- and summer-reproducing populations of *F. vesiculosus* in the Baltic was no greater than differentiation among populations of the same reproductive morph at similar distances. With our design, we would have been able to detect reproductive isolation that superseded the geographic variation within reproductive morphs, which would likely have been the case if differentiation between summer and autumn morphs would have qualified for a species or subspecies level. This possibility was, however, not the case, as is obvious from the fact that Baltic populations clustered not according to reproductive morph, but according to geographic proximity. Consequently, our conclusion is that genetic differentiation between autumn and summer morphs is minor at most.

The lack of major differentiation between Baltic summer- and autumn-reproducing plants is somewhat unexpected given that no overlap was observed in the reproductive periods of the morphs and that timing of reproduction was conserved over years for individual plants (Berger et al. 2001). One explanation might be that the origin of the alternative reproductive strategy (most likely autumn reproduction) may be so recent that very little stochastic differentiation has yet accumulated. This explanation requires an effective spread of the new morph over large areas during periods of time too short for generating genetic differentiation at neutral loci. However, as both morphs reveal geographic genetic differentiation, this explanation seems unlikely. Another possibility is that reproductive periods overlap to some extent, during particular years or owing to individuals with intermediate periods of reproduction, or even owing to the same individuals having reproductive activities during both periods. Indeed, only a low level of gene flow (generally, the successful transfer of genes by 1–5 individuals per generation) is enough to counteract the accumulation of genetic differences in neutral loci over time in isolated populations (Slatkin 1987). It is, in fact, possible that certain plants reproduce during both periods. Berger et al. (2001) recorded the proportion of plants reproducing during each period in 27 sites and reported that at three sites, some plants were reproductively active during both periods. Certainly, such exchange will not be very effective, but it may be enough to prevent accumulation of neutral genetic differences between summer- and autumn-reproducing populations. Intraspecific variation for reproductive season is reported in high-shore populations of *F. distichus*; a dwarf morph reproduces only during the autumn, while the normal form shows a bimodal reproductive cycle with peaks during both spring and autumn. Interestingly, both differences in morphology and in age of maturity persisted in progeny raised in a common

environment, indicating important genetic differences between the two morphs (Sideman and Mathieson 1983). Whether the partial reproductive barriers present in *F. distichus* and in *F. vesiculosus* (this study) have any effect on the genetic structure of the species is impossible to say without a more detailed genetic analysis. We cannot rule out the existence of a minor genetic isolation between the reproductive morphs of *F. vesiculosus*, as we did not include sympatric samples in our design. Some observations suggest minor differences between Baltic summer- and autumn-reproducing plants—for example, in number of alleles and in the pattern of genetic differentiation (within- vs. between-area differences). However, differences between Baltic summer and autumn morphs were smaller than the differences in these parameters between summer-reproducing plants from the Baltic and Skagerrak, again supporting our earlier conclusion of these differences being minor.

Local extinction of stands of *F. vesiculosus* in the Baltic Sea (Vogt and Schramm 1991, Nilsson et al. 2004) has already prompted a few restoration projects (L. Kautsky, personal observation). Knowledge about the genetic structure of *F. vesiculosus* populations is obviously crucial to support such initiatives and additionally important for assessing the value of the threatened populations in conservation. The considerable spatial genetic structure present in *F. vesiculosus* (the present study) and *F. serratus* (Coyer et al. 2003) indicates that levels of gene flow over distances >10 km are low, suggesting that natural recovery at these distances may be poor. Moreover, the strong spatial structure suggests possible effects of local genetic adaptation in selected traits, further impeding successful introduction of nonlocal individuals. An implication from these observations is that if a population is to be restored by artificial introduction, a rather dense planting is recommended to ensure fertilization and an effective local recruitment of germlings. Furthermore, planting from geographically close populations is to be preferred because the high level of subdivision may promote establishment of adaptive characters specific to a particular area.

High genetic variation in *F. vesiculosus* parallels its extensive phenotypic variation and is also likely to reflect diverse ecological effects of this habitat-forming species. Indeed, this species is more successful than most fucoid species in colonizing marginal marine environments such as low-salinity estuaries, showing a range of morphological (Kalvas and Kautsky 1993, Ruuskanen and Bäck 2002), physiological (Serrão et al. 1996b, Pearson et al. 2000), and ecological adaptations, including a unique way of clonal reproduction (Tatarenkov et al. 2005). It thus seems crucial to manage populations of this species in such a way as to conserve the broad range of genetic and phenotypic diversity, including populations locally adapted to particular environments.

We thank Paul O. Lewis for suggestions on hierarchical analysis implemented in GDA. This work was part of the MARBIPP research program funded by the Swedish Environmental Protection Agency.

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