Received: 7 July 2021 | Accepted: 28 August 2021

DOI: 10.1111/1365-2664.14023

Journal of Applied Ecology BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Inference of local invasion pathways in two invasive crayfish species displaying contrasting genetic patterns

Ivan Paz-Vinas[1] | Iris Lang[1] | Paul Millet[1] | Charlotte Veyssière[1] | Géraldine Loot[1,2] | Julien Cucherousset[1]

[1]Laboratoire Évolution et Diversité Biologique (EDB), UPS, CNRS, IRD, Université de Toulouse, UMR 5174, Toulouse, France

[2]Institut Universitaire de France, Paris, France

Correspondence
Ivan Paz-Vinas
Email: ivanpaz23@gmail.com

Funding information
UNPG; Centre National de la Recherche Scientifique; Agence de l'Eau Adour-Garonne

Handling Editor: Melinda Coleman

Abstract

1. The efficient management of invasive alien species (IAS) requires the identification of their introduction pathways. Genetic assessments have proven useful to inform invasion pathways at large (national to worldwide) scales, but studies at local scales are still rare, despite their importance for guiding management.
2. In this study, genetic analyses were used to identify local invasion pathways of two invasive crayfish species (the spiny-cheek crayfish *Faxonius limosus* and the red swamp crayfish *Procambarus clarkii*) in a dense network of artificial lakes. We first characterized the spatial patterns of genetic variability, effective population sizes (*Ne*) and among-lakes recent migration events for each species using neutral microsatellite markers. We then identified the environmental factors affecting genetic variability and inferred the potential local invasion pathways.
3. Results revealed different patterns of genetic variability between the two species: *F. limosus* displayed very low levels of genetic diversity, *Ne* and spatial structuring compared to *P. clarkii*, which displayed high genetic diversity, *Ne* and spatial genetic structuring. We also demonstrated context-dependent effects of different environmental factors (fishery management, spatial distribution and lake size) on genetic variability indices.
4. We did not identify local invasion pathways for *F. limosus* due to limited genetic variability, likely caused by a strong founder effect and potential parthenogenetic reproduction. Contrastingly, multiple invasion pathways (release, contaminant, unaided/corridor spread and stowaway) were identified for *P. clarkii*.
5. *Synthesis and applications*. Although limited in some particular cases (e.g. for species having experienced strong shaping events and/or displaying asexual reproductive modes), neutral genetic variation assessments can provide important insights for inferring local invasion pathways in complex landscapes for invasive alien species displaying short generation times and complex invasion histories.

KEYWORDS

artificial ecosystems, biological invasions, genetic baseline, gravel pit lakes, invasion genetics, invasion pathway, invasive crayfish, microsatellites

1 | INTRODUCTION

Rates of introduction, establishment and subsequent range expansion of invasive alien species (hereafter, IAS) are increasing, promoting a global rise in biological invasions (Seebens et al., 2017, 2018). Invasive species exert negative economic and ecological effects and act across levels of biological organization (Cucherousset & Olden, 2011; Jeschke et al., 2014; Simberloff et al., 2013). Characterizing and predicting biological invasions are crucial for efficient IAS management. Invasion genetics allow the study of the eco-evolutionary consequences of biological invasions and the reconstruction of complex colonization histories through the inferential and correlative analysis of the genetic footprints occurring during invasions (Barrett, 2015; Cristescu, 2015). Indeed, founder effects, intraspecific admixture or interspecific hybridization shape the genetic characteristics of invasive populations (Bock et al., 2015). Genetic analyses reveal the patterns of variation across invasive populations, facilitating hypotheses-driven reconstructions of invasion histories and informing global invasion pathways at large (national to global) spatial scales (Oficialdegui et al., 2019; Rey et al., 2015; Sherpa et al., 2019). Yet, genetic assessments identifying invasion pathways are scarce at local scales, despite being the spatial scale at which management actions are primarily performed. The limited capacity of most genetic markers to capture significant genetic structure at restricted spatial scales, and the short timeframes that often span between introduction and genetic sampling in local invasion contexts may preclude local-scale invasion genetic assessments (Fitzpatrick et al., 2012). Indeed, evolutionary processes such as mutation, genetic drift and migration might not produce detectable genetic footprints in short temporal scales for many IAS (Fitzpatrick et al., 2012). However, IAS with short generation times may overcome these limitations (Fitzpatrick et al., 2012), especially if they invade complex (e.g. patchy) landscapes, as their populations might produce sufficient numbers of generations to generate measurable—and differential—genetic changes (e.g. Bélouard et al., 2019). In this context, invasion genetic assessments can be valuable to reveal invasion pathways and inform IAS management actions at local scales.

The identification of invasion pathways and spread vectors is crucial for (a) containing ongoing invasions by reducing propagule pressure (Simberloff, 2009), (b) preventing invasive populations from acting as bridgehead populations (Bertelsmeier & Keller, 2018) and (c) hindering recolonization after successful eradication (Britton et al., 2011). Six major introduction pathways have been identified (Hulme et al., 2008): deliberate release as a commodity, escape from captivity, contaminant of a specific commodity, stowaway on a transport vector and spread through unaided dispersal from an invaded area or dispersal following anthropogenic corridors (CBD, 2014; Hulme, 2015; Hulme et al., 2008). When an invasion history is relatively simple (e.g. few introductions from identified sources; Simon et al., 2011), pathway identification is usually straightforward. However, biological invasions are often the result of complex socio-ecological interactions involving multiple and often unreported introductions, propagules *per* introduction event and source populations (Blackburn et al., 2015; Rey et al., 2015). The information contained in the genetic footprints left during invasion (e.g. genetically distinctive alleles, genotypes or individuals in a population due to introductions involving different genetic sources) depends on multiple factors. These can be intrinsic, like species traits (e.g. dispersal capacities, demography, reproductive traits), or extrinsic, like distances between populations, the physical configuration of ecosystems or the socio-economic activities occurring in the study area (Washburn et al., 2020). Consequently, the knowledge gained from population genetic analyses can be highly context-dependent and may modulate our ability to identify local invasion pathways.

Here, we used genetic analyses to identify local invasion pathways of two global invasive crayfish (the spiny-cheek crayfish *Faxonius limosus* and the red swamp crayfish *Procambarus clarkii*) characterized by short generation times, and exhibiting contrasting ecology and invasion histories in Europe (Filipová et al., 2011; Oficialdegui et al., 2019). We sampled a dense network of artificial gravel pit lakes harbouring a myriad of socio-economic activities within a restricted spatial scale (Evangelista et al., 2015; Zhao et al., 2016), in which the major invasion pathways may (co)-occur. Although located within a limited geographic area, these ecosystems are highly variable, notably in terms of age, size, use (from gravel extraction to water sports and recreational angling; Evangelista et al., 2015; Zhao et al., 2016) and management practices (e.g. from protected areas to lakes experiencing intensive fishery management involving intensive fish stocking; Závorka et al., 2020; Zhao et al., 2016). Importantly, they are disconnected from the hydrological network and therefore represent—from a biogeographical standpoint—a network of isolated aquatic 'islands' separated by a terrestrial 'ocean' (Hortal et al., 2014). These isolated ecosystems should promote differential effects of genetic drift among populations, while representing strong barriers to natural dispersal, hence providing a unique opportunity to test whether genetic-based local invasion pathways can be inferred for these IAS despite restricted spatial scale. We first quantified, for each species, the spatial patterns of genetic variability, their effective population sizes and among-lakes recent migration events using neutral microsatellite markers. We then assessed the effects of extrinsic environmental factors on genetic variation patterns. We finally interpreted our empirical results in the light of Hulme et al.'s (2008) invasion pathways classification, and discuss the added values and potential limitations of genetic assessments to identify local invasion pathways.

2 | MATERIALS AND METHODS

2.1 | Study organisms

We studied two invasive crayfish species (listed in the European Union Regulations EU 1143/2014 and EU 2019/1262) displaying contrasting life-history traits and invasion histories at the continental scale. *Faxonius limosus* is native to the Eastern Coast of North America (Filipová et al., 2011). Its only known successful introduction in Europe dates back to 1890 when 90 individuals were successfully

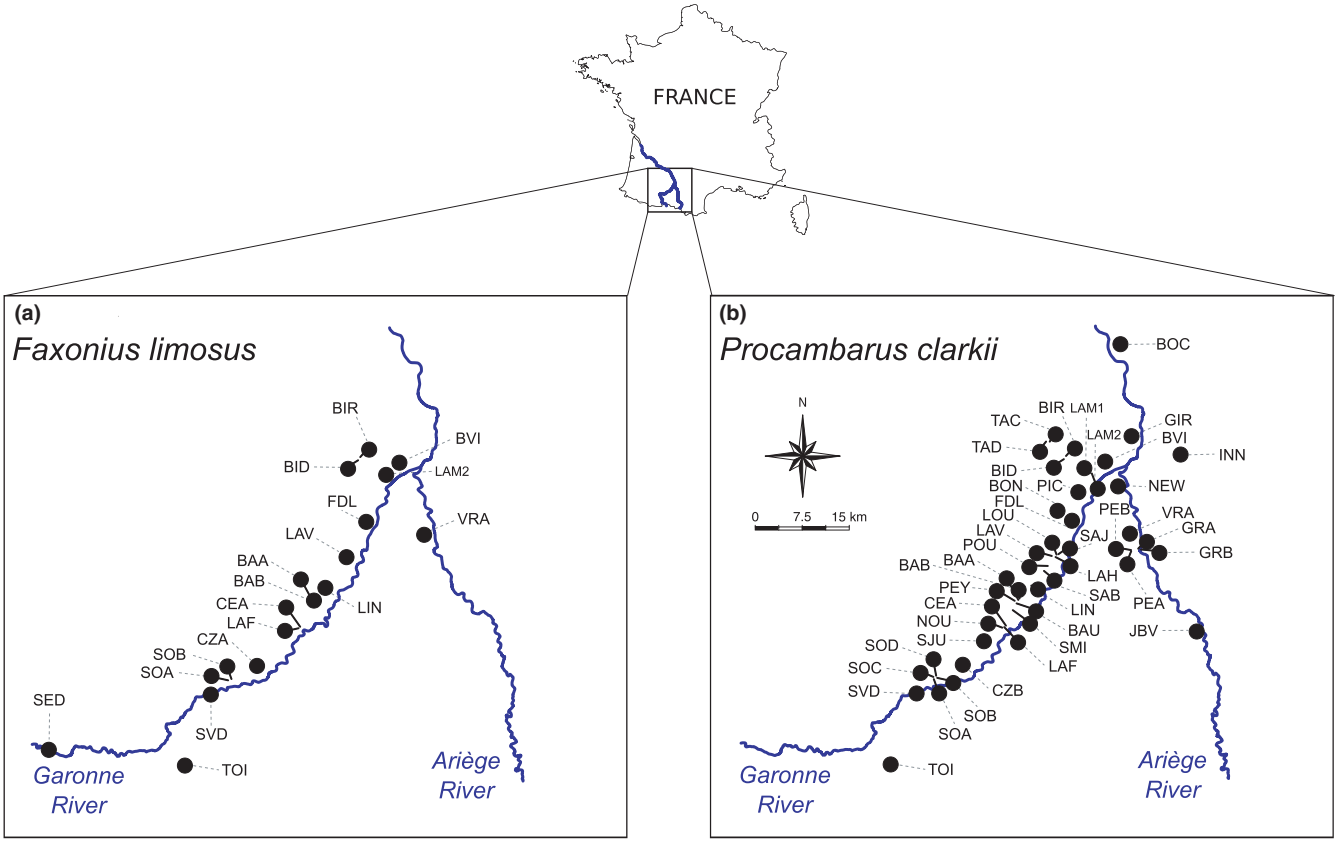

FIGURE 1 Study area with the location of studied populations for (a) *Faxonius limosus* and (b) *Procambarus clarkii*

introduced from the US Commission of Fish and Fisheries into western Poland to replace populations of native species that were decimated by crayfish plague (Filipová et al., 2011). The species subsequently spread across Europe and arrived in central France between 1911 and 1913, where 2,000 individuals from Germany were deliberately released (Laurent, 1997). It invaded the Garonne river basin in the early 1960s (Laurent, 1997) and its presence in the study area was first documented in 1988 (Magnier & Petit, 2016), that is, approximately 50–60 generations, considering that the species can reproduce twice a year (Buřič et al., 2013; Lang et al., 2021). *Procambarus clarkii* is native to southern United States and north-eastern Mexico and has been widely introduced worldwide due to its economical value for aquaculture, colonizing almost all continents (Oficialdegui et al., 2019). It was first introduced in Europe in 1973 from Louisiana to Spain, and the species rapidly spread across Europe (Oficialdegui et al., 2019). In France, the first introduction is reported in South-western France in 1976 with individuals from Spain (Laurent, 1997). Besides this introduction event, many individuals were imported from Spain and Kenya for aquaculture from the late 1970s to the early 1980s (Holdich, 1993; Oficialdegui et al., 2019, 2020). The presence of *P. clarkii* in our study area was first mentioned in 1995 (Changeux, 2003), that is, approximately 40–50 generations, considering two reproductions *per* year (Lang et al., 2021). Both crayfish species cause serious impacts on biodiversity, ecosystem functioning and services, through consumption of native prey, disease transmission and ecological engineering (e.g. burrowing). However, they also display contrasting life histories (e.g. distinct trophic niches, thermal tolerances and overland dispersal capacities; Lang et al., 2021; Puky, 2014; Thomas et al., 2019; Veselý et al., 2021).

2.2 | Study area and sampling design

The study area was composed of a network of artificial gravel pit lakes of different ages (from 10 to 60 years) and sizes (surface ranging from 1,280 to 474,274 m^2) spread across a 70 × 75 km area located within the Garonne floodplain and disconnected from the hydrographical network (South-western France; Figure 1; Tables S1 and S2 in Supporting Information). They are also distributed along a decreasing north to south urbanization gradient (i.e. Toulouse metropolitan area on the north and Pyrenees Mountains' piedmont on the south). Their fishery management in terms of angling practices and fish stocking can be categorized as *high management level* when managed by public and private angling clubs and as *low management level* when managed by municipalities or private owners (Zhao et al., 2016).

This area is particularly interesting for studying local invasion pathways because many major pathways identified by Hulme et al. (2008) can potentially (co-)occur here for *F. limosus* and *P. clarkii*:

deliberate releases in specific lakes for human consumption, contaminants of commodities (e.g. during fish stocking events), stowaways on transport vectors (e.g. dispersed from one lake to another by humans or aquatic birds; Anastácio et al., 2014; Coughlan et al., 2017) and unaided/corridor spread, by dispersing overland, through anthropogenic corridors or the riverine network (Puky, 2014; Thomas et al., 2019). We hypothesized that (a) high management level lakes should display higher genetic diversities for both species, as they are more prone to receive individuals through the contaminant pathway (e.g. during fish stocking events); (b) lakes near the main city should display higher genetic diversities for both species, as they are more prone to receive individuals through deliberate releases (e.g. for human consumption) due to high surrounding human densities; (c) big lakes are more prone to display higher genetic diversities and *Ne* due to their putatively higher carrying capacities and that (d) *P. clarkii* should exhibit higher genetic diversities than *F. limosus*, as a consequence of its more complex invasion history at the continental scale (which involves many introductions of individuals originating from the native area).

The sampling was conducted from mid-September to mid-October 2016–2019 primarily using pairs of baited traps set both overnight and during the day (Alp et al., 2016; Závorka et al., 2020). We also performed active sampling using dip nets when needed. Additional samples were provided by local anglers and agencies. We successfully sampled 18 populations of *F. limosus* (514 genotyped individuals) and 43 populations of *P. clarkii* (1,182 genotyped individuals; Figure 1). Almost all sampled *F. limosus* populations (16/18) were sympatric with *P. clarkii* populations. We targeted at least $N = 28$ sampled individuals *per* population *per* species for subsequent genetic analyses, although this number was not always reached (77.78% of *F. limosus* populations with $N \geq 20$, mean N across populations $= 29 \pm 13$ *SD*, Table S1, and 83.72% of *P. clarkii* populations with $N \geq 20$, mean N across populations $= 27 \pm 7$ *SD*, Table S2).

2.3 | Extrinsic factors

A set of extrinsic (environmental) factors was quantified to perform subsequent landscape genetic analyses. Lake *surface area* (km^2) was calculated using aerial pictures (using the web portal Geoportail; https://www.geoportail.gouv.fr/). We further determined *Euclidean distances* between (a) each pair of lakes and (b) between each lake and Toulouse city using the R package RASTER. Distances between lakes and Toulouse can be viewed as a proxy of an *urbanization gradient*, with decreasing urbanization pressure with increasing distance. Finally, we quantified the *level of fishery management* (i.e. high or low) from stakeholders and managers as previously described.

2.4 | Genotyping

Genomic DNA was extracted from abdominal muscle tissue using a modified salt extraction protocol (Aljanabi & Martinez, 1997). We co-amplified 9 and 14 microsatellite loci for *F. limosus* and *P. clarkii*, respectively (Hulák et al., 2010; Jiang et al., 2015), using two (*F. limosus*) or three (*P. clarkii*) multiplexed PCRs, 5–20 ng of genomic DNA and QIAGEN® Multiplex PCR Kits (Qiagen). Details on loci, primer concentrations, PCR conditions and multiplex sets are available elsewhere (Hulák et al., 2010; Jiang et al., 2015; Lang et al., 2020). Genotyping was conducted on an ABI PRISM™ 3,730 Automated Capillary Sequencer (Applied Biosystems) and allele size scoring using GENEMAPPER® v.4.0 (Applied Biosystems).

2.5 | Genotyping quality controls

For each species, we assessed (a) null alleles and potential scoring errors incidence with MICROCHECKER 2.3 (Van Oosterhout et al., 2004), (b) linkage disequilibria among loci within populations with FSTAT v2.9.3.2 (Goudet, 1995) and (c) departures from Hardy–Weinberg (HW) equilibrium with GENEPOP v4.0 (Rousset, 2008). Levels of significance for these multiple tests were adjusted using the false discovery rate (FDR) procedure of Benjamini and Hochberg (1995). Finally, we used BAYESCAN v.2.1 (Foll & Gaggiotti, 2008) to test the neutrality of the microsatellite datasets. We specifically ran four MCMC chains considering 100 prior odds for the neutral model, sample sizes of 10,000 (with thinning intervals of 50), burn-in periods of 50,000 and 20 pilot runs with lengths of 5,000 *per* chain. The convergence of the four chains *per* species was checked through a Gelman–Rubin analysis (Gelman & Rubin, 1992). We considered that chains reached convergence when values lower than 1.1 were obtained (Gelman & Hill, 2007). According to these quality controls, we removed one (for *F. limosus*) and two (for *P. clarkii*) loci (see details in Appendix S1). We finally conducted power analyses (i.e. random subsampling procedures) that confirmed that the selected number of loci and sample sizes was sufficient for capturing the genetic structure of both species within the study area (details in Appendix S2).

2.6 | Genetic diversity and structure

We assessed the genetic diversity over all loci and for each population and species by calculating both allelic richness (*AR*) and private allelic richness (*PA*) using the rarefaction procedures implemented in ADZE v.1.0 (Szpiech et al., 2008). We assumed minimum sample sizes for rarefaction of $N = 11$ and $N = 12$ for *F. limosus* and *P. clarkii* respectively (i.e. minimum sample size for each species; Tables S1 and S2). We also estimated (across loci for each species and population) expected heterozygosities (*Hexp*) using GENETIX v.4.05 (Belkhir et al., 1996). We estimated current effective population sizes (*Ne*) using the linkage disequilibrium method implemented in NeESTIMATOR v2.1 (Do et al., 2014). Finally, we calculated, for each species, pairwise genetic differentiation values (i.e. *F'st*; Meirmans & Hedrick, 2011) with the R package STRATAG (Archer et al., 2017). We used *F'st* instead of *Fst* as the former enables interspecific

comparisons. We then calculated for each population and species the average of all pairwise *F'st* values estimated between one given population and all the remaining to obtain a within-population *genetic uniqueness* value (i.e. $F'st_{UNI}$).

2.7 | Spatial patterns of genetic variability

We first mapped *AR*, *PA* and $F'st_{UNI}$ to visually inspect the spatial distribution of genetic diversity and uniqueness. As *Hexp* values were highly correlated with *AR* values (Pearson's $r = 0.958$ and 0.950 for *F. limosus* and *P. clarkii* respectively), they were not mapped. We then tested whether isolation by distance (IBD) patterns exist by exploring the relationship between pairwise Euclidean distances and pairwise *F'st* values by conducting single Mantel tests with 1,000 permutations with the R package VEGAN.

2.8 | Genetic clustering analyses

Genetically homogenous groups of individuals (i.e. clusters) were identified using the R package RMAVERICK (Verity & Nichols, 2016). This method relies on the same mixture modelling framework implemented in STRUCTURE (Pritchard et al., 2000) but uses a thermodynamic integration (TI) procedure to estimate the best *K* number of clusters in a dataset (Verity & Nichols, 2016). The TI procedure allows evaluating $K = 1$ and provides more accurate evidence for *K* than classical methods like Evanno's ΔK (Evanno et al., 2005) or Akaike, Bayesian and Deviance information criteria (Verity & Nichols, 2016). We explored values of *K* ranging from 1 to 18 for *F. limosus* and from 1 to 20 for *P. clarkii*, considering two different evolutionary models (i.e. with and without admixture). We ran MCMC chains considering burn-in periods of 10,000 iterations, 2,000 sampling iterations and rung parameter equal to 10. We then determined, for each species and evolutionary model (i.e. with/without admixture), the best *K* value according to the obtained TI posterior probabilities. Finally, we compared the evidence of the two tested evolutionary models to determine which one better fits our genotypic data. For *F. limosus*, the two evolutionary models generated qualitatively similar results (Figure S1a); we thus only report results obtained under the most parsimonious model (i.e. 'without admixture'). For *P. clarkii*, we will only report results for the 'without admixture' model, which had high support (Figure S1c).

2.9 | Recent migration

We identified first-generation migrants among lakes using GENECLASS 2 (Piry et al., 2004), by computing the frequency-based criterion of Paetkau et al. (1995) and by assessing the likelihood as the 'L_origin / L_max' ratio, using a Monte Carlo resampling method, 1,000 bootstraps and an assignment threshold $\alpha = 0.01$.

2.10 | Extrinsic factors explaining observed patterns of genetic variability

To identify the extrinsic factors affecting genetic variability, we built linear models using *AR*, *PA*, *Ne* and $F'st_{UNI}$ as dependent variables, and *surface*, *distance* and *management* as explanatory variables. All models were initially run with two-way interactions and the best models were selected using a backward selection procedure using R v.2.6.2. Marginal effects of significant interactions were plotted using the R package SJPLOT.

3 | RESULTS

3.1 | Genetic diversity and structure

Faxonius limosus displayed very low levels of genetic diversity (Figure 2; Table S1), with *AR* averaging 1.470 (±0.217 *SD*) and *Hexp* averaging 0.131 (±0.056 *SD*) across populations. Mean within-population *PA* values ranged between <0.001 and 0.333 (mean *PA* across populations of 0.054 ± 0.08 *SD*) and mean $F'st_{UNI}$ values ranged between 0.111 and 0.302 (mean $F'st_{UNI}$ across populations of 0.188 ± 0.056 *SD*). Effective population sizes were low (mean *Ne* across populations of 57.8 ± 152.9 *SD*; Table S1), except for CZA (*Ne* = 561.9). Five out of 18 *Ne* estimates were infinite, indicating a lack of evidence for variation in the genetic characteristics caused by genetic drift due to sampling error (Do et al., 2014). Contrastingly, *P. clarkii* populations displayed higher levels of genetic diversity (Figure 2; Table S2), with *AR* averaging 3.586 (±0.621 *SD*) and *Hexp* averaging 0.596 (±0.082 *SD*) across populations. Mean *PA* values ranged between <0.001 and 0.224 (mean *PA* across populations of 0.04 ± 0.05 *SD*) and $F'st_{UNI}$ ranged from 0.349 to 0.648 (mean $F'st_{UNI}$ value of 0.478 ± 0.081 *SD*). Effective population sizes were overall higher than for *F. limosus*, and ranged between 1.6 (for LAF) and 2,834.1 (for CEA), although 13 of 43 *Ne* estimates were infinite.

3.2 | Spatial patterns of genetic variability and genetic clustering

No significant IBD pattern was observed for *F. limosus* (Mantel $r = -0.095$, $p = 0.827$, Figure 3a), but there was a significant IBD pattern for *P. clarkii* (Mantel $r = 0.415$, $p < 0.00099$, Figure 3b). We found strong evidence for two different genetic clusters for this species (Figure S1b). Overall, there was no clear spatial distribution of these two clusters nor were populations exclusively belonging to one of these two clusters (Figure 4a). For *P. clarkii*, we found evidence for the occurrence of 15 different genetic clusters (Figure S1d). These clusters were highly spatially structured, with many single lake populations (e.g. INN, CEA, VRA or JBV, Figure 4b) or groups of neighbouring populations belonging to the same private owner or experiencing similar management practices (e.g.

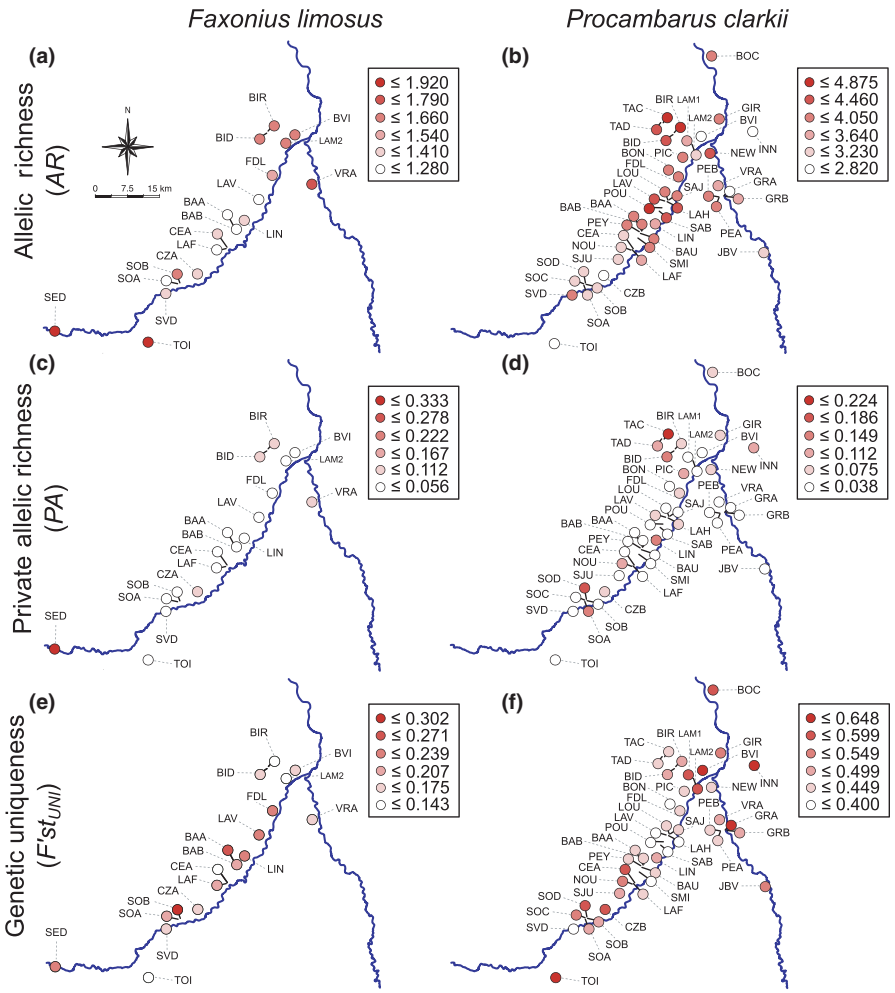

FIGURE 2 Spatial distribution of allelic richness (*AR*; a and b), private allelic richness (*PA*; c and d) and genetic uniqueness ($F'st_{UNI}$; e and f) for *Faxonius limosus* (a, c and e) and *Procambarus clarkii* (b, d and f)

SOD, SOC, SOB and SOA for cluster 7; BIR and BID for cluster 4; Figure 4b) assigned almost exclusively to specific clusters. We also identified (a) some distant populations composed of individuals assigned almost exclusively to the same cluster (e.g. LIN and BON for cluster 10; Figure 4b), (b) signals of introgression among clusters (e.g. from cluster 4, mainly represented in BIR and BID, into TAC and TAD, mainly represented by individuals assigned to cluster 1; Figure 4b) and (c) potential long-distance migrants (e.g. four individuals in JBV assigned to cluster 14, which is mainly represented by individuals from GRA and GRB lakes, situated 16 km away from JBV; Figure 4b).

3.3 | Recent migration

We identified a total of 14 migrants for *F. limosus* (Figure 5a), and almost all movements were long (mean = 32.240 km, range = 0.578–67.078 km). For *P. clarkii*, we detected 30 migrants (Figure 5b), which moved primarily between relatively close populations (mean among-populations distance = 11.573 km, range = 0.297–37.061 km), though some migrants moved long distances (Figure 5b).

3.4 | Extrinsic factors explaining observed patterns of genetic variability

We found a significant interaction term (Distance × Surface: $F_{(13)} = 8.0014$, $p = 0.0142$) for *AR* of *F. limosus* (Table S3). Specifically, *AR* decreased with increasing lake surface in lakes closer to Toulouse (Figure S2a), while *AR* increased with increasing lake surface in lakes farther from Toulouse (Figure S2a). We did not find significant effects of the tested variables on *PA*, $F'st_{UNI}$ or *Ne* of *F. limosus* (Table S3). For *AR* of *P. clarkii*, the three interaction terms were significant (Distance × Surface: $F_{(36)} = 1.0841$, $p = 0.013$; Distance × Management: $F_{(36)} = 19.9144$, $p < 0.001$; Management × Surface: $F_{(36)} = 3.0271$, $p < 0.001$; Table S3). Overall, *AR* increased with increasing surface and distance from Toulouse (Figure S3a), though these effects differed depending on the level of fishery management. Specifically, *AR* decreased with increasing distance in low management level lakes and inversely increased with increasing distance in high management level lakes (Figure S3b). Lake surface did not affect *AR* in low management level lakes, although it had a strong positive effect on *AR* in high management level lakes (Figure S3c). We did not find significant effects of the tested variables on *PA*, but we found two significant

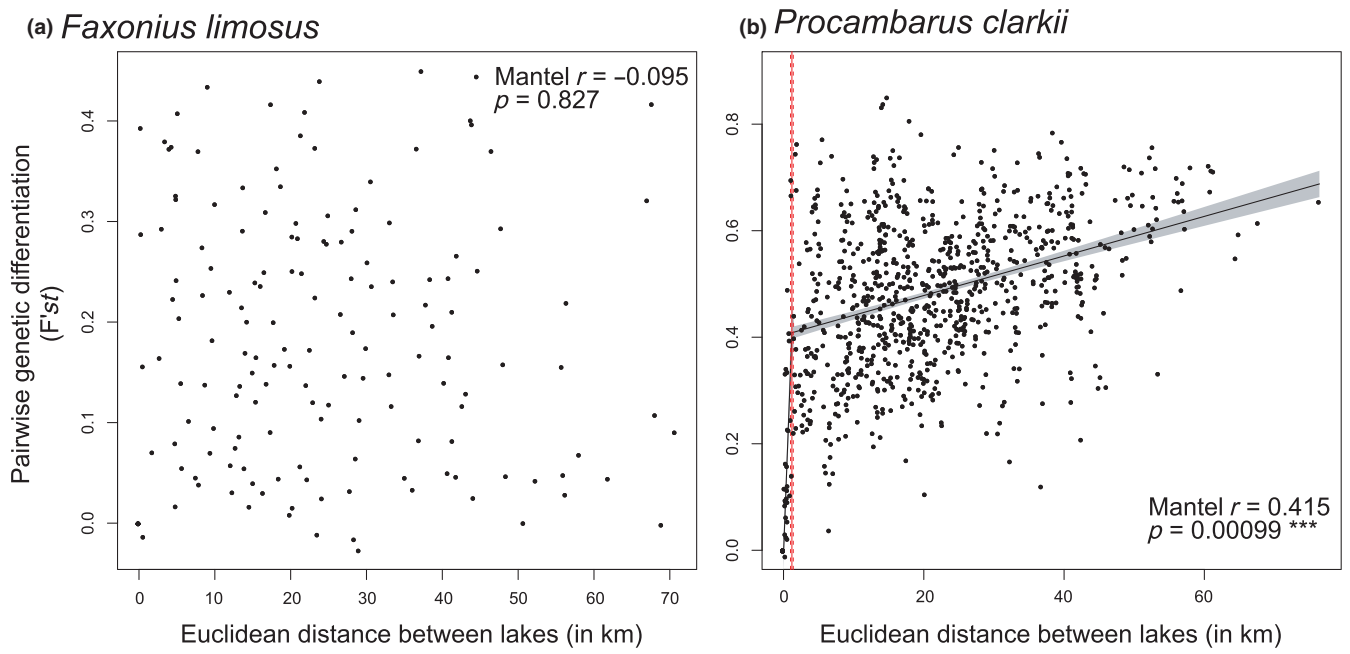

FIGURE 3 Pairwise *F'st* values among populations against Euclidean distances between lakes for *Faxonius limosus* (a) and *Procambarus clarkii* (b). A piecewise regression line (b) is represented in blue (95% CI in grey) as the Mantel test detected a significant IBD pattern for this species. The vertical red dotted line represents the breakpoint of the regression line at the distance of 1.290 km (95% CI = [1.011–1.565 km])

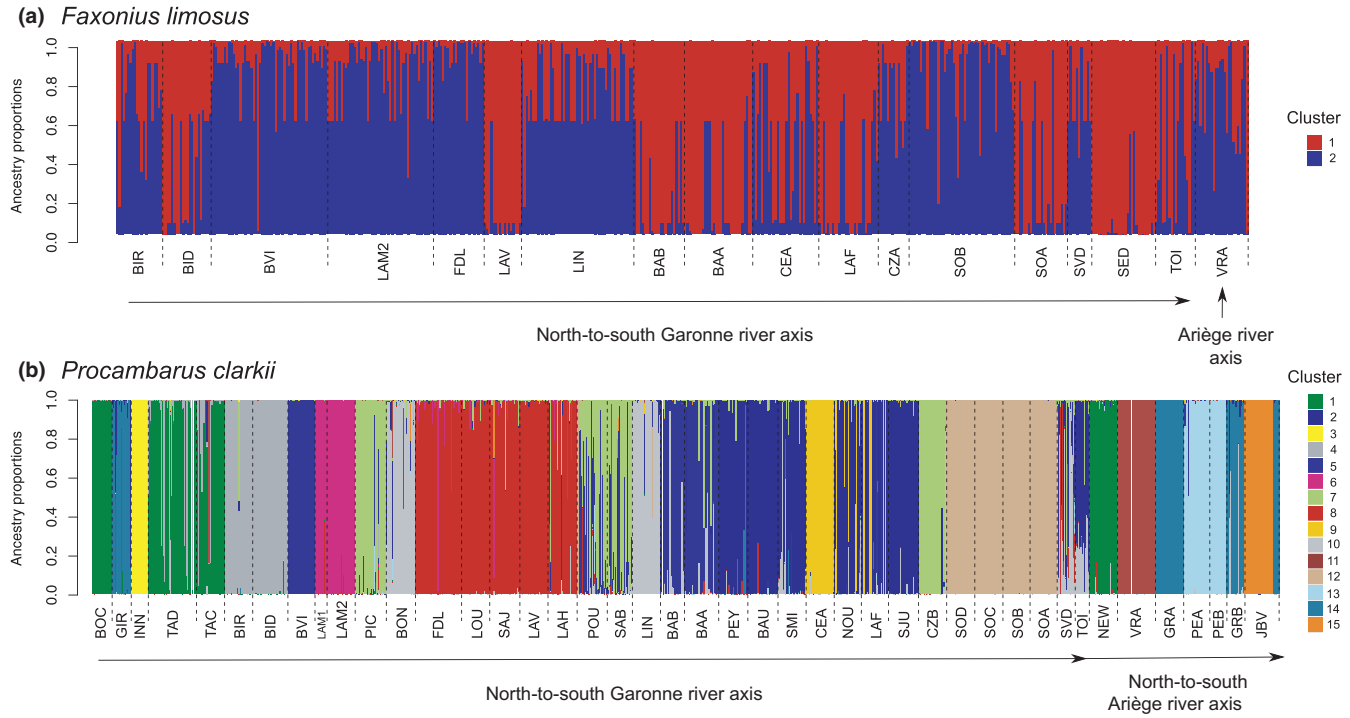

FIGURE 4 Proportion of ancestry of individuals to each cluster identified using 'rmaverick' procedure under a model assuming no admixture: (a) *Faxonius limosus* (2 clusters) and (b) *Procambarus clarkii* (15 clusters)

interactions for $F'st_{UNI}$ (Distance × Surface: $F_{(37)} = 5.4424$, $p = 0.025$; Distance × Management: $F_{(37)} = 29.2018$, $p < 0.001$; Table S3). Specifically, $F'st_{UNI}$ decreased with increasing lake surface and increasing distance from Toulouse in high management level lakes, but increased with increasing distance in low management level lakes (Figure S4). Finally, we found a significant effect of the fishery management level on *Ne* (Table S4), with higher *Ne* values in highly managed lakes (Figure S5).

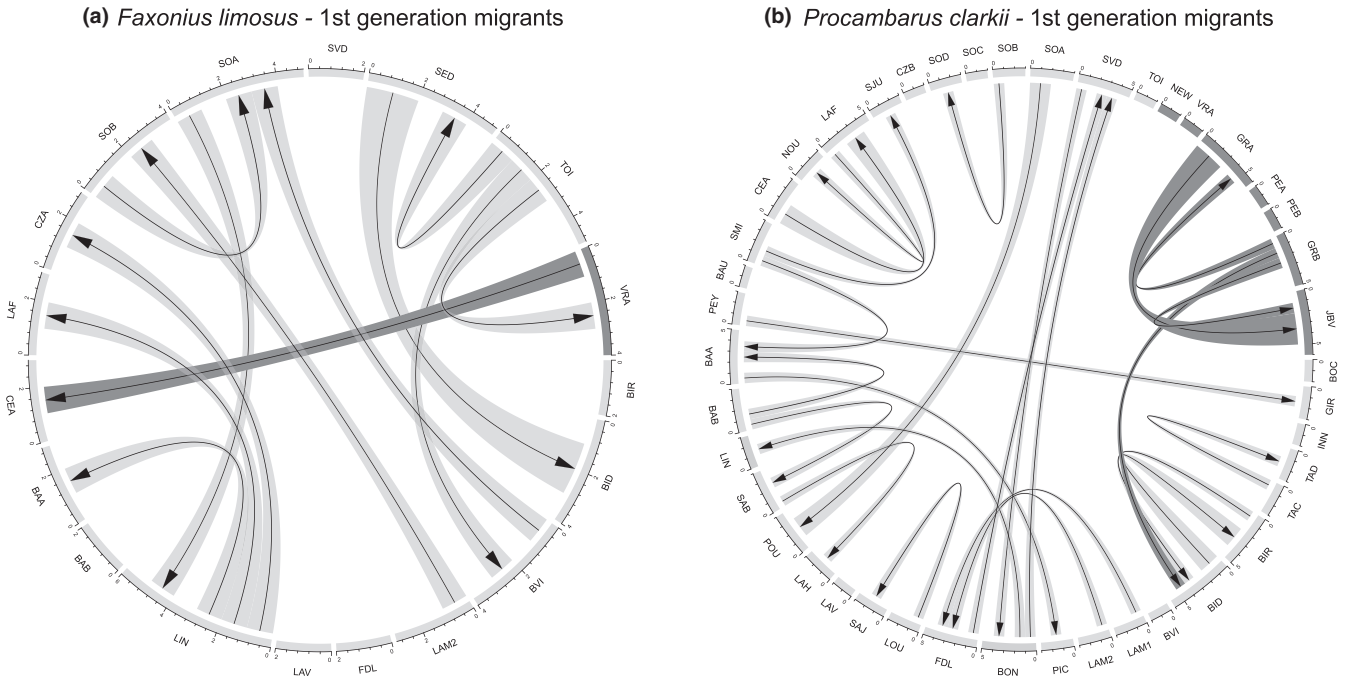

FIGURE 5 (a and b): First-generation migrants identified by Geneclass 2 for (a) *Faxonius limosus* and (b) *Procambarus clarkii*. Arrows indicate the direction of the migration. The width of the arrows is proportional to the number of migrants. Light grey for lakes from the Garonne river axis; dark grey for lakes from the Ariège river axis. Within all river axis, populations are ordered according to a clockwise north-to-south latitudinal gradient

4 | DISCUSSION

The present study revealed very different patterns of genetic variability between two invasive co-occurring crayfish. *Faxonius limosus* displayed very low levels of genetic diversity, unclear spatial patterns of genetic structure and little evidence to identify local invasion pathways. Contrastingly, *P. clarkii* displayed higher genetic variability and spatial genetic structuring, allowing the identification of specific genetic footprints suggesting that its invasion was driven by the co-occurrence of multiple local invasion pathways (release, contaminant, unaided/corridor spread and stowaway). An effect of fishery management practices (alone or in interaction with lake surface and distance to the main city) on genetic diversity and *Ne* was also observed. Overall, our results illustrate the usefulness—but also the limitations—of multilocus neutral genetic variation assessments for inferring local invasion pathways in complex environments for IAS with short generation times.

Some successful IAS have been reported to overcome the pervasive effects generally associated with low genetic diversity (e.g. high risk of inbreeding depression and extinction) and to persist in an invaded environment (Rollins et al., 2013), as observed for the Asian long-horned beetle (*Anoplophora glabripennis*; Javal et al., 2019) or the water hyacinth (*Eichhornia crassipes*; Zhang et al., 2010). This could be the case for *F. limosus*, which displayed very low genetic diversities and *Ne*. These patterns of genetic variability are congruent with the main hypothesis of *F. limosus* invasion history in Europe. A strong reduction in genetic diversity was likely caused by a strong founder effect in 1890 when the species was brought from North America to Europe, followed by multiple successive founder effects during subsequent introductions and colonization events across the continent (Filipová et al., 2011). Additionally, facultative parthenogenesis has been reported under controlled conditions for this IAS (Buřič et al., 2013). Indeed, parthenogenetic reproduction could contribute to maintain populations with low genetic diversities and *Ne* values like those we observed, though the occurrence of parthenogenesis in the wild remains unknown for *F. limosus*. Overall, our multilocus genetic analyses provided little evidence for the occurrence of specific local invasion pathways for *F. limosus*. Even though recent migration events among distant populations were detected (which may indicate the occurrence of human-mediated dispersal), we could not clearly infer any invasion pathway. It is more likely that the detected migration events and the lack of clear spatial genetic structuring observed may be just consequences of the very low genetic baselines observed for this IAS within the study area. We also found a context-dependent effect of lake surface and distance to the main city on allelic richness, though we did not detect any effect of fishery management practices on genetic metrics. No significant IBD pattern was detected, and pairwise *F'st* values were highly idiosyncratic, with pairs of populations separated by comparable distances displaying highly variable genetic differentiation. Although this idiosyncrasy may indicate the occurrence of multiple human-mediated invasion pathways (Zhan et al., 2012), the very low levels of genetic diversity observed for *F. limosus* have probably blurred our ability to clearly identify local invasion pathways occurring in the study area for this IAS.

Genetic diversities and *Ne* were overall higher for *P. clarkii* than for *F. limosus*. Our genetic analyses suggested the occurrence of multiple invasion pathways for *P. clarkii* at a local scale, hence mirroring the co-occurrence of multiple invasion pathways observed at the global scale (Oficialdegui et al., 2019, 2020). For instance, the high values observed for genetic variability metrics (notably for *PA*) for some populations, combined with the very high number of detected genetic clusters, suggest that many independent introduction events involving genetically distinct sources may have occurred despite the small spatial scale (~5,000 km²), probably through the release or contaminant pathways.

Furthermore, the sharp increase of pairwise *F'st* values in *P. clarkii* at short distances suggests that differential effects of genetic drift are fuelling population differentiation in lakes that are mostly isolated, despite the occurrence of some migration events, notably at short distances (probably through unaided overland dispersal). These results are in agreement with a recent study (Bélouard et al., 2019) which highlighted strong influences of genetic drift in highly structured *P. clarkii* populations within a three-decade-old invaded wetland covering a 15-km^2 area. We found nonetheless a high variability in pairwise *F'st* values for couples of populations separated by similar distances. Such complex genetic structure patterns have already been observed for many invasive species at both local and regional scales (Darling & Folino-Rorem, 2009; Zhan et al., 2012), suggesting the occurrence of many human-mediated invasion pathways (e.g. contaminant, release, stowaway). We also found that management practices may promote the genetic diversity and *Ne* of *P. clarkii* while reducing among-lakes genetic differentiation, probably through an increased occurrence of undeliberate releases as contaminants (e.g. during fish stocking events), with crayfish being moved as stowaways or through illegal, deliberate releases.

Interestingly, we identified a handful of individuals that may have (been) moved between distant lakes, suggesting the occurrence of human-mediated translocations (probably through deliberate releases and/or as contaminants of other commodities or angling equipment). However, waterbird-mediated passive dispersal (Anastácio et al., 2014) and/or active overland dispersal (Thomas et al., 2019) across the study area cannot be excluded and further investigations are needed to identify the drivers of such translocations.

5 | CONCLUSIONS

Our study illustrates both the insights and potential limitations of neutral genetic variation assessments for inferring local invasion pathways of IAS in complex environments. At local scales, genetic assessments are particularly useful in short generation-timed IAS having experienced complex invasion histories like *P. clarkii*, as they will tend to display high genetic variability baselines within a restricted spatial scale, providing a robust playground to identify the genetic footprints of many concomitant local invasion pathways. In these cases, key information such as *Ne*, ongoing migration and genetic structure can reliably be assessed and used to guide IAS management actions (e.g. prioritizing actions in bridgehead populations, or in populations with highest/lowest *Ne* or genetic diversities). However, this approach may only provide limited information on local invasion pathways for IAS displaying very low genetic variability baselines due to past strong shaping events (i.e. a severe founder effect after a primary introduction) and/or atypical reproductive modes (e.g. clonal and/or parthenogenetic reproduction), as for *F. limosus*. In such cases, combining neutral genetic assessments with other complementary approaches like studies focusing on other intraspecific diversity facets (e.g. phenotypic or functional diversities; Lang et al., 2021), surveys aimed at understanding the role of local socio-economic activities in promoting the spread of IAS and/or with broader scale model-based invasion history reconstructions (e.g. Javal et al., 2019; Rey et al., 2015; Sherpa et al., 2019) will also be helpful to reveal pathways that might remain obscure when solely using descriptive genetic approaches.

ACKNOWLEDGEMENTS

We are very grateful to the 'gravière' team for their help on the field during sample collection, to lake owners and managers for allowing sampling and to Melinda Coleman and three anonymous reviewers for their comments. Sampling was conducted under the Arrêtés Préfectoraux 20160706, 20170309, 20180717 and 20190814. This work has been funded by the 'Agence de l'Eau Adour-Garonne' (DISPERSINVA project). Additional funding was obtained from the UNPG and the CNRS-EC2CO (STOECHIOINVA project).

CONFLICTS OF INTEREST

Authors have no conflicts of interest to declare.

AUTHORS' CONTRIBUTIONS

J.C. and G.L. designed and supervised the study; P.M., I.L. and J.C. collected field data and samples; G.L., C.V., P.M. and I.L. conducted laboratory analyses; I.P.-V. analysed the data; I.P.-V. drafted the first version of the manuscript; all authors edited and revised the manuscript and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Genotypic data and dependent/explanatory variables data are available via the Figshare Repository at https://doi.org/10.6084/m9.figshare.16574243.v1 (Paz-Vinas et al., 2021).

ORCID

Ivan Paz-Vinas https://orcid.org/0000-0002-0043-9289
Iris Lang https://orcid.org/0000-0001-5329-5023
Julien Cucherousset https://orcid.org/0000-0003-0533-9479

REFERENCES

Aljanabi, S. M., & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, *25*(22), 4692–4693. https://doi.org/10.1093/nar/25.22.4692

Alp, M., Cucherousset, J., Buoro, M., & Lecerf, A. (2016). Phenological response of a key ecosystem function to biological invasion. *Ecology Letters*, *19*(5), 519–527. https://doi.org/10.1111/ele.12585

Anastácio, P. M., Ferreira, M. P., Banha, F., Capinha, C., & Rabaça, J. E. (2014). Waterbird-mediated passive dispersal is a viable process for crayfish (*Procambarus clarkii*). *Aquatic Ecology*, *48*(1), 1–10. https://doi.org/10.1007/s10452-013-9461-0

Archer, F. I., Adams, P. E., & Schneiders, B. B. (2017). STRATAG: An R package for manipulating, summarizing and analysing population genetic data. *Molecular Ecology Resources*, *17*(1), 5–11. https://doi.org/10.1111/1755-0998.12559

Barrett, S. C. H. (2015). Foundations of invasion genetics: The Baker and Stebbins legacy. *Molecular Ecology*, *24*(9), 1927–1941. https://doi.org/10.1111/mec.13014

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., & Bonhomme, F. (1996). GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. *Laboratoire Génome*, *Populations*, *Interactions*, *CNRS UMR*. Retrieved from https://kimura.univ-montp2.fr/genetix/

Bélouard, N., Paillisson, J., Oger, A., Besnard, A., & Petit, E. J. (2019). Genetic drift during the spread phase of a biological invasion. *Molecular Ecology*, *28*(19), 4375–4387. https://doi.org/10.1111/mec.15238

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate - A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B. Methodological*, *57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bertelsmeier, C., & Keller, L. (2018). Bridgehead effects and role of adaptive evolution in invasive populations. *Trends in Ecology & Evolution*, *33*(7), 527–534. https://doi.org/10.1016/j.tree.2018.04.014

Blackburn, T. M., Lockwood, J. L., & Cassey, P. (2015). The influence of numbers on invasion success. *Molecular Ecology*, *24*(9), 1942–1953. https://doi.org/10.1111/mec.13075

Bock, D. G., Caseys, C., Cousens, R. D., Hahn, M. A., Heredia, S. M., Hübner, S., Turner, K. G., Whitney, K. D., & Rieseberg, L. H. (2015). What we still don't know about invasion genetics. *Molecular Ecology*, *24*(9), 2277–2297. https://doi.org/10.1111/mec.13032

Britton, J. R., Gozlan, R. E., & Copp, G. H. (2011). Managing non-native fish in the environment. *Fish and Fisheries*, *12*(3), 256–274. https://doi.org/10.1111/j.1467-2979.2010.00390.x

Buřič, M., Kouba, A., & Kozák, P. (2013). Reproductive plasticity in freshwater invader: From long-term sperm storage to parthenogenesis. *PLoS ONE*, *8*(10), e77597. https://doi.org/10.1371/journal.pone.0077597

CBD. (2014). *Pathways of introduction of invasive species*, *their prioritization and management*. Convention on Biological Diversity. UNEP/CBD/SBSTTA/18/9/Add.1. https://www.cbd.int/doc/meetings/sbstta/sbstta-18/official/sbstta-18-09-add1-en.pdf

Changeux, T. (2003). Évolution de la répartition des écrevisses en France Métropolitaine selon les enquêtes nationales mensées par le conseil supérieur de la pêche de 1977 à 2001. *Bulletin Français de La Pêche et de La Pisciculture*, *370–371*, 15–41. https://doi.org/10.1051/kmae:2003002

Coughlan, N. E., Kelly, T. C., Davenport, J., & Jansen, M. A. K. (2017). Up, up and away: Bird-mediated ectozoochorous dispersal between aquatic environments. *Freshwater Biology*, *62*(4), 631–648. https://doi.org/10.1111/fwb.12894

Cristescu, M. E. (2015). Genetic reconstructions of invasion history. *Molecular Ecology*, *24*(9), 2212–2225. https://doi.org/10.1111/mec.13117

Cucherousset, J., & Olden, J. D. (2011). Ecological impacts of non-native freshwater fishes. *Fisheries*, *36*(5), 215–230. https://doi.org/10.1080/03632415.2011.574578

Darling, J. A., & Folino-Rorem, N. C. (2009). Genetic analysis across different spatial scales reveals multiple dispersal mechanisms for the invasive hydrozoan *Cordylophora* in the Great Lakes. *Molecular Ecology*, *18*(23), 4827–4840. https://doi.org/10.1111/j.1365-294X.2009.04405.x

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Molecular Ecology Resources*, *14*(1), 209–214. https://doi.org/10.1111/1755-0998.12157

Evangelista, C., Britton, R. J., & Cucherousset, J. (2015). Impacts of invasive fish removal through angling on population characteristics and juvenile growth rate. *Ecology and Evolution*, *5*(11), 2193–2202. https://doi.org/10.1002/ece3.1471

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Filipová, L., Lieb, D. A., Grandjean, F., & Petrusek, A. (2011). Haplotype variation in the spiny-cheek crayfish *Orconectes limosus*: Colonization of Europe and genetic diversity of native stocks. *Journal of the North American Benthological Society*, *30*(4), 871–881. https://doi.org/10.1899/10-130.1

Fitzpatrick, B. M., Fordyce, J. A., Niemiller, M. L., & Reynolds, R. G. (2012). What can DNA tell us about biological invasions? *Biological Invasions*, *14*(2), 245–253. https://doi.org/10.1007/s10530-011-0064-1

Foll, M., & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics*, *180*(2), 977–993. https://doi.org/10.1534/genetics.108.092221

Gelman, A., & Hill, J. (2007). *Data analysis using regression and multilevel/hierarchical models*. Cambridge University Press. https://doi.org/10.1017/CBO9780511790942

Gelman, A., & Rubin, D. B. (1992). Inference from iterative simulation using multiple sequences. *Statistical Science*, *7*(4), 457–472. https://doi.org/10.1214/ss/1177011136

Goudet, J. (1995). FSTAT (Version 1.2): A computer program to calculate F-Statistics. *Journal of Heredity*, *86*(6), 485–486. https://doi.org/10.1093/oxfordjournals.jhered.a111627

Holdich, D. M. (1993). A review of astaciculture: Freshwater crayfish farming. *Aquatic Living Ressources*, *6*(4), 307–317. https://doi.org/10.1051/alr:1993032

Hortal, J., Nabout, J. C., Calatayud, J., Carneiro, F. M., Padial, A., Santos, A. M. C., Siqueira, T., Bokma, F., Bini, L. M., & Ventura, M. (2014). Perspectives on the use of lakes and ponds as model systems for macroecological research. *Journal of Limnology*, *73*(s1), https://doi.org/10.4081/jlimnol.2014.887

Hulák, M., Kašpar, V., Kozák, P., Buřià, M., Filipová, L., & Petrusek, A. (2010). Cross-species amplification of microsatellite markers in the invasive spiny-cheek crayfish (*Orconectes limosus*): Assessment and application. *Journal of Applied Genetics*, *51*(1), 73–78. https://doi.org/10.1007/BF03195713

Hulme, P. E. (2015). Invasion pathways at a crossroad: Policy and research challenges for managing alien species introductions. *Journal of Applied Ecology*, *52*(6), 1418–1424. https://doi.org/10.1111/1365-2664.12470

Hulme, P. E., Bacher, S., Kenis, M., Klotz, S., Kühn, I., Minchin, D., Nentwig, W., Olenin, S., Panov, V., Pergl, J., Pyšek, P., Roques, A., Sol, D., Solarz, W., & Vilà, M. (2008). Grasping at the routes of biological invasions: A framework for integrating pathways into policy. *Journal of Applied Ecology*, *45*(2), 403–414. https://doi.org/10.1111/j.1365-2664.2007.01442.x

Javal, M., Lombaert, E., Tsykun, T., Courtin, C., Kerdelhué, C., Prospero, S., Roques, A., & Roux, G. (2019). Deciphering the worldwide invasion of the Asian long-horned beetle: A recurrent invasion process from the native area together with a bridgehead effect. *Molecular Ecology*, *28*(5), 951–967. https://doi.org/10.1111/mec.15030

Jeschke, J. M., Bacher, S., Blackburn, T. M., Dick, J. T. A., Essl, F., Evans, T., Gaertner, M., Hulme, P. E., Kühn, I., Mrugała, A., Pergl, J., Pyšek, P., Rabitsch, W., Ricciardi, A., Richardson, D. M., Sendek, A., Vilà,

M., Winter, M., & Kumschick, S. (2014). Defining the impact of non-native species. *Conservation Biology*, *28*(5), 1188–1194. https://doi.org/10.1111/cobi.12299

Jiang, H., Qian, Z., Lu, W., Xing, Z., Yu, H., & Li, J. (2015). Microsatellite marker identification from transcriptome derived sequences of the red swamp crawfish, *Procambarus clarkii*. *Conservation Genetics Resources*, *7*(3), 729–731. https://doi.org/10.1007/s12686-015-0447-1

Lang, I., Evangelista, C., Everts, R. M., Loot, G., & Cucherousset, J. (2020). Stable resource polymorphism along the benthic littoral–pelagic axis in an invasive crayfish. *Ecology and Evolution*, *10*(5), 2650–2660. https://doi.org/10.1002/ece3.6095

Lang, I., Paz-Vinas, I., Cucherousset, J., & Loot, G. (2021). Patterns and determinants of phenotypic variability within two invasive crayfish species. *Freshwater Biology*, *66*(9), 1782–1798. https://doi.org/10.1111/fwb.13792

Laurent, P. J. (1997). Introductions d'écrevisses en France et dans le monde, historique et conséquences. *Bulletin Français de La Pêche et de La Pisciculture*, *344–345*, 345–356. https://doi.org/10.1051/kmae:1997034

Magnier, J., & Petit, K. (2016). L'enquête nationale sur les écrevisses. *Office International de l'eau*, *Note Technique*, pp. 1–21. https://www.eaufrance.fr/publications/lenquete-nationale-sur-les-ecrevisses-edition-2016

Meirmans, P. G., & Hedrick, P. W. (2011). Assessing population structure: F_{ST} and related measures. *Molecular Ecology Resources*, *11*(1), 5–18. https://doi.org/10.1111/j.1755-0998.2010.02927.x

Oficialdegui, F. J., Clavero, M., Sánchez, M. I., Green, A. J., Boyero, L., Michot, T. C., Klose, K., Kawai, T., & Lejeusne, C. (2019). Unravelling the global invasion routes of a worldwide invader, the red swamp crayfish (*Procambarus clarkii*). *Freshwater Biology*, *64*(8), 1382–1400. https://doi.org/10.1111/fwb.13312

Oficialdegui, F. J., Sánchez, M. I., Lejeusne, C., Pacini, N., & Clavero, M. (2020). Brought more than twice: The complex introduction history of the red swamp crayfish into Europe. *Knowledge & Management of Aquatic Ecosystems*, *421*, 2. https://doi.org/10.1051/kmae/2019044

Paz-Vinas, I., Lang, I., Millet, P., Veyssière, C., Loot, G., & Cucherousset, J. (2021). Data from: Inference of local invasion pathways in two invasive crayfish species displaying contrasting genetic patterns. *Figshare Repository*, https://doi.org/10.6084/m9.figshare.16574243.v1

Piry, S., Alapetite, A., Cornuet, J.-M., Paetkau, D., Baudouin, L., & Estoup, A. (2004). GENECLASS2: A software for genetic assignment and first-generation migrant detection. *The Journal of Heredity*, *95*(6), 536–539. https://doi.org/10.1093/jhered/esh074

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959. https://doi.org/10.1093/genetics/155.2.945

Puky, M. (2014). Invasive crayfish on land: *Orconectes limosus* (Rafinesque, 1817) (Decapoda: Cambaridae) crossed a terrestrial barrier to move from a side arm into the Danube River at Szeremle, Hungary. *Acta Zoologica Bulgarica*, *7*, 143–146.

Rey, O., Fourtune, L., Paz-Vinas, I., Loot, G., Veyssière, C., Roche, B., & Blanchet, S. (2015). Elucidating the spatio-temporal dynamics of an emerging wildlife pathogen using approximate Bayesian computation. *Molecular Ecology*, *24*(21), 5348–5363. https://doi.org/10.1111/mec.13401

Rollins, L. A., Moles, A. T., Lam, S., Buitenwerf, R., Buswell, J. M., Brandenburger, C. R., Flores-Moreno, H., Nielsen, K. B., Couchman, E., Brown, G. S., Thomson, F. J., Hemmings, F., Frankham, R., & Sherwin, W. B. (2013). High genetic diversity is not essential for successful introduction. *Ecology and Evolution*, *3*(13), 4501–4517. https://doi.org/10.1002/ece3.824

Rousset, F. (2008). genepop'007: A complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, *8*(1), 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., Pagad, S., Pyšek, P., Winter, M., Arianoutsou, M., Bacher, S., Blasius, B., Brundu, G., Capinha, C., Celesti-Grapow, L., Dawson, W., Dullinger, S., Fuentes, N., Jäger, H., … Essl, F. (2017). No saturation in the accumulation of alien species worldwide. *Nature Communications*, *8*, 14435. https://doi.org/10.1038/ncomms14435

Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., Pagad, S., Pyšek, P., van Kleunen, M., Winter, M., Ansong, M., Arianoutsou, M., Bacher, S., Blasius, B., Brockerhoff, E. G., Brundu, G., Capinha, C., Causton, C. E., Celesti-Grapow, L., … Essl, F. (2018). Global rise in emerging alien species results from increased accessibility of new source pools. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(10), E2264–E2273. https://doi.org/10.1073/pnas.1719429115

Sherpa, S., Blum, M. G. B., Capblancq, T., Cumer, T., Rioux, D., & Després, L. (2019). Unravelling the invasion history of the Asian tiger mosquito in Europe. *Molecular Ecology*, *28*(9), 2360–2377. https://doi.org/10.1111/mec.15071

Simberloff, D. (2009). The role of propagule pressure in biological invasions. *Annual Review of Ecology*, *Evolution*, *and Systematics*, *40*(1), 81–102. https://doi.org/10.1146/annurev.ecolsys.110308.120304

Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E., & Vilà, M. (2013). Impacts of biological invasions: What's what and the way forward. *Trends in Ecology & Evolution*, *28*(1), 58–66. https://doi.org/10.1016/j.tree.2012.07.013

Simon, A., Britton, R., Gozlan, R., van Oosterhout, C., Volckaert, F. A. M., & Hänfling, B. (2011). Invasive cyprinid fish in Europe originate from the single introduction of an admixed source population followed by a complex pattern of spread. *PLoS ONE*, *6*(6), e18560. https://doi.org/10.1371/journal.pone.0018560

Szpiech, Z. A., Jakobsson, M., & Rosenberg, N. A. (2008). ADZE: A rarefaction approach for counting alleles private to combinations of populations. *Bioinformatics*, *24*(21), 2498–2504. https://doi.org/10.1093/bioinformatics/btn478

Thomas, J. R., Masefield, S., Hunt, R., Wood, M. J., Hart, A. G., Hallam, J., Griffiths, S. W., & Cable, J. O. (2019). Terrestrial emigration behaviour of two invasive crayfish species. *Behavioural Processes*, *167*, 103917. https://doi.org/10.1016/j.beproc.2019.103917

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). micro-checker: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, *4*(3), 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Verity, R., & Nichols, R. A. (2016). Estimating the number of subpopulations (*K*) in structured populations. *Genetics*, *203*(4), 1827–1839. https://doi.org/10.1534/genetics.115.180992

Veselý, L., Ruokonen, T. J., Weiperth, A., Kubec, J., Szajbert, B., Guo, W., Ercoli, F., Bláha, M., Buřič, M., Hämäläinen, H., & Kouba, A. (2021). Trophic niches of three sympatric invasive crayfish of EU concern. *Hydrobiologia*, *848*(3), 727–737. https://doi.org/10.1007/s10750-020-04479-5

Washburn, B. A., Cashner, M. F., & Blanton, R. E. (2020). Small fish, large river: Surprisingly minimal genetic structure in a dispersal-limited, habitat specialist fish. *Ecology and Evolution*, *10*(4), 2253–2268. https://doi.org/10.1002/ece3.6064

Závorka, L., Lassus, R., Britton, J. R., & Cucherousset, J. (2020). Phenotypic responses of invasive species to removals affect ecosystem functioning and restoration. *Global Change Biology*, *26*(10), 5693–5704. https://doi.org/10.1111/gcb.15271

Zhan, A., Darling, J. A., Bock, D. G., Lacoursière-Roussel, A., MacIsaac, H. J., & Cristescu, M. E. (2012). Complex genetic patterns in closely related colonizing invasive species: Genetic Complexities in *Ciona intestinalis*. *Ecology and Evolution*, *2*(7), 1331–1346. https://doi.org/10.1002/ece3.258

Zhang, Y.-Y., Zhang, D.-Y., & Barrett, S. C. H. (2010). Genetic uniformity characterizes the invasive spread of water hyacinth (*Eichhornia crassipes*), a clonal aquatic plant. *Molecular Ecology*, *19*(9), 1774–1786. https://doi.org/10.1111/j.1365-294X.2010.04609.x

Zhao, T., Grenouillet, G., Pool, T., Tudesque, L., & Cucherousset, J. (2016). Environmental determinants of fish community structure in gravel pit lakes. *Ecology of Freshwater Fish*, *25*(3), 412–421. https://doi.org/10.1111/eff.12222

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of the article at the publisher's website.

How to cite this article: Paz-Vinas, I., Lang, I., Millet, P., Veyssière, C., Loot, G., & Cucherousset, J. (2021). Inference of local invasion pathways in two invasive crayfish species displaying contrasting genetic patterns. *Journal of Applied Ecology*, 58, 2854–2865. https://doi.org/10.1111/1365-2664.14023