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Genetic diversity, population structure, and demographic history of exploited sea urchin populations (*Tripneustes gratilla*) in the Philippines

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ABSTRACT

The sea urchin, *Tripneustes gratilla* is ecologically and economically important in the Indo-Pacific region. We use population genetic methods to investigate the population structure and historical demography of exploited populations in the Philippines. Sea urchins were collected from 6 localities in western Luzon and 4 outgroup sites. Samples were sequenced for mitochondrial cytochrome oxidase-1 gene ($n = 282$) and genotyped for seven microsatellite loci ($n = 277$). No significant genetic structure was found for either class of markers, indicating either extensive gene flow across the archipelago, or that populations have high genetic diversity and have not yet attained equilibrium between genetic drift and migration following large changes in demography. Interestingly, demographic inferences from the two types of markers were discordant. Mitochondrial lineages showed demographic expansion during the Pleistocene while microsatellite data indicated population decline. Estimates for the date of each event suggest that a Pleistocene expansion could have preceded a more recent population decline, but we also discuss other hypotheses for the discordant inferences. The high genetic diversity and broad distribution of haplotypes in populations that recently recovered from fishery collapse indicate that this species is very resilient over evolutionary timescales.

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1. Introduction

The widely-distributed *Tripneustes gratilla* is a high-value sea urchin in the Indo-Pacific region. This ubiquitous herbivore occupies a key trophic position in nutrient cycling especially in tropical seagrass ecosystems (Alcoverro and Mariani, 2002; Klumpp et al., 1993; Koike et al., 1987). *T. gratilla*, being an opportunistic grazer, also functions as a keystone species for coral reef and seagrass communities by controlling invasive macroalgae (Conklin and Smith, 2005; Stimson et al., 2007). Population outbreaks in some areas, however, have resulted in overgrazing of seagrasses (Eklöf et al., 2008) and foliose algae (Valentine and Edgar, 2010). This is also an economically-valuable sea urchin species that is collected for its high quality gonad — a specialty food item primarily in Japan (Andrew et al., 2002; Lawrence and Agatsuma, 2001). Many fisheries for this species have declined over time due to indiscriminate harvesting and lack of management measures which resulted to economic losses (i.e. Andrew et al., 2002; Shimabukuro, 1991; Talaue-McManus and Kesner, 1995). While these cases documented significant fisheries impacts on *T. gratilla* populations, they need to be placed into a broader spatial and temporal context. What does the regional population structure of the species look like? What is the demographic history of the species?

A population genetic approach can potentially provide these much needed insights for exploited invertebrate species (Thorpe et al., 2000). Molecular methods were initially used with marine species to better understand the effects of larval dispersal on the population structure (e.g. Doherty et al., 1995; Waples, 1987) and determine the spatial scales of population connectivity (Cowen and Sponaugle, 2009; Hedgecock et al., 2007; Palumbi, 2003, 2004). Development of genetic markers and analysis have since extended the utility of genetic data to direct measurement of migration, estimation of effective population size, and examination of population demographic history (Emerson et al., 2001; Hare et al., 2011; Hellberg, 2009; Manel et al., 2005; Pearse and Crandall, 2004). To date, genetic assessments on *T. gratilla* populations have been limited to broad scale phylogeographic surveys of its genus (Lessios et al., 2003) and phylogenetic studies (Palumbi and Lessios, 2005; Zigler and Lessios, 2003). Mitochondrial sequence variation showed very weak regional divergence of *Tripneustes* populations across the Indo-Pacific region despite the significant local differentiation among the populations in this region. This implies that *T. gratilla* in this region belongs to a large *Tripneustes* metapopulation (Lessios et al., 2003). The only finer-scale genetic survey on *T. gratilla* populations was carried out in western Luzon and eastern Philippines (Malay et al., 2002). This was conducted soon after the collapse of a local artisanal sea urchin fishery (i.e. Talaue-McManus and Kesner, 1995) to aid management of the heavily-exploited *T. gratilla* populations in northwestern Luzon (Malay et al., 2002). Based on six allozyme

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markers, genetic differentiation of sea urchin populations within the western Luzon region was not significant, indicating extensive gene flow among populations (Malay et al., 2002). This lack of genetic structure of *T. gratilla* was initially explained by its potential for long-distance larval dispersal (Malay et al., 2002). This species has a relatively long planktonic larval duration of 20 to 52 days under culture conditions (Juinio-Meñez et al., 1998; Lawrence and Agatsuma, 2001; Shimabukuro, 1991). The planktonic stage or echinopluteus, though capable of movement using their ciliated bands (Emlet et al., 2006), is categorized as a weakly swimming larva and considered passive to the forces of oceanographic processes (Chia et al., 1984 cited in Weersing and Toonen, 2009). Thus, there is great potential for *T. gratilla* larvae to be dispersed over large distances before settlement. It is possible, though, that limited but significant genetic differentiation was not detected with the employed genetic marker, sampling design, or analysis (Ward, 2006). The availability of DNA markers for *T. gratilla* and recent developments in population genetics analysis presents an opportunity to re-examine the genetic variation of this exploited species.

Using multiple molecular markers, this study aims to obtain a more detailed characterization of *T. gratilla* populations in the Philippines to make inferences about population structure, effective size, and demographic history and gain insights on the vulnerability of the species to exploitation. Mitochondrial DNA has been noted for its relatively rapid development of population genetic structure due to its small effective population size. Based on this, it has become a preferred genetic marker over allozymes for initial examination of population differentiation (Bowen et al., in press). Given its non-recombining nature, mtDNA has been useful in gaining some insights on species evolutionary history and population demographic history based on maternal lineage (Avise et al., 1987; Liu and Cordes, 2004 but see also Ballard and Whitlock, 2004). On the other hand, microsatellites are inherited as codominant markers and thus provide insights on genetic differentiation of populations based on gene flow of both sexes (Liu and Cordes, 2004; Selkoe and Toonen, 2006). Highly polymorphic microsatellite loci also have the potential to reveal contemporary gene flow and effective population size (Ovenden et al., 2007; Saenz-Agudelo et al., 2011). The different characteristics of the DNA markers and new analytical approaches employed in this study should aid in elucidating historical and contemporary processes that influenced the current pattern of genetic variations in *T. gratilla*.

2. Materials and methods

2.1. Tissue collection and DNA extraction

Samples were collected from 6 sites along the western Luzon coast and 4 outgroup sites from other regions in the archipelago (Table 1). Only adult sea urchins with test diameter greater than 60 mm (Bangi, 2001) were collected on snorkel and tube feet and spines were sampled non-destructively. Individual tissue samples were kept in a vial and preserved in 90% ethanol. DNA was extracted from the tissue samples using a 10% Chelex™ (Biorad) solution (Walsh et al., 1991).

2.2. DNA sequencing of the CO1 region

A region of the mitochondrial cytochrome oxidase subunit 1 (CO1) gene was amplified using the universal forward primer: CO1f 5′ CCTGCAGGAGGAGGAGAYCC and a *Tripneustes*-specific reverse primer CO1TR1 5′GGCATTCCAGCTAGTCCTARAAA (Lessios et al., 2003). PCR reactions were carried out in a final volume of 25-μl containing 1 μl genomic DNA extraction, 2.5 μl 10× PCR Buffer (PE-II), 2.5 μl of 8 mM dNTPs, 2.0 μl of 25 mM $MgCl_2$, 1.25 μl of each primer (10 mM), 0.125 μl of Amplitaq Gold polymerase (Applied Biosystems, Inc.; 5 units/μl) and 14.5 μl of molecular grade water. The PCR temperature profile was as follows: initial denaturation for 7 min at 95 °C, 40 times of the main cycle: 30 s at 95 °C (denature), 30 s at 52 °C (anneal) and 1 min at 72 °C (extension), and 10 min of final elongation at 72 °C. After amplification, PCR products were run in 1% agarose gels stained with ethidium bromide to evaluate the quality and quantity of the amplified DNA. Excess dNTPs and primers were removed prior to cycle sequencing using an enzymatic method. Five (5) microliters of the PCR product was incubated with 0.5 μl (0.5 U) of shrimp alkaline phosphatase and 0.5 μl (5 U) of exonuclease (GE Healthcare) for 30 min at 37 °C, 15 min at 80 °C, and 1 min at 25 °C. Purified DNA products were cycle sequenced in both directions using BigDye 3.0 terminator chemistry (Applied Biosystems, Inc., Foster City, CA). The cycle sequencing reaction was carried out in a 12-μl volume reaction: 2.5 μl of ABI 5× sequencing buffer, 0.5 μl of 10 mM primer, 0.5 μl BigDye, 0.5 μl DMSO, 7.0 μl molecular grade water and 1 μl of cleaned PCR product. The sequencing program started with 25 cycles of 95 °C for 10 s, 50 °C for 5 s and 60 °C for 5 min and a final step of 5 min at 20 °C (Barber et al., 2006). Afterwards, the labeled DNA sequences were precipitated with ethanol, resuspended in formamide, and sent to Life Sciences Core Laboratories Center at Cornell University for sequencing on an Automated 3730 DNA Analyzer (Applied Biosystems, Inc.).

2.3. Microsatellite PCR amplification and genotyping

Seven out of 11 microsatellite loci developed for *T. gratilla* (Carlon and Lippé, 2007) were used (Supplementary Table S1). The other four loci were excluded due to persistence of null alleles in all subpopulations tested (Carlon and Lippé, 2007). PCR amplifications of three loci: Tgr-B11, Tgr-C117, and Tgr-D134 were carried out in multiplex while the remaining four loci (i.e. Tgr-24, Tgr-A11, Tgr-C11, and Tgr-D5) were individually amplified in separate PCR runs. The multiplex PCR

Table 1
Sampling location, population code, collection dates, and the number of individuals (n) screened at mitochondrial (CO1) and microsatellite markers.

Sampling sites	Lat (N)	Long (E)	Population code	Mitochondrial		Microsatellite	
				Collection date	n	Collection date	n
Study region: Western Luzon							
Bubon, Burgos, Ilocos Norte	18°30′31″	120°34′45″	Burgos (BUR)	Nov-08	29	Nov-08	34
Dadalaquiten, Sinait, Ilocos Sur	17°53′34″	120°26′28″	Sinait (SIN)	Dec-08	28	n.d.	n.d.
Lucero, Bolinao, Pangasinan	16°24′09″	119°54′30″	Lucero (LUC)	Nov-08	21	Nov-09	50
Victory, Bolinao, Pangasinan	16°23′35″	119°57′59″	Victory (VIC)	Nov-08	24	Nov-09	49
Panglit Is., Masinloc, Zambales	15°29′42″	119°55′09″	Masinloc (MAS)	Apr-09	30	Apr-09	48
Matuod, Lian, Batangas	13°59′11″	120°37′40″	Lian (LIA)	Feb-09	30	Feb-09	47
Outgroup sites							
Bacon, Sorsogon	13°02′24″	124°02′56″	Sorsogon (SOR)	Jul-08	21	n.d.	n.d.
Lawi, Jordan, Guimaras	10°32′45″	122°31′16″	Guimaras (GUI)	Jan-09	47	Dec-09	49
Cantaan, Guinsiliban, Camiguin	09°06′25″	124°48′14″	Camiguin (CAM)	Mar-09	32	n.d.	n.d.
Simunul, Tawi-tawi	04°54′03″	119°50′56″	Tawi-tawi (TAW)	May-09	20	n.d.	n.d.

n.d.: no data.

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