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SHORT COMMUNICATION

Population-genetic structure of the toxic dinoflagellate *Karenia brevis* from the Gulf of Mexico

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Blooms of *Karenia brevis*, the major bloom-forming dinoflagellate in the Gulf of Mexico, are thought to originate in the eastern Gulf. Single-cell polymerase chain reaction and five microsatellites were used to obtain genotypes for >1800 cells from 38 samples collected from six bloom events. A consistent pattern of genetic divergence between blooms from Florida and Texas was not detected, which supports the hypothesis of a common origin for blooms of *K. brevis* in the Gulf of Mexico.

KEYWORDS: dinoflagellate; Gulf of Mexico; microsatellite; HAB; *Karenia brevis*; population structure

The major, harmful bloom-forming dinoflagellate in the Gulf of Mexico (hereafter Gulf) is *Karenia brevis*. Blooms of *K. brevis* can occur throughout the northern Gulf and high concentrations of cells of *K. brevis* and their negative impacts (e.g. fish kills, respiratory irritation) are observed almost annually off the west coast of Florida (Brand and Compton, 2007). Blooms occur much less frequently off the coast of Texas (Magaña *et al.*, 2003; Walsh *et al.*, 2006), and it is not known why one area of the Gulf experiences more blooms than other areas (Steidinger *et al.*, 1998). Based in part on the frequency of bloom occurrence, Steidinger *et al.* (Steidinger *et al.*, 1998) suggested that the west and southwest coast of Florida might serve as points of origin for blooms of *K. brevis* in other parts of the Gulf. The conditions necessary for blooms to move across the Gulf to the Texas coast may occur only sporadically. An alternative hypothesis is the presence of multiple seed populations but where environmental conditions conducive to bloom formation occur more frequently in the eastern Gulf. We tested the hypothesis that there is only a single origin for blooms of *K. brevis* in the Gulf by determining whether bloom samples from Florida and Texas were distinct. Population-genetic structure and temporal stability among samples of *K. brevis* from Florida and Texas were assessed using single-cell haplotypes obtained from spatially and temporally varying bloom samples taken from six bloom events occurring across three different years.

A total of 45 samples were obtained from six bloom events (three offshore of Florida and three offshore of Texas) occurring between 2005 and 2009 (Fig. 1; Supplementary data, Table SI). Whole-water samples were collected from surface blooms, preserved with acidified Lugol's iodine (LI) solution and stored at 4°C. Isolation and DNA amplification protocols followed

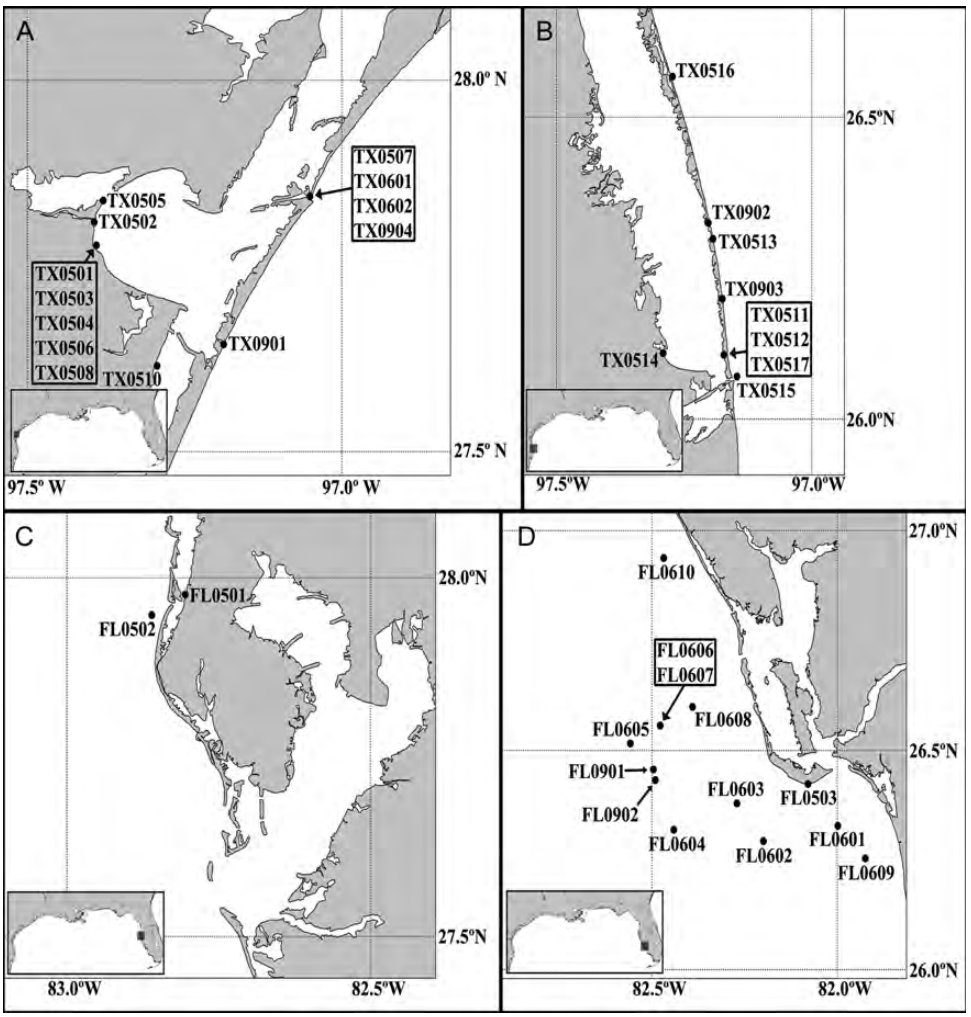

Fig. 1. Collection locations for samples from the Gulf of Mexico. Black boxes indicate samples that were collected from the same location. (**A**) Corpus Christi Bay, TX, USA. (**B**) South Padre Island near Brownsville, TX, USA. (**C**) Tampa Bay, FL, USA. (**D**) Charlotte Harbor, FL, USA.

Henrichs *et al.* (Henrichs *et al.*, 2008). Microsatellites were genotyped using both simplex and multiplex procedures. The five microsatellites amplified consisted of three dinucleotide repeats (*Kbr*5, *Kbr*8, *Kbr*10) and two trinucleotide repeats (*Kbr*7, *Kbr*9) from Renshaw *et al.* (Renshaw *et al.*, 2006). Resulting polymerase chain reaction (PCR) products were visualized on 5% polyacrylamide gels electrophoresed using an ABI Prism 377 Genetic Analyzer (Applied Biosystems, Inc.). Allele sizes were determined using Genescan 3.1.2 (Applied Biosystems, Inc.) and Genotyper version 2.5 (Applied Biosystems, Inc.). If the number of scored genotypes with allele information from at least four microsatellites was <10, the sample was removed from the study.

Number of alleles, allele frequencies and unbiased estimates of gene diversity (Nei and Chesser, 1983) for each sample were calculated using Arlequin 3.5 (Excoffier and Lischer, 2010). To account for differences in the number of cells per sample, estimates of allelic richness were determined by rarefaction (El Mousadik and Petit, 1996). Homogeneity of allelic richness and gene diversity among samples was tested using Friedman rank tests. Tests for genotypic disequilibrium between pairs of microsatellites were conducted using Genepop v4.0 (Rousset, 2008); sequential Bonferroni correction (Rice, 1989) was applied for all multiple tests performed simultaneously.

Pairwise estimates of genetic divergence between samples employed Jost's D (Jost, 2008), using custom Python scripts. Multilocus estimates of D were obtained by averaging H_S and H_T over loci, as suggested by Meirmans and Hedrick (Meirmans and Hedrick, 2011). Pairwise estimates of F_{ST} were calculated using Arlequin 3.5; significance of $F_{ST} = 0$ (and $D = 0$) was tested using 50 000 permutations of cells between all pairs of samples; sequential Bonferroni correction was applied for all multiple tests performed simultaneously. Pairwise values of genetic divergence were also calculated for samples grouped according to year and collection location. Friedman rank sums were used to test homogeneity of allelic richness and gene diversity among groups. Analysis of molecular variance (AMOVA) was performed using Arlequin to determine the amount of genetic variation partitioned into each hierarchical level. Samples were grouped according to (i) year and collection location (six groups); (ii) location (two groups) and (iii) year of collection (three groups).

Genotypes with allele information for at least four microsatellites were obtained from 1949 single cells, representing 38 samples. For a small subset of cells ($n = 284$; $\sim$14.5%), two alleles were scored at one or more microsatellites. It is not known whether the two alleles are the result of a gene duplication event or are PCR artifacts. For these cases, microsatellite(s) appearing to possess two alleles were coded as missing data, leaving 1804 cells for further analysis after removing cells with data for less than four microsatellites. Individual sample sizes ranged from 16 to 116 cells (47.5 ± 23.7 [mean $\pm$ SD]; Supplementary data, Table SI).

All samples were highly diverse genetically (Supplementary data, Table SII). The number of alleles per microsatellite ranged from 13 to 29 (20.2 ± 6.2 [mean $\pm$ SD]), while gene diversity ranged from 0.393 to 0.825 (0.682 ± 0.193 [mean $\pm$ SD]; Supplementary data, Table SII). Significant heterogeneity among samples was detected for both allelic richness ($P < 0.004$) and gene diversity ($P < 0.009$). No significant differences in allelic richness or gene diversity among groups were detected (Table I).

There were 24 significant ($P < 0.05$) tests for genotypic disequilibrium prior to Bonferroni correction; none of the tests were significant after Bonferroni correction. Jost's D among samples ranged from -0.055 to 0.316; pairwise F_{ST} values ranged from -0.075 to 0.162 (Supplementary data, Table SIII). Significant genetic divergence was detected among samples for both D (0.051; $P = 0.000$) and F_{ST} (0.026; $P = 0.000$).

Table I: Genetic diversity estimates of grouped samples for five microsatellites

	*Kbr*5				*Kbr*7				*Kbr*8				*Kbr*9				*Kbr*10			
Sample	n	N_A	A	$\hat{h}$	n	N_A	A	$\hat{h}$	n	N_A	A	$\hat{h}$	n	N_A	A	$\hat{h}$	n	N_A	A	$\hat{h}$
FL2005	67	11	10.1	0.809	65	4	3.8	0.479	73	11	9.3	0.837	68	6	5.6	0.611	54	9	8.2	0.836
FL2006	358	11	7.6	0.819	356	7	4.3	0.399	342	22	11.0	0.837	369	9	4.4	0.532	285	12	8.0	0.773
FL2009	162	11	8.6	0.827	172	7	4.5	0.387	165	20	12.8	0.871	176	10	6.0	0.544	153	14	9.4	0.816
TX2005	803	13	7.3	0.785	798	11	4.1	0.360	797	22	9.2	0.792	840	17	5.2	0.576	729	22	8.6	0.824
TX2006	49	7	7.0	0.820	46	6	6.0	0.526	46	10	10.0	0.863	49	4	4.0	0.558	35	7	7.0	0.716
TX2009	211	9	7.4	0.819	221	7	4.6	0.437	189	19	11.2	0.849	223	7	5.0	0.625	179	10	7.6	0.806
mean	275	10.3	8.5	0.813	276	7.0	4.2	0.431	269	17.3	10.1	0.841	288	8.8	5.4	0.574	239	12.3	8.1	0.795

n, number of cells with an allele for this locus; N_A, number of alleles; A, allelic richness estimate calculated by rarefaction; $\hat{h}$, unbiased estimate of gene diversity.

A total of 125 and 103 pairwise comparisons of D and F_{ST}, respectively, remained significant after Bonferroni correction (Supplementary data, Table SIII). The majority of significant comparisons (103 of 125 Jost's D; 92 of 103 F_{ST}) included at least one of four samples from the TX2005 group (TX0506, TX0508, TX0510, TX0516). Closer inspection revealed gene diversity and allelic richness estimates at *Kbr*7 (TX0506) and *Kbr*8 (TX0508, TX0510, TX0516) to be much lower in these four samples compared with the other 34 samples. Removal of the four samples resulted in the Friedman rank-sum test of homogeneity of gene diversity among samples to be nonsignificant ($P > 0.05$); the rank-sum test of homogeneity of allelic richness remained significant ($P < 0.024$). The low genetic diversity and allelic richness estimates from these four samples did not appear to be a product of small sample sizes and the samples were therefore included in further analyses. Genetic divergence among groups was low but significant for D (0.017; $P = 0.000$) and F_{ST} (0.003; $P < 0.001$). A total of nine and six pairwise comparisons of genetic divergence between groups remained significant after Bonferroni correction for Jost's D and F_{ST}, respectively (Table II). All significant pairwise comparisons included at least one of two groups, FL2005 and TX2005. Removal of the same four samples from the TX2005 group (TX0506, TX0508, TX0510, TX05116) resulted in lower estimates of genetic divergence for all pairwise comparisons involving the TX2005 group except for one (data not shown). The estimate of genetic divergence between FL2005 and TX2005 increased slightly from 0.022 to 0.025 and 0.008 to 0.010 for D and F_{ST}, respectively. No pairwise comparisons between groups from 2006 or 2009 indicated significant genetic divergence.

Regardless of how the samples were grouped (by year, by collection location, and by year and collection location), the results from the AMOVA indicated the majority of the genetic variation was found within samples (>97.3%; $P < 0.0001$ for all three grouping strategies) and very little genetic variation was found among groups in all cases (<0.3%; $P > 0.200$ for all three grouping strategies).

Table II: Pairwise estimates of genetic divergence between groups for Jost's D (above diagonal) and F_{ST} *(below diagonal)*

Sample	FL2005	FL2006	FL2009	TX2005	TX2006	TX2009
FL2005		*0.050*	*0.049*	*0.022*	*0.053*	*0.046*
FL2006	*0.022*		0.003	*0.015*	−0.007	0.006
FL2009	*0.019*	−0.001		*0.021*	0.001	0.011
TX2005	0.008	*0.005*	*0.010*		*0.028*	*0.018*
TX2006	0.020	−0.002	−0.005	0.007		0.001
TX2009	*0.015*	0.001	0.004	*0.006*	−0.002	

Italicized values indicate significant after Bonferroni correction for multiple tests.

Bloom samples of *K. brevis* are diverse genetically, which is consistent with other dinoflagellates that have been studied (e.g. Nagai *et al.*, 2009; Erdner *et al.*, 2011). Examination of cultured isolates revealed both broad-scale (Nagai *et al.*, 2009; Casabianca *et al.*, 2012) and fine-scale (Lowe *et al.*, 2010; Erdner *et al.*, 2011) genetic structure using microsatellites. Many studies of dinoflagellate population structure have made use of clonal cultures in order to increase the number of genetic markers due to larger quantities of useable DNA. The difficulty of establishing clonal cultures of *K. brevis* necessitated the use of preserved single cells in this study (Henrichs *et al.*, 2008).

No significant genetic divergence was detected among the eight samples from the FL2006 group, which suggests that this geographically large bloom which covered an area >500 km^2 may be a single population. This is in contrast to the genetic divergence detected among several samples in the TX2005 group. Samples from the Corpus Christi Bay area were collected over a 3-week period and indicate there may have been more than one genetically distinct population of *K. brevis* off the coast of Texas in 2005.

On a larger geographic scale, Steidinger *et al.* (Steidinger *et al.*, 1998) suggested that the west coast of Florida was a source for blooms of *K. brevis* throughout the Gulf. If this hypothesis is true, no significant genetic divergence should be detected between blooms from Florida and blooms from Texas. Although significant genetic divergence was observed between the FL2005 and TX2005 groups, the estimate of genetic divergence was low (D: 0.022; F_{ST}: 0.008) and may not be biologically significant. No significant genetic divergence was detected between FL2006 and TX2006 or FL2009 and TX2009, which supports the hypothesis that blooms of *K. brevis* in the Gulf have a common origin. The absence of significant genetic divergence among groups in AMOVA also supports occurrence of a single population of *K. brevis* in the Gulf. A spatial autocorrelation analysis showed no significant spatial structure among samples (data not shown).

While the number of genetic markers used in this study is lower than the number used by Nagai *et al.* (10 loci; Nagai *et al.*, 2009), Lowe *et al.* (9 loci; Lowe *et al.*, 2010) and Casabianca *et al.* (7 loci; Casabianca *et al.*, 2012) and equivalent to the number used by Erdner *et al.* (Erdner *et al.*, 2011), the number of genotyped individuals for this study (1804 cells) far exceeds these previous studies (maximum of 421 cultures, Nagai *et al.*,

2009). Increasing the number of loci often increases the power to detect genetic divergence. The low number of loci used in the present study means that a subtle signal of genetic divergence could be missed. However, increasing the number of sampled individuals and using highly polymorphic markers should provide enough power to detect genetic divergence if it is present (Kalinowski, 2005; Ryman *et al*., 2006).

While significant genetic divergence was observed between some samples, a consistent pattern of genetic divergence between samples from Florida and samples from Texas was not identified. There are at least two possible explanations for this result: (i) blooms have a common geographic origin and are transported to distant regions in the Gulf or (ii) the Gulf contains one large population of *K. brevis* and blooms can occur independently in different regions of the Gulf. Movement of a surface bloom from the eastern side of the Gulf to the western side is possible as shown by previous drifter studies (Lugo-Fernandez *et al.*, 2001; Morey *et al*., 2003). Determining whether whole blooms, or seed populations, are actually transported across the Gulf would require additional study. Another, more likely, scenario involves the movement of water by surface currents or eddies in the Gulf which would continuously mix cells of *K. brevis* from distant locations, thereby preventing the development of population genetic structure due to genetic drift and would explain the resulting presence of a single, large population of *K. brevis* in the Gulf (Elliott, 1982). Future work describing bloom initiation should focus on physical mechanisms of bloom (or cell) transport over long distances in the Gulf in addition to coastal areas.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

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