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Reconstructing routes of invasion using genetic data: why, how and so what?

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Abstract

Detailed knowledge about the geographical pathways followed by propagules from their source to the invading populations—referred to here as routes of invasion—provides information about the history of the invasion process and the origin and genetic composition of the invading populations. The reconstruction of invasion routes is required for defining and testing different hypotheses concerning the environmental and evolutionary factors responsible for biological invasions. In practical terms, it facilitates the design of strategies for controlling or preventing invasions. Most of our knowledge about the introduction routes of invasive species is derived from historical and observational data, which are often sparse, incomplete and, sometimes, misleading. In this context, population genetics has proved a useful approach for reconstructing routes of introduction, highlighting the complexity and the often counterintuitive nature of the true story. This approach has proved particularly useful since the recent development of new model-based methods, such as approximate Bayesian computation, making it possible to make quantitative inferences in the complex evolutionary scenarios typically encountered in invasive species. In this review, we summarize some of the fundamental aspects of routes of invasion, explain why the reconstruction of these routes is useful for addressing both practical and theoretical questions, and comment on the various reconstruction methods available. Finally, we consider the main insights obtained to date from studies of invasion routes.

Keywords: introduction, invasive species, molecular markers, outbreak, rapid evolution, statistical inferences

Introduction

Biological invasions are a major component of global change, with potentially huge detrimental effects on public health, agriculture and biodiversity (Sax *et al.* 2005). This has stimulated considerable interest among biologists in determining why some species become successful invaders (Cadotte *et al.* 2006; Sax *et al.* 2005). Paradoxically, there is still some debate about the definition of an 'invasive' species (Colautti & MacIsaac 2004; Valery *et al.* 2008), and current conceptual frameworks for biological invasions differ in the relative importance given to three major components: 'range extension' (Facon *et al.* 2006), 'high local abundance' (Suarez *et al.* 1999) and 'disruption of ecosystem function' (Mooney & Hobbs 2000). Here, we consider an invasive population to be a set of individuals that has been introduced into a new area, in which these individuals have established themselves, increased in number and spread geographically. The use of other definitions of biological invasion has no significant effect on most of the ideas developed in this study.

The reasons why some introductions lead to invasions whereas others do not result in the establishment or expansion of the invading population are currently being addressed by many researchers. A number of studies focus on determining the likelihood of a species

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being invasive based on the presence or absence of particular phenotypic characters, such as wide dispersal, high growth rate, generalist vs. specialist trophic relationships, asexual reproduction, tolerance to environmental heterogeneity, high levels of competitiveness (e.g. Kimberling 2004; Kolar & Lodge 2002; Sakai *et al.* 2001; Vall-Ilosera & Sol 2009). The species identity of a population may therefore be seen as an important factor determining the success or failure of invasions. However, invasiveness may vary among species with similar 'invasive characteristics' as well as within species (Kolar & Lodge 2001). A few intraspecific studies have shown that invasiveness outcome may differ considerably between introduced populations from the same species (e.g. Ciosi *et al.* 2008; Kang *et al.* 2007; Kelly *et al.* 2006). Clearly, comparing species does not allow the evaluation of the effects of intraspecific polymorphism on the outcome of an introduction. Moreover, it is difficult to study the sensitivity of invasion success to stochastic events or environmental variation in interspecific comparisons.

The new ecological conditions encountered by introduced individuals may differ considerably from the original conditions; therefore natural selection and adaptation may be key determinants of the success of invasion at the population level before or during the settlement phase (reviewed in Reznick & Ghalambor 2001; Voisin *et al.* 2005; Facon *et al.* 2006; Schierenbeck & Aïnouche 2006). Several factors determine the capacity of invasive populations to respond to selection, and genetic variability plays an important role (Drake & Lodge 2006; Facon *et al.* 2006; Lockwood *et al.* 2005; Memmott *et al.* 2005). Substantial genetic variability (or at least a high level of additive genetic variation) is expected to favour adaptation in remote territories and, thus, the settlement and spread of the introduced propagules (Facon *et al.* 2006, 2008; Kolbe *et al.* 2004; Lavergne & Molofsky 2007; Lee 2002; Roman & Darling 2007). However, there are many examples of successful invaders with very low levels of genetic variability, at least at selectively neutral loci (reviewed in Novak & Mack 2005; Wares *et al.* 2005). Good alleles of specific genes or good combinations of genotypes for those genes may nonetheless facilitate successful introduction (Facon *et al.* 2006).

One critical piece of the puzzle in the understanding of biological invasions is an understanding of the history of the invasion process, including the geographical pathways followed by the founders of the invading populations. Indeed, the genetic variability of invading populations, in terms of both molecular and quantitative traits, depends on the history of their populations of origin and the historical and demographical features of their introduction. Such features include the number and genetic composition of source populations, the number of introductions from each source, the number of individuals introduced during each introduction episode, the number of intermediate populations between the initial introduction and the spread of the invasive population and the dynamics of the geographical and demographic expansion following each introduction. Information about the history of the invasion process, including, in particular, a description of the geographical pathways of the propagules between the source and invading populations referred to here as routes of invasion thus provides useful information about the origin and genetic composition of the invading populations (Dlugosch & Parker 2008). As we will see below, it is important to retrace the routes of invasion, to generate and test hypotheses concerning the environmental and evolutionary factors responsible for biological invasions.

In this review, we will (i) summarize fundamental aspects of the routes of invasion, (ii) explain why reconstructing routes of invasion is a useful approach for addressing both practical and theoretical questions, (iii) comment on the various methods available for reconstructing invasion routes, and (iv) consider the main insights obtained to date through studies of invasion routes.

The fundamental aspects of invasion routes

Biological invasions vs. natural range expansions

The scientific literature is full of examples of natural range expansion over large distances with the colonization of remote geographical areas and new habitats (Wilson *et al.* 2009). Such natural range expansions are sometimes associated with extinction, speciation and the building of new ecosystems. For instance, oak has colonized Europe since the end of the last ice age, with a new species arising from hybridization between the pedunculate oak (*Quercus robur* L.) and sessile oak [*Q. petraea* (Matt.) Liebl.] (Petit *et al.* 2004). It has been argued that biological invasions simply represent an increase in natural dispersal events of a species outside its natural range (Vermeij 2005). However, invasions often involve complex routes, including multiple source populations and multiple introduction sites. Moreover, biological invasion occurs much more rapidly than the major colonizations and recolonizations of areas during evolution (e.g. after glaciations Valery *et al.* 2008; Wilson *et al.* 2009). One major consequence of these two features is that invasions are often associated with the introduction of considerable genetic variability over a short period of time (Dlugosch & Parker 2008; Roman & Darling 2007; Wares *et al.* 2005). This high level of initial genetic variation may increase the likelihood of encountering adaptive genetic variants and the response to natural selection of the offspring of the founders (Facon *et al.* 2006, 2008; Lavergne & Molofsky 2007; Roman & Darling 2007). This characteristic may be decisive if the conditions in the new environment are significantly different from those of the native area. We already have a full section devoted to this question in our ms.

With regard to the methods that can be used to make inferences about the routes of invasions vs. the routes of natural range expansions, we do not see any obvious reasons to consider that different methods should be used in either case. Therefore, many of the advantages and disadvantages of the methods detailed below for the routes of invasions (see Methods for reconstructing routes of invasion) should also hold for the routes of natural range expansions. However, the fact that the major colonizations of areas during evolution are older than biological invasion tends to suggest that DNA sequence data might be more informative to make inferences about the routes of natural range expansions than of invasions. On the other hand, nongenealogical-based methods such as STRUCTURE (Pritchard *et al.* 2000), BAPS (Corander *et al.* 2003) or GENELAND (Guillot *et al.* 2005) should be of lower interest to make inferences about the routes of natural range expansions than of invasions.

Role of humans in shaping invasion routes

It is now widely recognized that biological invasions are often because of fortuitous or intentional dispersal events linked to human activities (Lockwood *et al.* 2005). Human activities provide vectors for dispersion (air traffic, train networks, marine ballasts, canals, etc.), thereby promoting introductions into new remote areas and accelerating the spatial expansion of species after their introduction into new areas (i.e. stratified dispersal; Shigesada *et al.* 1995). However, it would be extremely restrictive to limit the influence of humans exclusively to 'extra-range species dispersal'. A second crucial, but indirect, role of human activities relates to the worldwide homogenization of the environment, through urbanization and agriculture in particular (Sax & Brown 2000; Tilman *et al.* 2001). For example, maize crops provide a relatively uniform habitat throughout the world, from Africa to Asia, and North and South America to Europe (Anonymous, 1993). This homogenization of the environment considerably decreases the magnitude of the evolutionary response required to adapt to the conditions found in new, geographically distant territories. This situation increases the likelihood of successful invasion by species living in maize crops, even if there is a very large distance between the native area and the area of introduction. In conclusion, humans not only provide efficient means of transport over increasingly large geographical distances, but also favour biological invasions by decreasing ecological differences between geographically remote areas.

The reconstruction of invasion routes is a useful approach for addressing both practical and theoretical questions

From a practical point of view, the reconstruction of invasion routes facilitates the design of strategies for preventing invasion. In cases of recurrent introductions, strategies based on the prevention of introduction may be more cost-effective than eradication or containment (Mack *et al.* 2000). If the geographical origin and the vector responsible for the recurrent introduction of an invader can be identified, then specific monitoring and quarantine measures targeting the source area and the means of dispersal can be designed. However, if an introduction has already occurred, eradication or containment may be the best solution if the introduction is a single event and the area invaded is not too large (Hulme 2006). In this context, the inference of invasion routes may also facilitate the design of measures for controlling invasive populations, because the efficacy of such measures depends on the genetic diversity and geographical origin of the genotypes introduced. For example, in the case of classical biological control against an invasive pest, in which a predator or a parasite is deliberately introduced into a new area with a view to its becoming stably established, it may be better to choose strains of the auxiliary agent with the same geographical origin as the invasive population. This is because local adaptation may lead to auxiliary agents being more efficient against target species from populations with which they coevolved within their native range (see Kang *et al.* 2007 for a complication of this simple case; Roderick & Navajas 2003). Methods developed to describe the introduction routes of invasive populations, and hence their origin within their native range, should therefore facilitate the design of effective control or prevention strategies.

From an academic point of view, the reconstruction of invasion routes is crucial for defining and testing different hypotheses concerning the environmental and evolutionary factors underlying biological invasions (e.g. Hufbauer & Sforza 2008). It is difficult to demonstrate that a specific adaptation is responsible for an invasion (see for example Keller & Taylor 2008). Many examples of evolution in the context of invasions have been described (Bossdorf *et al.* 2005). However, as noted by Keller & Taylor (2008), changes in the distribution of phenotypic and life history traits during dispersal, establishment and range expansion may reflect neutral phenotypic changes rather than adaptive evolution. Random sampling of the genetic diversity of the source population, the sudden and dramatic decrease in population size during the introduction phase, and the low density of the introduced population during the establishment phase may result in changes in the phenotype of the introduced population, resulting in differences between the introduced and source populations that are not because of selection.

Keller & Taylor (2008) noted that in cases in which the putative source populations differ in their phenotype distributions, the incorrect assignment of the introduced population to one of the putative sources may be erroneously interpreted as phenotypic evolution. They proposed to estimate phenotypic divergence between ancestral lineages in the native range and their descendants in the introduced range directly. This approach is based on a rationale of measuring phenotypic differences between comparable entities, i.e. between demes or populations derived from each other through introduction events. This approach thus requires a precise knowledge of the historical source introduction relationships of the populations studied and reconstructing introduction routes may be helpful in this respect. If the same type of phenotypic evolution is demonstrated in several independent introductions, then it is a strong indication that phenotypic evolution may be adaptive.

Another solution involves carrying out Q_{ST} F_{ST} analysis (McKay & Latta 2002). F_{ST} is a measurement of inter-population genetic variation based on the molecular variation observed at neutral or nearly neutral loci. It is supposed to estimate inter-population divergence because of stochastic and demographic events only. Q_{ST} is a measurement of inter-population genetic variation due not only to stochastic and demographic events, but also to selection. It is measured for quantitative traits, such as body size and fecundity. A significant difference between the estimates of Q_{ST} and F_{ST} for a given set of populations provides evidence for a response of the quantitative trait concerned to selection (significant differences are, however, often difficult to observed because of the usually large variance of F_{ST} and Q_{ST}; Keller & Taylor 2008). As discussed earlier, this quantitative genetics-based approach requires thorough knowledge of the source populations of the invasive populations studied. Using such approaches, it is possible to reject the hypothesis of neutrality in some cases, and to demonstrate rapid adaptation to new environmental conditions after introduction. For instance, populations of *Drosophila subobscura* introduced into America from Europe display the hallmarks of morphological (Huey *et al.* 2000) and chromosomal (Prevosti *et al.* 1988) adaptations to climatic conditions that have occurred in less than 20 years. Similarly, Lee *et al.* (2003, 2007) have documented rapid changes in the reaction norm to salinity in the invasive copepod *Eurytemora affinis,* introduced into low-salinity environments from high-salinity environments. Other examples can be found in Keller & Taylor (2008).

Tests of the hypothesis of non-neutral and nonrandom evolution because of an adaptive change in an invasive population i.e. separating the role of stochastic and demographic events from that of selection in shaping the evolution of the phenotype require comparisons between the introduced and source populations. The use of comparative approaches of this type requires selection of the most appropriate entities to be compared (the comparable entities). In this context, the description of invasion routes, providing information about the history of the invasion process and, specifically, about the sources of the introduced propagules, is crucial.

Methods for reconstructing routes of invasion

Two types of methods have traditionally been used to make inferences concerning routes of introduction: direct methods based on current and historical observations of invasive species and indirect methods based on patterns in population genetics data. Recently, a new model-based Bayesian method called approximate Bayesian computation (ABC, Beaumont *et al.* 2002) has been proposed and used to draw inferences about the complex evolutionary scenarios typically encountered in the introduction histories of invasive species. This method is based, at least partly, on population genetics data and may therefore be considered an indirect method. This approach constitutes a particularly important development in the field and will therefore be dealt with in a separate section.

Direct methods

Direct methods are based on records of the presence and absence of invasive taxa (e.g. the study of Argentine ant by Suarez *et al.* 2001). Routine controls carried out in airports and harbours by quarantine services and monitoring by environmental or agricultural agencies are particularly informative in this respect (Work *et al.* 2005). These records can be useful for retracing invasion routes, particularly when combined with ecological and bioclimatic data. For instance, Tatem *et al.* (2006) used a database of international ship and aircraft traffic movements, together with climatic information, to retrace the expansion of the range of the *Aedes albopictus* mosquito. However, it is rarely possible to infer the routes of invasion with a high degree of precision by these direct methods. Harbour and airport records provide information about the immediate geographical origin of the introduced individuals detected, but can provide no information about subsequent steps in the invasion process. Indeed, given the low rates of establishment and expansion recorded for introduced individuals (Williamson 2006), there is no guarantee that the individuals intercepted would have spearheaded a successful invasion.

Indirect methods

Indirect methods are based on the genetic patterns observed within and between populations at molecular markers (e.g. Ciosi *et al.* 2008; Darling *et al.* 2008; Davies *et al.* 1999; Facon *et al.* 2003; Fonseca *et al.* 2000; Kolbe *et al.* 2004; Lindholm *et al.* 2005; Thibault *et al.* 2009; Hoos *et al.* 2010). One indirect method that is still widely used is based on the construction of dendrograms (e.g. neighbour-joining trees) from matrices of genetic distances between populations (e.g. Lozier *et al.* 2009; Thibault *et al.* 2009). The main features of this approach are described in Box 1. Other methods are based on calculations of assignment likelihood (Ciosi *et al.* 2008; Genton *et al.* 2005; Paetkau *et al.* 2004; Pascual *et al.* 2007; Rannala & Mountain 1997; Tepolt *et al.* 2009; Thibault *et al.* 2009) or parsimony networks (e.g. Voisin *et al.* 2005; Hoos *et al.* 2010). More recently, a number of studies trying to retrace the introduction scenarios of invasive populations (mostly to determine their source) have used clustering methods like those implemented in STRUCTURE (Pritchard *et al.* 2000), BAPS (Corander *et al.* 2003) or GENELAND (Guillot *et al.* 2005). If the invasive population clusters clearly with one of the potential source populations, this is considered to provide fairly conclusive information about the origin of the invasive population (e.g. Darling *et al.* 2008; Eldridge *et al.* 2001; Marrs *et al.* 2008; Rosenthal *et al.* 2008; Rollins *et al.* 2009). A shared ancestry of the individuals of invading populations with various populations from the native area is sometimes interpreted as evidence for an admixture origin of the invasive population considered (Darling *et al.* 2008; Rosenthal *et al.* 2008). However, if the invasive population does not cluster clearly with any of the potential source populations and/or if the calculated coancestry coefficients indicate shared ancestry with several populations, then it is difficult to infer the invasion route from the pattern obtained. This may be because of the existence of multiple sources or unsampled sources, drift during and after introduction, or insufficient numbers of markers (e.g. Darling *et al.* 2008). Claims of admixture between source populations on the basis of coancestry coefficients may therefore be overstated in at least some cases.

Box 1: Inferring routes of invasion by traditional indirect methods based on genetic distances and trees

Invasion routes are frequently inferred from tree topologies reconstructed from a matrix of genetic distances between pairs of populations (e.g. Goldstein *et al.* 1999). Here, we summarize the main features of these inference methods based on tree topologies, highlighting their principal drawbacks. The main advantage of these methods is that they are straightforward and rapid to carry out with a number of user-friendly programs. Various types of genetic markers, genetic distances and tree reconstruction algorithms can be used. Below, we will focus on the most commonly used types of genetic markers, distances and tree algorithms in the context of invasion biology: microsatellite loci, the genetic distances proposed by Cavalli-Sforza & Edwards (1967) and Nei *et al.* (1983) and the neighbour-joining algorithm for tree construction (NJ, Saitou & Nei 1987) (see Takezaki & Nei 1996) for a comparative study of genetic distances on tree topologies). Most of the features and drawbacks described below are general and also apply to other genetic markers, distances and tree algorithms.

Small divergence times and bottleneck events. In invasion biology, evolutionary scenarios are often characterized by small divergence times between populations and by bottleneck events of various intensities after introduction. The effect of small divergence times on the likelihood of identifying the true topology is illustrated with microsatellite data simulated under a serial introduction scenario (Fig. 1 and Table 1). Short divergence times result in low levels of differentiation by drift and mutation, and these low levels of differentiation make it difficult to reconstruct the 'true' tree topology. In our simulation study, we found that about 40% of the topologies with short divergence times were reconstructed incorrectly, leading to the inference of false introduction routes (Table 1). The proportion of correctly inferred true topologies increases considerably, however, if bottleneck events occur after introductions (Table 2). This is because bottleneck events generate drift pulses in each of the introduced populations, which become genetically differentiated from each other whilst retaining their source introduction relationships. This makes it easier to reconstruct the true tree topology, provided that enough genetic polymorphism remains after the bottleneck events.

Independent introductions from the same source population. In the invasion scenario shown in Fig. 1,

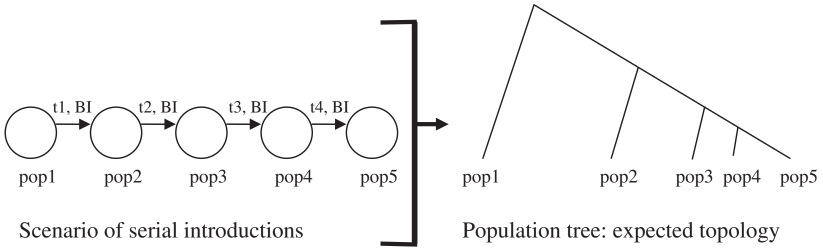


Fig. 1 Computer simulation of microsatellite data under a scenario of serial introductions and expected tree topology. t1, t2, t3 and t4: introduction times in number of generations since present. All five populations have the same effective population size (Ns = 500 diploid individuals). Each introduction involves the same number of effective founding individuals (Nf = 5, 50 or 500 diploid individuals) for five generations after introduction. Each introduction is characterized by the same bottleneck intensity index, BI = $\log_{10}(Ns/Nf)$. Three categories of divergence times are considered: short (t1 = 40, t2 = 20, t3 = 10, t4 = 5), medium (t1 = 200, t2 = 100, t3 = 50, t4 = 25) and long (t1 = 1000, t2 = 500, t3 = 250, t4 = 125). For each category of divergence time, three bottleneck intensities are considered: null (BI = 0), medium (BI = 1) and strong (BI = 2). Each simulated microsatellite data set includes 30 individuals genotyped at 10 independent loci evolving with a mutation rate of 5×10^{-4} under a generalized stepwise mutation model with the parameter of the geometric distribution of the length in number of repeats of mutation events equal to 0.20 (Estoup *et al.* 2002). Simulations were based on coalescence theory (Kingman 1982), and we simulated 10 000 data sets for each set of conditions. For each simulated data set, we constructed a population tree with pop 1 as root, using a matrix of genetic distances of Nei *et al.* (1983) or Cavalli Sforza & Edwards (1967), and the neighbour joining algorithm (NJ, Saitou & Nei 1987). The topology of each tree was compared with the expected topology (i.e. the true topology) and the number of topologies without error was recorded. All simulations and tree reconstructions were processed with custom written programs (available on request).

Table 1 Effect of divergence time on the proportion of correctly inferred topologies. The introduction scenario, true topology and simulated data sets are described in Fig. 1. All introductions are characterized by an absence of bottleneck (bottleneck intensity, BI = 0)

	Divergence time		
Genetic distance	Long	Medium	Short
Nei *et al.* (1983)	90.55	83.11	54.99
Cavalli Sforza & Edwards (1967)	93.76	91.43	59.32

Table 2 Effect of bottleneck intensity during introductions on the proportion of correctly inferred topologies. The introduction scenario, true topology and simulated data sets are described in Fig. 1. Only the scenarios with short divergence times (t1 = 40, t2 = 20, t3 = 10, t4 = 5) were considered

	Bottleneck intensity		
Genetic distance	Null	Medium	Strong
Nei *et al.* (1983)	54.99	99.09	99.80
Cavalli Sforza & Edwards (1967)	59.32	98.70	99.83

each introduced population originates from a different source population. In many cases, several populations have the same source population. For recent introductions, the genetic distances between source and introduced populations are due purely to genetic bottlenecks occurring during and soon after introductions. Therefore, two populations independently introduced from the same source would not be expected to be genetically more closely related to each other than to the source and they should not necessarily cluster together in a NJ tree. As the source population is assumed to be the root of the tree, the grouping of the introduced populations together is a constraint. As a consequence, low bootstrap values are expected for the nodes connecting the introduced populations. But how low? There is no rigorous statistical framework allowing the definition of threshold bootstrap values below which it is safe to conclude that the populations originate from the same source. The expected bootstrap values depend on the number of populations analysed and sample sizes (numbers of genotyped loci and individuals) and, probably, on the intensity of the bottleneck events. This is illustrated by the NJ trees obtained for European outbreaks of the invasive beetle *Diabotica virgifera virgifera* (Fig. 2), for which an analysis of the same samples by approximate Bayesian computation (ABC, Beaumont *et al.* 2002) suggests four independent introductions into Europe from North America (Miller *et al.* 2005; Box 2). Table 3 shows that the number of independent European introductions from North America deduced from tree topologies and bootstrap values largely depends on the threshold bootstrap values below which a trifurcation is inferred.

Direction of introductions. When considering two terminal branches connecting two populations (e.g. CSE Europe NE Italy or Eastern France Paris

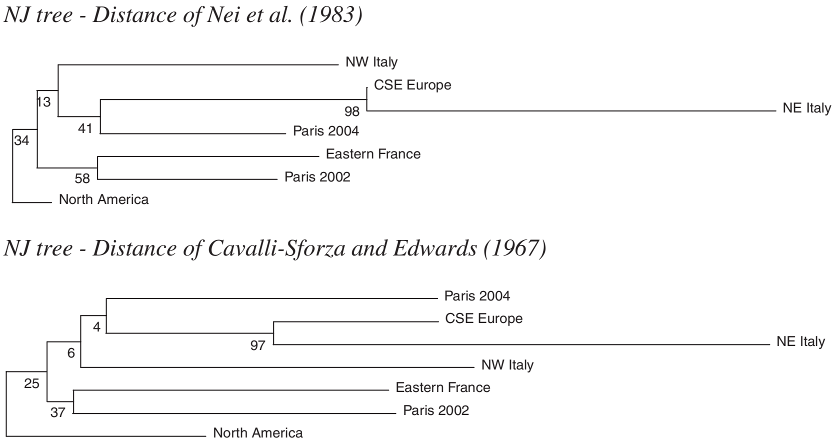
2004; Fig. 2), it is not possible to know which population is the source of the other. Only the consideration of historical data, such as dates of first observation, can resolve this problem. However, these dates are often uncertain and may therefore lead to incorrect conclusions, particularly when they are very close together, as in the case of *D. virgifera virgifera* (e.g. 2002 for Paris-2002 and 2003 for eastern France). As bottleneck events increase genetic distances, they would be expected to increase the length of the branch corresponding to the introduced population. However, branch lengths have a large variance and depend on many factors, including bottleneck intensity and genetic distance. In Fig. 2, the branch lengths of the Paris 2002 Eastern France population pair suggest that Paris 2002 is the source population if the distance of Nei *et al.* (1983) is used and that eastern France is the source population if the distance of Cavalli-Sforza & Edwards (1967) is used.

Ghost populations. When genetic relationships between populations are summarized by a tree, no inference can be made about the putative existence of a nonsampled introduced population (a so-called ghost population) acting as the source of one or several introduced populations (see Guillemaud *et al.* 2010 for details). This issue is particularly problematic when the nonsampled population plays the role of an invasion bridgehead (see main text and Box 2).

Conclusion. Traditional indirect methods based on genetic distances and trees are useful tools for making preliminary inferences about invasion routes. Their main advantage is that they are simple and rapid to carry out with a number of user-friendly programs. In addition to the limitations of such methods described earlier, the two most important issues associated with these techniques are that they reduce genetic data to a single summary statistic of genetic variation between populations and they do not adequately take into account the stochasticity of the demographic and genetic history considered. This drawback results from the lack of reliance of these methods on explicit demographic and molecular stochastic models. Approximate Bayesian computation (ABC, Beaumont *et al.* 2002) uses several to many summary statistics to describe most of the molecular information and is based on explicit demographic and molecular stochastic models. ABC thus represents a promising alternative methodology for the inference of invasion scenarios (see Box 2).

Table 3 Effect of the choice of threshold bootstrap value on the number of deduced independent introductions of *Diabrotica virgifera* into Europe from North America, as inferred from the tree in Fig. 2. Microsatellite data (eight loci) are those published by Miller *et al.* The number of independent introductions inferred from ABC analysis is four (Miller *et al.* 2005)

	Threshold bootstrap values				
Genetic distance	20%	30%	40%	50%	60%
Nei *et al.* (1983)	1	3	3	4	5
Cavalli Sforza & Edwards (1967)	1	4	5	5	5


NJ tree - Distance of Nei et al. (1983)

NJ tree - Distance of Cavalli-Sforza and Edwards (1967)

Fig. 2 The introduction of *Diabrotica virgifera* into Europe. The native population is called 'North America'. The other six populations are populations invading Europe. NW Italy = Northwest Italy; NE Italy = northeast Italy; CSE Europe = Central Southern East Europe (invaded area centred on Serbia). Microsatellite data (eight loci) are those published by Miller *et al.* (2005). Bootstrap values (with the locus as a unit) were calculated over 5000 iterations. (a) NJ tree Distance of Nei *et al.* (1983); (b) NJ tree Distance of Cavalli Sforza & Edwards (1967).

Box 2: Inferring routes of invasion by approximate Bayesian computation

In this box, we will not detail the general statistical features of approximate Bayesian computation (ABC; Beaumont *et al*. 2002). General statistical features of ABC have been reviewed in two recent studies (Bertorelle *et al*. 2010; Csilléry *et al*. 2010). We will rather focus on some practical aspects of ABC that are important when using this method to make inferences about invasion routes. Briefly, ABC is a Bayesian approach in which the posterior probabilities of different models and/or the posterior distributions of the demographic parameters under a given model are determined by measuring the similarity between the observed data set (i.e. the target) and a large number of simulated data sets; all data sets are summarized by statistics such as the mean number of alleles or heterozygosity per population and F_{ST} or genetic distances between pairs of populations. The ABC method was recently successfully used to make inferences from large data sets for complex models in population and evolutionary biology (e.g. Estoup *et al*. 2004; Fagundes *et al*. 2007; Jakobsson *et al*. 2006; Neuenschwander *et al*. 2008; Patin *et al*. 2009; Rosenblum *et al*. 2007; Toni *et al*. 2009; Verdu *et al*. 2009), infectious disease epidemiology (e.g. Luciania *et al*. 2009) and systems biology (e.g. Ratmann *et al*. 2009). It has already been successfully used to infer the invasion routes of a number of species (Lombaert *et al*. 2010; Miller *et al*. 2005; Pascual *et al*. 2007).The method is indeed well adapted to the complex historical and demographical models associated to biological invasions, in which bottlenecks, serial or independent introductions and/or genetic admixture events are often suspected. Under an ABC framework, such events can be modelled explicitly hence defining different models (often called scenarios) that can be formally tested against each another (see Fig. 3 for an illustration of competing scenarios traditionally considered in the study of invasion routes).

In practice, ABC users can base their analysis on simulation programs, such as SIMCOAL (Laval & Excoffier 2004), ms (Hudson 2002), Serial SimCoal (Anderson *et al*. 2005) or MaCS (Chen *et al*. 2009) and then use statistical software to postprocess their simulation outputs. Several ABC programs have recently been developed to provide nonspecialist users with solutions varying in the extent to which they are user-friendly (Cornuet *et al*. 2008; Foll *et al*. 2008; Hickerson & Meyer 2008; Jobin & Mountain 2008; Tallmon *et al*. 2008; Lopes *et al*. 2009; Wegmann *et al*. 2009). These programs can be used for data simulation and some postprocessing steps. To our knowledge, three of these ABC programs, popABC (Lopes *et al*. 2009), DIYABC (Cornuet *et al*. 2008) and ABCtoolbox (Wegmann *et al*. 2010), provide particularly useful operational solutions for treating the complex introduction scenarios typical of biological invasions. PopABC (Lopes *et al*. 2009) combines an isolation with migration model with microsatellite (under an SMM mutation model) or sequence data (under ISM) and recombination. It can be used to analyse several populations that have diverged with or without migration, making it possible to infer invasion routes. PopABC involves an element of pipelining at the end of data processing, making this software most suitable for users with some experience in computing (Lopes *et al*. 2009). Cornuet *et al*. (2008) developed DIYABC to provide a user-friendly interface, making it possible for biologists with little background in programming to perform inferences by ABC. DIYABC can consider complex population histories, including any number of divergence (without migration), admixture and population size variation events, for population samples that may have been collected at different times. The package initially assumes a GSM or SMM mutation model for microsatellites (Estoup *et al*. 2002). Recent developments of DIYABC include (i) inference from DNA sequence data in addition to or separately from microsatellite data, (ii) the possibility of analysing five categories of loci, taking into account balanced or unbalanced sex ratios: autosomal diploid, autosomal haploid, X-linked, Y-linked and mitochondrial, and (iii) the possibility to proceed model checking computation to assess the 'goodness-of-fit' of a model, a much-neglected facet of ABC analysis (Cornuet *et al*. 2010; DIYABC V1.0 available at http://www1.montpellier.inra.fr/CBGP/diyabc). An example of a set of introduction scenarios that can be analysed with DIYABC is given in Fig. 3. DIYABC is able to handle two types of scenario that may be frequent in invasion biology. First, it allows scenarios including intra-specific hybridization (or admixture) events to be considered, and such events are frequently reported in successful invasions (Facon *et al*. 2005; Gaskin & Schaal 2002; Gaskin *et al*. 2009; Rhymer & Simberloff 1996). Second, it also allows the modelling of ghost populations (Guillemaud *et al*. 2010; Miller *et al*. 2005), i.e. populations contributing to the invasion scenario but that are not sampled and thus not analysed (Fig. 4). The very recent computer package ABCtoolbox (Wegmann *et al*. 2009) includes a series of open source programs that can be pipelined and launched independently to perform ABC for various models (including migration) and different types of molecular markers (DNA sequences, single-nucleotide polymorphism (SNP) and microsatellites). The global flexibility of this package (ABCtoolbox can interact with virtually any command-line simulation programs), modulo its potentially lower computation speed, makes it a complementary if not an alternative tool to the more canned ABC software popABC and DIYABC for addressing questions dealing with invasion routes.

Theoretically, any number of populations and loci can be analysed with DIYABC, ABCtoolbox or popABC and there is no theoretical limit to the complexity of the scenarios considered. However, two problems emerge when the number of populations becomes too large. First, the number of summary statistics to be manipulated increases considerably with the number of populations (especially if different types of markers are considered in the same analysis). This may be of concern because ABC algorithms attempt to sample from a small multidimensional sphere around the observed statistics, and the probability of accepting a simulation decreases exponentially as dimensionality increases. This phenomenon is referred to as the 'curse of dimensionality' and increasing the number of simulations may not be

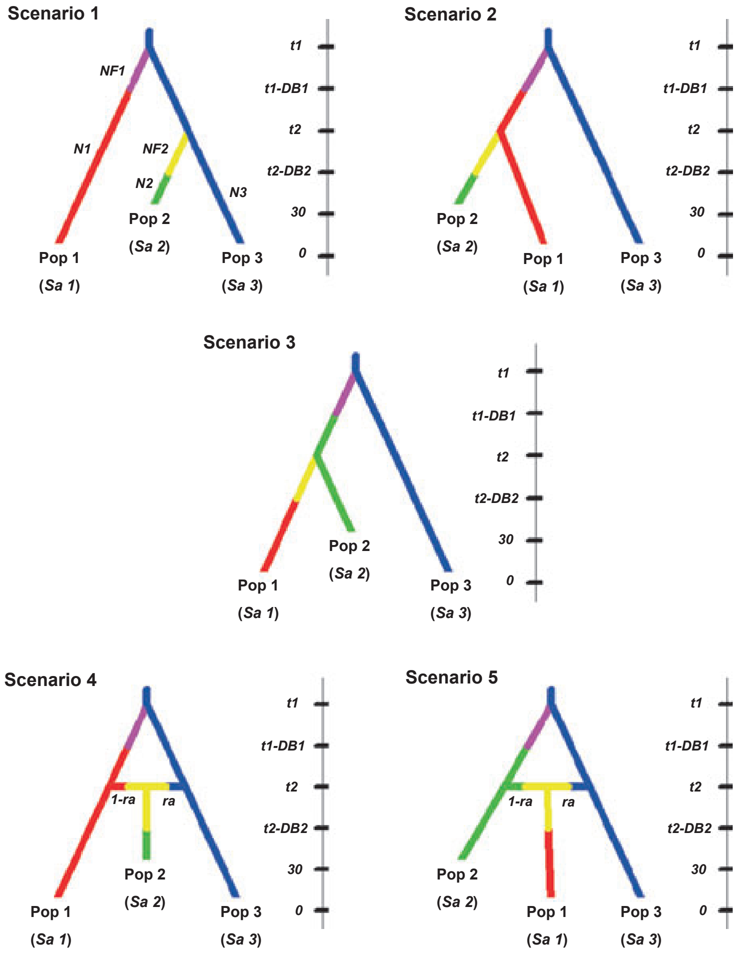


Fig. 3 Graphical representation of five invasion scenarios that can be considered with approximate Bayesian computation using the software DIYABC, for two invasive populations (Pop 1 and 2) and one native population (Pop 3). Scenario 1 corresponds to independent introductions of Pop 1 and 2 from the native population. In this example, Pop 1 is older than Pop 2, but Pop 2 can be older than Pop 1 if t2 > t1, depending on the values of t1 and t2 drawn from their prior distributions. Scenarios 2 and 3 correspond to serial introductions of Pop 1 and 2. In scenarios 4 and 5, the most recent invasive population is the result of an admixture between individuals from the native population at a rate ra and from the oldest invasive population at a rate 1 ra. N1, N2 and N3 are stable effective population sizes in Pop 1, 2 and 3, respectively. The introduction of population *i* occurred ti generations ago and includes a period of DBi generations of potentially small population size (NF2 and NF3 for Pop 2 and 3, respectively). Time 0 is the sampling time of populations 1 and 3; Pop 2 was sampled 30 generations ago.

sufficient to deal with it (Beaumont *et al.* 2002). Recent improvements of ABC get round this problem by using dimension reduction techniques, including a nonlinear feed-forward neural network (Blum & François 2010) and partial least squares regression (Wegmann *et al.* 2009). At least some algorithms of this type have been implemented in ABCtoolbox, but not in DIYABC or popABC. The added value of such algorithms in the context of complex models and large data sets remains, however, to be thoroughly tested. Second, if the number of invasive populations to be considered becomes too large, then the number of possible scenarios becomes too large to be treated in a single ABC treatment (e.g. a set of one native and six invasive populations corresponds to 6! = 720 different scenarios, in which each invasive population is successively derived from another invasive population without polychotomy or admixture). Historical information, like dates of first observation of the invasive populations, must be used in such cases, to reduce the number of possible scenarios. Historical information can also be used to define various nested subsets of competing invasion scenarios that are analysed sequentially. The first scenario considers the oldest invasive populations and determines their invasion routes. Step by step, subsequent analyses use the results obtained from the previous analyses, until the most recent populations are considered (see Lombaert *et al.* 2010 for an example).

All the indirect methods described earlier are subject to a major limitation: they take poorly into account the stochasticity of the demographic and genetic history considered. For a given set of demographic parameters, chance strongly affects the genetic composition of the samples studied, because (i) genetic drift affects the genetic composition of the source population; (ii) only part of the genetic variability of the source population is sampled during introduction; (iii) genetic bottlenecks often occur during the first few generations after introduction because of the limited number of founders and the small size of the newly founded population; (iv) mutational events occur at all stages in the introduction history of a species, and (v) field samples of populations for genetic analysis usually consist of a limited number of individuals for which only a small number of genetic loci are characterized (Muirhead *et al.* 2008). As a result, the level of stochasticity is usually high and is likely to have profound consequences: a single 'true' introduction history with a unique combination of genetic and demographic parameters may give rise to large numbers of potentially very different genetic compositions of the samples of the introduced populations collected (collection of a large number of different data

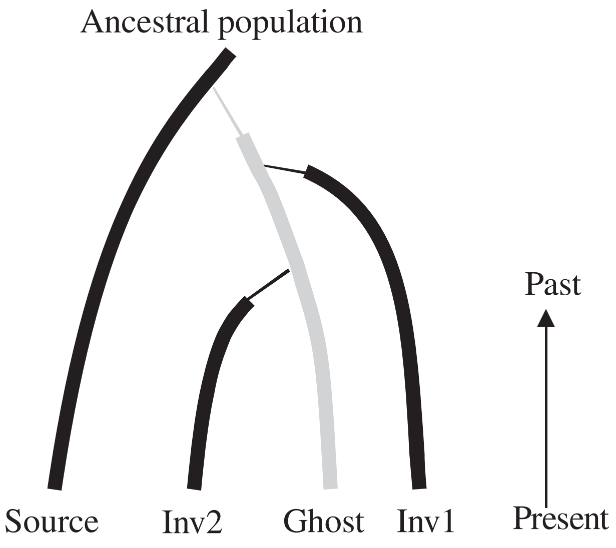


Fig. 4 Graphical representation of an invasion scenario in which an introduced ghost population, i.e. an unsampled population, derived from the source population, is the source of two invasive populations. The thin lines indicate the bottlenecks occurring in the first few generations following introductions. Genes were sampled in the source and invasive populations Inv1 and Inv2, but not in the ghost population. Considering scenarios with ghost populations, i.e. populations that participate in the invasion scenario but are not analysed, provides an opportunity to contrast scenarios with multiple introductions from the source in the native area with scenarios in which an unknown population already introduced into the invaded area is the source of various other invasive populations (bridgehead scenarios).

sets possible) and, reciprocally, different true introduction histories may produce similar data sets. This stochasticity hence limits the validity of the results obtained by the indirect genetic methods described earlier, for which there is no simple method for estimating the probability of error (e.g. Knowles & Maddison 2002).

A second general drawback of both direct and indirect methods is that they do not generally allow probabilistic estimations of competing introduction scenarios (but see e.g. Gaggiotti *et al.* 2004). In practice, a particular introduction scenario is chosen because the data (obtained directly or indirectly) are more consistent with that scenario than with other scenarios. The choice of introduction scenario is a binary decisional process (accept or reject) in which the likelihoods or weights of alternative scenarios are unknown. For instance, a tree based on genetic distances represents genetic relationships between populations (or haplotypes) that can be interpreted in terms of introduction sequences. It may be possible to calculate the likelihood of the tree topology and to carry out statistical tests against alternative tree topologies (Huelsenbeck & Crandall 1997), but it is not possible to determine the likelihood of the history suggested by the topology or the weighting of this history relative to other possible histories.

Despite the abovementioned limitations and those highlighted in Box 1, indirect methods have proved useful in many cases. If the putative source populations display substantial genetic differentiation, then reconstruction of the invasion scenario may be reasonably straightforward (Geller *et al.* 2010). The invasion of Florida by the Cuban lizard provides an example of the successful inference of invasion routes (Kolbe *et al.* 2004) made possible by the high degree of inter-population genetic differentiation and low levels of intra-population genetic variation in the source area. The extensive polymorphism found in Florida was interpreted as evidence for multiple introductions from Cuba. The invasion of America by the parthenogenetic snail *Melanoides tuberculata* is another case in which it was possible to retrace the invasion route by classical indirect methods (based on 12S and 16S mitochondrial DNA sequences; Facon *et al.* 2003). The native area of this freshwater snail contains several divergent, geographically structured clades with low levels of intra-population genetic variability, probably because of the clonal mode of reproduction in this snail. A large proportion of the genetic variability of the native area was found in the recently invaded New World, providing evidence for multiple introductions. Other convincing examples of the inference of invasion routes by indirect methods have been reported in other studies in which high levels of inter-population genetic variation are observed in the native area (e.g. Novak & Mack 2005; Roman 2006; Voisin *et al.* 2005).

Indirect model-based method based on approximate Bayesian computation

Approximate Bayesian computation (ABC; Beaumont *et al.* 2002) constitutes a new approach to carrying out model-based inference in a Bayesian setting in which model likelihoods are difficult to calculate (because of the complexity of the models considered) and must be estimated by massive simulations (see Bertorelle *et al.* 2010 and Csilléry *et al.* 2010 for recent reviews on theoretical and practical aspects of ABC, and Box 2 for further details on ABC in the context of inferences about invasion routes). The Bayesian nature of the method makes it possible to make use of prior historical, biological and genetic information about the system studied. Many ABC approaches treating genetic data are based on coalescent theory (Norborg 2001). Therefore, they take into account the stochasticity of the demographic and genetic processes involved. The ABC method can be used to calculate the relative probabilities of competing scenarios from a finite set of complex scenarios and hence choose one most likely scenario (see Fig. 3 in Box 2 for an illustration of competing scenarios traditionally considered in the context of biological invasions). Posterior distribution of demographic parameters of interest can be also estimated in a given scenario.

The ABC method has four main advantages over the more traditional indirect methods described earlier: (i) it uses all the data simultaneously in inference, unlike the methods based on trees, raw F_{st} or assignment likelihood values, (ii) it can be used to estimate probabilities, with confidence intervals for each of the scenarios compared (e.g. Cornuet *et al.* 2008), (iii) it can be used to evaluate the power for deciding between a set of invasion scenarios on the basis of controlled simulated data sets (Cornuet *et al.* 2008; Guillemaud *et al.* 2010), and (iv) it avoids the introduction of misleading biases, such as those because of nonsampled ghost populations (Guillemaud *et al.* 2010; Box 2) or genetic admixture between multiple sources (Lombaert *et al.* 2010), if included in the set of compared scenarios. The ABC method thus constitutes a real advance in the inference of invasion routes.

However, in addition to the limitations mentioned in Box 2, a number of difficulties in the application of this method remain in the context of inferences about invasion routes. For instance, it remains difficult to distinguish between a single introduction of a large number of individuals and repeated introductions of a small number of individuals originating from the same source population. It is also difficult to estimate some of the historical parameters of the invasion scenarios, such as recent dates of introduction. Finally, it should be stressed that use of the ABC method does not overcome the need to sample a large enough number of sites in both the native range and the range of introduction and to analyse these samples at a sufficiently large number of loci to be confident of obtaining a global picture of the population structure of the invasive species in both its native and invasive ranges. This general principle has been often neglected (Muirhead *et al.* 2008 but see Durka *et al.* 2005). Standard methods of population structure analysis, such as F_{st}-based analysis and/or STRUCTURE (Pritchard *et al.* 2000), BAPS (Corander *et al.* 2003) or GENELAND (Guillot *et al.* 2005) analyses, may be used for this purpose. In practice, population samples from the same population unit are pooled for subsequent inferences about invasion routes based on the ABC method (e.g. Lombaert *et al.* 2010; Pascual *et al.* 2007).

What molecular data have taught us about invasion routes?

Analyses of molecular markers to infer invasion routes have, in certain cases, confirmed previous suspicions based on historical observations. For instance, Pascual *et al.* (2007) retraced the history of the recent colonization of the New World by *D. subobscura* following its introduction from Europe. The most likely invasion route involves successive introductions, with a first founder event in South America, and then a second involving the introduction of the species into North America from South America. Moreover, Pascual *et al.* (2007) showed that the New World flies probably originated from the Mediterranean region (probably from the area around Barcelona). In other cases, indirect methods have generated unexpected results. Conclusions using mtDNA data about the sources of the invasion of California by the Atlantic amethyst gem clam *Gemma gemma* differ substantially from the historical records (Hoos *et al.* 2010). Two other examples of unexpected results regarding invasion routes, obtained with ABC methods, are detailed below (i.e. the invasion routes of the western corn rootworm *Diabrotica virgifera virgifera* and the Harlequin ladybeetle, *Harmonia axyridis*). In any case, indirect methods applied to genetic data helped to define general rules regarding invasions and to generate useful hypotheses regarding the environmental and evolutionary factors underlying biological invasions.

Multiple introductions

The examples of the Cuban lizard (Kolbe *et al.* 2004), the freshwater snail *M. tuberculata* (Facon *et al.* 2003), the shrub Scotch broom (Kang *et al.* 2007), the mosquito *Culex quiquefasciatus* (Fonseca *et al.* 2000), the amphipod *Gammarus tigrinus* (Kelly *et al.* 2006) and other cases reported elsewhere (Bossdorf *et al.* 2005; Roman & Darling 2007; Geller *et al.* 2010), together with the worldwide spread of *D. virgifera virgifera*, suggest that multiple introductions of invasive species might be a common phenomenon. This idea is not new in itself, but convincing demonstrations of this evolutionary scenario remained rare until recently. Until the 1980s, the western corn rootworm *D. virgifera virgifera* had a geographical range extending from Mexico to the east coast of North America. It was recently introduced into Europe, where it was first observed in 1992, near Belgrade, Serbia, and from where it rapidly spread. Several disconnected outbreaks of *D. virgifera virgifera* infestation have since been observed in Western Europe. The first of these outbreaks was detected in Venice in 1998, and further outbreaks have since been detected in northwest Italy and Switzerland in 2000, at two different sites in northeast Italy in 2002 and 2003, in northern Italy, eastern France, Switzerland, Belgium, the United Kingdom and the Netherlands in 2003, near Paris, France in 2002, 2004 and 2005 and, more recently, in Germany in 2007 (Ciosi *et al.* 2008). The explanation most frequently put forward for the distribution of the western corn rootworm in Europe was that the isolated outbreak populations were 'leap-frogging' out of the growing Eastern European population. Using the ABC framework presented in Box 2, Miller *et al.* (2005) showed that this hypothesis was very probably false and that at least three, and probably four disconnected Western European outbreaks had actually resulted from independent introductions from North America. It was not possible to reconstruct the routes of invasion for *D. virgifera virgifera* by classical indirect methods (Box 1), because the source population of all European outbreaks the northeastern American population is a single, genetically homogeneous population. In *D. virgifera virgifera*, one consequence of the multiple-introduction scenario is the transfer of genetic variation from the intra- to the inter-population level. This pattern is the inverse of that commonly observed for multiple introductions, which generally lead to there being more genetic variation, rather than less, within the areas of introduction (reviewed in Kolbe *et al.* 2004; Lavergne & Molofsky 2007; Lee 2002; Roman & Darling 2007). In the later cases, multiple introductions lead to a large genetic variation within the introduced area(s) which may favour adaptation to remote territories conditions and thus the settlement and spread of introduced propagules (Facon *et al.* 2006; Wares *et al.* 2005).

Invasive bridgehead effect

Recent studies of invasion routes have also suggested that a number of successful invasions have involved a specific evolutionary scenario that we call here the 'invasive bridgehead effect'. The concept of an 'invasion bridgehead' refers to widespread secondary invasions stemming from a particularly successful invasive population, which serves as a source of colonists for potentially remote new territories (Downie 2002; Floerl *et al.* 2009; Hanfling *et al.* 2002; Kolbe *et al.* 2004; Miller *et al.* 2005). The invasive bridgehead scenario is evolutionarily parsimonious: a single evolutionary shift in a single introduced population (the bridgehead) is required, whereas multiple changes are required in the case of multiple introduced populations, which must independently evolve traits conferring invasiveness. It is worth noting that the bridgehead population did not necessarily endured an important evolutionary shift as it could be (also) geographically favoured with respect to human vectors. Convincing demonstrations of such an invasive bridgehead effect remain scarce because (i) this scenario was only recently formalized (Lombaert *et al.* 2010), (ii) appropriate methods for reconstructing routes of invasion were previously lacking and (iii) too few invasive populations have been studied to capture the global picture of the worldwide invasion process for most species. The Harlequin ladybeetle, *H. axyridis*, whose native area is in Asia, is a worldwide invader that provides a striking illustration of the invasive bridgehead effect. This species has been used as a biological control agent against aphids since 1916 in North America, but only recently have remote established populations been observed. Invasive populations were first recorded in eastern North America in 1988 and in western North America in 1991. This beetle was then recorded in Europe and South America in 2001 and in South Africa in 2004 (reviewed in Lombaert *et al.* 2010). Using ABC methods applied to microsatellite and historical data, Lombaert *et al.* (2010) compared large sets of *H. axyridis* (HA) invasion scenarios covering all invaded areas, taking into account historical data (dates of first observation of the outbreaks and dates of initial collection of biocontrol strains) and all potential sources (native, older outbreaks and biocontrol), including genetic admixtures. The data and alternative models, analysed with DIYABC software (Cornuet *et al.* 2008), show that the recent sudden burst of worldwide invasions of HA outside its native Asian area have followed a bridgehead scenario, with the invasive population in eastern North America acting as the source of the colonists invading the European, South American and African continents. These analyses also showed that the HA population invading Europe was derived from an admixture between the bridgehead population and individuals used as biological control agents. The potential role of admixture with individuals used for biocontrol in Europe is unknown.

From an evolutionary biology perspective, the invasive bridgehead scenario is fundamentally different from scenarios in which the invasive populations originate directly from the source population in the native area (Guillemaud *et al.* 2010; Miller *et al.* 2005). It is therefore of crucial importance to distinguish between all these possible scenarios. One solution proposed by DIYABC (Cornuet *et al.* 2008) is the inclusion among the tested invasion routes of a scenario in which an unsampled bridgehead population acts as the source of the various invasive populations analysed (Box 2).

Conclusions and perspectives

For most invasive species, our knowledge about introduction pathways is based largely on historical and observational data, which are often sparse, incomplete and, sometimes, misleading. In this context, population genetics has proved to be a useful approach to the reconstruction of routes of introduction, highlighting the complexity of the real story and, in some cases, the extent to which it may be counterintuitive. The recent development of new model-based methods, such as ABC, has proved particularly useful and our ability to draw inferences about invasion routes is likely to increase further in the near future. The cost of producing large data sets of genetic markers (e.g. SNPs) using high-throughput methods is rapidly decreasing (Gupta 2008; Hert *et al.* 2008) and statistical methods for the efficient treatment of such data sets are being developed (Beaumont & Rannala 2004; Bertorelle *et al.* 2010; Csilléry *et al.* 2010). The conjunction of these two trends will soon make it possible to distinguish very precisely between invasion scenarios that are currently almost indistinguishable. For example, if several invasive populations originate from source populations with very low levels of differentiation, the use of tens of variable microsatellite markers is unlikely to be sufficient for the precise inference of introduction routes (e.g. the case of *D. virgifera virgifera* in the US Corn Belt (Ciosi *et al.* 2008)). The genotyping of individuals at hundred or thousand of markers should make it possible to identify the source of an invasion even if the levels of genetic differentiation are very low.

Elucidation of the routes of introduction of undesirable organisms is essential for the development of effective management strategies and sustainable science-based policies. It facilitates the design of strategies for preventing new accidental disseminations of alien organisms by promoting heightened vigilance (through specific monitoring and quarantine measures) against the identified key source populations, including invasive bridgehead populations (e.g. Sved *et al.* 2003; Miller *et al.* 2005; Rollins *et al.* 2009; Thibault *et al.* 2009; Lombaert *et al.* 2010). Knowledge of sources helps to define the ecological characteristics of introduced populations and hence predict the extent of ultimate distribution in the region of introduction (Kolar & Lodge 2001). Finally, a precise identification of the source population(s) within the native also aids in the choice of strains of auxiliary agents that can be used for biological control with the same geographical origin as the invasive population (Roderick & Navajas 2003). It is worth stressing, however, that the actual management outcomes of studies regarding invasion routes will largely depend on the suitability of the recommendations relative to other agenda (political, financial, operational ease, etc.).

Elucidation of the routes of introduction is also required for defining and testing useful hypotheses concerning the environmental and evolutionary factors underlying biological invasions. Once invasion routes have been inferred and hypotheses deduced, further laboratory-based studies can begin. Intensive quantitative genetics studies are required to test and to measure the key factors that may have evolved in response to natural selection during invasions (Facon *et al.* 2006; Keller & Taylor 2008). Some studies have convincingly demonstrated the occurrence of adaptive evolution after introduction for some traits, but there have been few demonstrations of the instrumental role of adaptation for such traits in the success of invasions (i.e. these studies have not demonstrated that the adaptation allowed the invasions). It generally remains unclear whether adaptation for the traits studied allowed the invasion, coincided with the invasion or was a consequence of the invasion (but see Lee *et al.* 2003). An approach allowing the comparison of the traits of introduced populations of a given species that invade and introduced populations of the same species that do not invade would facilitate identification of the environmental and/or evolutionary factors underlying successful invasion. In this context, species for which multiple introductions have occurred but for which only a subset of the introduced populations became invasive could prove to be the goose that laid the golden egg.

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