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High genetic homogeneity of an intertidal marine invertebrate along 8000 km of the Atlantic coast of the Americas

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Abstract

Phallusia nigra is a cosmopolitan solitary ascidian with a distinct niger-blue tunic that is considered to be an easy diagnostic feature, even in the field. The wide geographic distribution of this species may have been a result of the overconfidence of taxonomists and ecologists in the assignment of unknown specimens to this species, based on this simple diagnostic character. Indeed, there seems to be a correlation between cosmopolitanism and the presence of few, but very conspicuous diagnostic characters in benthic invertebrate species. Almost invariably, genetic studies have demonstrated that the cosmopolitanism of those species is artificial and that, rather, they are made up of groups of morphologically very similar, but genetically distinct, species. Thus, we decided to verify the specific status of Western Atlantic populations of *P. nigra*, from Miami (USA) to São Paulo (southeastern Brazil) by estimating genetic variation and population structure levels along the entire range of this ascidian in the Western Atlantic. The analysis of 10 allozyme systems shows high levels of mean heterozygosity ($H=0.28$) so that *P. nigra* has genetic variation levels more related with other invertebrates than to their, phylogenetically closer, chordate relatives (that show lower levels of heterozygosity). The genetic structure of the population was relatively high ($F_{ST}=0.083$) for conspecific populations, but much lower than what would be expected if they belonged to different species. High levels of gene identity also indicate that the Western Atlantic population of *P. nigra* constitutes a single species. This result suggests an extremely high dispersal capability of the larvae of this ascidian or a relatively recent range expansion of its populations. Since this species is commonly found in harbors, the

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anthropogenic transport (p.e., in ballast water) may have contributed for the observed genetic homogeneity.

Keywords: Phallusia nigra; Ascidian; Heterozygosity

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1. Introduction

Phallusia nigra Savigny 1816 is a common solitary ascidian in many tropical areas around the world. The tunic of *P. nigra* is smooth and, because of the accumulation of strong acids, it is seldom covered by fouling organisms. Its large size (up to 10 cm long (da Rocha et al., 1999)) and deep black colour (Hirose, 1999) makes *P. nigra* a very conspicuous ascidian in the benthic habitats wherever it occurs (da Rocha et al., 1999). *P. nigra* was originally described for the Red Sea and, since then, it has also been reported in the Indian, the Pacific and the Atlantic Oceans (da Rocha et al., 1999a; Meenakshi, 1998; Van Name, 1945).

Nevertheless, the cosmopolitanism of this ascidian is at odds with what has been observed for many benthic marine invertebrates. In most cases, wide distributional ranges of marine invertebrate species have been found to be the result of an over-conservative taxonomy (e.g., Knowlton, 2000; Thorpe and Solé-Cava, 1994; Klautau et al., 1999). Since the existence of cryptic species has been demonstrated in other ascidians (Aron and Solé-Cava, 1991; Dalby, 1997; Degnan and Lavin, 1995), it is possible that the cosmopolitanism attributed to *P. nigra* is also artifactual.

In this study, we used allozymes to compare populations of *P. nigra* over its entire distribution on the West Atlantic, from Florida, USA to São Paulo, Brazil. We found surprisingly high levels of gene similarity between the populations analysed and concluded that, at least for the Atlantic Ocean, a high genetic homogeneity for this ascidian species cannot be ruled out.

2. Material and methods

Samples of *P. nigra* ($N=157$) were collected by snorkelling or scuba-diving from five different locations over its entire range of distribution in the Western Atlantic. The locations (latitude and longitude) and sample sizes were as follows: Florida (Florida Keys: 25°46′ N, 80°16′ W—58 samples), Panama (Bocas del Toro: 09°19′ N, 82°17′ W—29 samples) and the Brazilian locations were Cabo Frio (22°45′ S, 42°01′ W—25 samples), Angra dos Reis (23°00′ S, 45°04′ W—25 samples) and São Paulo (23°27′ S, 45°04′ W—20 samples) (Fig. 1). All samples were transported live or on ice to the laboratory, where they were kept in liquid nitrogen until analysis.

Allozymes were extracted from the digestive gland in one volume of distilled water and analysed through 12.5% starch gel electrophoresis, using the Tris citrate pH 8.0 (Ward and Beardmore, 1977) and the lithium hydroxide pH 8.1 (Selander et al., 1971)

Fig. 1. Map of sampling area with collection sites.

buffer systems. After electrophoresis, the gels were sliced and stained using standard procedures (Manchenko, 1994). Twenty-five enzyme systems were initially essayed, but only 9 (10 loci) gave interpretable and reproducible results.

Genotype frequencies, obtained from the scoring of the gels, were used to estimate gene frequencies, fits to Hardy-Weinberg equilibrium, mean heterozygosities and inbreeding indices (Wright, 1978), using the BIOSYS 1.7 programme (Swofford and Selander, 1981). Pairwise genetic distances and their standard errors (Nei, 1972; Nei, 1978) were also calculated with this program.

A rough estimate of the expected effective number of migrants per generation was obtained from F_{ST} as $N_e m = (1 - F_{ST})/4F_{ST}$ (Wright, 1978). Although this estimate relies on a number of assumptions that often are not met in natural populations (Whitlock and

Table 1
Gene frequencies and average heterozygosities for populations of *P. nigra* studied

Locus	Florida	Panama	Brazil		
			Cabo Frio	Angra dos Reis	São Paulo
Pgi					
(*N*)	6	18	17	25	19
A	–	0.20	0.12	0.14	0.21
B	1.00	0.72	0.88	0.86	0.76
C	–	0.08	–	–	0.03
α-Est-1					
(*N*)	27	19	17	25	20
A	0.56	0.16	0.38	0.36	0.52
B	0.18	0.45	0.29	0.24	0.20
C	0.26	0.39	0.33	0.40	0.28
Adh					
(*N*)	5	16	17	25	10
A	0.60	0.94	0.65	0.96	0.55
B	0.40	0.06	0.35	0.04	0.45
Mdh					
(*N*)	17	11	16	17	20
A	0.91	0.59	0.81	0.88	0.83
B	0.09	0.41	0.19	0.12	0.17
Gdh					
(*N*)	23	18	17	25	20
A	1.00	1.00	1.00	1.00	1.00
α-Est-2					
(*N*)	38	20	17	25	20
A	0.68	0.40	0.88	0.86	0.85
B	0.32	0.60	0.12	0.14	0.15
β-Est					
(*N*)	23	17	17	18	20
A	0.72	0.82	0.85	0.83	0.85
B	0.28	0.18	0.15	0.17	0.15
Aldox					
(*N*)	46	28	17	25	20
A	1.0	1.0	1.0	1.0	1.0
Cat					
(*N*)	46	29	17	25	20
A	1.0	1.0	1.0	1.0	1.0
Got					
(*N*)	3	10	17	19	6
A	–	0.10	0.32	0.68	0.33
B	0.33	0.55	0.53	0.32	0.33
C	0.67	0.35	0.15	–	0.34
H_e	0.27	0.31	0.28	0.22	0.31

McCauley, 1999), it is fairly robust to many of those violations. It has co-varied positively with direct estimates of migration (Neigel, 1997) and can still be useful, if only to compare with similar estimates from other organisms (Neigel, 2002).

3. Results

Levels of genetic variability in the populations of *P. nigra* were consistently high and varied between 0.22 and 0.31 (Table 1). Similar values have been found in colonial ascidians and other marine invertebrates (Aron and Solé-Cava, 1991; Sole-Cava and Thorpe, 1991) that would indicate that shared ecological and physidogical characteristics among marine invertebrates play a more important role in the maintenance of variability than phylogenetic relationships, in which ascidians and vertebrates are closer.

A significant heterozygote deficiency ($0.01 < P < 0.05$) after Bonferroni's correction; (Lessios, 1992) was found for the α-Est2 locus in the population from Florida (data not shown). Heterozygote deficiencies are very common in marine invertebrates, and many mechanisms have been suggested to explain them (Zouros and Foltz, 1984). Since all individuals analysed here were genetically unique, i.e., had different multi-locus genotypes, the excess of homozygotes cannot be explained as the effect of asexual reproduction, as observed in other species of ascidians (Ayre et al., 1997). The high homogeneity of the populations studied (see below) also indicates that population mixing (the Wahlund effect; David et al., 1997; Wahlund, 1928) is also not an adequate explanation for these deficiencies. Alternative explanations, based on inbreeding, natural selection, aneuploidy or the presence of null alleles (Zouros and Foltz, 1984), cannot be discarded for *P. nigra* populations.

Levels of pairwise genetic distances (Nei, 1972; Nei, 1978) between the studied populations were remarkably low (between 0.000 and 0.078; Table 2). Wright's (1978) inbreeding index (mean $F_{ST} = 0.083$, $N_e m = 2.76$) indicates population structuring, but no major differentiation between samples along the 8000 km of Atlantic coast analysed. This suggests that there is a high genetic homogeneity of the populations of *P. nigra* over a large geographical distribution from southern Brazil to southern Florida in the U.S. Considering the average 10:1 ratio between nominal and effective migrants (Frankham, 1995), $N_e m = 2.8$ would translate into an average of about 28 larvae being exchanged between the populations per generation, along the 8000 km of the studied area. This result strongly suggests that *P. nigra*, contrary to most wide-ranged marine invertebrates studied to date, is a single species along the entire Atlantic Coast.

Table 2
Pairwise genetic distances (Nei 1972) for populations of *P. nigra* studied

	Florida	Panama	Cabo Frio	Angra dos Reis	São Paulo
Florida	–	0.062	0.026	0.078	0.009
Panama	–	–	0.055	0.073	0.067
Cabo Frio	–	–	–	0.018	0.000
Angra	–	–	–	–	0.029

4. Discussion

It is remarkable that populations of *P. nigra* could exchange so many individuals over such long geographical distances, particularly in face of the distinct surface ocean circulation cells of the North and South Atlantic oceans. Solitary ascidians, such as *P. nigra*, are known to show lower levels of genetic structure than colonial species (Ayre et al., 1997; Kano et al., 2001; but see also Astorga et al., 2002). For instance, the colonial ascidian *Botryllus niger* (Aron and Solé-Cava, 1991) was found to be in fact two distinct species along 400 km coast of Brazil. However, major sedimentation and low salinity barriers of the mouth of the Amazon and the Orinoco rivers (McCartney et al., 2000) make the genetic homogeneity of *P. nigra* still puzzling. It should be noted, though, that Indian and Pacific specimens of *P. nigra* were not sampled in the present study so the taxonomic status of those populations remains to be tested.

Interestingly, much of the cosmopolitanism attributed to benthic marine invertebrate species during the first half of the 20th century turned out to be, under closer scrutiny, a taxonomical artifact (for reviews, see Knowlton, 1993; Knowlton, 2000; Thorpe and Solé-Cava, 1994). This artifact seems to originate, basically, from the coupling of two facts. The first is that marine invertebrates often have high levels of morphological conservativeness compared to other animals. These may facilitate cryptic speciation events. The second fact is that taxonomists have many times dismissed, as intraspecific variation, subtle but clear morphological differences between samples from different geographical areas (Klautau et al., 1999; Solé-Cava and Boury-Esnault, 1999; Solé-Cava et al., 1991). Additionally, taxonomists that suggested broad geographical distributions to benthic marine invertebrate species overestimated the dispersal capabilities of their planktonic larvae (Knowlton and Keller, 1986; Pechenik, 1999; Silva and Russo, 2000; Todd, 1998).

One should expect, naturally, that truly cosmopolitan species do exist and exhibit extremely high dispersal capabilities. Although, it is difficult to imagine a larva that can frequently disperse over all major oceans, *P. nigra*'s populations are not necessarily interbreeding at the present time. If a single recent, naturally or antropogenically mediated, colonization event is responsible for the spreading of these ascidians over the Atlantic, populations are not expected to be in migration-drift equilibrium (Bohonak et al., 2002). In this case, the homogeneity along the Atlantic coast is transitory and it is a consequence of the short time that these populations may have had to diverge genetically (Holland, 2000; Lazoski et al., 2001).

In general, ascidians seem to be very efficient in colonizing new areas (Lambert and Lambert, 1998) and they are common invading species of the fauna of bays and harbours (Boyd et al., 1990; Ben-Shlomo et al., 2001; Stoner et al., 2002). *P. nigra* is one of the first colonizers on vertical slopes, grows fast and seems to have a short (< 2 years) life-expectancy at settlement (da Rocha et al., 1999b), characteristics often found in bio-invading species (Holland, 2000). Solitary ascidian species can be efficient bio-invaders. For example, the large Australian ascidian *Pyura praeputialis* has recently invaded the coast of Chile (Astorga et al., 2002). Nevertheless, in a single recent colonization event, populations are expected to present low of genetic variability, because of the founder effects related to the process of invasion (Holland, 2000). This is the case of *P. praeputialis*, where only 3 out of 17 allozyme loci analysed were polymorphic (18%

polymorphism). However, the populations of *P. nigra* studied here had high levels of genetic variability (70% polymorphism; data not shown). Therefore, if recent population expansion is the explanation for the observed genetic homogeneity, it is likely that it occurred through several recurrent colonization events.

Since it is hard to speculate on any major natural process that could promote gene flow over such long distances consistently, the events were probably antropogenically mediated, such as transport in ballast water of ships (Carlton, 1985; Carlton, 1996; Nishikawa et al., 2000). The impact of the 35,000 currently estimated cargo ships circulating on the oceans on the distribution of species is enormous, particularly considering that each of those ships can carry up to 150,000 tons of ballast water that may carry gametes, larvae and adults to the most distant shores (Holland, 2000; Solé-Cava, 2001). Most molecular systematic studies are concentrated on a restricted geographic region of a couple of hundred kilometers and their conclusions are naturally limited by this restriction. Broader range studies are necessary in order to verify the actual taxonomic status of cosmopolitan species.

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