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18 Applications of DNA-based Methods for the Study of Biological Invasions

18.1 Introduction

Numerous conceptual frameworks and methodologies have been developed for the study of the invasion of an area by a non-indigenous (syn. non-native) species (NIS) (Williamson, 1996; Shigesada & Kawasaki, 1997; Richardson, 2011). The issues addressed may be classified into three main research themes: 1) history of the invasion processes and subsequent dispersal (e.g. date(s) and location(s) of the introduction(s); source(s) and vector(s); pathway(s) of colonization); 2) biological impact of the invaders and factors favoring new species in colonizing new territories (e.g. ecological consequences for the native community; invasion dynamics; life history traits favoring foreign species); 3) predictions and control of biological invasions. Modeling, population dynamics, and field- or laboratory-based community ecology are scientific fields that have been extensively used to investigate these issues since the seminal book by Elton (1958).

Until the end of the 1990s, only a few studies based on genetic data specifically addressed non-native species. These early studies demonstrated the insights such approaches may offer, across a wide range of taxa and environments:

- to identify the invader (e.g. Geller *et al.*, 1997 for crabs)
- to determine the geographical origin of the NIS (e.g. Goff *et al.*, 1992 in algae; O'Foighil *et al.*, 1998 in the Portuguese oyster)
- to test for hypotheses of founder events or recurrent introductions (e.g. Davies *et al.*, 1999a for the medfly; Suarez *et al.*, 1999 for the Argentine ant) and examine dispersal strategies (e.g. Wilson *et al.*, 1999 for freshwater mussels)
- to investigate the genetic consequences of introductions (Stone & Sunnucks, 1993 in a gall wasp) or to look for hybridization with native species (e.g. Goodman *et al.*, 1999 in deer)
- to demonstrate changes in behavior (Tsutsui *et al.*, 2000 in ants) and reproductive systems in plants (Eckert *et al.*, 1996; Daehler, 1998) between the native and colonized areas.

With the emergence of DNA tools in the last two decades, population genetics and related scientific fields, like phylogeography or DNA barcoding (see definitions below), have been increasingly used to tackle these issues across many taxa, regions, and habitats. The number of papers dramatically increased, and several reviews summarize the main findings, highlight the usefulness of inferences from genetic data, provide warnings and recommend specific analytical designs, and link genetic data

with the evolutionary potential of non-native species (e.g. Holland, 2000; Darling & Blum, 2007; Roman & Darling, 2007; Estoup & Guillemaud, 2010; Geller *et al.*, 2010; Dormontt *et al.*, 2011; Fitzpatrick *et al.*, 2012; Lawson Handley *et al.*, 2011; Rius & Darling, 2014 ; Rius *et al.*, 2015). This book chapter does not aim at covering all the issues addressed in these reviews, and we encourage readers to benefit from these papers for their own study designs. Our aim is to point out some of the major applications of DNA-based approaches, each of them focused on a specific step of the invasion dynamics (Figure 18.1): 1) the primary introduction, where molecular tools are powerful for early detection of NIS; 2) the history of the introduction process (routes, pathways and spread); and 3) the study of the evolutionary and ecological consequences after establishment.

18.2 A Need for Powerful Non-Native Species Identification Tools

An accurate and rapid identification of non-native species is a pre-requisite for successful survey, monitoring, and management schemes (Pyšek *et al.*, 2013). This is particularly true at the early stages of an introduction (i.e. transport and primary introduction) for attempts to eradicate the new species and prevent its future successful proliferation (Simberloff *et al.*, 2013). To address this issue, molecular (DNA-based) tools have proven to be particularly relevant, having numerous advantages over traditional approaches (Darling & Blum, 2007; Le Roux & Wieczorek, 2009). These methods (1) are fast, (2) do not require expertise in different taxonomic fields, (3) can be applied to fragments of specimens or to particular life history stages for which morphological diagnostic characters are lacking, and (4) can be applied to complex matrices (e.g. environmental samples). Choosing among the various methods available depends on the sample to be analyzed and the questions to be answered (Darling & Blum, 2007; Bott *et al.*, 2010) (Table 18.1).

Many methods have been developed to identify a single target species and are used to confirm the identity of pre-identified specimens or to detect the presence of the target species: this is true for PCR-RFLP (e.g. Darling & Tepolt, 2008), PCR with species-specific primers (e.g. Harvey *et al.*, 2009), *in situ* hybridization (e.g. Le Goff-Vitry *et al.*, 2007; Mountfort *et al.*, 2007), sandwich hybridization assay (e.g. Harvey *et al.*, 2012), real-time quantitative PCR, or the new hybridization coupled with light transmission spectroscopy method (Egan *et al.*, 2013). Alternative approaches using DNA sequences, in which various DNA sequences from unknown specimens are compared to those in databases (e.g. Genbank), not only allowed confirmation of the identity of specimens, but also offered the possibility of detecting the presence of non-native species without any *a priori*, for example during regular surveys. This will be detailed below.

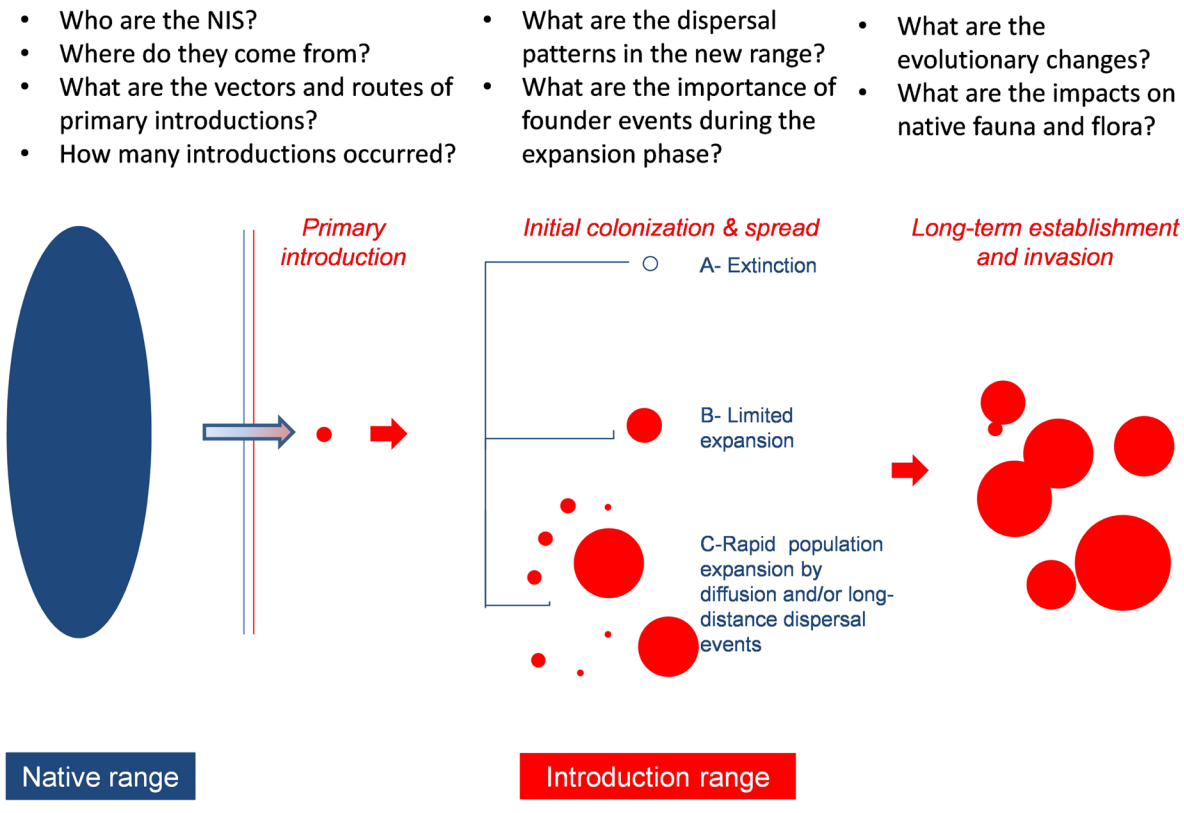

Fig. 18.1: Main issues of biological invasions commonly addressed by using genetic data. These issues are typically related to different stages of the introduction/invasion process. The blue ellipse depicts populations in the native range, and red circles represent populations in the introduction range. Ellipse and circle size are proportional to the population size.

Tab. 18.1: Overview of popular molecular approaches and tools with their most relevant applications (*, **, *** stand for low to high relevancy) in the study of biological invasions.

	Approach	Species-specific marker	DNA barcoding	DNA metabarcoding	Phylogeography	Population genetics
	Example of tools	**PCR-RFLP**	**Sanger sequencing with e.g. *coxI* for metazoans; *rbcL* for plants**	**Illumina or 454 sequencing**	**Organellar or nuclear DNA sequencing**	**Microsatellites, SNPs, RAD-Seq**
Theme	Question					
NIS identification	Targeting a specific NIS	***	***			*
	NIS inventories		***	***		
	Looking for cryptic NIS				***	
Pathways	Source identification				***	***
	Testing for single vs. repeated introductions				***	***
	Patterns of spread in the new range(s)				*	***
Evolutionary changes	Testing for selection on standing genetic variation					*** (many markers needed)
	Looking for admixture				** (if nuclear)	***
	Looking for founder events				*	***
	Studying hybridization				** (if nuclear)	***
Community level	Food web	***	***	***		
	Host-parasite interactions		***	***	*** (co-evolution studies)	** (co-evolution studies)
	Biodiversity assessment		* (time consuming)	***		

18.2.1 Molecular Barcoding: A Popular Approach for Aliens' Identification

The identification of NIS culminated in the use of DNA barcoding (Floyd *et al.*, 2002; Hebert *et al.*, 2003), which has proven to be particularly relevant in the context of biological invasions (e.g. Armstrong & Ball, 2005; Cross *et al.*, 2011; Comtet *et al.*, 2015). The main novelty that DNA barcoding has brought is the standardization of the identification process. DNA barcoding relies on short standard DNA sequence(s), called DNA barcodes, which can be applied to a wide range of taxa (such as *coxI* for most metazoans; *ITS* for fungi; *rbcL*, *matK*, and *ITS* for plants), whose design requires upstream research based on taxonomy and phylogeny. The power of DNA barcoding is based on the principle that the intraspecific barcode polymorphism is lower than the interspecific divergence, the difference between the two being known as the barcoding gap (Meyer & Paulay, 2005; Wiemers & Fiedler, 2007; Figure 18.2). The wider the barcoding gap is, the better the species discrimination is. Thanks to standardization, a lot of efforts have been and are made to feed international reference databases composed of barcodes recovered from voucher specimens identified morphologically. For example, the BoLD database (Ratnasingham & Hebert, 2007), with its wide taxonomic coverage, is the most comprehensive international database dedicated to DNA barcoding.

Such initiatives lead DNA barcoding to be particularly well-suited to the identification of species out of their native range, for which no *a priori* on the geographic origin is available. It is particularly useful in some taxonomic groups that are very difficult to identify due to a paucity of diagnostic morphological criteria, for example in algae (Geoffroy *et al.*, 2012) or ascidians (Callahan *et al.*, 2010; Bishop *et al.*, 2013). In these taxa, many non-native species may be overlooked because of poor taxonomy at the species level and poor knowledge of their biogeographic status (Bishop *et al.*, 2013). A further advantage of standardization is the possibility of using DNA barcoding in routine analyses in any laboratory where basic molecular equipment is available, but also in governmental agencies in charge of biosecurity and management strategies (Darling & Mahon, 2011). It is thus routinely used by government agencies in New Zealand and Australia for survey purposes, for example for the detection of the invasive ascidians *Didemnum vexillum* and *D. perlucidum* (Smale & Childs, 2012) or to control the presence at the border of high-risk insect species (Armstrong, 2010).

DNA barcoding applications are numerous. It first allows the discovery of new non-native species during regular surveys. For example, a solitary styelid tunicate was repeatedly observed along the coasts of Brittany during surveys in marinas. DNA barcoding showed it belonged to *Asterocarpa humilis*, a species that has never been reported and probably remained unnoticed for years (Bishop *et al.*, 2013). Similarly, the alien macroalga *Gracilaria vermiculophylla* was detected in British Columbia by using DNA barcoding (Saunders, 2009). Second, when applied to historical samples (e.g. museum specimens), DNA barcoding may reveal former misidentifications. For example, non-native calyptraeid gastropods from California, putatively identified as *Crepidula fornicata*, were later (20 years) identified through DNA barcoding as *C. convexa*, a species with different

dispersal abilities, with potential consequences in management strategies (McGlashan *et al.*, 2008). Finally, because DNA barcoding can be used on early life history stages, it can be used to detect invaders during the introduction step, for example during quar-

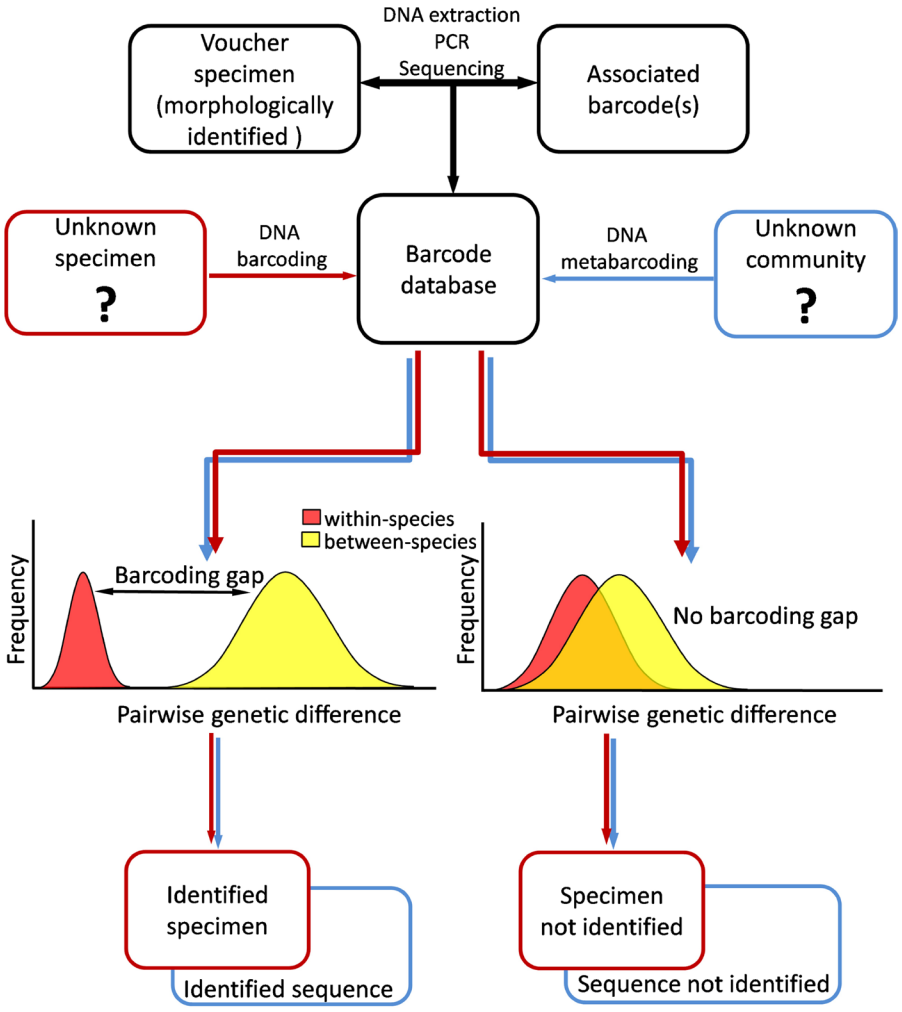

Fig. 18.2: Main steps of the DNA barcoding and metabarcoding approaches for the identification and inventory of non-native species. Barcoding (in red) and metabarcoding (in blue) rely on the availability of reference barcodes recorded in international databases. Such databases are established from upstream research (in black). The power of these approaches is conditioned by the existence of a barcoding gap (i.e. the lack of overlap between within-species and between-species polymorphism), which allows unambiguous identification of species. In case of such an overlap (i.e. no barcoding gap), identification to the species level will fail. However, identification at higher taxonomic levels is still valuable. Whereas barcoding consists in the identification of specimens based on their barcode, metabarcoding identifies sequences obtained from a mixture of many species (i.e. specimens are not observed).

antine procedures at the border (e.g. Armstrong & Ball, 2005), or through examination of introduction vectors (ships, planes...). In this context, DNA barcoding was used successfully to inventory invertebrates living in ships' ballast sediments, focusing on the diapausing eggs of rotiferans and crustaceans (Briski *et al.*, 2011).

18.2.2 Early Detection: A Challenging But Critical Task

One of the challenges of early detection of non-native species is the detection of a low number of specimens hidden within the local species pools. All the above molecular methods of identification are particularly relevant in that context, being typically more sensitive than traditional methods involving sorting and counting. For example, PCR-RFLP or PCR with species-specific primers allow the detection of single alien invertebrate larvae in plankton (Darling & Tepolt, 2008; Harvey *et al.*, 2009). Ultimately, alien species may be detected in the form of molecular imprints, i.e. free or particle-bound DNA molecules released by organisms (environmental DNA, eDNA), which may still be detected several weeks after the species has been removed (Ficetola *et al.*, 2008; Jerde *et al.*, 2011; Dejean *et al.*, 2012).

It is expected in the future that early detection of aliens will be further enhanced thanks to the development of next-generation sequencing techniques (NGS), which typically provide billions of sequence reads in a single run, quickly and at low cost. In particular, NGS allows assessing biodiversity from complex environmental samples through DNA metabarcoding, an extension of traditional barcoding (Shokralla *et al.*, 2012; Taberlet *et al.*, 2012; Cristescu, 2014) (Figure 18.2). DNA metabarcoding relies on the same principle as traditional barcoding, differing by the sequencing depth, which theoretically allows recovering the whole diversity of the sample, and offers the possibility to simultaneously analyze several samples. A recent study focusing on the detection of marine aliens showed that DNA metabarcoding allowed the detection of a single *Asterias amurensis* (an invasive seastar in New Zealand) larva in water and sediment samples containing a large array of environmental eukaryotes (Pochon *et al.*, 2013).

18.2.3 Revealing New Cryptic NIS

Identifying non-native species with molecular tools like DNA barcoding requires that their taxonomy is well understood, so that the developed markers indeed identify single species. However, in some taxa, taxonomy at the species level remains unclear, and many species are in fact species complexes composed of several species which looks identical, called cryptic species. The taxa for which molecular approaches are needed to help identification (because of the paucity of diagnostic characters) are often also those for which taxonomic status is unclear. As a result, many non-native species may be overlooked because of cryptism. Within-species sequencing approaches, like phylo-

geography approaches (see 18.3.1), are particularly relevant to reveal the existence of non-native cryptic species by showing unexpected large molecular divergence between some lineages. Many examples exist in the marine realm where taxa that were considered as worldwide invasive 'species' were recently shown to be comprised of several cryptic lineages, possibly corresponding to several sister-species or sub-species, like in the bryozoan *Watersipora subtorquata* (Mackie *et al.*, 2012) and the ascidians *Botryllus schlosseri* (Bock *et al.*, 2012) and *Ciona intestinalis* (Zhan *et al.*, 2010). Revealing cryptic non-native species is crucial because they may differ in their life history traits or their invasion histories and pathways. For example, the occurrence of two cryptic lineages of the non-native amphipod *Grandidierella japonica* suggested the existence of two independent introduction events on the Pacific coasts of North America (Pilgrim *et al.*, 2013).

18.3 Tracing Back Introduction and Expansion Processes

Tracing back the origin of an introduction to identify the dispersal routes and pathways (e.g. vectors, number of introduction events) and studying the spread of NIS in their new range are key issues to better understand the ecology and biology of an invader and to propose management strategies. DNA-based studies are widely used to tackle these issues.

18.3.1 Routes and Pathways of Colonization

One central feature of introduction processes is the disruption of natural dispersal pathways with new populations often established far away from their native range (Wilson *et al.*, 2009). For example, the Japanese kelp *Undaria pinnatifida*, native to the Northwest Pacific, has colonized new areas in the Northern Atlantic and Western Pacific within less than 30 years (Voisin *et al.*, 2005, and references therein). Such a rapid global spread would not have been possible through natural routes of dispersal, especially in a species characterized by low dispersal abilities. Determining routes, vectors or numbers of introduction events is difficult based only on direct observations or using logbooks of ships and planes or shellfish importation registers: the information is often difficult to get or incomplete, and such approaches are usually inadequate to document repeated and cryptic introductions. Investigating the routes and pathways of introductions has been central in many genetic studies of NIS (Holland, 2000; Estoup & Guillemaud, 2010; Geller *et al.*, 2010). Both phylogeography-based methods (Avise *et al.*, 1987; Avise, 2000; Hickerson *et al.*, 2010) and population genetics (Hartl & Clark, 1989; Weir, 1990; Hedrick, 2000) are commonly used to tackle this issue, using different molecular tools (Table 18.1): sequencing of mitochondrial or nuclear genes, microsatellites (SSRs), and single nucleotide polymorphisms (SNPs). Molecular markers are most often neutral, for examining migration and drift processes, and polymorphic enough in the native range at the population level to capture traces left during and after the introduction.

Despite some success in analyzing general features of the introduction processes (e.g. testing the propagule pressure hypothesis, testing competing introduction scenarios (Lawson Handley *et al.*, 2011; Geller *et al.*, 2010), many studies failed to identify the sources of the target invaders, because of rather restrictive conditions to be fulfilled (Figure 18.3). One critical aspect for determining the source is the sampling effort, with all putative sources being included (Muirhead *et al.*, 2008; Geller *et al.*, 2010) (see case II as compared to case I in Figure 18.3). Determining the source is particularly challenging for species lacking genetic structure in their native range (case II in Figure 18.3), like the numerous marine invertebrates characterized by a long-lived dispersal stage and large effective population size.

Even with a significant genetic structure in the native range (a distinct genetic footprint of each putative source population), determining the source of NIS can be difficult (Geller *et al.*, 2010). This may be due to the difficulty of getting a comprehensive sampling, and the post-introduction changes in the genetic composition of the introduced or native populations through genetic drift, if the time elapsed since the introduction is long. Attempts to determine the geographic origins of an introduction thus require an important sampling effort, ideally in the early stages of the process. If the precise origin cannot be determined, important information can nevertheless be obtained about introduction pathways and processes. For instance, Voisin *et al.* (2005) showed that populations of *U. pinnatifida* introduced in Europe were far less polymorphic and genetically similar to populations cultivated in Asia (native range) than populations introduced in Australasia. This supported earlier hypotheses explaining the different introduction pathways of this species in Europe (through aquaculture) and Australasia (through shipping).

Gene sequencing and parsimony networks (Posada & Crandall, 2001) are commonly used in invasion studies in light of phylogeography approaches (which examine the geographical distribution of gene lineages (Avise *et al.*, 1987)). For species in their natural distribution range, the geographical distribution of gene lineages is analyzed to understand the historical and natural demography and migratory changes (Avise, 2000; Maggs *et al.*, 2008). Common interpretation keys (e.g. star-like networks featuring demographic expansion) are, however, misleading in non-native species, as the observed patterns cannot be associated to natural long-term vicariant, demographic or migratory processes, given the short time elapsed since the introduction, and the disruption of natural migration routes due to human-mediated activities. Parsimony networks are nevertheless useful as descriptive tools to highlight genetic mixing between evolutionary divergent lineages in introduced populations, an indication of repeated introductions from several sources (e.g., Kolbe *et al.*, 2004; Simon-Bouhet *et al.*, 2006). Besides, unexpected patterns observed in phylogeography studies of a given species across its supposed native range can reveal unreported introductions. For example, in two annelid species, Jolly *et al.* (2006) explained the presence of haplotypes typical of Northern European clades in Southern Europe by human-mediated colonization.

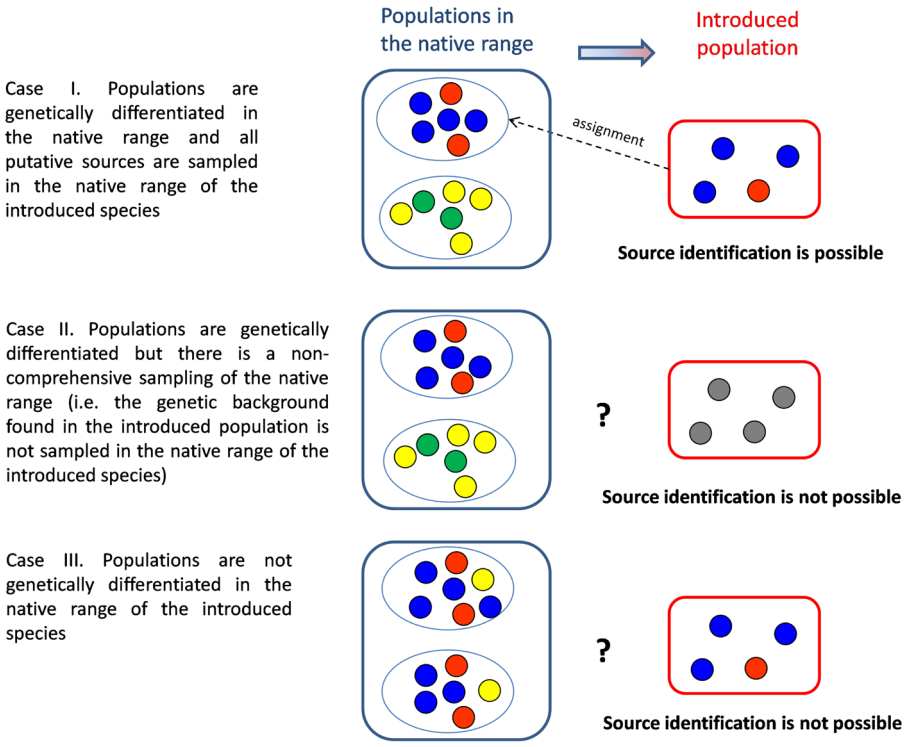

Fig. 18.3: Tracing back the sources: success and failure in assigning sources of introduction. Figures illustrate conditions under which a correct identification of the source may or may not be possible. Blue rectangles and ellipses (left) feature the native range and populations (here 2 populations), respectively, of the introduced species. Red rectangles (right) feature an introduced population of this species. Filled circles represent individuals; each color refers to a different genetic background.

Assignment analyses, based on multilocus genetic data and maximum likelihood calculations, have been largely used to trace back introductions. They provide a means of assigning individuals to particular putative sources and assessing to some extent population structure (Cornuet *et al.*, 1999; Davies *et al.*, 1999b; Manel *et al.*, 2005; Broquet & Petit, 2009). Using such methods, Davies *et al.* (1999a) demonstrated the difficulty of controlling the infestation by the medfly *Ceratitis capitata*, by showing that a single individual captured in California was most likely an immigrant from an unnoticed re-introduction rather than a remnant individual produced locally. New analytical methods, like Approximate Bayesian Computations (ABC) have also brought much to the study of colonization history (Cornuet *et al.*, 2008; Lawson Handley *et al.*, 2011). Their main advantage is to allow a probabilistic approach of different competing introduction scenarios. Using a simulation-based approach and a case study with the western corn rootworm, *Diabrotica virgifera virgifera*, Guillemaud *et al.* (2010) showed the higher

efficiency of ABC compared to traditional genetic distance-based methods (e.g. F_{st}), or assignment-likelihood statistics, in testing the hypothesis that two invasive populations have the same origin. The usefulness of these methods was also pointed out by Rius *et al.* (2012), who showed that the colonization pathways of the ascidian *Microcosmus squamiger* over the whole introduction range followed the historical taxonomic records.

Altogether, despite the above limitations inherent to the use of DNA-based methods (Geller *et al.*, 2010; Fitzpatrick *et al.*, 2012), many studies shed light on the routes and pathways of invasions and their associated vectors or processes. These studies showed that for a given non-native species, the introduction pathways can differ between regions of the introduction range, as exemplified above in *U. pinnatifida* (Voisin *et al.*, 2005). They also documented that both single and multiple introductions can be observed in successful introduced species (Dlugosch & Parker, 2008). In terrestrial plants (Dormontt *et al.*, 2011) and marine species (Roman & Darling, 2007; Rius *et al.*, 2015), a genetic diversity in the introduced populations equal or higher than in the native ones was the most frequently observed pattern. It is explained by multiple and repeated introductions that may be unnoticed based only on field observations, i.e. cryptic introductions (Geller *et al.*, 2010). New statistical methods, like ABC, and molecular tools, like SNPs and DNA-seq, which deliver an enlarged set of markers, are now available for deeper investigations of colonization histories under complex scenarios (Estoup & Guillemaud, 2010).

18.3.2 Spread Dynamics and Temporal Changes

Population genetics studies are analyzing the way genetic diversity is distributed in space or time, to determine the relative importance of various evolutionary forces (migration, genetic drift, selection, mutation) in shaping the evolutionary trajectories of populations. Invasive species may, however, be challenging when using population genetics approaches (Fitzpatrick *et al.*, 2012), for instance because introduced populations did not yet reach equilibrium. Some of the basic assumptions of classical population genetics are thus expected to be violated as, for instance, the relationship between *Fst*, an estimator of the genetic differentiation between populations, and *Nm*, the effective number of migrants. Note, however, that such deviations from the model assumptions also hold for many species in their native range, as pointed out by Whitlock & McCauley (1999). An increasing number of population genetics studies of non-native species thus go beyond the usual measurements of genetic distance, like *Fst*-estimates, using for instance ABC analyses or assignment tests, which are less or non-sensitive to equilibrium assumptions.

One issue commonly addressed with DNA-based studies is the way NIS spread into their new range. Patterns of dispersal may follow several models (Figure 18.1), from 'simple' diffusion from the primary site(s) of the introduction, up to long-distance, jump or saltatory dispersal events with or without diffusion around the new site (Shigesada & Kawasaki, 1997). DNA-based studies have been used to discrimin-

ate these scenarios, to assess the importance of long-distance dispersal events, or to estimate dispersal rates. Based on the compilation of several studies of non-native insects, Lawson Handley *et al.* (2011) pointed out that the spread of non-native species is often a combination of short- and long-distance events, a pattern named 'stratified dispersal' (Shigesada *et al.*, 1995). The importance of long-distance and jump dispersal can often be related to human activities, particularly for species with low dispersal abilities, like many algae with short-lived spores or tunicate species with short-lived gametes and larvae. For example, using microsatellites, Lacoursière-Roussel *et al.* (2012) showed the importance of regional recreational boating in spreading the invasive colonial tunicate *Botryllus schlosseri* from its primary introduction sites located in commercial ports. Genetic studies also demonstrated that mechanisms involved in secondary spread (i.e. within the introduction range) of non-native species may differ from those involved in primary introduction (i.e. from the native area). For instance, using assignment tests with microsatellites, Grulois *et al.* (2011) investigated the genetic structure of populations of the kelp *Undaria pinnatifida*, first introduced deliberately in Brittany (France, English Channel) for aquaculture, and which subsequently established sustainable populations in the wild. The lack of isolation-by-distance patterns and the significant genetic divergence between wild and cultivated populations suggested that although farming initiated the escape into the wild, it was likely not the main source of the long-term establishment and population renewal of this species. Long-distance dispersal, through drifting thalli or fouling on buoys, ships, etc., more likely explained its rapid regional spread. The comparison between modeling of the natural dispersal (e.g. oceanic currents for larval dispersal in marine species) and genetic data can help disentangle natural and human-mediated patterns of spread of aliens (e.g. Viard *et al.*, 2006; Dupont *et al.*, 2007).

Invasion processes are obviously dynamic and fast-evolving processes. As such, monitoring the changes in the genetic composition of an invader along its spreading range can shed light on the vectors of spread (see above) and the invasion dynamics (e.g. additional propagule pressure), and help assess the efficiency of management actions. In the western corn rootworm, *Diabrotica virgifera virgifera*, Ciosi *et al.* (2011) analyzed established populations from the center towards the edge of its expansion route: they observed an interesting genetic pattern characterized by an increase of the genetic variation that was unexpected under the hypothesis of serial founder events during the expansion. They suggested that this may result from control measures in the center of the invasion zone, which could have significantly reduced the population size with consequences on both the demography and genetic composition of the primary introduced populations (i.e. demographic and genetic bottlenecks). Surprising patterns have also been evidenced, in particular 'gene surfing' patterns, where singular clines in allele frequencies have been observed in relation to genetic drift events along the colonization wave (Excoffier & Ray, 2008; Excoffier *et al.*, 2009). Temporal analyses are another way to follow the invasion dynamics; for example, by using herbarium or museum specimens, it is possible to compare modern and historical specimens. In this

context, Saltonstall (2002) showed the high invasive potential of a specific non-native lineage of the common reed *Phragmites australis*, which invaded and even displaced previous lineages over 50 years in the USA. Alternative approaches consist of repeated sampling over time at the same location. Such an approach allowed Pérez-Portela (2012) to show a sequential loss of genetic diversity in one population of the introduced ascidian *Perophora japonica*, which suggested either serial bottlenecks or selection effects.

The study of invasion dynamics in both space and time has greatly benefited from DNA-based methods. The studies mentioned above, however, highlighted the importance of combining several approaches like modeling, field observations, and demography studies with genetic data to better describe the expansion wave, to evaluate the effectiveness of management strategies, and to investigate the likely evolution of non-native species.

18.4 Long-Term Establishment and Consequences on the Ecosystems

18.4.1 Insights about the Evolutionary Potential of the Invaders

Genetic studies are increasingly used to help understand the evolution of the invaders and their invasiveness (Figure 18.4). Studying invasive species adaptation and evolution is, however, still a very challenging issue (Sax *et al.*, 2007; Keller & Taylor, 2008; Dormontt *et al.*, 2011; Rius *et al.*, 2015).

18.4.1.1 Evidence in Favor of the Emergence of Rapid Adaptation

Evidence of rapid adaptive evolution associated with phenotypic shifts has been documented in several invasive species. In the cane toad *Bufo marinus*, for example, the rate of progress of the invasion front was related to changes in toad morphology (e.g. length of the legs; Phillips *et al.*, 2006) although other phenotypic changes were not adaptive but likely determined by stochastic processes (Shine, 2012). Although rapid adaptation may occur, other mechanisms may explain the sudden invasion by non-native species, in particular the introduction of new lineages from new sources. This is exemplified by the sudden expansion of the European crab *Carcinus maenas* in the Canadian Maritimes, where water temperatures are sub-optimal for this crab, although it was established there for a long time. This sudden expansion was first explained by a putative adaptation of the crab to cold-water conditions, combined with an increase in local water temperatures. A cryptic introduction by a new genetic lineage originating from Northern Europe was later revealed based on genetic analyses (Roman, 2006), suggesting that individuals better adapted to cold conditions could have been introduced. Among the approaches available to examine the emergence of adaptive evolution is the joint analyses of the genetic structure at neutral markers (*Fst* measures) and at

quantitative traits (*Qst* measures), provided that a comprehensive examination of the traits exist over the native and invasive ranges (Keller & Taylor, 2008). Following such an approach, Xu *et al.* (2010) showed that phenotypic shift in the perennial herb *Phyla canescens* was due to selective rather than stochastic processes.

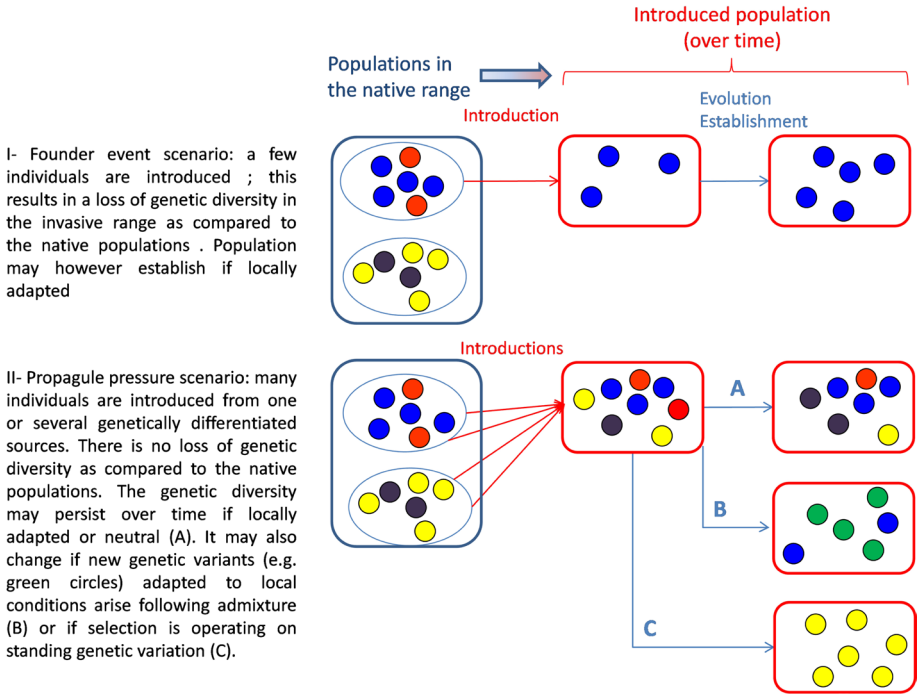

Fig. 18.4: Schematic diagram illustrating the relationships between introduction patterns, genetic diversity, and possible evolutionary trajectories of invader non-native species. Blue rectangles and ellipses depict the native range and populations, respectively. Red rectangles represent an introduced population (i.e. introduction range). Filled circles depict individuals; each color represents a different genetic background.

18.4.1.2 Studying Mechanisms That May Promote Rapid Adaptation

Two major mechanisms have been proposed as drivers of rapid evolutionary changes in invaders at the within-species level: selection of standing genetic variation (Barrett & Schluter, 2008; Figure 18.4-II-C) and genetic admixture (Rius & Darling, 2014; Figure 18.3.II-B). Many studies showed that introduced populations have similar or even higher genetic diversity than native populations (Dormontt *et al.*, 2011; Roman & Darling, 2007; Rius *et al.* 2015). This genetic diversity offers a large basis on which selection may operate over short time scales, as compared to selection on new mutations (Barrett & Schluter, 2008). Genome scan analyses, in which outlier loci (i.e. loci showing atypical patterns because they are influenced by selection processes) are

looked for, are classical ways to study adaptation (Beaumont & Balding, 2004), but should be used and discussed with care (Bierne *et al.*, 2011). In European introduced populations of the Pacific oyster *Crassostrea gigas*, based on outlier identification, Rohfritsch *et al.* (2013) found two groups of populations established in fjord-like environments, a result which may reveal parallel adaptations in similar environments. Conversely, Riquet *et al.* (2013) did not find any outliers between native American and introduced European populations of the slipper limpet *Crepidula fornicata*, while their study revealed outliers between populations in the native range. They suggested that genome scans may not be always efficient for identifying selection between native and introduced ranges because of the short time elapsed since the introduction. Rapid evolution through genetic admixture is supported by observations of repeated introductions of many individuals from several genetically-diversified sources. Through reproduction between individuals with different genetic background, genetic admixture may lead to evolutionary novelties that confer selective advantage in the new environments. Examples supporting this mechanism are still scarce (Rius & Darling, 2014); a particularly convincing example was shown through the invasion by the freshwater snail *Melanoides tuberculata* (Facon *et al.*, 2008). Genetic admixture may, however, be rare in the wild because of the underlying costs, like outbreeding depression due to reproduction between individuals with evolutionary-divergent genetic backgrounds.

It is possible that the genetic diversity of invaders is an important component of their invasiveness and adaptive potential. And yet, invaders suffering genetic bottlenecks (with associated loss of genetic diversity) have been successful (Figure 18.4-I). In addition, adaptive shifts require a substantial amount of adaptive genetic diversity, i.e. genetic variation on which selection may act and that determines fitness traits, and in most of the examples cited in the preceding sections, neutral genetic diversity is measured. There are thus debates about 1) the importance of founder events for preventing, or conversely enhancing, the rapid adaptation of non-native species to their new environments; and 2) the relevance of neutral genetic diversity as a proxy for adaptive genetic diversity (Sax *et al.*, 2007; Dlugosch & Parker, 2008; Dormontt *et al.*, 2011). The emergence of -omics technologies (Hohenlohe *et al.*, 2010; Rius & Darling, 2014) may be helpful in the future for a better characterization of the genomic architecture of the traits under selection as well as detailed investigations of how recombination is operating between genetic lineages in contact after their introductions.

18.4.2 Linking Hybridization and Invasiveness

Hybridization can be viewed as an extreme case of genetic admixture extended to different species when they are not fully reproductively isolated. Such events are common in plants, and hybridization has long been investigated as the main mechanism explaining their invasiveness (Abbott, 1992; Ellstrand, 2000). It has been studied more recently in invasive animals (but see Hedrick, 2013, and references therein).

Hybridization may occur even between highly divergent cryptic species, as exemplified by the successful matings observed between *Ciona robusta* and *Ciona intestinalis*; these two tunicate species were shown to hybridize in the English Channel, a sympatric area where *Ciona robusta* was putatively introduced recently (Nydam & Harrison, 2010). Introgression of *Carcinus maenas* into *C. aestuarii* in Japan, recently documented by Darling (2011), also shed light on the importance of these processes in the history of the introduction. In this specific case, the most parsimonious explanation of the observed genetic patterns, especially a similar genetic diversity in all the studied populations and no genetic structure between them, was a single introduction event into Japan from a source where hybridization between the two species occurred earlier. Adaptive introgression was recently emphasized as a major mechanism by which hybridization may favor the establishment success of an introduced species with potential risks for native species (Fitzpatrick *et al.*, 2010). Adaptive introgression is defined by the transfer of native species alleles into the genome of the introduced species, thus providing the latter with genes that are locally-adapted. Such processes may lead to a mosaic genome made of native and introduced species genes. Like for genetic admixture and selection on standing genetic variation, next-generation sequencing approaches can be useful to examine the way genes are transferred from one species to another, and how the whole genome architecture is modified in the donor and recipient species. To what extent hybridization is widespread in nature and may facilitate invasions is one important direction for future research on invasions to take (Dormontt *et al.*, 2011).

18.4.3 Examining Species Interactions

Once a non-native species has been established, it becomes part of the local community and thus interacts with native species. In particular, it enters the food web both as a potential consumer and as potential prey. Molecular methods are one of the existing approaches available to decipher the trophic links within ecosystems (e.g. King *et al.*, 2008; Traugott *et al.*, 2013). As such, various DNA-based approaches were used to assess the trophic role of aliens, from PCR with species-specific primers to identify target prey (Sheppard *et al.*, 2004; Gorokhova, 2006) to barcoding-like approaches (Kasper *et al.*, 2004). In this latter example, the inventory of prey of one native and one non-native social wasp by molecular identification (16S mtDNA sequences) allowed the determination of prey overlap between the two species. As in all other potential applications, metabarcoding approaches (see 18.2.1) may enhance the assessment of the diet of non-native species, and then their role into the food web (e.g. Pompanon *et al.*, 2012).

DNA barcoding and metabarcoding may also help elucidate the role of parasites in the introduction and invasion processes (Roy & Lawson Handley, 2012), allowing the identification of parasites and pathogens of aliens. For example, 18S rDNA

sequences allowed the identification of larvae of the sea anemone *Edwardsiella*, a parasite of the highly invasive marine ctenophore *Mnemiopsis leidyi* in the Northeast Atlantic (Selander *et al.*, 2010). Parasitic anemone larvae are common in the native habitat, suggesting that this may result from a co-introduction. However, the discriminatory power of 18S is not sufficient to identify sea anemones at the species level, so the parasite may also be a local species (Selander *et al.*, 2010). This first occurrence of an endoparasite in *M. leidyi* in its introduced area might help control its establishment and spread, and has been proposed as a biological control agent.

18.4.4 Monitoring Community Diversity and Structure

Invasions by non-native species greatly impact biodiversity, sometimes with drastic consequences on the structure and functioning of the recipient communities, like community displacement or extinction of native species (Nichols *et al.*, 1990; Simberloff *et al.*, 2013). Documenting the changes in the composition of a community after one alien species has been introduced requires temporal surveys. In this context, molecular tools may help identify the NIS as reported in section 18.2.1. In some cases, both alien and native species may be difficult to identify, and both may benefit from molecular identification tools (e.g. soil and marine sediment meiofauna, pelagic protists, insect larvae). A few multiple-taxa surveys using DNA barcoding were applied to soil fauna (Porco *et al.*, 2013) and lepidopteran communities (deWaard *et al.*, 2009), but such approaches are time-consuming and expensive. For example, in the latter study, 190 species, including 31 non-natives, were identified from more than 900 barcoded individual insects. Next-Generation Sequencing technologies would allow us to overcome these drawbacks, being time- and cost-efficient. They offer the possibility of assessing the composition of the whole community through DNA metabarcoding (Cristescu, 2014), including rare species (Pochon *et al.* 2013; Zhan *et al.* 2013). In addition to species richness, DNA metabarcoding may also allow estimation of the relative abundances of different taxa (Porazinska *et al.*, 2010; Hajibabaei *et al.*, 2011; Comtet *et al.*, 2015), even if it may sometimes fail (Porazinska *et al.*, 2009), and the development of new technologies would certainly help improve these estimates. DNA metabarcoding will thus help with monitoring of the changes that occur after the introduction of new species, or after the implementation of management procedures.

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In a nutshell: about DNA-based studies of non-native species

- In the last two decades, molecular tools have become very popular for addressing questions relating to biological invasions.
- Knowing earlier is better: DNA-based identifications are critical for early and accurate detection of non-native species.
- An ever-increasing number of non-native cryptic species are recognized; revealing these cryptic invasions is a prerequisite to trace back introduction pathways.
- When studying colonization and spread by non-native species, particular care has to be given to sampling design and the statistical framework. The study design is guided by the question addressed (e.g. assignment tests to determine the geographic origin require a comprehensive coverage of all putative sources). New statistical frameworks (e.g. Approximate Bayesian Computations, Maximum-likelihood methods) have to be considered.
- The success in tracing back introduction processes, particularly for assigning sources, depends on the time elapsed since the introduction (the shorter the better) because of post-introduction changes in the genetic composition of native and invasive populations. In this context, the knowledge of the life history traits of the NIS is also crucial.
- Despite their inherent limitations, DNA-based studies shed light on introduction pathways and processes; for instance, by showing that the same NIS can invade different regions through different routes and vectors.
- DNA-based studies have shown that introduced populations can be genetically as or even more diversified than native ones. This underlined the importance of propagule pressure and recurrent events in biological invasions.
- Genetic admixture between different genetic pools has been documented in non-native species. Such a process may lead to evolutionary novelties in sexually reproducing species.
- Hybridization between native and non-native species could facilitate adaptive introgression and thus long-term establishment of the invader.
- Evolutionary outcomes of invasions in both non-native and native species have yet to be examined in detail, particularly the effects of genetic admixture at the species level and hybridization processes.
- Next-Generation Sequencing technologies offer new opportunities to study biological invasions, from the development of huge numbers of polymorphic markers for population genetics, to biodiversity assessments through DNA metabarcoding. New statistical and theoretical frameworks are, however, required.

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