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ORIGINAL ARTICLE

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Contrasting genetic population structures in acorn weevils (*Curculio* spp.) in expanding forests: The effects of differences in resource-tracking strategies

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Abstract

1. Woody vegetation spread over former croplands in Europe has created new unexploited habitats for forest organisms. Their ability to colonise them and thrive depends on life-history traits including fecundity, dormancy and dispersal ability.
2. The effects of these traits on species distribution, abundance and community assembly have been extensively studied in fragmented landscapes. However, their consequences for genetic diversity and connectivity in local populations remain largely unknown.
3. We investigated the genetic population structure and diversity of *Curculio elephas* and *Curculio glandium* (Coleoptera: Curculionidae), two sympatric acorn weevils with contrasting life-history strategies, in a landscape with mature oak stands and plots of new expanding forests.
4. Using a fragment of a mitochondrial gene cytochrome oxidase subunit 1 and nuclear DNA (80 single nucleotide polymorphisms [SNPs]), we found that gene flow between populations was significantly weaker in the poor disperser *C. elephas*, especially in isolated new forests. However, genetic neutrality tests did show population expansion in *C. elephas*, which suffers frequent population bottlenecks (probably linked to extended dormancy) and is a poor coloniser of isolated new forests. However, its greater fecundity allows it to recovery quickly if the number of reproductive individuals falls. Its populations are thus larger but genetically less diverse than those of *C. glandium*
5. Within foraging guilds, the most fecund species will outcompete the others under a context of constrained dispersal. Hence, new landscapes of expanding forests represent a good opportunity for more mobile but less fecund species to colonise new habitats and so be temporarily released from competition.

KEYWORDS

dispersal ability, dispersal/dormancy trade-offs, environmental stochasticity, forest fragments, *Quercus ilex*

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INTRODUCTION

One of the most striking landscape shifts linked to global change in Europe is the establishment of patches of woody vegetation on abandoned croplands (Baśnou et al., 2016; Jacquemyn et al., 2003; Palmero-Iniesta et al., 2021). These patches provide an opportunity for forest organisms, which find there new favourable habitats with as-yet unexploited food resources. Resource availability may, however, be subject to strong fluctuations, which can have dramatic effects on the survival of populations colonising small forest plots (Schmidt & Roland, 2006). Certain life-history traits (fecundity, dormancy and dispersal ability) affect species' ability to colonise and persist in these habitats (Ruiz-Carbayo et al., 2018), which in turn will determine community assemblages and the genetic diversity found in populations (Arias-Leclaire et al., 2018).

Organisms that feed on pulsed resources in patchy habitats have evolved a variety of life-history strategies to enable them to track unpredictable food availability over space and time. Two major strategies in short-lived organisms such as insects for coping with environmental stochasticity are spatial dispersal and dormancy (i.e. dispersal over time) (Venable & Brown, 1988; Venable & Lawlor, 1980). Both strategies imply specific adaptations, and the emergence of trade-offs between them is therefore expected due to energetic constraints (Zera & Harshman, 2001). In addition, species fecundity will determine the speed of local population growth upon arrival in a newly colonised patch. Species with greater dispersal ability can more readily colonise new patches and maintain a better landscape-scale population connectivity (Baguette et al., 2003; Doligez & Part, 2008; Ruiz-Carbayo et al., 2018). Poor dispersers, on the other hand, cannot escape adverse conditions by moving to other patches. Extended dormancy may help them to avoid periods of resource scarcity to some extent (Venner et al., 2011)––but it hinders individual survival (Menu & Desouhant, 2002) and so effective population size falls after lean years (Suez et al., 2013). Nonetheless, populations can recover quickly if species fecundity is sufficiently high.

Dispersal- and dormancy-centred strategies will affect the spatial distribution and relative abundance of species, as well as the genetic structure and diversity found within their populations. Thus, species with good dispersal ability should have higher within-population genetic diversity, better across-patch connectivity and, consequently, weaker population genetic structures than less mobile species. Species fecundity may have a serious effect too, as quick population growth derived from few individuals will result in populations with less genetic diversity than expected given their size. This pattern, known as 'sweepstake reproduction', is common in marine organisms in which, due to stochastic processes, only a fraction of the adult population reproduces successfully (Christie et al., 2010; Hedgecock & Pudovkin, 2011).

Although global forest loss and fragmentation threaten biodiversity conservation, the decline in world forest cover has begun to slow in recent decades (FAO, 2020) and, particularly in the northern hemisphere, in recent years forest cover has even increased (Kauppi et al., 2018). Although the species assemblies in these new forest patches have been explored in a number of studies (Cruz-Alonso et al., 2021; Jacquemyn et al., 2003; Valdés-Correcher et al., 2019; Verheyen et al., 2003), we are still largely unaware of how this assembly process interacts with food-tracking strategies and the fecundity of forest insects, and how it shapes their population dynamics and viability. It has been shown that the species composition of new forest patches depends on their degree of isolation and age (Fuller et al., 2018; Jeffries et al., 2006; Maldonado-López et al., 2015). However, we know much less about the effects of species' key life-history traits (dispersal and fecundity) on the genetic composition of colonising populations (but see Arias-Leclaire et al., 2018).

In this study, we analysed how the establishment of new forest stands affects population size, genetic structure and diversity in *Curculio elephas* (Marsham, 1802) and *Curculio glandium* (Gyllenhal 1836) (Coleoptera: Curculionidae), two specialist insects with contrasting life histories that predate on oak (*Quercus* spp.) acorns. *C. elephas* has a poorer dispersal capacity than *C. glandium* (Pélisson et al., 2013), but a more flexible dormancy (Pélisson et al., 2013; Soula & Menu, 2003; Venner et al., 2011). Both strategies enable them to cope with the great spatial and interannual variation in acorn crops that are typical of oaks (i.e. masting; Espelta et al., 2017). In terms of potential fecundity, the mean number of eggs per female is 30% higher in *C. elephas* than in *C. glandium* (Desouhant et al., 2000; Forrester, 1990; Menu, 1992) and larval survival during development in the former species is higher (Bonal et al., 2011). The two species follow different breeding strategies: *C. elephas* is a larger-sized capital breeder, able to start reproducing just after adult emergence, whereas in the smaller *C. glandium* females need to feed for a few months before acorns are available for oviposition (i.e. income breeder) (Pélisson et al., 2012)

C. elephas and *C. glandium* co-occur in our study area, which is characterised by landscapes with abundant holm oak (*Quercus ilex*) woodlands (Catalonia, NE Spain). The forest cover in the study area has increased by 20% over the past 50 years (Baśnou et al., 2013) and has generated a mosaic including mature forest stands, newly established forest patches adjacent to mature forests, and new isolated forest patches. *C. elephas* and *C. glandium* are the most important pre-dispersal acorn predators in these oak woodlands (Espelta et al., 2009). Given that *C. elephas* is able to survive situations with low acorn abundance by adapting its temporal behaviour (i.e. dormancy) and *C. glandium* its spatial behaviour (i.e. higher dispersal ability), landscape features have different impacts on their population dynamics and the resulting population genetic structure. This is also to be expected given the differences in their potential fecundity, since populations of the more fecund *C. elephas* are more likely to experience sweepstake reproduction events.

We combined extensive field sampling with the use of molecular techniques (mitochondrial DNA sequences and nuclear single nucleotide polymorphisms [SNPs]) to assess how life-history traits (dispersal ability, dormancy and potential fecundity) affect these two species' ability to colonise and persist in dynamic patchy landscapes. Our specific objectives were to investigate (i) whether or not the abundance and genetic diversity of these two species differ depending on the age and connectivity of the forest patches and (ii) how the contrasting key life-history traits of these two species influence the population genetic structure at landscape scale. According to their life histories,

we expect differences between the two acorn weevil species in genetic diversity and differentiation. The poor dispersal abilities of *C. elephas* could result in a lower gene flow between forest patches and a stronger population structure compared to *C. glandium*. Moreover, *C. elephas* poorer dispersal could also make this species more susceptible to local bottlenecks and loss of genetic diversity, as the arrival of immigrants from other forest patches would also be less likely than in *C. glandium*. However, *C. elephas* higher fecundity could promote a quick population growth from fewer surviving adults after extended dormancy.

MATERIALS AND METHODS

Acorn weevil biology

C. elephas and *C. glandium* (Coleoptera: Curculionidae) are specialist pre-dispersal seed predators whose larvae feed on oak acorns. Oviposition occurs in early autumn and larvae complete their development in a single acorn by feeding on its cotyledons (Bonal et al., 2010). Female potential fecundity averages 44 eggs per female in *C. elephas* (Desouhant et al., 2000; Menu, 1992) and 34 eggs in *C. glandium* (Drekic & Mihajlovic, 2007; Forrester, 1990). The greater fecundity of *C. elephas* is due to its larger body size (Hughes & Vogler, 2004); at intraspecific level, body size and female fecundity are also positively related (Desouhant et al., 2000; Forrester, 1990). Infested acorns drop prematurely and larvae leave them as soon as they have completed their growth. Subsequently, the larvae bury themselves in the ground and build overwintering earth cells within which they enter diapause. In *C. elephas*, diapause length is variable and the larvae of a single cohort may emerge after 1, 2 or 3 years, whereas in *C. glandium* larvae spend 2 years buried before pupating and emerging to the surface as adults (Pélisson et al., 2013; Soula & Menu, 2003; Venner et al., 2011). The timing of adult emergence differs between species too: in *C. elephas* it occurs from late summer to early autumn and in *C. glandium* in spring. Importantly, emergence in the late-emerging *C. elephas* is triggered by late-summer rains and a lack of precipitation provokes weevil mortality (Bonal et al., 2015; Espelta et al., 2017). This phenological difference affords *C. glandium* adults several months in which to disperse to other habitat patches, whereas *C. elephas* only has a very short period of time between adult emergence and oviposition; as a result, *C. elephas* adults typically reproduce in the same tree under which they emerged (Bonal et al., 2012; Menu & Debouzie, 1993). *C. elephas* dispersal abilities are poorer than those of *C. glandium*: wind-tunnel experiments have revealed maximum potential dispersal distances of up to 600 m in the former and 1200 m in the latter (Pélisson et al., 2013). Although such laboratory tests may overestimate natural dispersal, they match field observations, whereby local populations of *C. elephas* but not *C. glandium* exhibit a colonisation lag from old forests (OFs) patches to recently established ones (Ruiz-Carbayo et al., 2018). *C. elephas* are poorer dispersers but proovigenic capital breeders: females do not need to feed before starting oviposition, which occurs as soon as they have emerged and mated. In contrast, synovigenic *C. glandium* females cannot mate if they do not feed in the period between spring and early autumn (when acorns are available for oviposition) (Pélisson et al., 2012).

Study area and experimental design

The sampling was carried out in 2014—a year of abundant acorn crops—in the Vallès lowland (Barcelona province, Spain, 41°33′ N, 2°2′ E; Figure 1). The climate of the area is Mediterranean with annual average rainfall of about 650 mm and monthly mean temperatures ranging from 6°C in winter up to 23°C in summer. The landscape is a mosaic of croplands, built-up areas and forest patches dominated by pines (*Pinus pinea* L. and *P. halepensis* Mill.) and oaks (*Quercus ilex* L. and *Quercus pubescens* L. Mill.).

We selected six sampling locations that served as replicates, and each contained three forest patches of different ages and connectivity (18 forest patches in total) (Figure 1). Patch selection was based on a comparison of land-cover maps and orthoimages dating from 1956 and 2009. Forest patches already present in 1956 are hereafter referred to as 'old forests' (OF) and younger patches as 'new forests' (NF). The latter were further classified as isolated (INF) or connected (CNF) new forests based on the forest cover within a 600-m-radius buffer around each patch (a distance corresponding to the observed maximum dispersal distance of *C. elephas*). Patches with less than 20% of forest cover were considered to be isolated and those with more than 20% to be connected. We did not determine connectivity classes for OF patches because they are present as large continuous areas in the study area.

In late August 2014, we randomly selected five seed-producing trees within each forest patch (90 in total). We sampled each tree three times to cover the beginning, peak and end of the fruiting season and to thus control for eventual differences in oviposition phenology (Bonal et al., 2011). During each sample event, we gathered a random sample of 30 acorns. Back in the laboratory, we separated sound from infested acorns according to the presence or absence of *Curculio* oviposition scars. Acorns were placed in individual trays and checked every 2 days for emerged larvae (see Bonal & Muñoz, 2009 for further details on this methodology). All larvae were preserved in 99% ethanol and stored at low temperature (4°C) before genetic analyses.

Molecular analyses

We randomly selected a subsample of 540 larvae (10 per forest patch and sampling event) for the molecular analyses. We extracted DNA from a small piece of larval tissue using NucleoSpin® Tissue (Macherey-Nagel GmbH and Co. KG, Düren, Germany) following the manufacturer's protocol. As the two species cannot be distinguished from their larval morphologies, we used DNA barcoding to perform species identification (Ruiz-Carbayo et al., 2018). For each individual, we sequenced a fragment (length 826 bp) of the mitochondrial gene cytochrome oxidase subunit 1 (cox1) and compared it with reference DNA barcodes from adult individuals previously identified to species level (see Bonal et al., 2011). The same sequence was also subsequently used for population-level analyses.

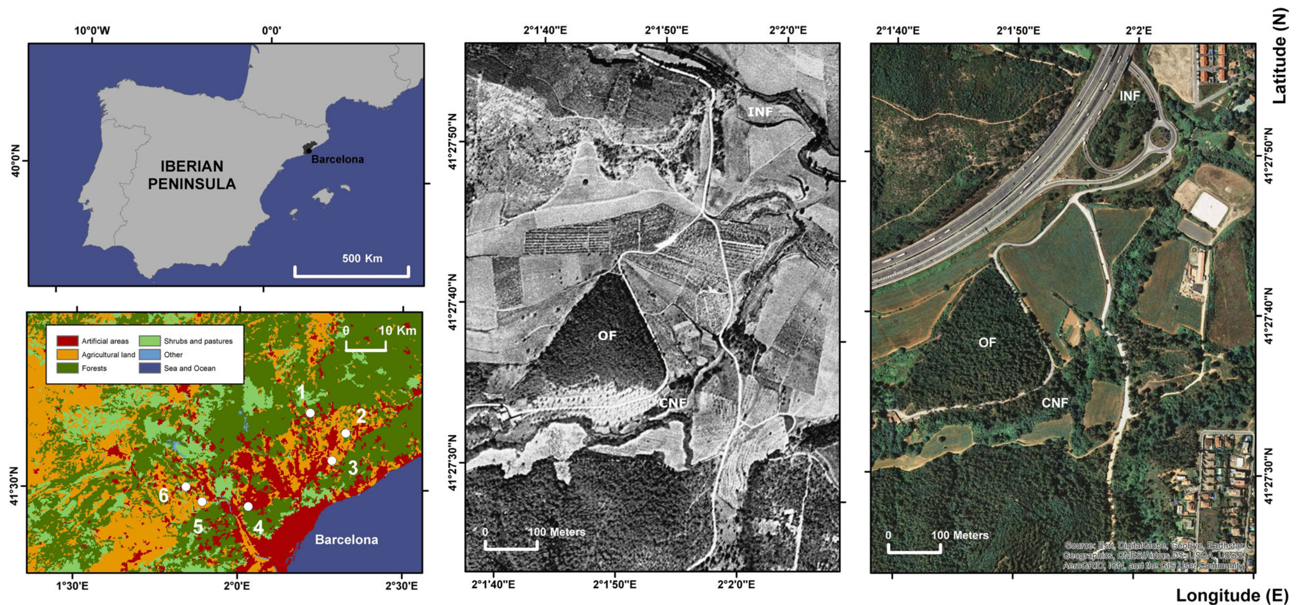

FIGURE 1 Location of the study sites. Top left: context and study area. Bottom left: location of the sampling replicates. Centre and right: one replicate to exemplify the experimental design with an aerial photograph from 1956 (centre) and 2005 (right). CNF, connected new forest; INF, isolated new forest; OF, old forest

The fragment was amplified via PCR using Pat and Jerry primers following the methods described by Hughes and Vogler (2004). The 14-μl PCR reactions contained 1.5 μl of template DNA sample, 11.2 μl of double-distilled water, 1.5 μl of PCR buffer, 0.6 μl of $MgCl_2$, 0.25 μl of deoxyribonucleotide triphosphate (100 mmol/L), 0.2 each of primer (10 μmol/L) (forward [F] and reverse [R]), and 0.06 μl of Taq polymerase (Linus). PCRs were run in a PTC-100® Thermal Cycler (Bio-Rad Laboratories, Hercules, California) under the following conditions: a 9-min denaturation at 95°C, 40 cycles of 30-s denaturation at 94°C, a 45-s annealing at 50°C, and a 45-s min elongation at 72°C, with a final extension step of 10 min at 72°C. The presence of the 826-bp DNA fragment in the sample was determined by electrophoresis on 3% (w/v) agarose gels with 1× Tris–borate–EDTA buffer at a voltage of 60 V for 25 min, and 70 V for 15 min. Finally, we looked for the DNA band in the agarose gel stained with 0.005% Midori Green nucleic acid staining solution. Sequencing was performed using Big-Dye (Perkin-Elmer) technology and an ABI3700 sequencer.

We accomplished forward- and reverse-strand editing using SEQUENCHER 4.1 (Gene Codes Corp., Ann Arbor, MI, USA), and aligned with CLUSTALW supplied via http://align.genome.jp. We collapsed alignment sets into single haplotypes and compared them with the *Curculio* spp. reference sequences available for Holarctic *Curculio* (see Hughes & Vogler, 2004 for accession numbers at GenBank). The comparison showed that all sequences belonged to either *C. elephas* or *C. glandium*. The raw genetic divergence (total number of differences divided by the total sequence length) between our samples and the corresponding reference sequences was consistently below 1%, that is, much lower than the divergence between the two species (Bonal et al., 2011).

The same larval samples were subjected to a second analysis using a set of nuclear SNP markers designed for this study. For this purpose, we used four *C. elephas* larvae from four distinct study patches, whose DNA was used for ddRAD library preparation (Peterson et al., 2012). Genomic DNA was quantified using a Qubit Fluorometer (Invitrogen). We carried out sample dilutions to 20 ng/μl using Biomek® NXP Laboratory Automation Workstation (Beckman Coulter). The protocol for library preparation followed the methods described by Pukk et al. (2015) with minor modifications. The final library was diluted to 10 pM before sequencing on an Ion Torrent PROTON (Thermo Fisher Scientific). About 10 Gb of data were generated and analysed using the Ion Torrent Suite Software to detect SNPs distributed across the genome. Using stringent technical (depth of coverage >10×) and biological (high heterozygosity) criteria, we selected in silico 160 SNPs. Specific primers were designed for amplifying 100-bp long fragments that contained these 160 SNPs. These were then used to genotype a subset of 96 individuals using the Mass Array Sequenom iPLEX Gold assay (Sequenom 2008). The scatter plots of all genotyped SNPs were inspected using TYPER 4.0 and a subset of 80 di-allelic SNPs were chosen according to their amplification success and polymorphism across individuals. These 80 SNPs were further arranged in two multiplexes of 40 SNPs each, which were used to genotype all the samples, again using the Mass Array Sequenom iPLEX Gold assay (Sequenom 2008) (data on sequences of SNPs and primers to be uploaded to GenBank if this article is accepted for publication).

Analyses of molecular data

Mitochondrial DNA

For each identified group of *C. elephas* and *C. glandium* samples stemming from the same forest patch (hereafter referred to as a 'local population'), we calculated rarefied haplotype richness (A_r), gene diversity (H) and nucleotide diversity (π). We also tested for signals of recent

TABLE 1 Posterior summaries of predictors from linear mixed models (lmm) fitted to detect effects on local population size, haplotype richness, gene and nucleotide diversity and pairwise Fst for both, *C. elephas* and *C. glandium*, and for each forest type: old forests (OF), connected new forests (CNF) and isolated new forests (INF); the study site (i.e. the replicate group) was included as a random factor

Response		Predictor		Coefficient	df	*p* value	R^2 marginal	R^2 conditional
		Fixed						
Population size							0.360	0.360
		Species	***C. glandium***	**−7.667**	**32**	**<0.001**		
		Forest type	CNF	−0.333	32	0.877		
			INF	−1.000	32	0.642		
Haplotype richness							0.286	0.510
		Species	***C. glandium***	0.684	23	**0.004**		
		Forest type	CNF	−0.324	**21**	0.211		
			INF	−0.612	**21**	**0.023**		
Gene diversity							0.253	0.426
		Population size		−0.009	25	0.133		
		Species	***C. glandium***	**0.113**	**23**	**0.068**		
Nucleotide diversity							0.701	0.793
		Population size		−0.013	25	0.539		
		Species	*C. glandium*	1.826	23	**<0.001**		
Pairwise F_{st}	COI						0.251	0.251
		Forest type pair	OF-CNF	−0.039	23	0.544		
			CNF-INF	−0.075	23	0.242		
		Species	*C. glandium*	−0.147	23	**0.013**		
	SNP						0.189	0.478
		Forest type pair	OF-CNF	−0.014	10	0.027		
			CNF-INF	−0.007	10	0.211		

Note: Values in bold indicate significant differences. Haplotype richness, gene diversity and nucleotide diversity compared between *curculio* species and forest types were calculated on mitochondrial DNA (fragment of gene cytochrome oxidase I). Pairwise gene flow (F_{st}) between plots was calculated in both curculio species using the same mitochondrial gene.

local population bottlenecks or expansions using Tajima's *D* (Tajima, 1989) and Fu's F_S tests (Fu, 1997). In addition, we plotted haplotype networks for each study replicate and species with the *haploNet* function of the *pegas* package (Paradis, 2010) in R version 3.4.1 (R Core Team, 2017). For all analyses, we removed all forest patches in which we had collected and sequenced less than five individuals of a given species (see recommendations in the study by Papadopoulou et al., 2011) and so all 18 *C. elephas* local populations but only 12 *C. glandium* local populations were retained (see Table S1).

To quantify the historical gene flow between local populations, pairwise F_{ST} values were computed and their significance was assessed using a permutation test with 1000 permutations. To avoid potential confounding effects that might result from the use of just F_{ST} for between species comparisons (Meirmans & Hedrick, 2011), we also calculated pairwise Nei's average number of differences between populations (Nei, & Li., 1979). All these analyses were performed in ARLEQUIN version 3.0 (Excoffier et al., 2005).

We further tested both species for the existence of genetic structure between populations by analysing molecular variance (AMOVAs) implemented in ARLEQUIN. The presence of geographical patterns in the genetic structure was assessed using the software SAMOVA 1.0 (Dupanloup et al., 2002). We simulated different numbers of populations (*K*) in the range of $K = 2–17$ for *C. elephas* and $K = 2–11$ for *C. glandium* to search for the optimal grouping option that maximises the among-group component (F_{CT}) of the overall genetic variance.

Nuclear DNA SNPs

We could only analyse the SNP data for *C. elephas* because of the low amplification success and polymorphism in *C. glandium*. We calculated observed heterozygosity (H_O) and expected heterozygosity (H_E) for each marker in each local population, whose deviation from the Hardy–Weinberg equilibrium was tested with 1,000,000 permutations. We also calculated pairwise F_{ST} values between populations using the same procedure as for the mtDNA sequences. We tested for isolation by distance in using a Mantel test as implemented in GenAlEx 6.5 (Peakall & Smouse, 2012), with 1000 permutations with the pairwise F_{ST} values as genetic matrix and simple Euclidean distances as geographical distances.

We carried out Bayesian clustering using STRUCTURE (Pritchard et al., 2000) to assess the global genetic population structure of *C. elephas*. We assumed correlated allele frequencies and admixture and set the number of runs to $K = 1–21$ (corresponding to the number of forest patches plus 3) to estimate the number of clusters with 100,000 MCMC cycles following a burn-in of 10,000 iterations. We performed 20 iterations for each *K*. The number of local populations best fitting the data set was defined using the ΔK method (Evanno et al., 2005). In addition to STRUCTURE, we performed a principal coordinate analysis (PCoA)—covariance standardised method—, as implemented in GENALEX 6.5 (Peakall & Smouse, 2012) to evaluate spatial genetic structure. We first computed a pairwise F_{ST} comparison matrix based on the DNA SNPs, which was used to perform the PCoA after calculating the Euclidean distance between all pairs of patches on the basis of their geographical coordinates.

Statistical analyses

Linear mixed models (LMMs) were built to test for differences in local population size among forest types and between species; the study site (i.e. the replicate group) was included as a random factor. We also used LMMs to test the effects of local population size, species and forest type on haplotype richness, gene diversity and nucleotide diversity. Saturated models included second-degree interactions of the predictors. In addition, we evaluated whether or not the age and the isolation of patches influenced gene flow. For each study replicate, we set three different types of pairwise comparisons: old versus connected new forests (OF-CNF), old versus isolated new forests (OF-INF), and connected versus isolated new forests (CNF-INF). We assessed whether or not gene flow differed in terms of the type of pairwise comparison, and whether or not it differed between the two species; the interaction between both factors was also tested. We used the inverse of the pairwise F_{ST} values (with negative F_{ST} values set to zero) as a proxy of gene flow (Toju & Sota, 2006). Models were performed with the *lmer* function implemented in the R package *lme4* using the REML method (Bates et al., 2015). Model selection followed a backward procedure in which the least significant variables, based on the output of the *anova* function (package *lmerTest*), were progressively eliminated from the saturated model. Validation of the model included the calculation of the coefficient of determination (R^2) and the normality of model residuals.

RESULTS

Molecular analyses

Mitochondrial DNA

We sequenced and identified a total of 392 weevils. The remaining samples were not used because the PCR failed or the sequences did not meet the minimum quality/length for use in further population genetic tests. Of these 392 individuals, 265 belonged to *C. elephas* and 127 to *C. glandium*. Irrespective of the age and isolation of the forest patch, *C. elephas* was the most abundant species (Table 1).

Haplotype richness was significantly higher in the OFs than in the isolated new ones (INF) (mean ± SD: OF $= 3.27 \pm 0.95$; CNF $= 2.96 \pm 0.69$; INF $= 2.66 \pm 0.64$. lmm: $t = -2.454$, df $= 21$, $p = 0.023$) and also lower in *C. elephas* than in *C. glandium* (mean ± SD: *C. elephas* $= 2.64 \pm 0.66$; *C. glandium* $= 3.44 \pm 0.74$. lmm: $t = 3.184$, df $= 23$, $p = 0.004$; see Figure S1 and Table 1). Conversely, genetic diversity did not differ among forest types and only the factor *species* was significant. Both nucleotide (π_n) and gene (*H*) diversity were lower in *C. elephas* ($\pi_n = 0.001 \pm 0.001$ and $H = 0.605 \pm 0.183$) than in *C. glandium* (mean ± SD: $\pi_n = 0.012 \pm 0.01$ and $H = 0.796 \pm 0.106$; see Table S1 and Figure 2). In the best model, only the factor *species* was significant in the case of nucleotide diversity ($t = 8.113$, $p < 0.001$) and marginally significant for gene diversity ($t = 1.915$, $p = 0.068$; Table 1). Regarding local population dynamics, neutrality tests only showed significant values for *C. elephas* (Table S2).

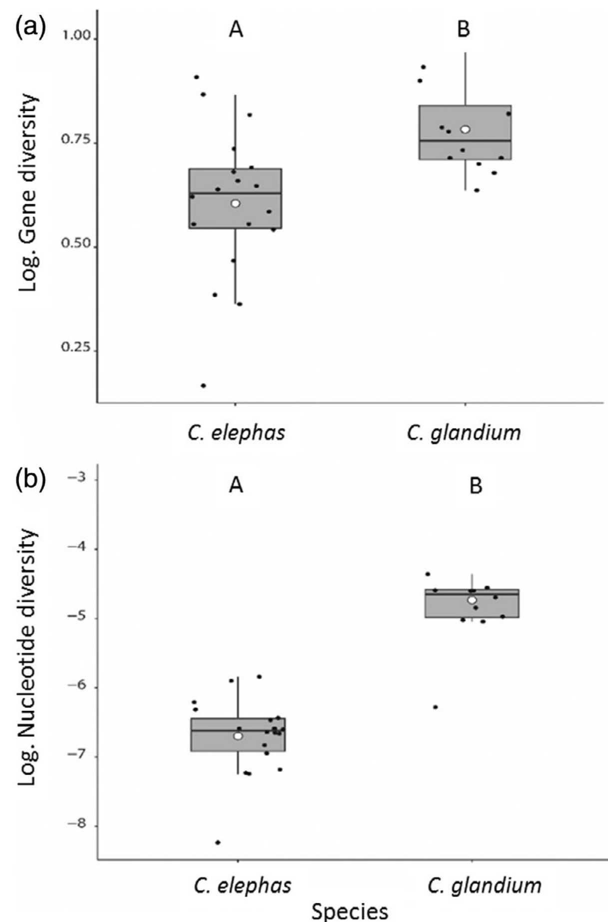

FIGURE 2 Boxplots illustrating differences in mitochondrial DNA (cytochrome oxidase I) gene (a) and nucleotide (b) diversity between *C. elephas* and *C. glandium*. Box plots show minimum, lower quartile, median, upper quartile and maximum values. Means are depicted as white dots. Different letters on the box plots indicate significant differences.

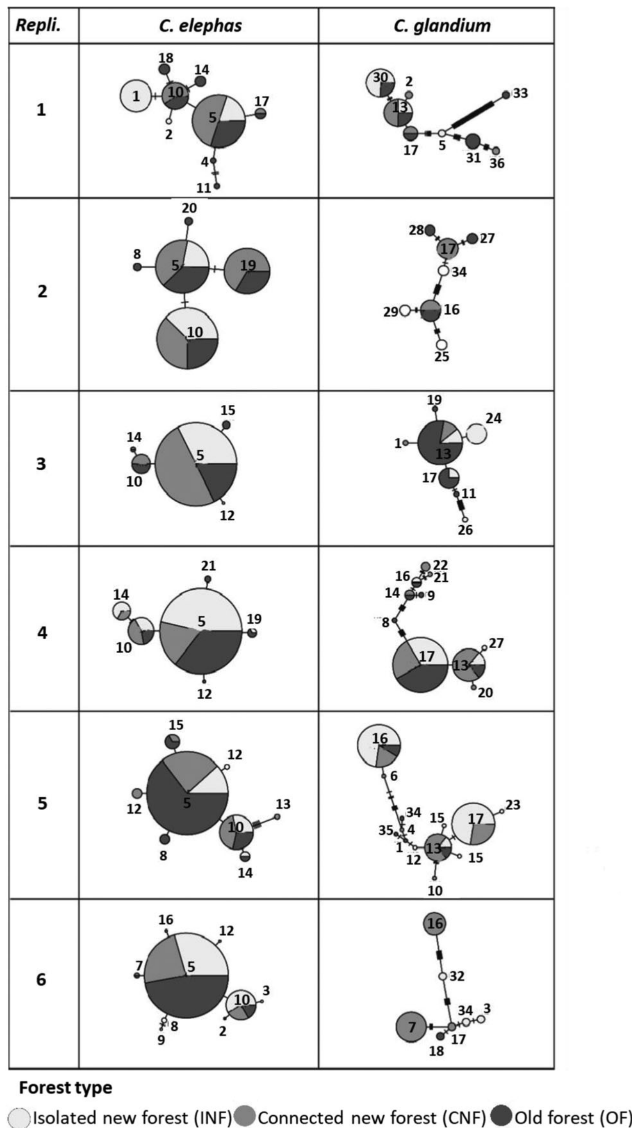

FIGURE 3 Parsimony network for mtDNA haplotypes of *C. elephas* and *C. glandium* in each study replicate. The size of the circles represents haplotype frequencies in each site. Bars represent unique mutation steps.

The analyses of haplotype networks showed that *C. elephas* local populations were in most cases dominated by one or few haplotypes, with the less frequent ones differing in just one nucleotide base pair (Figure 3). In accordance with the significant negative values in the neutrality tests (Table S2), these networks showed the typical star-like shape of populations undergoing expansion (Figure 3). In *C. glandium*, local populations within replicates contained haplotypes with similar frequencies that commonly differed in several base pairs.

The analysis of molecular variance (AMOVA) revealed a highly significant population structure in *C. elephas* (Table 2). Differences between local populations explained 10.5% of the total genetic variance when taking all 18 forest patches into account, and 12.3% when considering the 12 patches that could also be used in the analysis for *C. glandium*. Pairwise F_{ST} values were significant in three OF-INF patch comparisons (Table S3). In contrast, only a marginally significant ($p = 0.06$) population structure with a global F_{ST} value of 5.6% was found in *C. glandium* (Table 2). We detected no significant pairwise F_{ST} values in this species. Population differentiation was also stronger in *C. elephas* than in *C. glandium* according to pairwise Nei's average number of differences. A higher number of pairwise differences were significant in *C. elephas* and two of them were OF-INF patch comparisons. None of the fewer significant pairwise differences corresponded to these comparisons in *C. glandium* (Table S4). The SAMOVA analysis did not retrieve any significant geographical genetic structure at landscape scale for either *C. elephas* or *C. glandium* ($p > 0.05$).

Nuclear SNPs

The mean observed and expected heterozygosity of all *C. elephas* populations ranged from 0.368 to 0.443 and from 0.400 to 0.430, respectively. We found deviations from Hardy–Weinberg Equilibrium (HWE) (after a Bonferroni correction) in nine populations, with a maximum of three deviating loci (3.75% of the total) per population. Average gene diversity over loci did not differ between the different types of forest plots, namely, OF, CNF or INF ($F_{1,15} = 0.56$, $p = 0.56$).

Pairwise F_{ST} values were lower than those measured with mtDNA markers; more F_{ST} pairwise comparisons were significant between OF and INF than between OF and CNF or between CNF and INF (two and three significant comparisons, respectively; Table S3). F_{ST} values for the OF-INF comparisons exceeded those for OF-CNF (linear mixed model: $t = -2.48$, $p = 0.027$; see Figure 4). However, at the larger scale of the total study area, the Mantel test showed no significant isolation by distance ($R^2 = 0.002$, $p = 0.36$).

The ΔK statistics conducted for the STRUCTURE analysis suggested an optimal value of $K = 2$ (Figure S2). However, it is possible that this was an artefact given that (i) $K = 2$ is the minimum number of divisions allowed by the software, (ii) all local populations had a very high degree of genetic admixture under $K = 2$ (Figure S3), and (iii) there was no discernible trend between the different replicates or between the different types of forest within each replicate. The PCoA retrieved a similar result. There was not a clear grouping either among replicates or patches within replicates. There was a large group that included most of the patches and only three were clearly segregated from it. However, the distance between them was large and, moreover, they belonged to different replicates (Figure S4).

DISCUSSION

As expected, the different life-history strategies of *C. elephas* and *C. glandium* for tracking acorn availability affect their abundance and population genetics in fragmented forest landscapes. Our results fulfilled our predictions on the positive relationship between species dispersal abilities and gene flow among populations. In agreement with its poorer dispersal ability observed in laboratory assays (Pélisson et al., 2013), the population genetic structure of *C. elephas* was significant and twice as

TABLE 2 Results of the intraspecific AMOVA analyses (mitochondrial DNA) showing the partition of the total genetic variance between local populations (i.e. forest plots) for both *C. elephas* and *C. glandium*

Type of analysis	Source of variation	df	Sum of squares	Percentage of variation
C. elephas (18 populations)	Among forest plots	17	18.43	**10.52**
	Within forest plots	247	98.47	89.48
C. elephas (12 populations)	Among forest plots	11	14.47	**12.27**
	Within forest plots	142	67.24	87.73
C. glandium (12 populations)	Among forest plots	11	43.18	5.58
	Within forest plots	99	256.99	94.45

Note: Two analyses are reported for *C. elephas*: Including all 18 populations and including only those 12 populations in which *C. glandium* was also present. Values in bold indicate significant differences ($p < 0.01$).

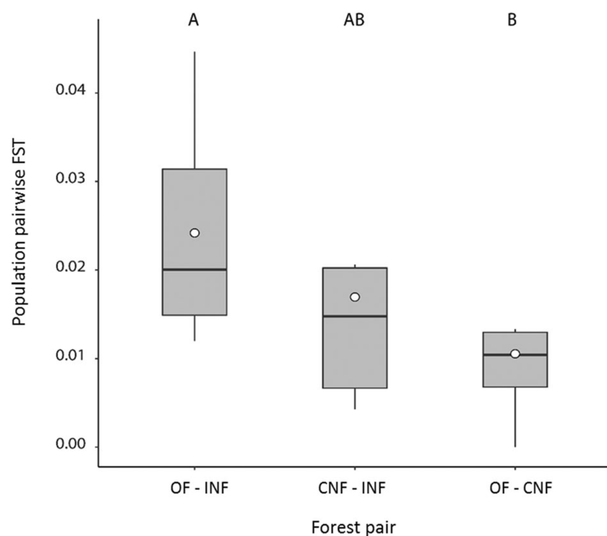

FIGURE 4 Boxplot illustrating differences in the three possible pairwise F_{st} comparisons (calculated using *C. elephas* DNA SNPs) between forest types for SNPs (CNF, connected new forest; INF, isolated new forest; OF, old forest). Box plots show minimum, lower quartile, median, upper quartile and maximum values. Means are depicted as white dots. Different letters on the box plots indicate significant differences.

strong as *C. glandium*. *C. elephas* also exhibited increased population divergence between mature oak stands and isolated new forest patches. These findings agree with the colonisation credits previously reported for *C. elephas* in isolated new forests in the same area, as the proportion of *C. glandium* is higher in these plots than in old forests (Ruiz-Carbayo et al., 2018). *C. elephas* is thus a poorer coloniser of isolated oak patches than *C. glandium*, despite being in absolute numbers the more abundant of the two species in all plots. This observation suggests that this species employs other strategies to compensate for its poorer ability to track food resources spatially.

The success of *C. elephas* could be linked to its greater resilience, which could help it to persist in isolated new forests even if only a very small number of colonisers arrive. Compared to other acorn weevils, this species is considered to rely more on variable dormancy to cope with the extreme fluctuation of acorn crops between years, and it is more likely to disperse 'in time' (sensu Venable & Brown, 1988) than 'in space' when tracking food resources. However, *C. elephas* overall has less genetic diversity, and the neutrality tests show that population expansion occurs from one or a few common mitochondrial haplotypes in most plots, and, strikingly, also in mature forest stands. Thus, dormancy apparently does not completely prevent *C. elephas* from suffering sporadic reductions in its effective population size.

Flexible dormancy (i.e. variable length diapause) in *C. elephas* assures that at least some individuals will emerge once adverse environmental conditions have improved. However, its effective population size will sharply decrease in the process. In any cohort, more than 60% of the individuals will be ready to emerge after 1 year (Pélisson et al., 2012). If they fail to reproduce, population genetic diversity will be significantly reduced. Such reproductive failure may be relatively common given the interannual variability in acorn production (Espelta et al., 2008). In addition, *C. elephas* adults emerge in late summer and many may die in dry years because they cannot leave their subterranean overwintering cells due to the hardened soil (Bogdziewicz et al., 2020; Bonal et al., 2015; Espelta et al., 2017). Those individuals that do not emerge after 1 year will face additional risks as prolonged diapause is costly in energetic terms (Menu & Desouhant, 2002); some will die before completing their diapause (Menu & Desouhant, 2002), thereby leading to a further loss of genetic diversity (Suez et al., 2013). Together, all these factors may help override the positive effects of increased generation overlap in population genetic diversity in *C. elaphas*. *C. glandium*, on the other hand, emerges in spring when adult mortality linked to drought is less likely. This phenology, along with its better dispersal ability, may partially explain its greater genetic diversity in the forest plots.

As predicted from its higher fecundity, *C. elephas* had larger populations than *C. glandium*; however, they were less genetically diverse. This result provides a clue as to the factors that might underlie its demographic dynamics. The pattern shown by *C. elephas* matches that of species in which very few individuals within a population contribute most offspring to the next generation, a phenomenon known as the sweepstake reproductive strategy (SRS) (Hedgecock, 1994). So far, this type of reproduction has almost only ever been described in marine animals inhabiting highly unpredictable habitats (Christie et al., 2010; Hedgecock & Pudovkin, 2011; Riquet et al., 2017). In these species, each female produces a large number of offspring,

which in many cases go through a pelagic life stage in which the vast majority fail to develop into adults and reproduce. However, the offspring of one or a few females will survive and so the new reproductive population will to a large extent be composed of genetically similar individuals. *C. elephas* is more likely to suffer population bottlenecks (Bogdziewicz et al., 2020; Bonal et al., 2015; Espelta et al., 2017) but *C. elephas* females lay, on average, more eggs than *C. glandium* females (Forrester, 1990; Menu, 1992) and, moreover, their larval survival will also be higher (Bonal et al., 2011).

Our results matched our predictions on the lower genetic diversity of *C. elephas* compared to *C. glandium*, which would also be affected by the restrictions for conspecific immigration due to the poorer dispersal abilities. In this sense, we acknowledge that one of the limitations of our study arises from the failure of the nuclear SNPs in *C. glandium*. Interspecific comparisons based on mitochondrial DNA could be biased as these genes are maternally inherited and only reflect female movements, which could be misleading if dispersal behaviour differs between sexes (Petit & Excoffier, 2009; Scribner et al., 2001). However, the concordance between mitochondrial and nuclear markers in *C. elephas* (i.e. higher gene-flow restriction in isolated new forests) suggests that such divergences do not exist in this species and hence probably do not affect the results of the species comparison.

The differences in parameter estimations may be also a result of the characteristics of the markers. Mitochondrial DNA is haploid and maternally inherited, and its effective population size is approximately one-fourth that of nuclear DNA (Hui et al., 2017; Keeney et al., 2005). Thus, mitochondrial DNA is more susceptible to genetic drift and inter-population differentiation. In our study, population pairwise F_{ST} values were higher in mitochondrial DNA. Accordingly, the AMOVA showed only significant population structure with this marker type. Such limitations could be overcome using a much larger number of nuclear markers (e.g. Nowland et al., 2019). The use of different tests of pairwise population differentiation also produced slight disparities. In *C. glandium*, there were no significant F_{ST}, whereas some of them were so when we computed pairwise Nei's average number of differences. However, this did not alter the marked contrast between species in this regard, as with both tests the number of significant pairwise comparisons was notably higher in *C. elephas* than in *C. glandium*. Beyond population level, no structure was detected using either type of marker. Neither SAMOVA, STRUCTURE or the PCoA found any genetic affinity between groups of populations at the geographical scale of this the study.

To conclude, our study shows that the life histories of insect species inhabiting expanding forest landscapes can leave a detectable genetic signature. In our study, a genetic signature of population expansion was found in the poor disperser *C. elephas* in both expanding new forests and mature stands. Hence, in this species, founder effects could be further reinforced by subsequent bottlenecks that will reduce the effective population size in all types of forest plots. Nonetheless, our data show that *C. elephas* numbers can recover quickly from a low number of reproductive individuals (i.e. via sweepstake reproduction). Accordingly, in most plots its populations were larger, albeit less genetically diverse, than those of the less fecund but more mobile *C. glandium*. The Neutral Theory of Biodiversity (Hubbell, 2001) states that, if the fitness of co-occurring species is not similar, those with lower fitness will be displaced. In this context, Zhou et al. (2015) have shown that limitations on dispersal favour more fecund species. Hence, fragmented landscapes with new isolated forest patches may well provide a competitive advantage for less fecund but more mobile species.

AUTHOR CONTRIBUTIONS

Helena Ruiz-Carbayo: Conceptualization (equal); formal analysis (lead); investigation (lead); methodology (equal); writing – original draft (lead). **Josep M Espelta:** Conceptualization (lead); formal analysis (equal); funding acquisition (lead); investigation (equal); methodology (lead); writing – original draft (equal); writing – review and editing (equal). **Joan Pino:** Conceptualization (equal); formal analysis (equal); funding acquisition (lead); investigation (supporting); writing – review and editing (equal). **Arndt Hampe:** Conceptualization (equal); formal analysis (equal); funding acquisition (lead); writing – review and editing (lead). **Raul Bonal:** Conceptualization (lead); formal analysis (lead); funding acquisition (lead); investigation (equal); methodology (lead); writing – original draft (lead); writing – review and editing (lead).

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CONFLICT OF INTEREST

There are no conflicts of interest to be declared among the authors of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1 Supporting Information.

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