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Population genetic structure and identification of loci under selection in the invasive tunicate, *Botryllus schlosseri*, using newly developed EST-SSRs

Yaping Lin [a, b], Aibin Zhan [a, b, *]

[a] Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Haidian District, Beijing, 100085, China

[b] University of Chinese Academy of Sciences, 19A Yuquan Road, Shijingshan District, Beijing, 100049, China

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ABSTRACT

Botryllus schlosseri, also known as golden star tunicate, is generally considered of European origin and has successfully invaded coastal waters of all continents except Antarctica. Owing to its significantly negative ecological impacts, it is crucial to understand its dispersal dynamics and mechanisms of invasion success. Here, we identified 1020 microsatellite-containing sequences from 98,626 expressed sequence tags (ESTs), and developed and characterized 17 polymorphic microsatellites (i.e. EST-SSRs) based on populations both from native (French coast) and invasive ranges (Canadian coast). The number of alleles, observed heterozygosity, and expected heterozygosity ranged from 2 to 12, 0.200 to 0.783, and 0.523 to 0.888 for the French population, and from 2 to 10, 0 to 0.524 and 0.043 to 0.827 for the Canadian population, respectively. We found significant population genetic differentiation between the native and invasive populations (pairwise $F_{ST} = 0.1712$). Moreover, principal coordinates analysis and Bayesian clustering test suggest long-distance dispersal between distant populations. When all loci were subjected for selection analyses, two loci (BS3244 and BS5339) were under selection based on the LOSITAN test. The results obtained in this study can help understand how ecological and evolutionary processes shape population genetic structure, and further how these processes contribute to invasion success.

1. Introduction

Biological invasions have become a global ecological and conservation crisis in many ecosystems such as marine and coastal waters (Willis and Birks, 2006). As many estuaries, bays and near-shore waters are increasingly affected by human activities, coastal waters represent one of the most invaded habitats on the Earth (Rilov and Crooks, 2009). Among many notorious invaders in marine and coastal ecosystems, the golden star tunicate *Botryllus schlosseri* represents one of the most widespread marine invasive species, resulting in huge ecological damages and economic loss in world's oceans (Zhan et al., 2015).

* Corresponding author. Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Haidian District, Beijing, 100085, China.
E-mail addresses: zhanaibin@hotmail.com, azhan@rcees.ac.cn (A. Zhan).

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Botryllus schlosseri, which is generally considered of European origin, was likely introduced *via* shipping to the east coast of North America in the early 1900s (Bock et al., 2012; Zhan et al., 2015). Since then, this species has successfully colonized coastal zones of all non-polar continents, for example, New Zealand in 1922, Australia in 1928, San Francisco in the early 1940's, South California in 1960's, Gulf of Maine in 1978, and Prince Edward Island of Canada in 2001 (Carver et al., 2006; Zhan et al., 2015). Established populations in new invaded areas are being reported frequently (Ben-Shlomo et al., 2010). Multiple vectors are responsible for its introduction and wide spread, such as hull fouling on a large number of boats, especially small pleasure crafts. Also, aquaculture transfers can provide new breeding stocks to colonize uninfested areas (Zhan et al., 2015). Natural dispersal is usually considered to be limited, only *via* rafting on eelgrass, algae or other forms of floating debris (Carver et al., 2006).

Although phylogenetics and population genetics studies have been performed on this highly invasive species (Bock et al., 2012), many questions remain poorly investigated, such as ecological and/or evolutionary changes of population genetic structure and genetic loci under selection during biological invasions. In order to deeply investigate these issues, we developed and characterized 17 polymorphic gene-associated microsatellite markers for *B. schlosseri*. We further assessed the population genetic structure of representative populations collected from both native and invasive distribution ranges. Finally, we employed multiple methods to identify loci under selection during biological invasions of *B. schlosseri*.

2. Materials and methods

2.1. Data mining and microsatellite identification

We obtained the expressed sequence tags (ESTs) of *B. schlosseri* from the NCBI database (http://www.ncbi.nlm.nih.gov/nucest/?term=Botryllus%20schlosseri). All downloaded ESTs were assembled into non-redundant sequences using web-based CD-HIT-EST with default parameters (Huang et al., 2010). Non-redundant EST sequences were used for microsatellite mining with SCIROKO version 3.4 (Kofler et al., 2007) based on the parameters described by Zhan et al. (2005) and Lin et al. (2016).

2.2. Microsatellite validation and polymorphism assessment

Amplification success and polymorphism were assessed using two populations collected from the coast of Canet-en-Roussillon, France (native habitat, $N = 24$) and Sydney, Nova Scotia, Canada (invasive habitat, $N = 24$). Genomic DNA was extracted from zooids using the standard phenol/chloroform method. DNA concentration and quality was assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The microsatellite genotyping was performed using the methods of Zhan et al. (2010). Briefly, the PCR amplification was carried out in 12.5 μL reaction volume containing approximately 40 ng of genomic DNA, 1× PCR buffer, 0.2 mM of each dNTP, 1.5 mM of Mg^{2+}, 0.5 pmol M13-tailed (5′-CAC-GACGTTGTAAAACGAC-3′) forward primer, 1 pmol reverse primer and 1 pmol fluorescently (6-FAM, HEX, TMR, ROX) labelled M13 primer, and 0.25 unit of *Taq* DNA polymerase (Takara Bio Inc.). The PCR profile consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. Amplified fragments were analyzed on an ABI 3730xl automated sequencer (Applied Biosystems, Foster City, CA, USA) with GeneScan™-500 LIZ™ as the internal size standard (Applied Biosystems). Alleles were scored using GeneMapper™ version 4.0 (Applied Biosystems).

2.3. Genetic diversity

Genetic diversity, including the number of alleles (A), observed heterozygosity (H_o), and expected heterozygosity (H_e) were calculated using GENEPOP online version (http://www.genepop.curtin.edu.au/). The polymorphic information content (PIC) value of each locus was estimated using CERVUS version 3.0.3 (Marshall et al., 1998). The presence of null alleles at all loci was evaluated using the software MICRO-CHECKER version 2.2.0 (van Oosterhout et al., 2004). The probability of significant deviation from Hardy–Weinberg equilibrium (HWE) was assessed using the Markov chain-based method implemented in GENEPOP. Significance criteria were adjusted for the number of simultaneous tests using the standard Bonferroni correction.

2.4. Loci under selection

To identify loci under selection between two populations, we adopted two theoretical approaches based on the F_{ST}-outlier test for modeling neutral loci. The first method that we used here was developed by Beaumont and Nichols (1996). This method detects the loci under selection based on the distributions of heterozygosity and F_{ST} under neutral expectations (Antao et al., 2008). As recommended by Antao et al. (2008), we used the program LOSITAN to perform 100,000 coalescent simulations to generate F_{ST} values under the infinite alleles model (IAM) with 99% confidence intervals and false discovery rate (FDR) of 0.05 for both 'neutral mean F_{ST}' and 'force mean F_{ST}' options. The second method that we used here was suggested by Foll and Gaggiotti (2008). This method decomposes locus population F_{ST} values into locus-specific components (α) and population-specific components (β). We performed this test using BAYESCAN program. Following 10 pilot runs of 5000 iterations with a 50,000 burn-in and a thinning interval of 20, we used the prior odds of 10 in favour of a neutral mode.