

The genetics of Lessepsian bioinvasions

Giacomo Bernardi, Daniel Golani and Ernesto Azzurro

INTRODUCTION

Bioinvasions are increasingly having an impact on ecological and economic balances in both terrestrial and marine habitats (Kolar and Lodge, 2001) and, as such, are becoming the focus of attention. At the same time however, invasive phenomena are increasingly seen as a unique opportunity to explore ecological (Lockwood et al., 2007) and evolutionary processes in the marine environment (Lee, 2002; Rice and Sax 2005; Wares et al., 2005). Much work has been dedicated to the understanding of the ecological consequences of introductions and resources have been allocated to prevent and control those invasions. Recently, genetic tools have been added to the approaches used to assess bioinvasions and together with those new results, theoretical predictions have been formulated. Genetic studies have been traditionally employed to answer a variety of questions such as to determine invasive patterns, to reconstruct the route, the source and the timing of invasion and to explore the historical biogeography, as evidenced by a growing amount of literature.

Invasive species colonizing a new environment typically face new selective pressures. Following fundamental principles of population genetics, their genetic variability can be considered integral to their capability to adapt. Indeed empirical evidence supports the link between invasion success and genetic attributes, such as additive genetic variance, epistasis, hybridization, genetic tradeoffs, the action of small numbers of genes and, possibly, genomic rearrangements (see Lee, 2002 for review). Nevertheless, bioinvaders generally harbor a sub-set of the original genetic pool, due to founding effects, which are evidenced by a genetic bottleneck. With some exceptions (e.g. Tsutsui, 2000), a reduction in genetic variability is predicted to make populations vulnerable, especially in their capability to adapt to environmental conditions, in contrast to the general success of bioinvaders.

The level of genetic loss during the colonization process will be determined by the propagule size and the diversity of founder individuals, which is related to the diversity

of the source population. In some cases, initial genetic bottlenecks can be dampened by multiple invasions, accompanied by high gene flow, that eventually allow invading populations to exhibit sustainable genetic diversity (Dlugosh and Parker, 2007).

One of the major problems that plagued the study of bioinvasions derives from the fact that bioinvaders are usually observed a long time after their original invasion and only once they have successfully colonized the new habitat. This is a problem because, in most situations, it is difficult to determine when the invasion originally occurred and if it is the result of a single event or successive ones. Failed invasions tend also not to be accounted for. In that respect, the case of Lessepsian bioinvasions is quite unique.

Lessepsian invaders are organisms originating from the Red Sea that entered the Mediterranean Sea via the Suez Canal, opened in 1869 under the supervision of the engineer Ferdinand de Lesseps. With more than 300 new species added to the Mediterranean (Galil, 2009), including 71 species of fishes (Bilecenoglu et al., 2008; Lipej et al., 2008; Golani, this volume), i.e., approximately a quarter to one half of the world's marine fish invaders (Lockwood et al., 2007), the Lessepsian migration represents the 'most important biogeographic phenomenon witnessed in the contemporary oceans' (Por, 1978). It is an ongoing process with new species regularly entering every year and certainly a massive human-mediated 'experiment' (Féral, 2002), with unique opportunities to study rapid evolutionary changes.

The vast majority of Indo-Pacific organisms that are currently present in the Mediterranean can be considered as resulting from Lessepsian migrations. Only few of them might possibly have a different origin, following two main hypotheses. Firstly, it has been suggested that Tethys Sea remnants may have been present in the Mediterranean during the Messinian Salinity Crisis (MSC). Approximately 5.5 Mya, a desiccation event dried up the Mediterranean (the MSC), which later refilled at the opening of the Strait of Gibraltar with the Atlantic Ocean (Bianco, 1990). Some authors have suggested that a few species, originally from the Red Sea (which was part of the Tethys Sea), may have been present in the Mediterranean and survived the desiccation period. In fact, this hypothesis is highly unlikely but needs to be kept in mind. A more likely scenario derives from the fact that a connection between the Red Sea and the Mediterranean has been tampered with for a very long time, starting in ancient Egyptian, and later, Roman times. Besides those extreme cases, the vast majority, if not all, of species with Red Sea affinities that are present in the Mediterranean did invade after 1869.

Interestingly, the dates of Lessepsian invasion span a very long period. In the case of the species that will be presented here, the date of first invasion varies from the late 1900s to the present (Table 1). Because the time of invasion is variable for different Lessepsian invaders, a precise record of the first occurrence of invasion is of crucial importance to fully appreciate genetic data. This information is often available for Lessepsian species, together with a detailed spatio-temporal picture of their spread in the Mediterranean (Golani et al., 2002).

Therefore, Lessepsian migrants offer some definite advantages for scientists. Since the timing of invasion, the invasion route, and the invader's geographic source are known, theoretical predictions seemed fairly simple. Some individuals from the Red Sea would enter the Mediterranean via the Suez Canal, and would later expand in the novel environment. This situation would predict a likely genetic bottleneck due to an invading sub-sample of the original populations, followed by a fast range expansion, a pattern that is consistent with other documented invasions (e.g. Sax et al., 2005).

Our goal here is to review and compare genetic studies of Lessepsian migrants in order to determine if this unique bioinvasion follows some general patterns. We also want to assess if Lessepsian invasions can be used to test specific theoretical predictions.

Specifically, we want to determine if (1) invasions were accompanied by bottlenecks, (2) success could be associated with genetic diversity, and if (3) bottlenecked populations displayed rapid population expansions.

METHODS AND APPROACHES

The genetic approaches used to study Lessepsian migrants reflect the general evolution of methodological toolboxes used in the field of molecular ecology and evolution. Early studies of Lessepsian invaders capitalized on the rapid, easy, and relatively cheap allozyme assays (e.g. Lavee and Ritte, 1994; Golani and Ritte 1999; Safriel and Ritte, 1986). These studies allowed the establishment of the bases of understanding for the genetic patterns displayed by marine bioinvaders. However, while genetic bottlenecks and their associated lowered genetic diversity were expected, these early studies showed that Lessepsian migrants did not show such genetic signatures. As for the rest of the field, criticisms of allozymic methods mostly focused on the lack of resolution and the potential for the presence of selective pressure. Therefore the logical next step was to use neutral DNA markers. The workhorse of population genetics being the use of mitochondrial DNA sequences, the second wave of studies used such markers. Unexpectedly, these studies confirmed previous results based on allozymes. Indeed, the surprising lack of bottlenecks in Lessepsian migrants was found not to be due to an artifact of the allozymic methods, but was real and confirmed by neutral mitochondrial markers (e.g. Bucciarelli et al., 2002; Hassan et al. 2003; but see Golani et al., 2007 for a bottleneck). It is clear that DNA sequences have provided some unique insight in the understanding of the genetics of Lessepsian bioinvaders, yet, it is also clear that more power could be gained by using larger datasets and more variable markers. Microsatellites and SNPs seem to be ideal candidates and the obvious choices for such approaches. They have not yet been used on Lessepsian migrants, but it is likely that they will soon be, and it is also likely that new light will then be shed on the Lessepsian system.

GENETIC STUDIES

Electrophoretic analysis of Red Sea and Mediterranean populations are available for *Aphanius dispar* (Rüppell, 1829). This Teleost fish was considered for many years to be a Lessepsian migrant but in all probability was in fact present in the Mediterranean prior to the opening of the Suez Canal (Kornfield and Nevo, 1972) and hence it will be not included in our review.

Genetic approaches focusing on bona-fide Lessepsian migrants started in 1994. Currently 14 species have been investigated (which is less than 5% of the known invaders) (Table 1). These 14 species include 1 marine angiosperm, *Halophyla stipulacea*, 6 invertebrates, and 7 fish species.

In the field of Lessepsian invasion, molecular techniques have been mainly employed to contrast the levels of genetic diversity between native and invasive populations (Golani and Ritte, 1999; Bucciarelli et al., 2002; Karako et al., 2002; Bonhomme et al., 2003; Hassan et al., 2003; Azzurro et al., 2006) and to test for genetic structuring within invasive populations (Karako et al., 2002; Azzurro et al., 2006; Terranova et al., 2006). Genetic studies have been also used to explore demographic aspects within the Mediterranean (Azzurro et al., 2006; Iannotta et al., 2007), to support taxonomy (Golani and Ritte, 1999; Kasapidis et al., 2007) and to unveil cryptic sibling species (Bucciarelli et al., 2002)

Many Lessepsian species tend to be quite cryptic (such as interstitial polychaetes and flatworms), and are likely to be overlooked for a long time before being recorded. However, the 14 species that were used in this analysis are large and conspicuous and are therefore likely to have been noticed very soon after their first occurrence in the Mediterranean.

GENETICAL VARIABILITY IN LESSEPSIAN INVADERS

Population bottleneck, often associated with a loss of genetic variability, seems to be a common feature of biological invasions (Allendorf and Lundquist, 2003). In this review, the presence of bottlenecks could be addressed for 12 out of 14 studied species. Noticeably, most of Lessepsian invaders (10 out of 12) did not display any sign of founder effect, with any apparent loss of genetic diversity from the Red Sea to the Mediterranean (see Table 1).

Nevertheless, sampling was biased since it was conducted mostly in the areas of major abundances for these species (the Eastern Mediterranean). In addition, bias was also introduced in sampling the source populations, because specimens were mainly collected in the northern Red Sea, particularly Eilat (Israel).

In the majority of the studied cases, the original invasion occurred a long time ago (Table 1). Older and successive waves of invaders might have added to incipient populations, making it difficult to detect specific signatures of single founding episodes. A chance to do away with these confounding effects was to focus on early invasive events, possibly

Table 1. Date of invasion, 1. Fritsch, 1895, 2. Fuchs, 1878, 11. Keller, 1883, 3. Tenerelli, 1962, 4. Tortonese, 1966, 10. Lai et al., 2008, 5. Steinitz, 1927, 6. Kosswig, 1950, 12. Haas and Steinitz, 1947. 13. Tillier 1902, 7. Ben-Tuvia, 1964, 8. Golani, 2000 9. Akyol et al., 2005.

Genetic study 1. Procaccini et al., 1999; 2. Ruggiero and Procaccini, 2004, 3. Lavée and Ritte, 1994, 4. Safriel and Ritte, 1986, 5. Shefer et al., 2004, 16. Sirna Terranova et al., 2006, 17. Lavie and Nevo, 1986, 6. Iannotta et al., 2007, 7. Karako et al., 2002, 15. Lai et al., 2008., 8. Hassan et al., 2003, 9. Golani and Ritte, 1999, 10. Hassan and Bonhomme, 2005, 11. Bucciarelli et al., 2002, 12. Azzurro et al., 2006, 13. Golani et al., 2007, 14. Kaspidis et al., 2007.

Organism	Species	Estimated date of invasion	Data type	Evidence of bottleneck	Reached western basin	Genetic study
Plants	<i>Halophyla stipulacea</i>	1895 (1)	RAPD	N/I		(1)
			ITS	no	yes	(2)
Invertebrates	<i>Brachydontes pharaonis</i>	1876 (2)	alloz., SSCP, CO1	no	yes	(3,4,5,16)
	<i>Cerithium scabridum</i>	1883 (11)	alloz.	n.d.	yes	(17)
	<i>Modiolus auriculatus</i>	early 1900 (2)	alloz.	no	no	(3)
	<i>Lysidice collaris</i>	1962 (3)	ITS, CO1	N/I, but low diversity	yes	(6)
	<i>Asterina burtoni</i>	1966 (4)	RAPD	yes	no	(7)
	<i>Minona ileanae</i>	not yet* (10)	ISSR	no	no	(15)
Fishes	<i>Siganus rivulatus</i>	1927 (5)	SSCP, RFLP, ISSR	no	no	(8)
	<i>Upeneus pori</i>	1950 (6)	alloz.	no	no	(9)
	<i>Upeneus moluccensis</i>	1947 (12)	alloz., n and m SSCP	no	no	(9, 10)
	<i>Atherinomorus lacunosus</i>	1902 (13)	DL	no	no	(11)
	<i>Siganus luridus</i>	1956 (7)	SSCP, RFLP, ISSR, DL	no	yes	(8, 12)
	<i>Fistularia commersonii</i>	2000 (8)	DL	yes	yes	(13)
	<i>Lagocephalus sceleratus</i>	2003 (9)	Cyt b	N/I	no	(14)

* potential Lessepsian invader sampled along the whole length of the Suez Canal

far from the source of invasion. In the last few years, three different papers (Azzurro et al., 2006; Terranova et al., 2006; Golani et al., 2007) presented such kinds of data, thus overcoming pragmatic difficulties in studying recently founded populations and making a step forward into the exploration of the Lessepsian invasive dynamics.

In *Brachidontes pharaonis*, no difference in both haplotype and nucleotide diversity was detected between the youngest populations (of Sicily) and the oldest populations of the Eastern Mediterranean Sea (Terranova et al., 2006). Within the Mediterranean, some regional clustering and the existence of unique haplotypes have been evidenced for this species, but the input of larvae from ballast waters seemed to be the most likely explanation, with no reference to the Lessepsian process (Shefer et al., 2004).

The absence of genetic differentiation between Mediterranean and Red Sea populations of *B. pharaonis* (Shefer et al., 2004; Terranova et al., 2006) and mostly of some fish species, i.e. the hardyhead silverside *Atherinomorus lacunosus* (Bucciarelli et al., 2002), the rabbitfishes *Siganus luridus* and *S. rivulatus* (Bonhomme et al., 2003; Hassan et al., 2003) and the goatfishes *Upeneus pori* and *U. maluccensis* (Golani and Ritte, 1999; Hassan and Bonhomme, 2005), contributed to develop the idea that Lessepsian migration involves many individuals since its early phases and continuous gene flow from the Red Sea.

These findings were later confirmed by Azzurro et al. (2006), which showed no partitioning between the Mediterranean and the Red Sea populations of *S. luridus*. Importantly, mitochondrial diversity appeared to be preserved also in the youngest and westernmost population of Linosa, with no traces of founder events, suggesting that the genetic variability was 'there' from the very beginning of the process. This could indicate that recruitment processes occur "en masse" with relatively high levels of genetic diversity within a cohort. At the same time, these authors showed a weak but detectable structure between Red Sea and Mediterranean populations, with a slight lowering of the genetic diversity in the latter. These findings were based on a wider geographic sampling with respect to previous studies (Bonhomme et al., 2003; Hassan et al., 2003) and highlighted the importance to sample along the entire introduced range of Lessepsian invaders, not only close to their entry point. Many other marine invaders have showed little or no genetic erosion during the colonization process (Holland, 2001; Wares, 2005) and this was mainly due to very large propagule sizes or even to repeated introductions from different source areas.

In contrast, the Blue-spotted cornetfish displayed very strong evidence of a genuine bottlenecking event, with only 2 haplotypes being present in the Mediterranean (Fig. 1), suggesting that a single invasion event by as few as two females had generated its invasion in the Mediterranean Sea (Golani et al., 2007). The Blue-spotted cornetfish, *Fistularia commersonii*, is now considered one of the 100 'worst' invasive species of Europe (Daisie, 2008. <http://www.europe-aliens.org>), as it only recently entered the Mediterranean (Golani, 2000) yet it is rapidly spreading through both the eastern and western basin.

So far, no similar episodes are available for the Mediterranean Sea but the migration of Red Sea species in the form of a small number of individuals is likely to have occurred several times. In fact, several Lessepsian species have been tallied on the basis

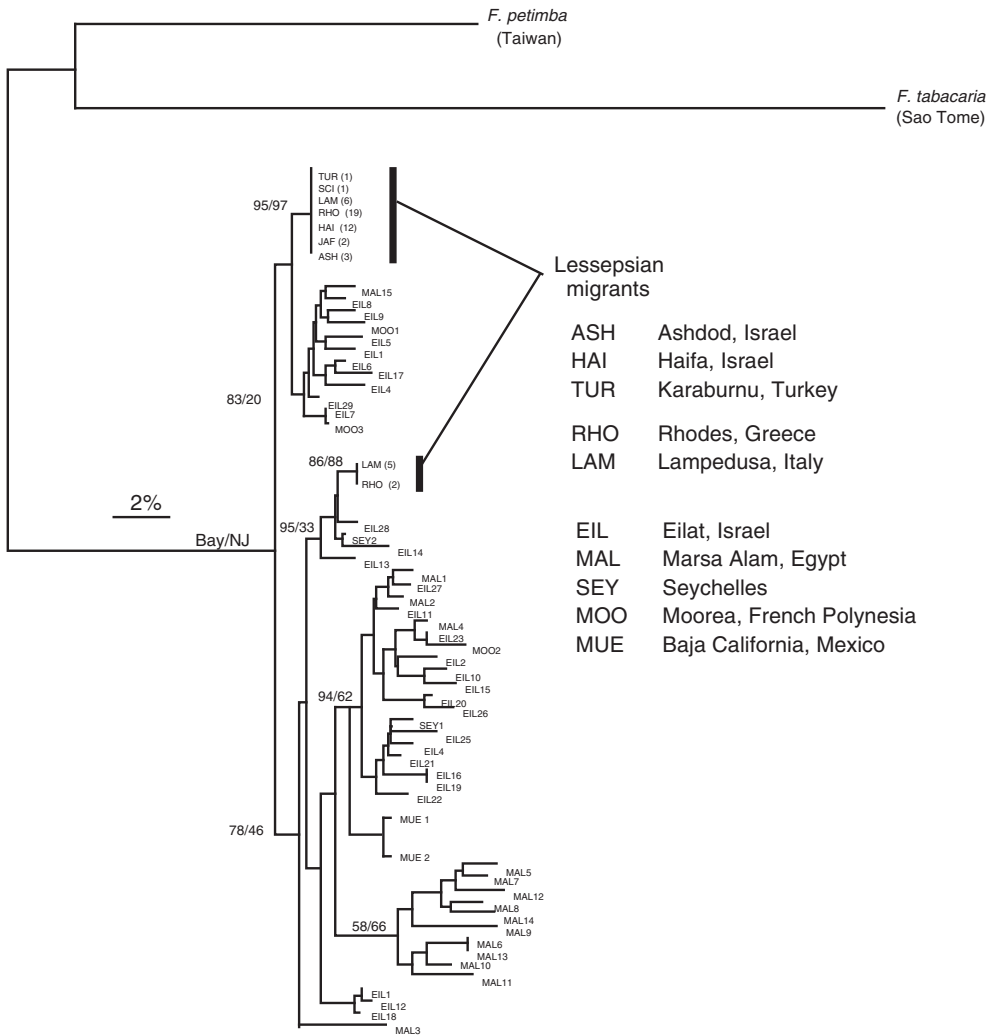


Fig. 1. Phylogenetic relationships of *Fistularia commersonii* samples based on mitochondrial control region sequences based on Bayesian and Neighbor-Joining reconstruction methods. Numbers next to the main nodes correspond to Bayesian consensus numbers (left figures) and Neighbor-Joining bootstrap support (right figures, 2000 replicates). Sample codes and sampling locality, and the two Mediterranean (Lessepsian) haplotypes are identified in the figure. Two outgroups were used, *F. petimba*, collected in Taiwan, and *F. tabacaria*, collected in Sao Tomé.

of only one or few specimens, with no further signs of population growth or expansion (Golani et al., 2002). In these cases, it is difficult to support the idea of a massive and continuous migration of individuals, since it would result in repeated sightings, or even in sustaining the incipient population during its establishment process.

Only one other Lessepsian species, the sea star *Asterina burtoni* (Karako et al., 2002) displayed a significant loss in genetic diversity, but this was attributed to differences in the reproductive mode adopted by this species in the Mediterranean and in the Red Sea (fissiparity *vs.* sexual reproduction).

GENETIC DIVERSITY, TEMPORAL DYNAMICS AND INVASION SUCCESS

Many invaders undergo variable periods of time between initial establishment and subsequent population growth and expansion. Such lags of time are a common feature in biological invasions (Kowarik, 1995) and it may have diverse ecological and demographic causes or even it may be determined by time needed for evolutionary adaptation to the new environment (Holt et al., 2005).

As far as Lessepsians are concerned, genetic studies did not support any conclusion on their temporal dynamics and documented time lags have been justified on the basis of alternative ecological reasons. The only available examples are the case of the recently settled population of *S. luridus* in Linosa, which appeared three decades after its first settlement in the Sicily Channel (Azzurro and Andaloro, 2004; Azzurro et al., 2006), and of the mussel *B. pharaonis*, which underwent population explosion (massive formations of beds) after a lag of about 120 years since its first establishment in the Israeli coasts (Rilov et al., 2004). This lack of knowledge is not surprising among Lessepsians, since the role of evolutionary changes in the colonization process is seldom explored in invasive species (Sakai et al., 2001).

The success of an exotic species, apparently not genetically adapted to its new environmental conditions, is always difficult to explain. All the analyzed studies deal with successful invaders but (after the case of *F. commersonii*) we have seen that their genetic variability may span between opposites, from the absence of genetic loss to severe bottleneck. The case of *F. commersonii* provides a clear example that an extreme bottleneck does not preclude population growth and rapid geographical expansion.

This also draws attention to an emblematic paradox of invasion biology: “how bottlenecked populations that typically have low fitness can become invasive?” (Frankham, 2004). Actually, the observed contradiction between the decline in genetic diversity and invasive success seems to be a rule rather than an exception in introduced species (Dlugosh and Parker, 2007).

The invasion success of *F. commersonii*, regardless of its genomic uniformity, also debunks the apparently coherent pattern in the dynamic of Lessepsian invasions, as probably expected from previous works (e.g. Golani and Ritte, 1999; Bonhomme et al., 2003; Hassan et al., 2003; Hassan and Bonhomme, 2005; Azzurro et al., 2006).

To sum up, there is no apparent association between genetic diversity and invasive success in Lessepsian migrants and our findings reaffirm a well-known difficulty of predicting the success of new invaders on the basis of this information. Yet, it is

possible that neutral genetic markers were poor indicators of heritable variation in adaptive traits (McKay and Latta, 2002), but the discussion of this hypothesis goes far beyond the purposes of this paper. Alternatively, high genetic variability may have little significance for Lessepsians, as hypothesized by Golani and Ritte (1999) and ecological traits could be much more important in determining the success of these organisms (Golani, 1993).

During the establishment process, a plethora of biotic and abiotic variables, together with demographic and environmental stochasticity probably interplay their roles (Lockwood et al., 2007) making it difficult to rationalize the factors of success for invasive species. Considering the young history of the Lessepsian invaders, it is possible that some of them had had enough phenotypic plasticity (the ability to cope with a range of environmental conditions) to survive, reproduce and succeed in their novel environment, with 'no need' of evolutionary adaptation. This can be likely at least for those species for which there was no lag of time between the initial colonization and subsequent population explosion. According to Allendorf and Lundquist (2003) and to Sax and Brown (2000) some species may be intrinsically better competitors because they evolved in a more competitive environment. Hence, the possibility of a competitive superiority of these colonists coming from a species rich region (the Red Sea), should be also taken into appropriate account.

It is equally true that successful Lessepsian migrants, which seem adapted or even 'preadapted' (Sakai et al., 2001) to the variety of the conditions of coastal Mediterranean habitats, might reasonably have some limits to their performances, with relation to biotic and abiotic variables, such as temperature (noteworthy are the mortalities which have been observed for *F. commersonii* during the coldest winter times: Azzurro, personal information).

Ultimately, the new biotic and abiotic conditions encountered in the Mediterranean Sea represent new selective forces for Lessepsian migrants and selective effects are expected in these populations, even if the studies available up to date do not provide evidence of that. Selection might occur in response to environmental forces, such as temperature and photoperiod, or to biological variables such as competitors, predators, prey and parasites.

RESEARCH PERSPECTIVES

Molecular techniques represent a new approach in invasion biology and certainly much has to be done in this field. Moreover the Lessepsian phenomenon has a relatively young history with only a small fraction of the Red Sea species that have already established in the Mediterranean. For the majority of these colonists, we have no information on their genetic structure and we have already lost the opportunity to study their colonization from the beginning. Nevertheless, other species are currently still in the process of

invading the Mediterranean and many other organisms are extending their distribution range into the Mediterranean, thus offering new study opportunities.

Genetic differences in invasive and source population might be masked by sampling biases. Therefore it is important to focus on the early phases of the invasive process and to sample the largest possible geographical area for comprehensive genetic studies. Marginal populations at the westernmost edge of the distribution range, and the study of the colonization at its earliest stages, turned out to be particularly informative (Azzurro, 2006; Terranova et al., 2006; Golani et al., 2007). Early settled populations are unique events that would allow to simplify theoretical work and help to determine the fundamental variables of the colonization process, such as the propagule size, one of the least documented aspect in invasion biology (Lockwood et al., 2007).

Our ability to detect the effects of founder events will also depend upon the measure that we use for genetic variation. Techniques such as microsatellites and SNPs have seldom been employed with Lessepsians and it is likely that they will yield new and exciting results.

Clearly, genetic information on a great number of Lessepsians would give us a better and more comprehensive understanding of this phenomenon. Nevertheless, the monitoring of selected key species at the genetic level may be used to test directly our hypothesis. For instance, given the large contemporary size of the Mediterranean population of *F. commersonii*, new migrants from the Red Sea are predicted to have little effects in altering haplotype frequencies and this would deserve to be assessed in the future.

CONCLUSIONS

Our review certainly failed to reconstruct a uniform pattern for the genetic of Lessepsian invaders, which likely includes a variety of different invasive models. However we have enough information to conclude that the *passage* used by larvae and/or adults to enter the Mediterranean (the Suez Canal) had the potential to sustain great numbers of migrants and high gene flow, at least for most of these colonists. Interestingly, there is also a homogeneous phylogeographical pattern for the species that have migrated from the Atlantic Ocean into the Mediterranean during geological times (Patarnello et al., 2007). Thus, the biogeographical relationships between the Mediterranean and its oceanic connections remain somehow controversial at the genetic level.

As far as Lessepsian migrants are concerned, beside spatial and temporal biases of previous studies, uncertainty may arise from the uniqueness of single species but also from the rapid environmental changes that are now happening in the Mediterranean Sea. The same Lessepsian pathway is far from steady in its function. In fact, the Suez Canal has changed much in the course of its history, having experienced drastic environmental modifications (e.g. the decline in salinity of the Bitter Lakes; see Golani, this volume) together with important human-induced alterations, which occurred at the release areas

(i.e. the damming of the Nile). Therefore its capacity to act as a genetic barrier and to produce phylogeographical breaks between native and donor populations has significantly changed since its opening.

Above all, the warming trend of the Mediterranean is providing more suitable ecological conditions for Lessepsian migrants (CIESM, 2008). These species, which have entered a temperate sea, are typically thermophilic, with tropical or subtropical origin (Golani et al., 2002) and their increasing number and success represent one of the most visible consequences of climate change within the Mediterranean realm (Bianchi, 2007; Azzurro, 2008 and references therein included). The occurrence of evolutionary adaptive processes in Lessepsian invaders highlights the fact that the Mediterranean environment may be changing towards the requirements of these tropical species, rather than the opposite.

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