The success of invasive species in the Mediterranean basin: molecular tools to identify non indigenous benthic species

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SUMMARY

The increase of non indigenous species (NIS) into the Mediterranean Sea in the last years, has had a significant effect on the economy and the ecology of the basin. The reasons that allow introduced species to become invasive are however still unclear. The classic ecological analyses, based on photometric methods and through direct observations are often unable to clarify the real taxonomic identity. The presence of cryptic species and of species with different dispersal capability, do not often give the possibility of identifying the origin of populations, the occurring of metainvasions and/or the genetic diversity (Geller, 1996). With the modern techniques of molecular biology it is however easier to give answers to the fundamental ecological questions of the bioinvasions phenomenon, producing at the same time useful data to develop models to prevent and to predict the invasive event. The use of specific molecular markers, like Microsatellites and SNPs has stressed that between the introduced populations there is a loss of genetic diversity, while the native populations are genetically more variable. Even if the reduction of the genetic diversity is usually considered disadvantageous at population level, the genetic reduction can lead to the ecological success of the invasive species (Lee, 2002). In this contest, through the analysis of the bio-ecology and genetic characteristics, the possibility to identify general models to discover and to study non indigenous species will be discussed.

INTRODUCTION

Marine ecosystems are populated by species that disperse between localities, sometimes sending propagules far away from their parental population, sometimes retaining eggs and larvae close to the point of origin. This potential dispersal, and its variation, is one of the critical elements of marine life history dynamics, and influences the aspect of ecosystem functionality including stability of populations, resilience to extinction, and the degree to which species can adapt to local conditions, establishing the bases for new colonizations.
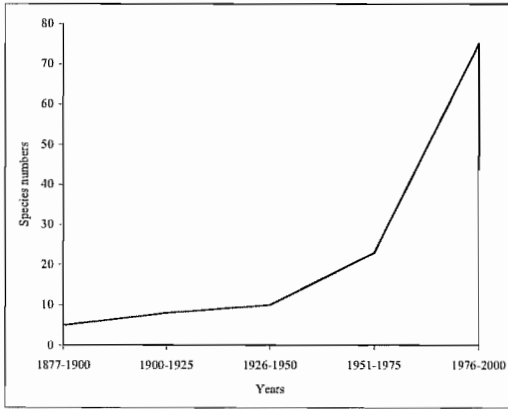
In the last 25 years the number of Mediterranean aliens species has increased with about 50 new species (Fig. 1) (Carlton, 1999; Ceccherelli and Piazzi, 2001; Famà et al., 2002; Boudouresque and Verlaque, 2002). New species have been described, based on ecological, morphological, and genetic traits. More often it has been possible to clarify the true taxonomic identity of incorrectly considered non indigenous species (NIS) due to misidentification and/or synonyms cases (Tab. I).

Fig. 1 - Mediterranean introduced species, plotted over a time scale

Tab. I - List of Molluscs excluded from the Mediterranean exotic species due to misidentification and/or synonymy cases. In the table are also indicated Indo-Pacific species recorded in the Mediterranean. (NM = Native Mediterranean species; AI = Accidental Introduction; MIS = Misidentification; SYN = Synonyms).

Acanthochitona penicillata (Deshayes 1863)	MIS	*Acanthochitona* sp.	Barash and Danin, 1973
Alvania dorbignyi (Audouin, 1827)	NM		Barash and Danin, 1973
Strombus decorus (Roeding, 1798)	MIS	*Strombus persicus*	Swainson, 1821
Monetaria moneta (Linnaeus, 1758)	AI		
Murex tribulus (Linnaeus, 1758)	MIS	*Murex forskoehli*	Röding, 1798
Murex ponderosus (Sowerby, 1879)	AI		
Aspella anceps (Lamarck, 1822)	NM		
Chrysallida maiae (Hornung & Mermod, 1924)	MIS	*Chrysallida dentata*	Nordsieck, 1972
Chrysallida pirinthella (Melvill, 1910)	MIS	*Chrysallida* sp.	Buzzurro and Nofroni, 1994
Syrnola massauensis (Hornung e Mermod,1924)	MIS	*Syrnola fasciata*	Jickeli, 1882
Cylichnina mongii (Audouin,1827)	MIS	*Cylichnina multiquadrata*	Oberling,1970
Petalifera gravieri (Vayssière,1906)	SYN	*Petalifera petalifera*	Rang, 1828
Fulvia australis (Sowerby GB, 1834)	SYN	*Fulvia fragilis*	Forsskål in Niehbur, 1775

Indo-Pacific species recorded in the Mediterranean
Cerithium scabridum (Philippi, 1848)
Hipponix conicus (Schumacher,1817)
Erronea caurica (Röding, 1798)
Rapana venosa (Valenciennes, 1846)
Coralliobia madreporarum (Sowerby, 1832)
Bursatella leachii (Blainville, 1817)

The main flow of invaders resulted from the opening of the Suez Canal in 1869 allowing the entry of Indo-Pacific species. Exotic macrophytes, invertebrates and fish are now in many coastal areas of the Mediterranean Sea, competing or replacing native species and becoming locally pests for species of commercial value.

Sluggish waters and high pollutants present in Mediterranean harbors (ballast waters intraductions) also played an important role establishing new colonizations of opportunistic species.

The situation is further more complicated by the fact that many invasions occur as a dynamic series of different time events commonly clustered together with the term metainvasion.

High resolution genetic data are useful to measure the interactions between marine organisms and to determine invasion pathways that are playing an important role in the Mediterranean economy.

The combination of new biotechnology to collect more exhaustive data sets with the attention of dynamics of marine communities, makes the impact of genetic studies on marine ecology stronger now than ever.

GENETIC DRIFT AND POPULATION CHANGE

In order to better clarify the invasive processes, it is necessary to emphasize a little about marine population genetics. In general, marine genetics is hard to interpret and it is difficult to understand the ecological implications because inferences about gene flow are usually made on evolutionary time scale, not the ecological time frame over which most management decisions are made.

Genetic exchange is usually monitored in terms of the average number of migrants (this is also the case of propagules and/or juveniles mediated by ballast waters) that move between populations. This value is a product of the average population size (N) and the average fraction of each population that are immigrants (m). Gene flow is estimated from the proportion of genetic variation geographically structured (F_{ST}), using models of population structure that are reliable only when gene flow is relatively slow.

There are different analytical frameworks for understanding genetic structure that may have greater power to describe marine population connectivities and invasions success.

Most of the applications of genetic data to population questions uses Sewell Wright's island model (Wright, 1951) as a way of relating the geography of gene frequency variation to levels of gene flow. Typically, Nm is estimated from F_{ST} using island model assumptions (all populations are linked by equal gene flow, with a proportion of migrants (m) every generation) (Fig. 2a). Under these conditions: $4Nm = (1/F_{ST})-1$ at equilibrium.

There is no intrinsic geography to these populations, they are all equally distant from one another, and thus the island model probably does not describe the real population structures very well.

Because of the reciprocal relationship between F_{ST} and Nm, as F_{ST} becomes smaller, the estimate of Nm increases very quickly (Fig. 3a). For example, a F_{ST} of
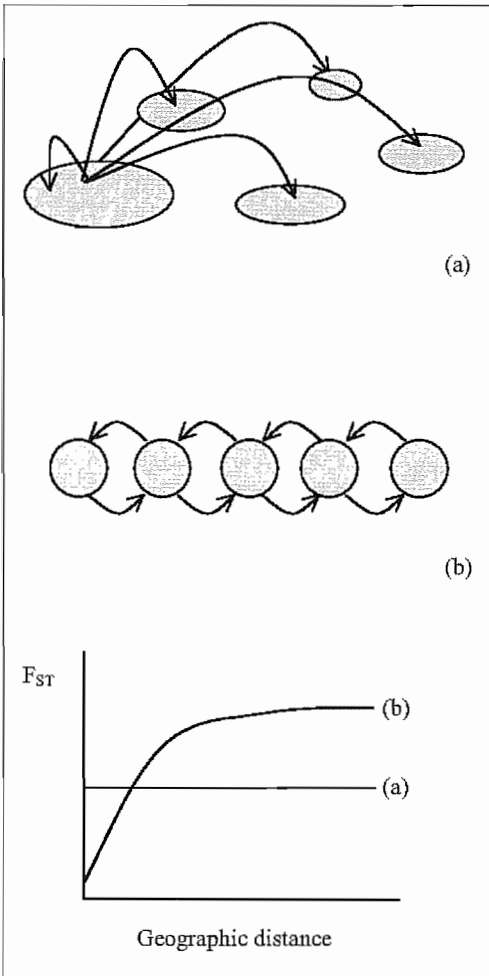
Fig. 2 - Two common models of population structure. The island model assumes equal migration among populations (a). The stepping stone model assumes populations exchange propagules only with adjacent populations (b) (see text).

0.025 suggests an Nm value of 10 but an F_{ST} of 0.005 suggests an Nm of 50 (Fig. 3b).

Alternative models to better explain the genetic drift and the exchange of information between populations are the stepping stone models in which populations assumed to be close to each other in a linear dimension, exchanging members only with adjacent populations (Fig. 2b). In such circumstances, there is a clear geography, and populations that are closer are linked by larger amounts of genetic exchanges. This seems to better reflect the pattern of natural invasions, in which dispersal between localities is probably related to geographic distance, rather than the island models where similar genetic identity over a long scale may be interpreted with a founder effect and man-mediated introduction.

Stepping stone models of isolation by distance have been less extensively studied than island models, but have shown that geographic and genetic distance are highly related. (Slatkin, 1993).

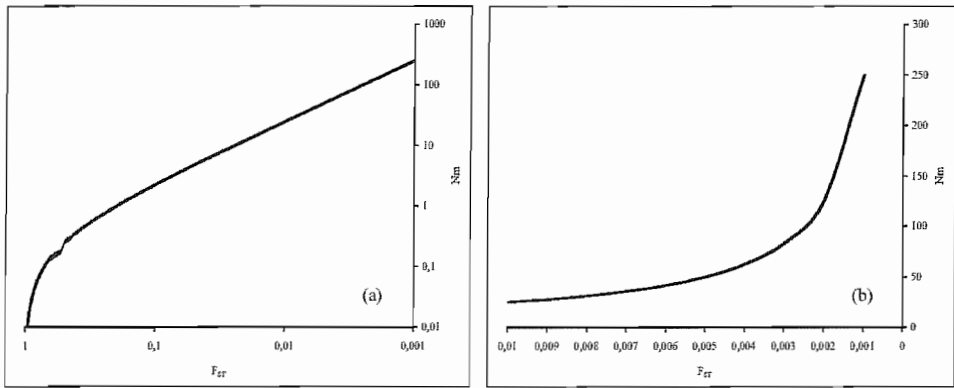When more realistic dispersal scenarios are allowed, genetic distance builds up quicker when average dispersal is low. For example, for populations where dispersal probability declines exponentially with distance, simulations show that an index of genetic differentiation (F_{ST}) increases quicker with increasing distance between populations for low dispersal species than high dispersal species (Fig. 4) (Slatkin and Barton, 1980). These values suggest a general method to obtain dispersal distance through the use of high resolution population genetic data that relates genetic differences between populations to the geographic distances between them, allowing at the same time to assess the relationship between different metapopulations and the genetic core of invasive species.

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Fig. 3 · Relationship between genetic differentiation (F_{ST}) and estimates of gene flow (Nm). Because of reciprocal relationship between F_{ST} and Nm (see text) small changes in F_{ST} can lead to large differences in Nm (a). This is especially true when F_{ST} is small, as is typical of many marine species with high dispersal potential (b)

BIOINVASIONS AND MOLECULAR MARKERS

Identify the source of marine invasions and reconstruct their invasion pathways is however not so easy.

New populations are in general genetically drained due to the population bottleneck effect associated with colonization (Nei et al., 1975) (Fig. 5). Introduced species evidence a reduction of the genetic diversity, while the native populations are genetically more variable. Although reduction in genetic diversity is usually considered disadvantageous at population level, a genetic bottleneck can lead to the ecological success of the small proportion of species that become established and then potentially invasive (Tsutsui et al., 2000).

To date most empirical progress in the genetic of biological invasion has been made on post-zygotic effects, like hybrid male sterility (Holway et al., 1998; Hatfield and Schluter, 1999). Few data are available on the genetics of ecological differentiation and how this relates to invasiveness. Studies of allozyme function showed that adaptive differences could be found across environmental
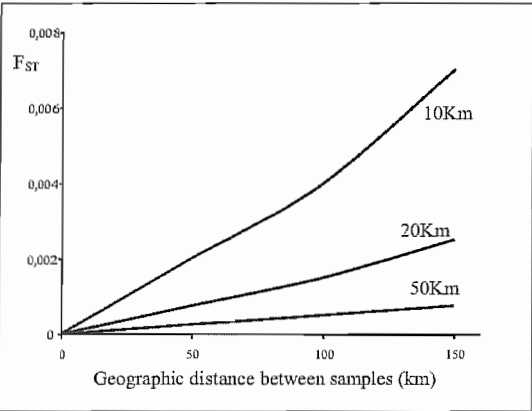

Fig. 4 - Increase in genetic distance (F_{ST}) with increasing geographic distance between samples in simulations with three increasing average larval dispersal (10Km, 20Km, 50Km, mean dispersal per generation)
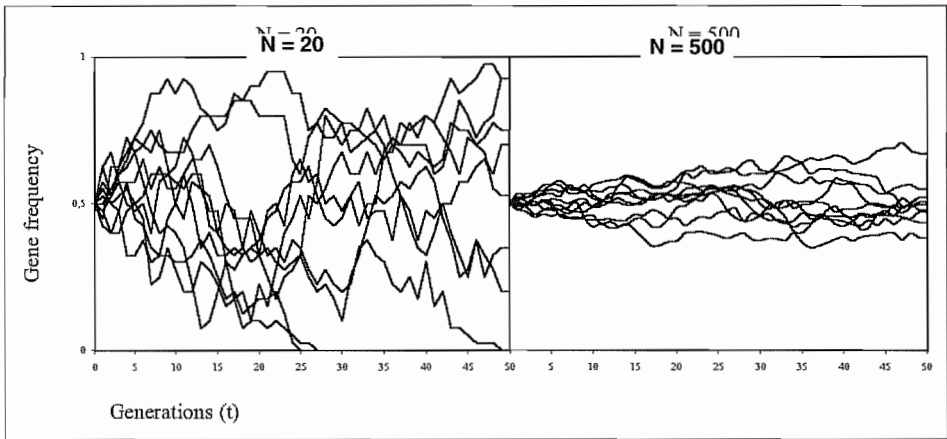
Fig. 5 - Genetic drift in different sized populations. Ten trials (different alleles or several independent founder events) with the same N are plotted on top of one another on the same grid. Where N is larger (N=500) divergence occurs more slowly and average time to fixation is slower. When the population size is smaller (N=20) and the time to fixation is independent of the population size, the populations diverge from one another as a result of genetic drift and the genetic compositions of newly founded populations might differ.

clines, sometimes determined by one or a few amino acid changes (Bernardi et al., 1993).

Although almost all cases of rapid speciation without obvious allopatric barriers involve clear ecological shift, corresponding to requirements of recent models of allopatric speciation, there are no general approaches to discovering these genes.

Mitochondrial DNA has been the most commonly used genetic marker in population studies (Avise, 1994). Unfortunately mitochondrial genes may exasperate the reduction in diversity associated with colonization bottlenecks, due to the quarter of the effective population size of nuclear genes (Moore, 1995).

Allozymes were also used to assess the similarity of populations and cryptic species, however only few variations at any given locus can be recognized using electrophoresis.

New breakthroughs in biotechnology and new markers, such as microsatellites, introns, single nucleotide polymorphisms (SNPs) and restriction length polymorphisms (RFLPs), for the first time allow intensive data collection, revealing population structure over a finer scale.

Genetic variation at neutral loci can be measured through sequence analysis of intron variation to discover single nucleotide polymorphisms. Essay of SNP's from different localities using rapid primer extension methods is a useful technique that will allow getting the data faster and efficiently (Patti and Palumbi, in prep.).

Tools like SNPs identification and in addition the general approach (nDNA sequences revealing intron single nucleotide polymorphism that are used to develop rapid genetic screening tests) are applicable to all marine species, and can show how parallel data sets can be used to investigate similar questions in other species, representing a beginning in the weaving together of genetic tools and ecological field research.

The new genetic markers, combined with ecological data, might become a predictive tool, able to identify the pathways that allow species to spread and to colonize new areas, becoming potential invaders.

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