Molecular Ecology (2008) **17**, 773–782 doi: 10.1111/j.1365-294X.2007.03622.x

# Population genetics reveals origin and number of founders in a biological invasion

GENTILE FRANCESCO FICETOLA,*‡ AURELIE BONIN† and CLAUDE MIAUD*
*Laboratoire d'Ecologie Alpine, UMR CNRS 5553, Université de Savoie. 73376 Le Bourget du Lac cedex France, †Laboratoire d'Ecologie Alpine, UMR CNRS 5553, Université Joseph Fourier, 38041 Grenoble cedex 9, France*

**Abstract**

**Propagule pressure is considered the main determinant of success of biological invasions: when a large number of individuals are introduced into an area, the species is more likely to establish and become invasive. Nevertheless, precise data on propagule pressure exist only for a small sample of invasive species, usually voluntarily introduced. We studied the invasion of the American bullfrog, *Rana catesbeiana*, into Europe, a species that is considered a major cause of decline for native amphibians. For this major invader with scarce historical data, we used population genetics data (a partial sequence of the mitochondrial cytochrome *b* gene) to infer the invasion history and to estimate the number of founders of non-native populations. Based on differences between populations, at least six independent introductions from the native range occurred in Europe, followed by secondary translocations. Genetic diversity was strongly reduced in non-native populations, indicating a very strong bottleneck during colonization. We used simulations to estimate the precise number of founders and found that most non-native populations derive from less than six females. This capability of invasion from a very small number of propagules challenges usual management strategies; species with such ability should be identified at an early stage of introduction.**

*Keywords*: alien species, biological invasion, demography, invasion risk, mitochondrial DNA, propagule pressure, *Rana catesbeiana*

*Received 2 August 2007; revision accepted 26 October 2007*

## Introduction

Alien invasive species (AIS) are usually described as one of the main causes of biodiversity loss, as they can negatively affect native biota through predation, parasitism, exploitation of resources, and diffusion of diseases (Diamond 1989; Stewart 1991; Strayer *et al*. 2006). Understanding the factors that allow successful establishment of non-native species and subsequent invasions is a key task for conservation biology, and a necessary prerequisite for plans of prevention, screening controls, monitoring, and management (Hulme 2006; Strayer *et al*. 2006). Although several hypotheses have been proposed to explain differences in invasion success among species (Kolar & Lodge 2001; Rejmánek *et al*. 2005; Wilson *et al*. 2007), it has been difficult to find general patterns. Propagule pressure (i.e. the number of individuals introduced and/or the number of introductions performed) is one of the few factors over which a general consensus has been reached: when a high number of individuals are introduced and/or introductions occur on several occasions, the species is more likely to establish and become invasive, for both demographic and genetic reasons (Kolar & Lodge 2001; Lockwood *et al*. 2005; Von Holle & Simberloff 2005; Colautti *et al*. 2006).

Unfortunately, accurate information on propagule pressure is sparse, or exists only for those AIS voluntarily introduced. In animals, information is frequently limited to conspicuous vertebrates, mainly birds and mammals (Forsyth & Duncan 2001; Cassey *et al*. 2004), while for most species, only very rough data are available, if any (e.g. Jeschke & Strayer 2006). Moreover, historical data from which propagule pressure is estimated are sometimes inaccurate (Thulin *et al*. 2006), and the minimum propagule pressure leading to a successful establishment and invasion

Correspondence: G. F. Ficetola, Fax: +33-(0)479758880; E-mail: francesco.ficetola@unimi.it
‡Present address: Dipartimento di Scienze dell'Ambiente e del Territorio, Università di Milano Bicocca. Piazza della Scienza 1, 20126 Milano Italy.

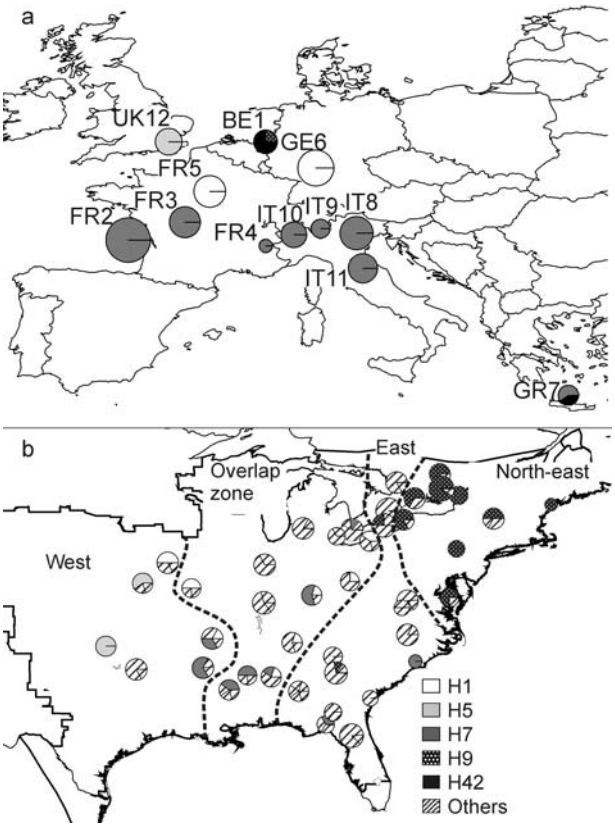


**Fig. 1** Geographical distribution and haplotypes observed in bullfrog populations sampled within the non-native (a) and native range (b). The size of circles is proportional to the number of individuals sampled per population (from 1 to 37). Data for North America are from Austin *et al*. (2004). The native range is divided in the four geographical groups individuated by the nested clade analysis (Austin *et al*. 2004). Note that the native population monomorphic for haplotype H7 was so because only one individual was sampled in this population.

(thereafter, *critical pressure*) varies strongly among taxa. These differences have been explained on the basis of features such as life-history traits and ecology (Forsyth & Duncan 2001; Kolar & Lodge 2001). It is therefore difficult to extrapolate the critical pressure of taxa for which records are not available. For such animals, experiments can be performed to estimate the critical pressure (Berggren 2001; Memmott *et al*. 2005), but these experiments cannot cover the complexity of conditions encountered during real invasions.

Even in the absence of historical data, population genetics has the potential to trace the demographic history that determined the genetic diversity of populations. In the context of biological invasions, it is therefore possible to specify the number of founders, a very important component of propagule pressure, as well as other important features such as the geographical origin of invaders and secondary translocations and, thus, to delineate the scenario of invasions.

Here, we studied the American bullfrog, *Rana catesbeiana* Shaw, 1802 (= *Lithobathes catesbeianus*), which is invasive in many European countries and is a major cause of native amphibian decline (Lever 2003; Ficetola *et al*. 2007c). We used extensive genetic data on both native and non-native ranges to assess the history of invasion and to infer the propagule pressure for a major invader for which historical information is missing. We determined the origin of each founding population and the number of distinct founding events. Moreover, we estimated the number of founders of currently invasive populations. Our results show that invasive populations of bullfrog in Europe descend from a handful of founders. The number of founders of invasive populations can be very small and can challenge the monitoring and biological control commonly set up for invaders.

## Methods

### *Study species and samples*

The American bullfrog is native to western North America, but has been introduced in about 40 countries over four continents since the 19th century (Lever 2003). It is considered among one of the 'hundred world's worst invasive species', since it is responsible for the decline of native amphibians by direct predation, competition, spread of diseases and complex biotic interactions (Lowe *et al*. 2000; Blaustein & Kiesecker 2002; Kats & Ferrer 2003; Garner *et al*. 2006). In Europe, bullfrog has been introduced in at least eight countries and naturalized populations are present in Belgium, France, Germany, Greece, and Italy (Ficetola *et al*. 2007a). We analysed mitochondrial DNA from samples from all European areas where populations were present in 2005–2006 (nine populations), and from three localities in France, the UK, and Italy where *Rana catesbeiana* is now extinct (Fig. 1; Table 1). Our data set was completed with data on 42 bullfrog populations covering the entire native range (Austin *et al*. 2004) and included sequences from 397 individuals.

### *DNA analysis*

For each sample, genomic DNA was extracted from tail (tadpoles), finger or skin (adults) with the DNeasy Tissue Kit (QIAGEN) following the manufacturer's instructions. We studied a 591-bp fragment corresponding to the first to the 591st nucleotide of the published complete sequence of cytochrome *b* of *R. catesbeiana* (GenBank Accession no. AF205089). This fragment includes the 408-bp fragment previously sequenced by Austin *et al*. (2004) to investigate the phylogeography of the species in its native range.

**Table 1** Populations of *Rana catesbeiana* sampled in Europe

| Site code | Country | Locality | Number of sampled individuals | Number of haplotypes | π (nucleotide diversity) ± SE | $H_d$ (haplotype diversity) ± SD |
|---|---|---|---|---|---|---|
| BE1 | Belgium | Geel, Antwerpen | 8 | 2 | 0.0028 ± 0.0019 | 0.429 ± 0.169 |
| FR2 | France | Gironde | 37 | 1 | 0 ± 0 | 0 ± 0 |
| FR3 | France | Dordogne | 16 | 1 | 0 ± 0 | 0 ± 0 |
| FR4 | France | *Savoie | 1 | 1 | 0 ± 0 | 0 ± 0 |
| FR5 | France | Loir-et-Cher | 17 | 1 | 0 ± 0 | 0 ± 0 |
| GE6 | Germany | Baden-Württemberg | 23 | 1 | 0 ± 0 | 0 ± 0 |
| GR7 | Greece | Crete | 5 | 2 | 0.0082 ± 0.0045 | 0.600 ± 0.175 |
| IT8 | Italy | River Po Lowland | 19 | 1 | 0 ± 0 | 0 ± 0 |
| IT9 | Italy | *Cameri, Piedmont | 4 | 1 | 0 ± 0 | 0 ± 0 |
| IT10 | Italy | Valfenera, Piedmont | 19 | 1 | 0 ± 0 | 0 ± 0 |
| IT11 | Italy | Tuscany | 14 | 1 | 0 ± 0 | 0 ± 0 |
| UK12 | U.K. | *Kent-Sussex border | 11 | 1 | 0 ± 0 | 0 ± 0 |

*Currently extinct population.

The polymerase chain reaction (PCR) amplifications were performed with primers 5′-AATGGCCCACACAATACG-3′ and 5′- GTGGATCATACTTGCTGC-3′ especially designed for these experiments using OLIGO 4.0 (National Biosciences). The PCRs were conducted in a 25-μL total volume with 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2.5 mM MgCl2, 0.8 μM of each primer, BSA (5 μg), 0.5 U of Ampli*Taq* Gold DNA polymerase (Applied Biosystems) and 10–30 ng of DNA. The PCR programme included an initial 10-min denaturation step at 95 °C; 45 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 40 s and elongation at 72 °C for 40 s; followed by a final extension step at 72 °C for 5 min. PCR products were purified with the QIAquick PCR purification kit (QIAGEN) and sequencing reactions were performed in both directions with the amplification primers and the BigDye Terminator Cycle Sequencing Kit version 1.1 (Applied Biosystems) following the manufacturer's instructions. Fluorescently labelled sequencing products were analysed on an ABI PRISM 3100 capillary DNA sequencer (Applied Biosystems) and corrected with SEQSCAPE version 2.0 (Applied Biosystems).

### *Data analysis*

We used the program TCS 1.21 (Clement *et al*. 2000) to build the 95% statistical parsimony cladogram network and to visualize the phylogenetic relationships among haplotypes. To build the network, we used only the 408 bp sequenced for all the native individuals, because the results can be unreliable when sequences have different lengths. We calculated $\phi_{ST}$ values as measures of pairwise differences between introduced populations using ARLEQUIN 3.01 (Excoffier *et al*. 2005). Significances of pairwise $\phi_{ST}$ values were estimated by performing 10 000 permutations. The absolute values of $\phi_{ST}$ might be inaccurate, because of the violation of assumptions such as equilibrium. However, they can be useful for the comparison of different pairs of populations. We also estimated the nucleotide diversity (π) and the haplotype diversity ($H_d$) for every native and introduced population.

We used two methods to determine the most likely area of origin of introduced European populations within the native range of *R. catesbeiana.* First, the source areas were inferred on the basis of the geographical distribution of haplotypes in native populations, and of the phylogeographical relationships among haplotypes. Moreover, we used analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) to estimate the amount of genetic covariation among introduced and native populations (May *et al*. 2006). AMOVA allows to measure the genetic differentiation among groups of populations, and is thus a more objective approach for the identification of source areas (May *et al*. 2006), but requires the a priori division of the native range into meaningful areas. The native range of *R. catesbeiana* was thus divided in four homogeneous areas, based on the geographical distribution of cyt *b* clade groups previously identified using the nested clade analysis (Austin *et al*. 2004). These four areas were the western group (clade 3–1 and subclades 2–1 and 2–2; native populations 19, 20, 21, 31, 32, 34 in Austin *et al*. 2004), the eastern group (clade 3–2 and subclades 2–3 and 2–5; populations 5, 7, 9, 25, 26, 28, 35, 36, 37, 38, 39, 40, 41, 42), the northeastern group (presence of the subclade 2–5 only within the clade 3–2; populations 1, 2, 3, 4, 6, 15, 16, 17, 24), and the area of overlap between western and eastern haplotypes (contemporary presence of haplotypes from both clades 3–1 and 3–2, and several different subclades; populations 8, 10, 11, 12, 13, 14, 18, 22, 23, 27, 29, 30, 33). The genetic substructuring within these

four groups was scarce. The location of these four groups is depicted in Fig. 1(b); see also Fig. 8 in Austin *et al*. (2004).

AMOVAS were performed with ARLEQUIN 3.01 (Excoffier *et al*. 2005). European populations that likely arose from the same introduction event inferred from sequence data were grouped together. Then, we assessed the percentage of molecular covariation between each European group and each native group, among populations within groups and within populations. Significance was tested by carrying out 10 000 permutations. For the three European groups consisting of only one population (see Results), significance of covariation among groups was not calculated, because the power of AMOVA is very low when a group includes only one population.

### *Simulating the effect of founding population size*

We used individual-based simulations to track the persistence vs. stochastic loss of mtDNA haplotypes during the demographic growth of populations after an introduction. Introduced populations were assumed to come from a parental population situated in the area of origin previously identified and possessing the same frequency of haplotypes (Vincek *et al*. 1997). This analysis was performed twice. In a first analysis, we assumed that the parental population had the same allele frequencies of the source area identified by AMOVA. In a second analysis, we created an ad hoc group including all native populations where the invasive haplotype was present (Fig. 1), and we averaged allele frequencies across these populations. The starting frequencies of haplotypes where remarkably different between the two parental populations used. This allowed us also to evaluate the robustness of our results to the estimation of area of origin and to the haplotype frequencies in an unknown parental population.

From the parental population, $N$ haplotypes were drawn randomly, and we simulated the growth of a population originating from $N$ females for $M$ generations. At the end of the simulations, the number of haplotypes retained in the population was determined. This procedure was repeated 500 times for each combination of demographic parameters of the introduced populations. Then, we built a 95% confidence interval of the number of retained haplotypes and compared the results of simulations with the number of haplotypes observed in real populations to determine which combinations of demographic parameters produced results corresponding to the observed patterns. Simulations were run using the package RMETASIM 1.08 (Strand 2002) under the R 2.2 environment (www.r-project.org). We considered the following demographic parameters: (i) number of introduced females (values tested: 2, 4, 6, 8, 10, 20); (ii) growth rate of the introduced populations (λ, values tested: 1.5, 2.0, 2.5, 3.0); (iii) number of generations (values tested: 6, 9, 12, 18).

The values for λ may seem very large for free-ranging populations; however, they are plausible during the first stages of the invasion of bullfrog in Europe. For example, during the invasion in Italy, the range of *R. catesbeiana* expanded from a single ditch to some 6500 km² in about nine generations (Albertini 1970; Albertini & Lanza 1987). Similarly, in France, the range expanded from a single pond to about 2000 km² in six generations (Ficetola *et al*. 2007a). Analogous values for λ are commonly found in populations of unmanaged invasive animals (Hansen & Wilson 2006).

The number of generations can be estimated based on generation time (GT). In both the French and Italian populations, tadpoles develop in 2 years (Albertini & Lanza 1987; Detaint & Coïc 2006), and we assumed that adult demographic traits are close to those observed in North American populations having similar larval development, such as in Illinois, Missouri, and British Columbia (Durham & Bennett 1963; Schroeder & Baskett 1968; Govindarajulu *et al*. 2005). The GT of females can be estimated as the mean age of females, that is (age at maturity + longevity)/2, or, if life tables are available (with survival and reproductive rates), as:

$$GT = \sum x.lx.mx / R0$$

where $x$ = age, $lx$ = number of individuals alive at age $x$, $mx$ = fecundity at age $x$, and $R0$ = population replacement rate (Caughley 1977). Based on data reported in the above studies, we estimated GT = 6 years for both French and Italian populations. French bullfrogs were introduced in 1968 (Ficetola *et al*. 2007a), while Italian bullfrogs were introduced around 1935 (Albertini 1970); therefore, about 6 and 12 generations have occurred after introduction, respectively. To evaluate the robustness of the results to variation in the estimation of GT, we also performed simulations assuming GT = 4 years. This corresponds to the age at maturity of females, and should be viewed as a lower limit for GT. Assuming GT = 4 years, 9 and 18 generations from introduction occurred for French and Italian bullfrogs, respectively. To avoid outgrowing computational resources and an unrealistic unlimited growth of populations, we assumed a carrying capacity of 10 000 females per population. Preliminary trials assuming different carrying capacities yielded identical results.

## Results

The analysis of mitochondrial DNA revealed the presence of five different haplotypes in the 12 introduced European populations (Fig. 1, Table 1), while 41 haplotypes were present in the 42 native populations (Austin *et al*. 2004). Four European haplotypes were identical to haplotypes

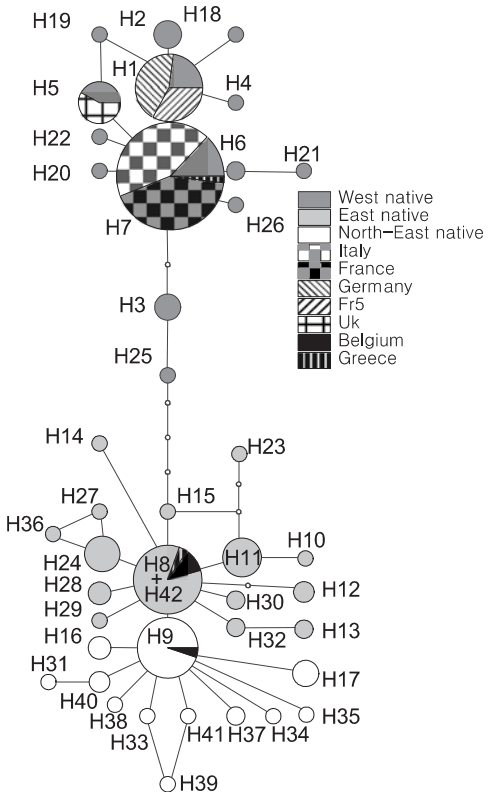


**Fig. 2** Ninety-five per cent statistical parsimony haplotype network. Circle size is proportional to the number of individuals (from 1 to 130). Each line represents a single mutation; empty circles represent nonsampled or extinct haplotypes.

previously identified in native populations (H1, H5, H7, and H9). A fifth haplotype (H42, GenBank Accession no. EF221759) was found in eight individuals from introduced populations of Belgium and Greece, but was not reported in native populations (Fig. 1). In most introduced populations, we found a single haplotype. Only haplotype H7 was detected in all Italian populations and three French populations. In the German population and the French population FR5, we identified only haplotype H1, and in the extinct UK population, only haplotype H5. We found two haplotypes in both the Belgian and Greek populations (Fig. 1).

The haplotype H7, found in Italy, France, and Greece, was detected in nine native populations, mainly in the southwest of the native distribution (Fig. 1). The haplotype found in Germany and in population FR5 was detected in eight native populations, mainly in the west and in the central part of native distribution. The haplotype found in UK was detected in two populations at the western limit of the specie's range. The haplotype H9 found in Belgium was detected in 11 populations, mainly in the northeastern range of the specie (Fig. 1).

The haplotype network (Fig. 2) showed two main groups: the first one included haplotypes found mainly in the west of the native distribution, and the second one haplotypes found mainly in the east of the distribution. A subgroup within the Eastern group represented the haplotypes found mainly at the northeastern limit of the species' range (Fig. 2). Within the network, all haplotypes found in the populations of France, Italy and UK clustered in the group of western haplotypes, while the haplotypes found in Belgium clustered with the eastern and the northeastern haplotypes. One of the two haplotypes found in Greece clustered with the western haplotypes, while the second one clustered in the eastern group (Fig. 2). For the 408 bp used to build the network, the haplotype H42 (Belgium and Greece) was identical to the Eastern haplotype H8. However, these two haplotypes differed in three positions in subsequent portions of the sequence. See Austin *et al*. (2004) for a complete discussion of phylogeographical relationships among haplogroups and for a nested clade analysis.

The number of haplotypes per population was remarkably lower in introduced than in native populations, although sample size was generally larger for introduced populations. In almost all native populations where more than a single individual was sampled, at least two haplotypes were found. Nucleotide diversity ($\pi$) of European populations (range: 0–0.0082; mean = 0.001; median = 0) was significantly lower than that of native populations (range: 0–0.0106; mean = 0.0036; median = 0.0021) (Mann–Whitney $U_{11,40} = 82.0$, $P = 0.001$; populations where only one individual was sampled were excluded from the analysis).

Pairwise $\phi_{ST}$ values (Table 2) confirm that all Italian and three French populations were genetically homogeneous and distinct from all other European populations. The French population from Loir-et-Cher and the German population constituted a second homogeneous subset, while the Belgian, the UK, and the Greek populations were genetically distinct and distinct from all other populations. The pairwise $\phi_{ST}$ values between the Greek population and the populations FR4 and IT9 were not significant (Table 2); however, this was probably due to the small number of individuals available for these two latter populations, which are now extinct (Table 1).

This pattern suggests that at least five independent bullfrog introductions occurred in Europe. However, as the available historical records report that Italian and French populations originated from independent importations from the USA (Ficetola *et al*. 2007a), we considered them as distinct introductions. In absence of any detail about the circumstances of importation, we clustered FR5 and the German populations together for subsequent analyses, based on their genetic similarity. As a result, we assumed six independent introductions: Italy; France (excluding FR5); FR5 + Germany; Belgium; Greece, and the UK (Table 3).

**Table 2** Pairwise $\phi_{ST}$ (lower triangle) and associated *P* values (upper triangle) between introduced populations

| | BE1 | FR2 | FR3 | FR4 | FR5 | DE6 | GR7 | IT8 | IT9 | IT10 | IT11 | UK12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BE1 | | **< 0.0001** | **< 0.0001** | **< 0.0001** | **< 0.0001** | **< 0.0001** | **< 0.0001** | **< 0.0001** | **< 0.0001** | **< 0.0001** | **< 0.0001** | **< 0.0001** |
| FR2 | 0.944 | | 0.999 | 0.999 | **< 0.0001** | **< 0.0001** | **< 0.0001** | 0.999 | 0.999 | 0.999 | 0.999 | **< 0.0001** |
| FR3 | 0.919 | 0 | | 0.999 | **< 0.0001** | **< 0.0001** | **< 0.0001** | 0.999 | 0.999 | 0.999 | 0.999 | **< 0.0001** |
| FR4 | 0.852 | 0 | 0 | | **< 0.0001** | **< 0.0001** | 0.08 | 0.999 | 0.999 | 0.999 | 0.999 | **< 0.0001** |
| FR5 | 0.940 | 1 | 1 | 1 | | 0.999 | **< 0.0001** | **< 0.0001** | **< 0.0001** | **< 0.0001** | **< 0.0001** | **< 0.0001** |
| DE6 | 0.954 | 1 | 1 | 1 | 0 | | **< 0.0001** | **< 0.0001** | **< 0.0001** | **< 0.0001** | **< 0.0001** | **< 0.0001** |
| GR7 | 0.527 | 0.606 | 0.486 | 0.222 | 0.674 | 0.741 | | **< 0.0001** | 0.283 | **0.0004** | **< 0.0001** | **< 0.0001** |
| IT8 | 0.899 | 0 | 0 | 0 | 1 | 1 | 0.405 | | 0.999 | 0.999 | 0.999 | **< 0.0001** |
| IT9 | 0.846 | 0 | 0 | 0 | 1 | 1 | 0.175 | 0 | | 0.999 | 0.999 | **< 0.0001** |
| IT10 | 0.868 | 0 | 0 | 0 | 1 | 1 | 0.295 | 0 | 0 | | 0.999 | **< 0.0001** |
| IT11 | 0.899 | 0 | 0 | 0 | 1 | 1 | 0.403 | 0 | 0 | 0 | | **< 0.0001** |
| UK12 | 0.944 | 1 | 1 | 1 | 1 | 1 | 0.740 | 1 | 1 | 1 | 1 | |

The pairwise $\phi_{ST}$ values significant after Bonferroni correction (66 comparisons, $P < 0.00076$) appear in bold.

**Table 3** Results of AMOVAs evaluating the amount of genetic covariance between groups of native and introduced populations. The most likely area(s) of origin for each European group is indicated in bold. The significance of covariation was not estimated for groups including only one population (Belgium, Greece, and UK). The distribution of non-native and native groups is shown in Fig. 1. The amount of covariation among populations within groups and within populations was also calculated, but is not shown for brevity

| | | Native groups | | | |
|---|---|---|---|---|---|
| Non-native groups | | Northeast | East | Overlap zone | West |
| Belgium | percentage covariation | 58.33 | **0** | 3.01 | 64.70 |
| France (FR2 + FR3 + FR4) | percentage covariation | 96.45 | 75.30 | 29.59 | **0** |
| | *P* | 0.0046 | 0.0026 | 0.003 | **0.890** |
| Germany + FR5 | percentage covariation | 95.29 | 78.62 | 36.41 | **15.03** |
| | *P* | 0.017 | 0.009 | 0.011 | **0.255** |
| Greece | percentage covariation | 86.08 | 42.01 | 0 | **0** |
| Italy (4 pop.) | percentage covariation | 95.87 | 74.97 | 30.02 | **0** |
| | *P* | 0.0009 | 0.0012 | 0.0004 | **0.785** |
| UK | percentage covariation | 96.30 | 76.67 | 33.86 | **0** |

These introductions were subsequently followed by secondary translocations of individuals from successfully established populations, which considerably expanded the non-native range (Ficetola *et al*. 2007a). Considering FR5 and Germany as two independent introductions did not change the qualitative results of the analyses (data not shown).

Within the native range, most genetic covariance was distributed among the four geographical groups detected using the nested clade analysis (Austin *et al*. 2004) and depicted in Fig. 1 (AMOVA: percentage of covariation among groups = 46.01%, $P < 0.0001$). Covariation was significant also among populations within group (26.40% of covariation, $P < 0.0001$) and within populations (27.59%, $P < 0.0001$). These results confirm that such a division of the native range is meaningful.

AMOVAs between native and introduced groups revealed low levels of genetic covariance between most European populations and western native populations (Table 3). The French, Italian, German, and UK populations thus probably originated from the western part of the native range. The Belgian and Greek populations showed little genetic covariation with two different areas within the native range (Belgian: East and overlap zone; Greek: West and overlap zone; see Table 3); therefore, the origin of these introduced populations is still unresolved.

### *Estimating the number of founders*

By reproducing the demographic conditions most likely to have produced the observed genetic features of non-native populations (Hoelzel *et al*. 1993; Vincek *et al*. 1997), we simulated the effect of founding population size on the maintenance of haplotypes during introductions. We performed these simulations to estimate the number of founders for the French (FR2-FR4) and Italian (IT8-IT11) populations. For both groups, the number of sampled individuals was large (≥ 54), which probably allowed accurate estimation of the number of retained haplotypes.

In our simulations, the number of haplotypes retained in the introduced populations clearly increased with the number of introduced females. Assuming a given number

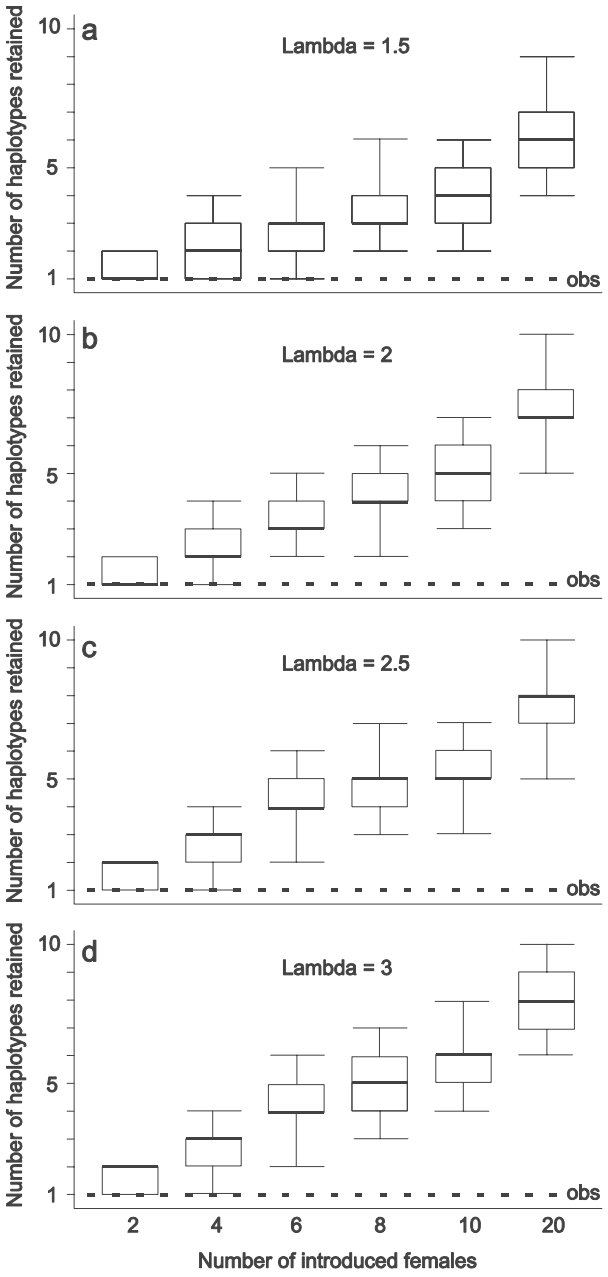


**Fig. 3** Number of haplotypes retained after six generations as a function of the number of introduced females for different values of the growth rate (Lambda): results of simulations. The bold lines represent the median, the boxes include the second and the third quartile of simulation results; the error bars represent 95% CI. The dashed line represents the number of haplotypes observed in populations of Southern France (FR2 + FR3 + FR4).

of introduced females, the number of haplotypes retained in the population slightly increased at high values of the population growth coefficient (λ), as a consequence of reduced genetic drift. Nevertheless, simulations assuming different values of λ showed small differences for the number of haplotypes retained, suggesting that our results are robust to variations of λ (Fig. 3; Fig S1, Supplementary material).

To simulate the introduction in France, we assumed that the parental population originated in the western area of the native range and we allowed the simulated population to grow for six generations, as the population was introduced in 1968. We found only a single haplotype in the French group (Table 1). According to simulations, assuming $\lambda = 2$ or 2.5 (see below for justification), an introduced population may retain only one single haplotype if the number of founding females is less than six (Fig. 3). If six or more females are introduced, at least two haplotypes will be retained after six generations and, in most simulations, this number reaches three or more (Fig. 3). Larger numbers of introduced females produce outcomes that are completely different from the observed pattern. For example, if 20 females are introduced, the number of haplotypes retained will always be equal to or greater than five. The real value for λ is certainly larger than 1.5, the lower value used in simulations. For example, assuming $\lambda = 1.5$, with a sex ratio of 1:1, and six introduced females, 140 adults would be present after six generations (such as in France). The exact number of bullfrogs present in France is unknown, but estimated to be several thousand adults (M. Detaint, personal communication); therefore, λ is very likely greater than two.

Our results were robust also in regard to variations of the number of simulated generations. The results obtained assuming 9, 12 (such as in the Italian populations), and 18 generations were virtually identical (Fig. S1). Therefore, the situation observed in Italy (only one haplotype, Table 1) is similar to the French. Our results were also robust in regard to the estimation of the source area and of the frequency of haplotypes in native populations, as simulations performed assuming a different parental population (see Methods) yielded almost identical results (Fig. S2, Supplementary material).

## Discussion

We found an extremely low genetic diversity in invasive bullfrog populations. Such low genetic diversity is possible if these populations originated from a very small pool of founders, less than six females presenting the most likely demographic parameters. Techniques analogous to those used in this study have been used successfully to estimate the number of founders for insular populations and to investigate historical bottlenecks (Hoelzel *et al*. 1993; Vincek *et al*. 1997), but this is the first instance in which they have been applied to invasive species. The reliability of our results is strengthened by the historical information available. Early records report that the Italian invasive populations descend from just two females and one male introduced during the 1930s. These figures are in agreement

with our results, but generally they have been considered impossible and a larger propagule pressure, either for the number of founders or for the number of introductions performed, has been presumed (Albertini 1970; Lever 2003; Ferri 2006). It is very unlikely that the reduced genetic diversity of invasive populations arose because the source was monomorphic, as high haplotype diversity was found in all native populations that where not at the limit of the species' range and where more than one individual was sampled (Fig. 1), and the haplotype observed in Italy and France was absent at the limit of the native range. Moreover, our results are very robust to variations in demographic parameters; therefore, they are likely applicable not only to the two European areas where the major bullfrog invasion is ongoing (France and Italy), but also to other invasive populations where only one haplotype is present (Germany and the UK). This multiple evidence means that an invasion originated by a handful of founders cannot be considered as an unfortunate, anecdotic event, but might, instead, be a rather frequent pattern of invasion for this species. A potential caveat on the use of simulations is that they assume a continuous expansion of the population, since the introduction. This is probably the case of bullfrogs, which immediately expanded from the localities of introduction (Albertini 1970). However, other species maintain a small population size for several generations before the rapid invasions (Crooks & Soulé 1999). In these cases, this complexity should be considered in the estimation of past demography.

A reduced genetic variability is usually thought to affect negatively individual fitness and the capacity of populations to adapt to a novel environment (Reed & Frankham 2003; Schmitt & Hewitt 2004; Reusch *et al*. 2005). For example, in introduced birds, high hatch failure has been detected in populations founded by less than 150 individuals, and this has been attributed to the negative effect of inbreeding depression (Briskie & Mackintosh 2004). However, bullfrogs seem capable of successful expansion and invasion even if their genetic diversity is very low (i.e. only a single haplotype present). In amphibians with similar demographic traits, population dynamics are regulated more by survival after metamorphosis (metamorphs and adults) than by larval survival (Vonesh & De la Cruz 2002; Govindarajulu *et al*. 2005). Bullfrogs have a very high fertility (average clutch size > 13 000 eggs; Govindarajulu *et al*. 2005); thus, invasive populations can quickly overcome demographic bottlenecks. Moreover, in species with large fertility and in populations with high growth rate, selection has more potential to purge inbreeding depression (Wang 2000; Miller & Hedrick 2001; Ficetola *et al*. 2007b).

Several non-native populations originated from independent introduction events, as there are strong genetic differences among them (Table 2). Therefore, if new introductions are performed in the future, or if bullfrogs are translocated across European countries, populations coming from different native sources areas can be mixed. In turn, mixing different non-native populations might increase their genetic diversity and the possibility of post-introduction recombination. This can enhance their potential to adapt to new conditions, boosting invasiveness (Kolbe *et al*. 2004; Lockwood *et al*. 2005; Lavergne & Molofsky 2007). Bullfrogs are already invasive despite low genetic variability. It is therefore imperative to halt both new introductions from the native range and translocations of individuals across European countries. While regulations on AIS usually hamper new introductions, intraboundary translocations are frequently overlooked (McGeoch *et al*. 2006) and the negative effects of mixing different non-native stocks are rarely considered. For example, the introduction of bullfrogs is forbidden within the European Union, but to date, translocations within Europe are not regulated. In regulating problematic AIS, it is thus urgent to include not only a ban on importation, but also an interdiction of translocation between areas within the non-native range.

It is generally accepted that establishment and invasion risk increases with large propagule pressures (Colautti *et al*. 2006). This could lead to the assumption that the introduction of a few individuals will not pose a risk. Similar reasoning may occur during eradication programmes. Eradication can be proportionally more expensive and difficult when individuals of an AIS become rare or of low density, and it might be argued that if an eradication effort can drive the population size of AIS down, the population will disappear. Clearly, however, species capable of colonization and invasion in spite of very small propagule pressure represent a challenge to management practices intended to control their introduction or to remove them from non-native environments. For example, if during the ongoing bullfrog eradication (Detaint & Coïc 2006), just a handful of adults remain, a new invasion is highly probable in a few generations, rendering previous management action ineffective. Bullfrogs are an emblematic species, but probably not the only one with the ability to establish invasive populations from a handful of founders. We suspect that other major invaders share this feature. It is therefore a priority for conservation to distinguish species that can become invaders in spite of very small propagule pressure so that special care may be devoted to their management, as traditional practices may not suffice for their control.

Populations of invasive species are sometimes treated as demographic and genetic black boxes, and the early stages of invasions are the least known, although they are crucial for subsequent stages of the invasion process (Lee 2002; Puth & Post 2005). Population genetics can help to fill this gap (Estoup *et al*. 2001; May *et al*. 2006; Thulin *et al*. 2006) and improve both the understanding of invasion processes and decision making in management. In studies evaluating which factors influence invasion success, the relationship

between propagule pressure and likelihood of establishment can be considered a null model over which evaluating the relative invasiveness of different species (Suarez *et al*. 2005; Colautti *et al*. 2006). Genetic tools can provide unbiased estimates of propagule size, even in absence of direct information. Moreover, for management practice, genetics can provide figures as a target for conservation plans; for example, if during eradications two to five females remain in the field, a very high risk of a new invasion exists. These figures can be more helpful than simple qualitative estimates which suggest the capture of as many individuals as possible.

## Acknowledgements

We thank T. J. C. Beebee, T. Garner, D. Seglie, R. Jooris, P. Lymberakis, S. Scali, A. Nistra, S. Mazzotti, H. Laufer, C. Coïc, M. Detaint, M. Berronneau, T. Dejean, C. Epain-Henry for providing specimens. We also thank the Museum of Natural History of Milan, Ferrara, Genoa, and Leiden, and the Museum of Zoology of Florence for providing samples from their collections. We are also grateful to J. Prunier and C. Miquel for laboratory assistance, to J. D. Austin for helpful information and to O. Gaggiotti, W. Thuiller, P. Taberlet, S. Lavergne and one anonymous reviewer for constructive discussion. G.F.F. was funded by a postdoctoral grant of the French Ministry for Research for young foreign researchers.

## References

Albertini G (1970) Sulla diffusione della rana toro (*Rana catesbeiana* Shaw) importata nel mantovano. *Atti E Memorie Della Accademia Di Agricoltura Scienze E Lettere Di Verona*, **20** (1968–69), 67–106.

Albertini G, Lanza B (1987) *Rana catesbeiana* Shaw, 1802 in Italy. *Alytes*, **6**, 117–129.

Austin JD, Lougheed SC, Boag PT (2004) Discordant temporal and geographic patterns in maternal lineages of eastern north American frogs, *Rana catesbeiana* (Ranidae) and *Pseudacris crucifer* (Hylidae). *Molecular Phylogenetics and Evolution*, **32**, 799–816.

Berggren A (2001) Colonization success in Roesel's Bush-Cricket *Metrioptera roeseli*: the effects of propagule size. *Ecology*, **82**, 274–280.

Blaustein AR, Kiesecker JM (2002) Complexity in conservation: lessons from the global decline of amphibian populations. *Ecology Letters*, **5**, 597–608.

Briskie JV, Mackintosh M (2004) Hatchling failure increases with severity of population bottlenecks in birds. *Proceedings of the National Academy of Sciences, USA*, **101**, 558–561.

Cassey P, Blackburn TM, Sol D, Duncan RP, Lockwood JL (2004) Global patterns of introduction effort and establishment success in birds. *Biology Letters*. doi: 10.1098/rsbl.2004.0199.

Caughley G (1977) *Analysis of Vertebrate Populations*. John Wiley & sons, New York.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1660.

Colautti RI, Grigorovich IA, MacIsaac HG (2006) Propagule pressure: a null model for biological invasions. *Biological Invasions*, **8**, 1023–1037.

Crooks J, Soulé ME (1999) Lag times in population explosions of invasive species: causes and implications. In: *Invasive Species and Biodiversity Management* (eds Sandlund OT, Schei SJ, Viken A), pp. 103–125. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Detaint M, Coïc C (2006) La Grenouille taureau *Rana catesbeiana* dans le sud-ouest de la France. Premiers résultats du programme de lutte. *Bulletin de la Société Herpetologique de France*, **117**, 41–56.

Diamond J (1989) Overview of recent extinctions. In: *Conservation for the Twenty-First Century* (eds Western D, Pearl MC), pp. 37–41. Oxford University Press, Oxford, UK.

Durham L, Bennett GW (1963) Age, growth, and homing in the bullfrogs. *Journal of Wildlife Management*, **27**, 107–123.

Estoup A, Wilson IJ, Sullivan C, Cornuet JM, Moritz C (2001) Inferring population history from microsatellite and enzyme data in serially introduced cane toads, *Bufo marinus*. *Genetics*, **159**, 1671–1687.

Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Excoffier L, Laval G, Schneider S (2005) ARLEQUIN ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Ferri V (2006) *Rana catesbeiana* Shaw, 1802. In: *Atlas of Italian Amphibians and Reptiles* (eds Sindaco R, Doria G, Razzetti E, Bernini F), pp. 330–333. Polistampa, Firenze, Italy.

Ficetola GF, Coïc C, Detaint M *et al*. (2007a) Pattern of distribution of the American bullfrog *Rana catesbeiana* in Europe. *Biological Invasions*, **9**, 767–772.

Ficetola GF, Garner TWJ, De Bernardi F (2007b) Genetic diversity, but not hatching success, is jointly affected by post glacial colonization and isolation in the threatened frog, *Rana latastei*. *Molecular Ecology*, **16**, 1787–1797.

Ficetola GF, Thuiller W, Miaud C (2007c) Prediction and validation of the potential global distribution of a problematic alien invasive species — the American bullfrog. *Diversity and Distributions*, **13**, 476–485.

Forsyth DM, Duncan RP (2001) Propagule size and the relative success of exotic ungulate and bird Introductions to New Zealand. *American Naturalist*, **157**, 583–595.

Garner TWJ, Perkins MW, Govindarajulu P *et al*. (2006) The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*. *Biology Letters*, **2**, 455–459.

Govindarajulu P, Altwegg R, Anholt BR (2005) Matrix model investigation of invasive species control: bullfrogs on Vancouver Island. *Ecological Applications*, **15**, 2161–2170.

Hansen MJ, Wilson SD (2006) Is management of an invasive grass *Agropyron cristatum* contingent on environmental variation? *Journal of Applied Ecology*, **43**, 269–290.

Hoelzel AR, Halley JM, O'Brien SJ *et al*. (1993) Elephant seal genetic variation and the use of simulation models to investigate historical population bottlenecks. *Journal of Heredity*, **84**, 443–449.

Hulme PE (2006) Beyond control: wider implications for the management of biological invasions. *Journal of Applied Ecology*, **43**, 835–847.

Jeschke JM, Strayer DL (2006) Determinants of vertebrate invasion success in Europe and North America. *Global Change Biology*, **12**, 1608–1619.

Kats LB, Ferrer RP (2003) Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Diversity and Distributions*, **9**, 99–110.

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution*, **16**, 199–204.

Kolbe JJ, Glor RE, Rodriguez Schettino L *et al*. (2004) Genetic

variation increases during biological invasion by a Cuban lizard. *Nature*, **431**, 177–181.
Lavergne S, Molofsky J (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Sciences, USA*, **104**, 3883–3888.
Lee CE (2002) Evolutionary genetics of invasive species. *Trends in Ecology & Evolution*, **17**, 386–391.
Lever C (2003) *Naturalized Amphibians and Reptiles of the World*. Oxford University Press, New York.
Lockwood JL, Cassey P, Blackburn TM (2005) The role of propagule pressure in explaining species invasions. *Trends in Ecology & Evolution*, **20**, 223–228.
Lowe S, Browne M, Boudjelas S, De Poorter M (2000) *100 of the World's Worst Invasive Alien Species. A selection from the Global Invasive Species Database*. ISSG, SSC and IUCN, Auckland, New Zealand. Available from URL: http://www.issg.org/booklet.pdf.
May GE, Gelembiuk GW, Panov VE, Orlova MI, Lee CE (2006) Molecular ecology of zebra mussel invasions. *Molecular Ecology*, **15**, 1021–1031.
McGeoch MA, Chown SL, Kalwij JM (2006) A global indicator for biological invasion. *Conservation Biology*, **20**, 1635–1646.
Memmott J, Craze PG, Harman HM, Syrett P, Fowler SV (2005) The effect of propagule size on the invasion of an alien insect. *Journal of Animal Ecology*, **74**, 50–62.
Miller PS, Hedrick PW (2001) Purging of inbreeding depression and fitness decline in bottlenecked populations of *Drosophila melanogaster*. *Journal of Evolutionary Biology*, **14**, 595–601.
Puth LM, Post DM (2005) Studying invasion: have we missed the boat? *Ecology Letters*, **8**, 715–721.
Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. *Conservation Biology*, **17**, 230–237.
Rejmánek M, Richardson DM, Higgins SI, Pitcairn MJ, Grotkopp E (2005) Ecology of invasive plants — state of the art. In: *Invasive Alien Species: a New Synthesis* (eds Mooney HA, Mack RN, McNeely JA *et al*.), pp. 104–161. Island Press, Washington, D.C.
Reusch TBH, Ehlers A, Hämmerli A, Worm B (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences, USA*, **102**, 2826–2831.
Schmitt T, Hewitt GM (2004) The genetic pattern of population threat and loss: a case study of butterflies. *Molecular Ecology*, **13**, 21–31.
Schroeder EE, Baskett TS (1968) Age estimation, growth rates, and population structure in Missouri bullfrogs. *Copeia*, **1968**, 583–592.
Stewart JE (1991) Introductions as factors in diseases of fish and aquatic invertebrates. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 110–117.
Strand AE (2002) METASIM 1.0: an individual-based environment for simulating population genetics of complex population dynamics. *Molecular Ecology Notes*, **2**, 373–376.
Strayer DL, Evinver VT, Jeschke JM, Pace ML (2006) Understanding the long-term effects of species invasions. *Trends in Ecology & Evolution*, **21**, 645–651.
Suarez AV, Holway DA, Ward PS (2005) The role of opportunity in the unintentional introduction of non-native ants. *Proceedings of the National Academy of Sciences, USA*, **102**, 17032–17035.
Thulin C-G, Simberloff D, Barun A *et al*. (2006) Genetic divergence in the small Indian mongoose (*Herpestes auropunctatus*), a widely distributed invasive species. *Molecular Ecology*, **15**, 3947–3956.
Vincek V, O'Huigin C, Satta Y *et al*. (1997) How large was the founding population of Darwin's finches? *Proceedings of the Royal Society B: Biological Sciences*, **264**, 111–118.
Von Holle B, Simberloff D (2005) Ecological resistance to biological invasion overwhelmed by propagule pressure. *Ecology*, **86**, 3212–3218.
Vonesh JR, De la Cruz O (2002) Complex life cycles and density dependance: assessing the contribution of egg mortality to amphibian declines. *Oecologia*, **133**, 325–333.
Wang J (2000) Effects of population structures and selection strategies on the purging of inbreeding depression due to deleterious mutations. *Genetical Research*, **76**, 75–86.
Wilson JRU, Richardson DM, Rouget M *et al*. (2007) Residence time and potential range: crucial considerations in plant invasion ecology. *Diversity and Distributions*, **13**, 11–22.

---

G. Francesco Ficetola is interested in conservation and ecology of amphibians, and in the importance of recent evolutionary processes for conservation. Aurélie Bonin is a postdoctoral researcher specialized in population genetics and evolutionary biology, with a special interest in amphibians. Claude Miaud's research focus on population structure, functioning and adaptation of amphibians exposed to environmental gradients.

---

## Supplementary material

The following supplementary material is available for this article.

**Fig. S1** Number of haplotypes retained after nine, 12 and 18 generations as a function of the number of introduced females for different values of the growth rate (Lambda): results of simulations. The bold lines represent the median, the boxes include the second and the third quartile of simulation results; the error bars represent 95% CI. The dashed line represents the number of haplotypes observed in populations of Southern France (FR2 + FR3 + FR4) and Italy (IT8 + IT9 + IT10 IT11).

**Fig. S2** Number of haplotypes retained after six generations as a function of the number of introduced females for different values of the growth rate (Lambda): results of simulations. For these simulations, we assumed as parental population an ad hoc group including all native populations where the invasive haplotype H7 was present (Fig. 1). The bold lines represent the median, the boxes include the second and the third quartile of simulation results; the error bars represent 95% CI. The dashed line represents the number of haplotypes observed in populations of Southern France (FR2 + FR3 + FR4).

This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-294X.2007.03622.x
(This link will take you to the article abstract).

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.