A genomic scanning using AFLP to detect candidate loci under selection in the finless porpoise
( *Neophocaena phocaenoides* )

Lian Chen and Guang Yang *

Jiangsu Key Laboratory for Biodiversity and Biotechnology, College of Life Sciences, Nanjing Normal University, Nanjing 210046, China

(Received 15 May 2009, accepted 24 August 2009)

Identifying loci under natural selection from genomic surveys is of great interest in different research areas, stimulated by the increasing ease with large numbers of markers to gain a genome-wide perspective on differentiation. In this study, we searched for the genetic signatures of selection by screening 114 amplified fragment length polymorphism (AFLP) markers in three finless porpoise populations inhabiting contrasting natural environments (freshwater and marine habitat). Comparing among three populations, four AFLP loci exhibited $F_{ST}$ values higher than 0.975 quantile which might be inferred to be under divergent selection and two loci fell below the 0.025 quantile which might be affected by balancing selection. Although these loci were not supported with statistical significance in false discovery rate (FDR) analysis, the present study illustrated the potential of genome-wide surveys to identify specific genome regions or genes associated with freshwater adaptation of the finless porpoise.

Key words: AFLP, finless porpoise, outlier loci, population genomics, selection

INTRODUCTION

Understanding the genetic basis of ecologically important traits—traits that increase an organism’s ability to survive and reproduce in natural environments—has been and continues to be a central goal for ecological and evolutionary genetics (Feder and Mitchell-Olds, 2003). The identity of genes underlying evolutionary change is still largely unknown especially in wild populations (e.g., Mackay, 2001; Orr, 2005). To identify key genes involved in adaptation to local environments or speciation, it is necessary to investigate the whole nuclear genome because it contains most of the genes related to these processes (Tsumura et al., 2007). However, analysis of genes under selection has been largely limited to a few model organisms in which candidate genes for traits of interest are known.

Recently, ‘Population genomics’ has become a powerful approach to identify genome regions or genes associated with adaptation or speciation. Population genomics can be narrowly defined as using genome-wide sampling to identify and to separate locus-specific effects (recombination, selection, mutation and so on) that affect one or a few loci at a time from genome-wide demographic effects (genetic bottlenecks, founder events, inbreeding and so on) (Stinchcombe and Hoekstra, 2008). The underlying idea of population genomics is that within a population, most of the loci are expected to respond similarly to demography and phylogenetic history, whereas a few loci that are important for fitness or adaptation will behave differently and are therefore detectable as ‘outliers’ (Luikart et al., 2003). These outliers can be of great interest because they provide unique insights into the genomic architecture of differential adaptation (Luikart et al., 2003; Schlötterer, 2003; Storz, 2005).

Population genetic theory predicts that when the frequency of a beneficial allele in a population is increased by selection, the patterns in differentiation and diversity at linked sites are changed as well in a predictable fashion (Smith and Haigh, 1974). Directional selection is expected to decrease within-population diversity and increase between-population differentiation in comparison to neutral expectations. In contrast, balancing selection tends to homogenize allele frequencies and increase the within-population diversity (Nielsen, 2005; Charlesworth, 2006). Thus, genomic regions showing such patterns of genetic diversity could be considered as candidates for containing loci involved in evolutionary change (Schlötterer, 2003).

Recently, with the breakthrough of the amplified fragment length polymorphism (AFLP) technique which allows the genotyping of hundreds of markers across the
genome with no previous genetic information on studied species, population genomics aiming to track adaptive divergence has also become feasible for studies of non-model organisms. This recent expansion into studies of non-model systems allows further development of evolutionary inferences (Luikart et al., 2003), such as the role that selection, mutation, gene flow, and drift play in adaptation (Wang et al., 2003). A powerful approach to understand genome-wide adaptation is to investigate independent natural populations that inhabit environments with strong selective pressures (Williams and Oleksiaik, 2008).

The finless porpoise (Neophocaena phocaenoides) inhabits a wide range of tropical and temperate waters in the Indo-Pacific region (Reeves et al., 1997; Kasuya, 1999). Based on obvious skeletal differentiation (i.e. the width of denticulate region, the height of dorsal ridge, and the number of rows of dorsal denticles) (Gao and Zhou, 1995a), and stepwise discriminate analysis of skeletal morphology (Gao and Zhou, 1995b, c), three populations of finless porpoises in Chinese waters have been identified: the Yellow Sea population distributed in the Yellow/Bohai Sea and the northern East China Sea, the South China Sea population distributed in the South China Sea and the southern East China Sea, and the freshwater-adapted Yangtze River population which is endemic to the middle and lower reaches of the Yangtze River (Gao and Zhou, 1995a). The Yangtze finless porpoise is the sole freshwater population of this species. Due to its small and rapidly declining population and highly endangered status, and its unusual distribution in and adaptation to freshwater environment, the Yangtze River population has attracted extensive attention in recent years.

To date, several studies have been conducted on the population genetic variation of finless porpoises in Chinese waters mainly using molecular markers such as mitochondrial DNA (Yang et al., 2002, 2003, 2008). These studies revealed a generally low level of genetic diversity within populations and a significant overall differentiation between populations. Unexpectedly, such significant differentiation was not found between the freshwater Yangtze River and the marine Yellow Sea populations. However, considering that these studies only used a single neutral marker (i.e. mitochondrial control), the adaptive significance or implication for adaptation of these genetic differentiations is unclear. Distinction in water temperature between different seas (e.g. the East China Sea, the South China Sea, and the Yellow/Bohai Sea, etc.) and gradual change in salinity between seas and freshwaters (the Yangtze River) (Gao and Zhou, 1995a) might be barriers restricting gene flow not only between different marine populations but between marine populations and the Yangtze freshwater population. If this was the case, it would be of great interest in particular to investigate isolating mechanism among different populations and especially adaptive differentiation between freshwater finless porpoises and their marine counterparts using more gene loci or genomics surveys. Because the AFLP technique generates a large numbers of markers to gain a genome-wide perspective on differentiation, it allows the signature of selection to be detected at the genetic level through the identification of ‘outlier loci’ that show unusually high levels of genetic differentiation between populations. These ‘outlier’ AFLP loci are candidate markers for genomic regions involved in local adaptation.

The main goal of this study, therefore, was to perform a genome scan analysis based on a large number of AFLP polymorphisms in three finless porpoise populations in Chinese waters, to assess whether evidence for natural selection acting on individual markers or linked genes can be found, or whether divergence between freshwater population and marine populations can be accounted for by neutral processes.

**MATERIALS AND METHODS**

**Sampling and DNA extraction** Blood and muscle samples (n = 61) were collected from finless porpoises from different geographic locations throughout the coast of China, as well as from the middle and lower reaches of the Yangtze River (Fig. 1). Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN) following the manufacturer’s instruction.

---

**Fig. 1.** Schematic map showing the sampling localities of this study, sampling size for each sampling locality was shown in the parenthesis. Sampling localities abbreviations are as follows: DL(Dalian), LS(Lusi), ZS(Zhoushan), HZ(Hangzhou), NJ(Nanjing), TL(Tongling), PT(Pingtan), and DS(Dongshan).
Scanning of AFLP loci under selection in finless porpoises

AFLP protocols  Restriction enzymes EcoRI and TaqI were used for the AFLP procedure, according to the protocol indicated in Bonin et al. (2005). Table 1 shows EcoRI and TaqI adapters and primers used. An initial screening of 20 combinations of selective primers (See details in Table 3 of Kingston and Rosel, 2004) on ten individuals from different populations was performed. Five combinations were then selected that yielded clear and evenly distributed bands: EcoRI-ATC/ TaqI-AGA; EcoRI-AAC/ TaqI-AGT; EcoRI-ATG/ TaqI-AGA. Instead of a 5′ dye-labelled primer, the EcoRI primers were with M13 tail at its 5′ end (5′-CACGACGTTGTAAAACGAC-3′). To produce labelled DNA fragments, fluorescently labelled M13 primer [either IRD700 or IRD800 (LI-COR)] was added to the selective PCR amplifications. AFLP products were analyzed on LI-COR Genetic Analyzer 4300. The presence (1) or absence (0) of unambiguous AFLP bands was visually scored from the digitized gel images. Only clear bands ranging from 100 to 500 bp that could be scored unambiguously were retained for the subsequent analyses. Bands which were too faint, migrating too close to other bands, or showing large differences in intensity across samples, were discarded. All subsequent data and statistical analysis were based on the 0/1 matrix obtained. A locus was defined as polymorphic if at least one individual showed a variant pattern (see Wilding et al., 2001).

Data analysis  Detection of outlier loci  Identification of outlier loci was carried out following the approach of Beaumont and Nichols (1996) and Beaumont and Balding (2004) as implemented in the computer program DfDsit (available at http://www.rubic.rdg.ac.uk/~mab/stuff/), which is suitable for the analysis of dominant data. The procedure of identifying outlier loci is based on the idea that loci under selection exhibit higher \(F_{ST}\) values than the great majority of neutral markers and are thus called positive outliers, whereas markers under balancing selection are expected to show substantially reduced \(F_{ST}\) values and are thus called negative outliers. DfDsit implements the Bayesian method of Zhivotovsky (1999) for the estimation of allele frequencies using the proportion of recessive phenotypes in the sample. For each analysis, DfDsit first calculates empirical \(F_{ST}\) values for each locus. From the empirical distribution, the trimmed mean \(F_{ST}\) is determined by removing the 30% highest and lowest \(F_{ST}\) values observed in the empirical dataset as recommended in the DfDsit program manual. The trimmed mean \(F_{ST}\) is then used as an estimate of the average neutral \(F_{ST}\) value uninfluenced by outlier loci (Bonin et al., 2006). Next, a null distribution of \(F_{ST}\) values was generated based on 50,000 simulated loci, with the mean \(F_{ST}\) similar to the trimmed mean \(F_{ST}\). DfDsit implements a hierarchical Bayesian approach to compute \(F_{ST}\) values conditional on heterozygosity in a subdivided population under Wright’s (1951) symmetrical island model. From the resulting null distribution of \(F_{ST}\) values, upper and lower confidence intervals are then identified. Because of our particular interest in freshwater-associated divergence, we used three combinations of populations (Yangtze River population vs. Yellow Sea population; Yangtze River population vs. South China Sea population Yangtze River population vs. two marine populations) with two demes (i.e., populations) during the simulation step to corroborate the freshwater-specific outlier status of loci. The 95th quantile of simulated \(F_{ST}\) values was adopted as the threshold for outlier acceptance. The significance level was set at 95% because the Bonferroni correction and the 99% confidence level were too conservative, with no loci detected in most pairwise comparisons. A locus was interpreted as being under directional selection if its locus effect ≥ 2.5% quantile was positive and under balancing selection if its 97.5% quantile was negative. Rather than applying the conservative Bonferroni correction, the false discovery rate (FDR) was used to assess the statistical significance of the

<table>
<thead>
<tr>
<th>Name</th>
<th>Function</th>
<th>AFLP stage</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>EcoRI-F</td>
<td>Adaptor</td>
<td>Digestion-ligation</td>
<td>5′-CTCCTGAGACTGCTAGTC-3′</td>
</tr>
<tr>
<td>EcoRI-R</td>
<td>Adaptor</td>
<td>Digestion-ligation</td>
<td>5′-AACTGGAGATTACGCTAGTC-3′</td>
</tr>
<tr>
<td>TaqI-F</td>
<td>Adaptor</td>
<td>Digestion-ligation</td>
<td>5′-GACGATGACTGCTAGTC-3′</td>
</tr>
<tr>
<td>TaqI-R</td>
<td>Adaptor</td>
<td>Digestion-ligation</td>
<td>5′-CGGTAGGACTGCTAGTC-3′</td>
</tr>
<tr>
<td>EcoRI-A</td>
<td>Primer</td>
<td>Pre-selective PCR</td>
<td>5′-GACTGGGATCAAATTTCA-3′</td>
</tr>
<tr>
<td>EcoRI-AAC</td>
<td>Primer</td>
<td>Selective PCR</td>
<td>5′-GACTGGGATCAAATTTCAAC-3′</td>
</tr>
<tr>
<td>EcoRI-ATC</td>
<td>Primer</td>
<td>Selective PCR</td>
<td>5′-GACTGGGATCAAATTTCACT-3′</td>
</tr>
<tr>
<td>EcoRI-ATG</td>
<td>Primer</td>
<td>Selective PCR</td>
<td>5′-GACTGGGATCAAATTTCATG-3′</td>
</tr>
<tr>
<td>TaqI-L</td>
<td>Primer</td>
<td>Selective PCR</td>
<td>5′-GATGAGTCCTGACTGGAA-3′</td>
</tr>
<tr>
<td>TaqI-AAC</td>
<td>Primer</td>
<td>Selective PCR</td>
<td>5′-GATGAGTCCTGGACGAA-3′</td>
</tr>
<tr>
<td>TaqI-AGG</td>
<td>Primer</td>
<td>Selective PCR</td>
<td>5′-GATGAGTCCTGACGAAAG-3′</td>
</tr>
<tr>
<td>TaqI-ACA</td>
<td>Primer</td>
<td>Selective PCR</td>
<td>5′-GATGAGTCCTGGACGAA-3′</td>
</tr>
<tr>
<td>TaqI-AGT</td>
<td>Primer</td>
<td>Selective PCR</td>
<td>5′-GATGAGTCCTGGACGAAATG-3′</td>
</tr>
<tr>
<td>TaqI-AGA</td>
<td>Primer</td>
<td>Selective PCR</td>
<td>5′-GATGAGTCCTGGACGAAATCC-3′</td>
</tr>
</tbody>
</table>
outlier loci (Storey and Tibshirani, 2003). The QVALUE package (Storey and Tibshirani, 2003) was used for estimation of false discovery rates to the set of F-values for individual loci provided by Dfdist.

Genetic differentiation - the distribution of genetic variation among populations In order to quantify the genetic differentiation with an alternative method not assuming Hardy–Weinberg equilibrium or independence of markers, the proportion of molecular variance within and among populations, AMOVA, was performed with ARLEQUIN 3.11 (Excoffier et al., 2005). AMOVA based on pairwise distances between individuals were carried out for the whole data set considering three levels, among regions, among populations within regions and within populations. The significance of the resultant statistics: \( \Phi_{CT} \), fixation index among groups; \( \Phi_{SC} \), fixation index among populations/within group; and \( \Phi_{ST} \), fixation index within population, was tested with 10,000 permutations.

RESULTS

Levels of polymorphism A total of 163 AFLP loci in the range 100–500 bp could be unambiguously scored from the five primers combinations for the 61 individuals sampled. All individuals exhibited unique AFLP profiles. Different primer pairs amplified variable number of bands, from 23 to 39. The percentage of polymorphic bands between primer pairs varies from 52.2% to 76.9%. There was little variation among primer combinations in both the number of scorable loci and levels of polymorphism except for the primer combination of EcoRI-AC/ TaqI-AC. After those monomorphic loci that were for the entire data set (n = 49) were excluded, 114 polymorphic loci were employed in subsequent analyses.

Outlier Detection Three comparisons performed with Dfdist led to the characterization of six different loci out of 114 polymorphic loci at the 95% confidence level (Fig. 2). Three loci (Loci 65, 73 and 105) had values above the 0.975 quantile in pairwise comparison between Yangtze River population and South China Population. Locus 22 appeared in pairwise comparison between Yangtze River population and Yellow Sea population. Loci 50 and 78 fell below the 0.025 quantile in comparison among three populations. However, applying FDR-level of 0.05 to all comparisons implied that these six outlier loci were not statistically supported.

Fig. 2. Results of Dfdist analyses. Each of the three plots illustrates the empirical distribution of \( F_{ST} \) values for individual AFLP loci (circles) in relation to a simulated 95th quantile for neutrally evolving loci, for a given population comparison. The red loci with \( F_{ST} \) values exceeding this quantile are ‘outlier’ loci putatively evolving under divergent selection. The blue loci with \( F_{ST} \) values exceeding this quantile are ‘outlier’ loci putatively evolving under balancing selection.

Table 2. Hierarchical AMOVA within and among groups of finless porpoise populations

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>Percentage of variation</th>
<th>Fixation index</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>One-group: all populations</td>
<td>Among population/within group</td>
<td>2</td>
<td>57.944</td>
<td>1.009</td>
<td>9.69</td>
<td>( \Phi_{SC} = 0.097 )</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>(YR,YS,SS)</td>
<td>Within populations</td>
<td></td>
<td>545.925</td>
<td>9.413</td>
<td>90.31</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Two-group: freshwater and marine populations</td>
<td>Among group</td>
<td>1</td>
<td>42.112</td>
<td>0.767</td>
<td>7.24</td>
<td>( \Phi_{CT} = 0.072 )</td>
<td>( P = 0.335 )</td>
</tr>
<tr>
<td>(YR),(YS,SS)</td>
<td>Within populations</td>
<td></td>
<td>545.925</td>
<td>9.413</td>
<td>88.85</td>
<td>( \Phi_{SC} = 0.042 )</td>
<td>( P = 0.017 )</td>
</tr>
<tr>
<td>Two-group: (YS),(YR,SS)</td>
<td>Among group</td>
<td>1</td>
<td>15.832</td>
<td>0.413</td>
<td>3.90</td>
<td>( \Phi_{CT} = 0.111 )</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td></td>
<td>545.925</td>
<td>9.413</td>
<td>91.96</td>
<td>( \Phi_{SC} = 0.113 )</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Two-group: (SS),(YR,YS)</td>
<td>Among group</td>
<td>1</td>
<td>19.595</td>
<td>–0.370</td>
<td>–3.62</td>
<td>( \Phi_{CT} = –0.036 )</td>
<td>( P = 1.000 )</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td></td>
<td>545.925</td>
<td>9.413</td>
<td>91.96</td>
<td>( \Phi_{SC} = 0.080 )</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Two-group: (SS),(YR,YS)</td>
<td>Among group</td>
<td>1</td>
<td>38.349</td>
<td>1.194</td>
<td>11.66</td>
<td>( \Phi_{CT} = 0.110 )</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td></td>
<td>545.925</td>
<td>9.413</td>
<td>90.80</td>
<td>( \Phi_{SC} = 0.092 )</td>
<td>( P &lt; 0.001 )</td>
</tr>
</tbody>
</table>
Genetic diversity and population differentiation

When the detected outlier loci were excluded, the “neutral” data set included a total of 108 loci that were not outliers in any comparison. Hierarchical AMOVA analysis using these neutral loci showed that the overall genetic variation among finless porpoise populations was low but significant, with $\Phi_{ST}$ ranging from 0.08 to 0.11 (Table 2). Especially, variation between the freshwater Yangtze River population and marine populations (i.e. the Yellow Sea and South China Sea populations) accounted for 7.24% of the total variation but was not supported by a significant fixation index ($\Phi_{CT} = 0.072, P = 0.331$).

DISCUSSION

Evaluating how natural selection explains genetic variation has been a central theme of population genetics for decades. Broad scale insights into this issue are now being provided by “genome scans” that can survey hundreds of gene regions scattered across the genome and thus allow the identification of those that are differentiating under divergent selection.

Elucidating the genetic basis of adaptation to different environments represents a goal of central importance in evolutionary biology (Storz, 2005). Therefore, past few years have witnessed the multiplication of genome scans aiming at exploring adaptation or speciation in a variety of organisms (e.g. Bonin et al., 2006; Campbell and Bernatchez, 2004; Namroud et al., 2008; Wilding et al., 2001). For example, the study of the genetic frame of adaptation to a gradient of altitude in the common frog (*Rana temporaria*) by Bonin et al. (2006) showed that approximately 2% of the AFLP loci they screened exhibited elevated altitudinal differentiation. In the case of spatially separated populations that inhabit different environments or sympatric populations but exploit different ecological niches, it is possible to identify chromosomal regions involved in adaptive divergence by comparing relative levels of differentiation among multiple, unlinked loci (Charlesworth et al., 1997; Stephan et al., 1998). Because local adaptation and directional selection should have locus-specific effects of reducing genetic variability within populations and increasing differentiation between populations, loci that are outliers for these characteristics are strong candidate regions for involvement in adaptation. Interpreting outlier loci in a genome scan in terms of selection assumes that the observed genomic mean $F_{ST}$ is a good approximation of the average level of differentiations among neutral loci. Loci under divergent selection would upwardly bias the initial estimate of global $F_{ST}$, creating a more conservative test for this form of selection, whereas balancing selection acting on some loci in the sample would have the opposite effect.

The amplified fragment length polymorphism (AFLP) technique, which allows genotyping of hundreds of markers across the genome without any prior sequence information, allows population genomics feasible for studies of non-model organisms. The finless porpoise is a special cetacean species which inhabits completely distinct environments for different population (i.e. freshwater vs. marine waters). Thus, finless porpoise populations provide an excellent system for genome scan studies to address interesting questions about the genetic basis of divergent adaptation (especially for those second aquatic animals such as marine mammals): Is there any adaptive differentiation for the freshwater finless porpoises and their marine counterparts? How many genes are involved in the evolution of adaptive traits?

In the present study, the outlier analysis revealed that although differentiation for the majority of markers did not significantly deviate from neutral expectations, a small number of markers ($n = 6$) were identified as outlier loci. Recent studies using AFLP markers for population genomics to identify outlier loci involved in differentiation between habitats reported the percent of differentiated loci ranging from 1.4%–4.9% (Bonin et al., 2006; Campbell and Bernatchez, 2004; Wilding et al., 2001). However, as mentioned in Beaumont (2005), there is a potentially little power with biallelic markers to detect outlier loci when examining populations in pairwise comparisons, which is due to substantial variability and skew in estimates of $F_{ST}$ using biallelic markers. However, in multi-population surveys, the larger number of samples, potentially collected over a wide geographical area, reduces the variability in $F_{ST}$ among loci and thus facilitates detection of outlier loci (Beaumont and Nichols, 1996). Considering that only a relatively small number of polymorphic AFLP loci ($n = 114$) and a small sample size of the Yellow Sea population ($n = 12$) were analysed in the present study, it is not surprising that only four locus were detected to be candidate loci for divergent selection. Of course, as suggested in Gallavotti et al. (2004), Mcvean et al. (2005), Przeworski et al. (2005), and Teshima et al. (2006) etc., it is also possible for other loci (at least some of them) to be under selection even without yielding statistically significant results in a test for selection. To date, most previous genome scan studies (e.g. Bonin et al., 2006; Egan et al., 2008) have used a conservative criterion that loci appearing only once among several analyses are discarded as false positives. However, different mutations/loci may also be involved in the divergent adaptation of different pairs of populations, which reduced the number of repeated outliers that are detected relative to the actual frequency of parallel selective divergence. Furthermore, for the finless porpoise, there is only one freshwater finless porpoise population in the world, thus it is not possible to sample a new freshwater population to further detect these loci in at least an additional and independent pair of populations.
Only two loci fell below the 0.025 quantile in comparison among three populations. Beaumont and Balding (2004) argue that it can be difficult to detect balancing selection using the Beaumont and Nichols (1996) approach, because the lower 95% confidence limit is typically close to zero. Another plausible explanation for the small number of detected loci affected by balancing selection is that significant but low differentiation was estimated in the present studied populations, which would result in an overall low mean $F_{ST}$ value, and thus would unable to provide a good opportunity to identify loci with exceptionally low differentiation especially when the adaptive divergence is not significant enough.

Although no truly significant outliers were detected by FDR, it still cannot exclude the possibility of those outlier loci. The most reliable way is to validate these outliers with functional genomics analyses or try to find candidate genes that could correspond to these AFLP markers by sequencing them. Then if the results are encouraging there, even if the statistical signals are weak in the genome scan, their functional significance should be assessed independently, and that is just what should be done next. Currently, revealing outlier loci in genome scans most depends on statistical test, one of the main concerns is to highlight truly significant loci to avoid the detection of false positives as much as possible. However, the detection methods used lack power for biallelic data, especially when examining populations by pairs (Beaumont, 2005).

Based upon the limitation of the present sampling design (i.e. only one freshwater population was included in the analyses), it is hard to conclude that the four outliers detected between the freshwater and marine populations are definitely due to freshwater adaptation which at most time was referred to osmoregulation at different salinity (freshwater adaptation in the narrow sense). However, besides freshwater-sea difference in salinity, many other factors (for example, water temperature, availability of preys, local pollution, etc.) may act on distinct differentiation for the freshwater population, and thus can be attributed to freshwater adaptation in a broad sense.

Despite only four loci contributing to divergent selection was detected and the extent of adaptive differentiation was unavailable in the present study, it provided a good clue for adaptive selection between the freshwater population and its marine counterparts. The present study could be a starting point for population genomic survey for revealing freshwater adaptation in finless porpoises, and anyway, some findings in this study could show potential for using AFLP and other genomics approaches to detect how selection operates in the finless porpoise.

Our results suggest the potential of genome-wide surveys to reveal selection signatures in subtle cases of adaptation, where the association between environmental variables and fitness-related traits may be complex and/or cryptic.

As AFLP technique requires high quality of genome DNA, degraded genomic DNA might bias the results, thus, only a small number of samples from three finless porpoise populations were examined in this study. From twelve primer combinations, only five could be used to generate unambiguous and polymorphic bands. The present total number of 114 polymorphic bands was relatively smaller than those of other population genomics studies (e.g. 392 loci in Bonin et al., 2006; 447 loci in Egan et al., 2008). Therefore, analyses using more samples collected in a wider geographical area and more polymorphic loci across the genome might identify more reliable outlier loci in the future. More markers such as single nucleotide polymorphisms (SNP) and microsatellites are also good choice to perform a genome-wide scan in finless porpoise populations for further study to identify candidate genes or genomic regions underlying adaptation.

We thank Dr. Aurélie Bonin for her valuable advice and kind assistance on analytical issues. This research was financially supported by the National Natural Science Foundation of China (NSFC) key project grant no. 30830016, NSFC grant nos 30670294 and 30470253, the Program for New Century Excellent Talents in University (NCET-07-0445), the Ministry of Education of China, the Specialized Research Fund for the Doctoral Program of Higher Education (SRFDP 20060319002), the Ministry of Education of China, and the major project of the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (07KJA18016).

REFERENCES


