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Fragmentation shapes nest density and social structure but not genetic diversity of *Temnothorax crassispinus* (Formicidae)

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Abstract

Human activities affect biodiversity by reducing the area of habitats, altering their shape, and increasing their isolation. Ants are particularly sensitive to habitat fragmentation, as it may locally change abiotic conditions, the availability of food and nest sites, the abundance of mutualists, competitors and predators, and also restrict gene flow between patches. As a result, the genetic population and colony structure of ants is expected to show signs of fragmentation. In the present study, we investigated the impact of fragmentation on the ant *Temnothorax crassispinus* in 45 forest patches across the Franconian Jura, Germany. Based on 283 colonies, of which 156 were genetically analyzed, we evidenced the presence of two putative distinct genetic clusters in the study area. Both the nest densities and the presence of queen were impacted by the number of adjacent forest patches within 400 m around the focal patch, but neither by patch shape nor patch size. We could not detect any effect of fragmentation on the genetic diversity, probably because the high dispersal abilities of *T. crassispinus* counterbalance any detrimental genetic consequences of fragmentation. Nevertheless, fragmentation still impacts the species density as well as the social structure of its colonies. Further investigations regarding the drivers of occurrence at a finer spatial scale might clarify the role of edge effects on the occurrence of this species.

KEYWORDS

connectivity, gene flow, landscape fragmentation, patch size, spatial structure

1 | INTRODUCTION

Biodiversity faces growing pressures from human actions, including fragmentation of natural habitats, that is, the landscape-scale habitat loss resulting in the division of large, continuous habitats into smaller and more isolated remnants (Didham et al., 2012; Fahrig, 2003; Wilcove, 1986). Fragmentation leads to the degradation of habitats and is one of the main processes contributing to the extinction and loss in species diversity (Ehrlich, 1988; Fahrig, 2003).

Social insects are among the ecologically most important organisms on earth (Hölldobler & Wilson, 1990). Among them, ants are particularly sensitive to habitat fragmentation, which may induce changes in species

composition and decrease species richness and population abundance (Bestelmeyer & Wiens, 1996; Brühl et al., 2003; Carvalho & Vasconcelos, 1999; Crist, 2009; González et al., 2018; Leal et al., 2012; Suarez et al., 1998). In fragmented patches of habitat, the changes in ant communities are often associated with an alteration of abiotic conditions such as temperature, a lower availability of food and nest sites, and a change in the abundance of mutualists, competitors, and predators (Braschler & Baur, 2003; Crist, 2009; Levings, 1983; Smallwood, 1982; Wiescher et al., 2012). As reviewed by Crist (2009), several studies did not find negative consequences of habitat fragmentation on ants. For instance, Mitchell et al. (2002) documented that decreasing patch size, associated with warmer and drier microclimate, led to an increased abundance of *Camponotus* spp. and *Aphaenogaster rudis*. In contrast, Suarez et al. (1998) documented a negative relationship between ant species richness and time since fragment isolation and Mäki-Petäys et al. (2005) showed that fragmentation induced a rapid genetic change and loss of variation in *Formica aquilonia*.

Fragmentation also reduces gene flow between populations due to changes in space use and dispersal within and between patches (Andren, 1994; Duelli et al., 1990; Fahrig & Merriam, 1994; Mader et al., 1990). Species requiring highly specific habitats to subsist should thus present a strong genetic structure between isolated populations and the remaining populations may be reduced in size, increasing the risk of inbreeding and accelerating the loss of genetic variability (Hedrick & Kalinowski, 2000). Ecological conditions and habitat characteristics also influence many aspects of social and genetic structure of ant colonies (Bernasconi et al., 2005), such as the number of queens per colony (Heinze, 1993).

In the present study, we investigated the impact of fragmentation on the forest ant *Temnothorax crassispinus* (Karavajev, 1826) in a highly fragmented landscape across the Franconian Jura. We examined how the fragmentation of its habitats affect the density of this species and the genetic structure of its populations and colonies. We simultaneously considered different descriptors of fragmentation of forest patches (size, shape, isolation). More precisely, the study aimed to answer the following questions: (i) What is the genetic structure of *T. crassispinus* population in the study area, and is this structure correlated with landscape? (ii) Do nest densities, genetic diversity and social structure vary among forest patches with different size and connectivity? We addressed these questions using a sample of 283 nests collected in 45 forest patches, of which 156 were used for genetic analyses. We hypothesized that the population of *T. crassispinus* should be genetically structured depending on the spatial organization of forest patches, with isolated sub-populations in remote patches, and that the genetic diversity and the density of the nests would decrease with smaller patch sizes, as well as in patches with the lowest connectivity.

2 | MATERIALS AND METHODS

2.1 | Biological model, study area, and sampling scheme

Temnothorax crassispinus (Karavajev, 1826) is among the most common ant species in deciduous forests throughout Eastern Europe. This small ant (~3 mm) has colonies with a single queen and up to 300 workers (Strätz & Heinze, 2004; Tichá & Štys, 2002) nesting in rotting branches and hollow acorns, often reaching extremely high densities (up to 10 nests per square meter; Strätz & Heinze, 2004), making it a good model to study the effects of habitat changes at small spatial scales. Colonies may inhabit multiple nest sites during the reproductive period in summer and move together for hibernation ("seasonal polydomy," Giehr, Senninger, et al., 2020; Giehr, Wallner, et al., 2020; Strätz & Heinze, 2004; Tichá & Štys, 2002). The study was carried out in March and April 2021 in the Franconian Jura, district Neumarkt, Southern Germany. The sampling area covered hundred square kilometers and included 45 woody patches of at least 0.5 ha each, separated by large areas of agricultural land and settlements. The sampling area is located west of the Hohenfels military training Natura 2000 area, a complex of limestone grasslands and beech forests with a particularly high biodiversity (Warren et al., 2007; Warren & Büttner, 2008). For each patch of forest, we documented size, shape (perimeter/surface), and isolation (distances to the five closest patches). This information was obtained by averaging three repeated measures obtained per independent observers on the German Geoportal interface. In each patch, ant sampling was performed by two to four peoples over 30 min. Sampled nests had a minimum distance of 10 m. This standardized sampling effort allowed to define a nest density proxy for each patch (corresponding to the number of nests found by one person in 30 min). The presence of queen(s) in each nest was systematically recorded. Though *T. crassispinus* is a monogynous ant (e.g., Strätz & Heinze, 2004), that is, nests contain only a single fertile queen, occasionally young female sexuals stay in the nest rather than leaving for a mating flight and eventually shed their wings. As we did not dissect individuals we cannot distinguish between proper fertile queens and these supernumerary wingless female sexuals and refer to all of them as queens. We therefore considered nests as queenright when one or more queen(s) were present, and queenless when no queen was collected. Complete nests were

collected and stored in 99% ethanol. Individuals from 283 colonies were collected for this study.

2.2 | Species identification

In parts of the study area, *T. crassispinus* cooccurs with its Western sibling *Temnothorax nylanderi* and these two species occasionally hybridize (Pusch, Seifert, et al., 2006; Seifert, 1995). Although these species can be distinguished by detailed morphometry (Seifert, 1995) or sequencing of the mitochondrial genes *cyt* and *COI* (*Cytochrome b* and *Cytochrome oxidase I*; Pusch, Seifert, et al., 2006;), identification might be erroneous in the case of hybridization (Cordonnier, Gayet, et al., 2019; McKendrick et al., 2017; Whitworth et al., 2007). In previous studies, hybrids were therefore identified by electrophoresis of the enzyme glucose-6-phosphateisomerase (GPI; P. Douwes, cited in Pusch, Seifert, et al., 2006; Seifert, 1995). However, approaches based on population assignment can allow a better detection of species and hybrids in morphologically similar taxa using multilocus genotypes to identify groups of genetically different individuals that might constitute potential species (e.g., Yang & Rannala, 2010).

We therefore combined the microsatellites genotyping and Sanger sequencing of mitochondrial genes *Cytochrome oxidase I* and *II* (*CO I* and *CO II*) to identify species, following the procedure described in Cordonnier, Gibert, et al. (2019). DNA was extracted using a CTAB method (cetyltrimethylammonium bromide; modified from Sambrook & Russell, 2001) from one worker per colony and for four colonies from each forest patch (except when fewer colonies where available), resulting in a total of 156 genotyped workers (Table S1). In all individuals, DNA at 13 highly variable microsatellite loci were amplified (primers available in Table S2). Twelve loci were polymorphic and showed considerable variation with an average of 26 alleles per locus (2MS46: 25; L-18: 41, L-5: 43, Ant11893: 37, LX GT 218: 12, Ant3993: 10, 2MS82: 33, 2MS87: 7, 2MS17: 38, 2MS29: 6, 2MS34: 12, LXA GT 1: 44). The last locus (primer 2MS91) could be amplified only in *T. nylanderi* and was therefore discarded from further analyses. Individuals that did not give reliable genotypic information in at least 10 successful loci were removed from subsequent analyses ($n = 23$; Table S1), resulting in 133 remaining samples. Genetic clusters were identified based on Bayesian clustering algorithm implemented in the software STRUCTURE v. 2.3.1 (Pritchard et al., 2000). Within each of the K clusters identified by genotyping, a set of individuals having a Q-value higher than 0.95 were Sanger sequenced for a stretch of mitochondrial gene covering the genes *CO I* and *II* to confirm species identification (corresponding to 46 sequences in total; primers available in Table S3; Genbank accession numbers [OP297180-OP297197 and OP322940-OP322967]). The complete species identification method (details available in Figure 1) resulted in the identification of 104 *T. crassispinus* colonies. Individuals, which could not be determined (unidentified individuals, interspecific hybrids, or otherwise unidentified species; $n = 11$), and individuals identified as *T. nylanderi* ($n = 18$) were removed from subsequent analyses. The complete genotypes of the 104 *T. crassispinus* individuals are available on the Zenodo repository (https://doi.org/10.5281/zenodo.7358105).

2.3 | Impact of the fragmentation on *T. crassispinus*

To investigate the population genetic structure within the species using microsatellite data, the Bayesian clustering algorithm implemented in the software STRUCTURE was used on the 104 genotyped individuals, based on the admixture model with correlated allele frequencies with a number of a priori unknown clusters (K) varying from $K = 1$ to $K = 5$, running 10 iterations for each K-value, and using a LOCPRIOR model (Hubisz et al., 2009) with prior location of samples corresponding to the sampled forest patches. Each run consisted of 500,000 replicates of the MCMC (Markov chain Monte Carlo) after a burn-in of 500,000 replicates. To investigate the 10 independent runs, clustering results were analyzed using CLUMPAK (Kopelman et al., 2015) based on a Markov clustering algorithm which identifies sets of highly similar runs grouped together in modes and separating these distinct groups of runs to generate a consensus solution for each distinct mode. For any given K, the different runs were either consensual with a single mode or resulting in both a majority mode consisting of most of the iterations and one or more minority modes consisting of the remaining iterations. Next, we used CLUMPAK to identify an optimal ordering of inferred clusters across different values of K, and then to define the optimal K-value using the method of Evanno et al. (2005). Based on the consensus solution of the majority mode, we obtained K distinct Q-values for each individual corresponding to their membership coefficient for each cluster. Individuals were grouped in a population assuming a membership coefficient of at least 50% to belong to a cluster (Balkenhol et al., 2014). To confirm the result and assess differentiation between clusters defined using Bayesian clustering, Fixation index F_{ST}-values were calculated between the sub-populations using GENALEX (Peakall & Smouse, 2006).

Global relationships among the 27 mitochondrial haplotypes of *T. crassispinus* were based on consensus sequences of the *CO I* and *CO II* sequences, corresponding to fragments ranging from 1094 to 1164 bp lengths.

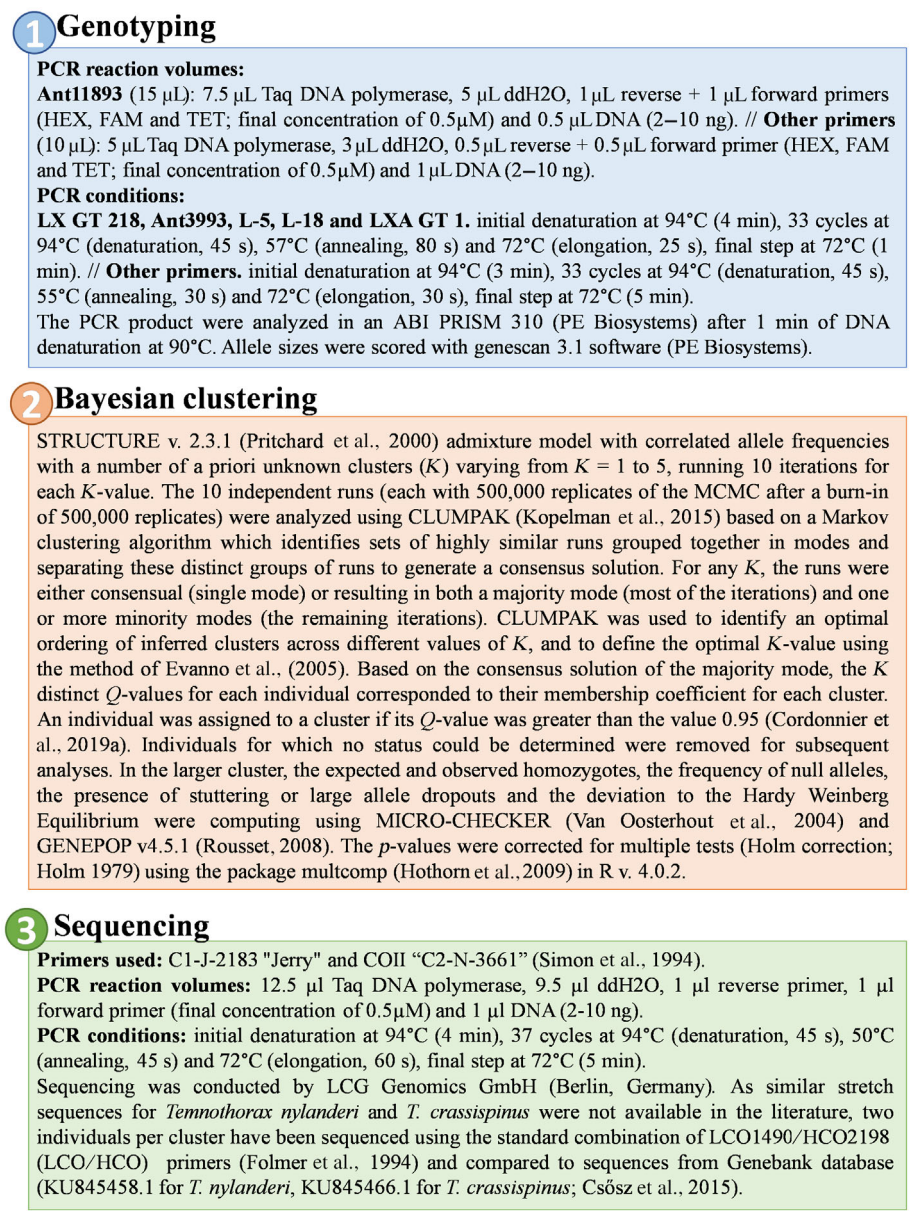

FIGURE 1 Detailed species identification process (references cited in the figure: Cordonnier, Gayet, et al., 2019; Csősz et al., 2015; Evanno et al., 2005; Holm, 1979; Hothorn et al., 2016; Kopelman et al., 2015; Pritchard et al., 2000; Rousset, 2008; Van Oosterhout et al., 2004). [Color figure can be viewed at wileyonlinelibrary.com]

The sequences were aligned using the default options in MUSCLE v3.8.31 (Edgar, 2004) as implemented in SeaView v4.2.9 (Gouy et al., 2010). The relationships were evaluated based on a tree constructed using the PhyML algorithm with the nucleotide substitution model GTR without invariable sites, optimized nucleotide equilibrium frequencies, and tree-searching operations involving best of NNI & SPR. PopART v. 1.7 (Leigh & Bryant, 2015) was used to build a haplotype network using the Median Joining inference under default settings and applying a provided trait file coding for genotypes clusters.

The effects of fragmentation on the densities and genetic structure of nests of *T. crassispinus* were investigated using a model averaging approach with R v. 4.2 software (R Development Core Team, 2019). Three different (generalized) linear models were set up to describe the impact of fragmentation on (i) a nest density proxy (number of *T. crassispinus* nests sampled per people in 30 min in each forest patch), (ii) the presence of queen(s) in the nests in each forest patch (successes/trials binomial data where each response is a number of queen-right nests from a number of trials, binomial family), and (iii) the genetic diversity (averaged number of alleles per *T. crassispinus* nest of each forest patch). Only the 41 patches with at least one nest of *T. crassispinus* were included in the two last models featuring queen presence and genetic diversity. Several independent variables were investigated in each model: the size of the patch, the shape of the patch (perimeter/surface), the distance to the closest patch, the mean distance to the five closest patches, the number of neighboring forest patches at

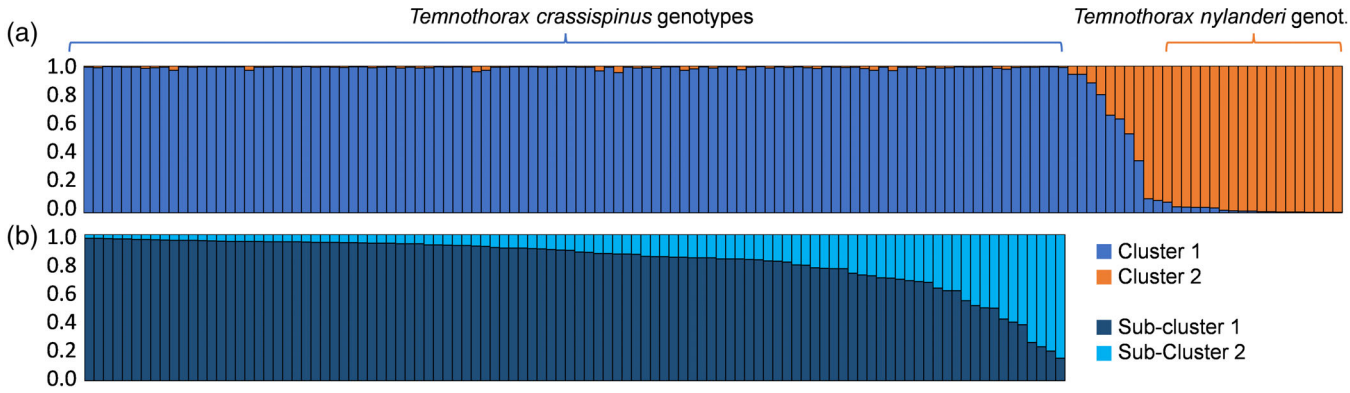

FIGURE 2 Structure bar plot. Each individual is represented by a vertical line, which is partitioned into K colored segments that represent each individual's estimated membership fractions in K clusters (Q-values) from the consensus solution of the majority mode for the $K = 2$. (a) Bayesian clustering conducted at the interspecific level ($n = 133$ individuals). (b) Bayesian clustering conducted at the intraspecific level, within *Temnothorax crassispinus* cluster (104 individuals). [Color figure can be viewed at wileyonlinelibrary.com]

200 and 400 meters from the focal forest patch (describing the bounds in the dataset with at least one patch having zero and one neighbor, respectively). Latitude and longitude were incorporated as adjustment variables in the model to test for a structured spatial pattern. In the three full models, variance inflated factors (VIFs) were used to test for potential multicollinearity issues (O'Brien, 2007), leading to the removal of the mean distance to the five closest patches from the models, and ensuring that only variables with VIFs lower than 2.5 were integrated. Model parameters were estimated through a model averaging procedure incorporating models with delta AICc ≤2 (small sample corrected Akaike information criterion; Burnham & Anderson, 2004; Symonds & Moussalli, 2011) using the MuMIn package (Bartoń, 2016). The significance of each explanatory term was tested using a Wald test on the full model and considering the confidence intervals on the estimates. Homoscedasticity, independence, and normality of residues were checked for each model. The dataset used for these analyses is available on the Zenodo repository (https://doi.org/10.5281/zenodo.7358105).

3 | RESULTS

Among the 133 colonies genotyped, 18 were identified as *T. nylanderi*, 11 had ambiguous genotypes and 104 colonies were identified as belonging to *T. crassispinus* based on their genotypes. Bayesian clustering analysis of the 104 multilocus microsatellite genotypes of *T. crassispinus* colonies revealed a significant genetic structure with two distinct genetic clusters (Delta($K = 2$) = 2.469; Prob($K = 2$) = 0.945; Figure 2). Most of the samples ($n = 95$) were grouped in one of the genetic clusters whereas the second cluster (indicated in dark gray in Figure 3) consisted of only nine colonies from

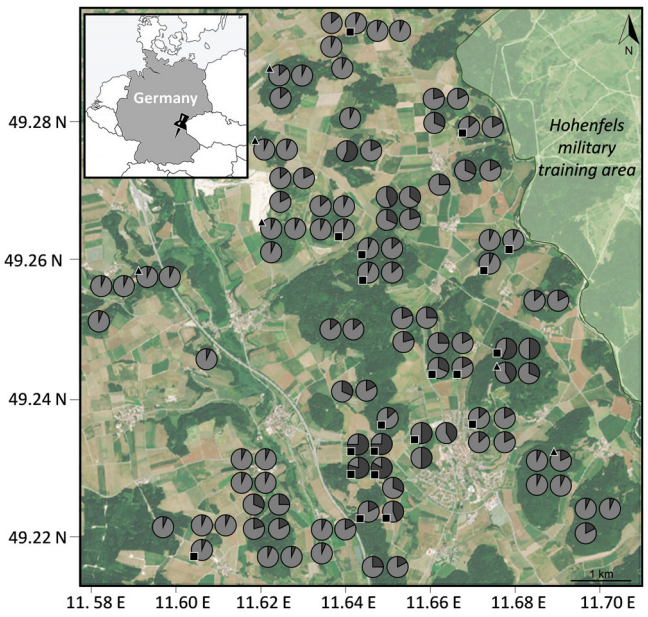

FIGURE 3 Top left: Location of the studied area in the Franconian Jura, Germany, Europe. Main map: representation of the 104 genotyped colonies of the ant *Temnothorax crassispinus*. In each patch one to four colonies were genetically identified. Color of circles indicates the Q-values obtained based on the Bayesian clustering process of microsatellite genotypes (light gray: *T. crassispinus*, Cluster 1; dark gray: *T. crassispinus*, Cluster 2). Squares and triangles correspond to the two main mtDNA haplotypes (see Figure 4) of *T. crassispinus* found in the colony. ©GeoBasis-DE/BKG 2022. [Color figure can be viewed at wileyonlinelibrary.com]

four different forest patches (Patches 4, 5, 22, and 29 in Figure S1). Few individuals had rather high Q-values for the second cluster (>0.7; Figures 2 and 3), suggesting a continuum between Cluster 1 and 2 rather than separated genetic populations. The investigation of sub-structuring within the

clusters did not reveal a genetic structure (only one homogeneous genetic cluster, Prob[$K = 1$] > 0.97), suggesting no genetic differentiation between these two genetic clusters, as confirmed by the weak fixation index ($F_{ST} = 0.05$; Wright, 1984).

The phylogenetic tree (Figure S2) revealed several groups of variants, of which the two main haplotypes were widely distributed, but the haplotype network did not show a structure corresponding to the genotype clusters (Figure 4). The two most frequent haplotypes were not spatially structured in the landscape (indicated by squares and triangles in Figure 3).

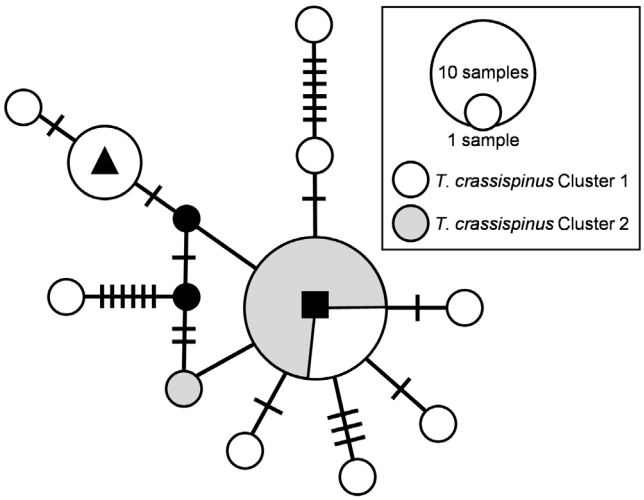

FIGURE 4 Haplotype networks based on sequences of the mitochondrial genes *CO I* and *CO II* in the ant *Temnothorax crassispinus* in the Franconian Jura. Each disk represents a haplotype. Disk surface is proportional to the number of individuals. The color of the disk indicates the genetic cluster of the individuals' genotypes based on the results provided by the Bayesian clustering process implemented in structure. Black disks correspond to missing haplotypes. The number of hatch marks corresponds to the number of mutations between two haplotypes. Square and triangle indicate the two main haplotypes also shown in Figure 1.

Both the nest density proxy and the presence of queens within nests were significantly impacted by the number of neighboring forest patches within 400 m of the focal forest patch, with more nests (est. = 0.386; *p*-value = 0.044; Figure 5a) and more queenright nests (est. = 0.760; *p*-value = 0.008; Figure 6a) in the patches surrounded by numerous other forest patches. We also evidenced higher nest densities in the eastern part of the study area, close to the Hohenfels protected area (est. = 26.17; *p*-value < 0.01; Figure 5b). The proportion of queenless nests was slightly higher in the larger patches (est. = -1.259×10^{-6}; *p*-value = 0.04; Figure 6b), although this result should be treated with caution as it is strongly influenced by three of the largest patches that constituted influent values in the model (Patches 8, 16 and 22 in Figure S1; Cook's distances > 0.1). Finally, the social structure was impacted by patch shape, with a higher proportion of queenless nests in the elongated patches (est. = −4.02; *p*-value < 0.01; Figure 6c). The complete results of the models are summarized in Table 1.

4 | DISCUSSION

In the present study, we investigated the impact of fragmentation on the ant *T. crassispinus* in 45 forest patches across the Franconian Jura. *T. crassispinus* was found in most of the study area, and we also confirmed the presence of its sibling species *T. nylanderi* and putative

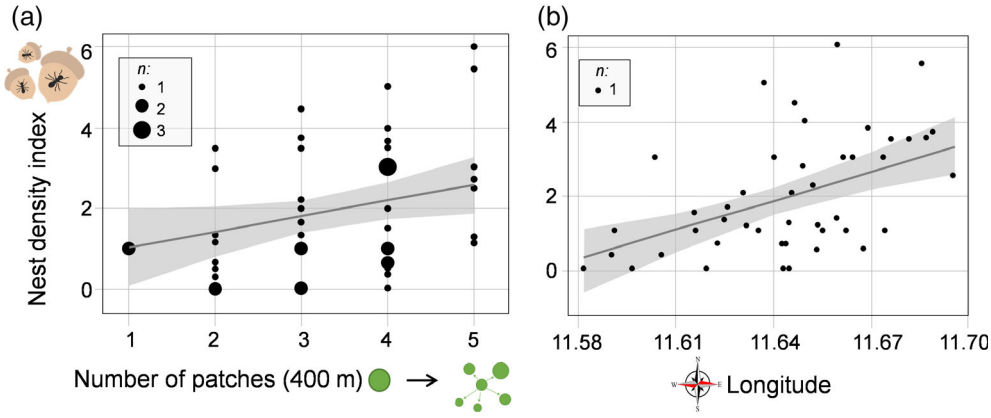

FIGURE 5 Relationships between the nest density of the ant *Temnothorax crassispinus* in the Franconian Jura and (a) the number of forest patches within 400 m of the focal patch and (b) the longitude. The confidence bands (shaded—95% CI) and the regression lines (gray) have been calculated based on the values predicted by the models. [Color figure can be viewed at wileyonlinelibrary.com]

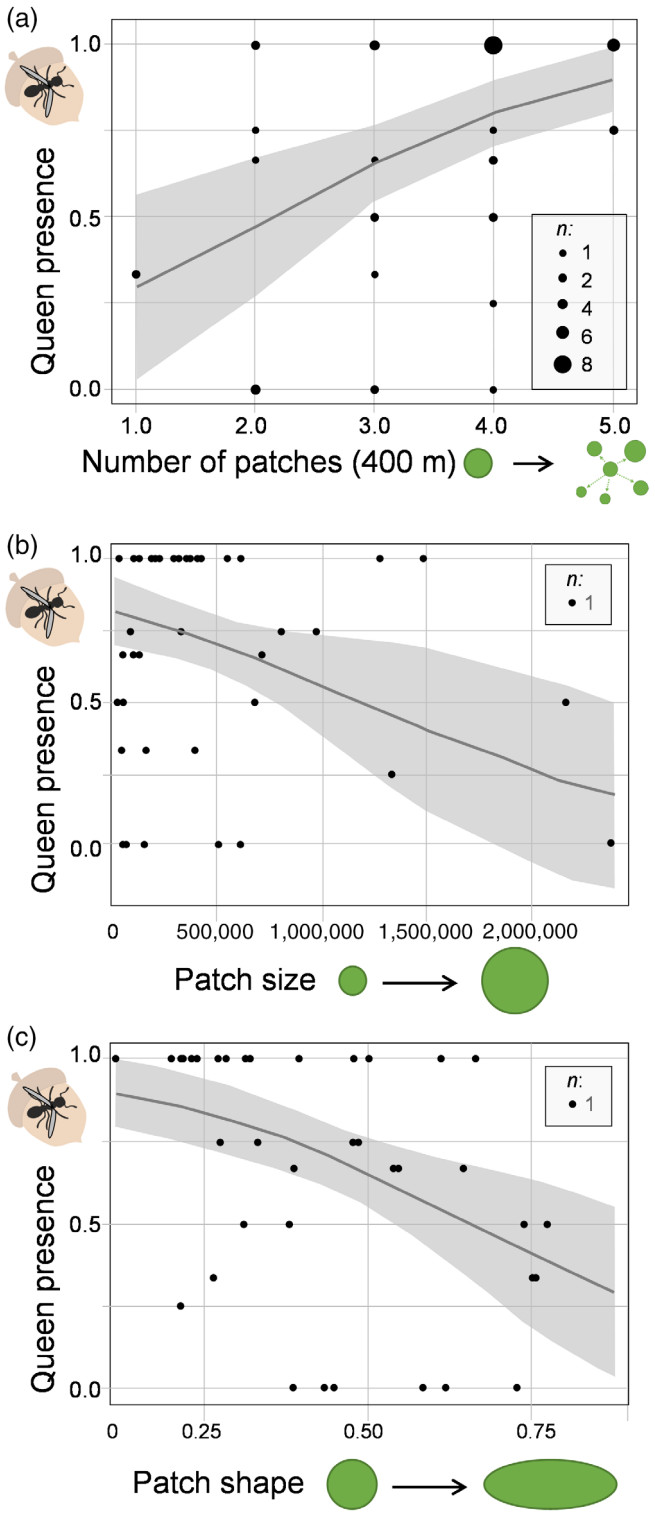

FIGURE 6 Relationships between the proportion of queenright nests of the ant *Temnothorax crassispinus* in the Franconian Jura and (a) the number of forest patches within 400 m of the focal patch, (b) the size of forest patches, and (c) the shape of forest patches. The confidence bands (shaded—95% CI) and the regression lines (gray) have been calculated based on the values predicted by the models. [Color figure can be viewed at wileyonlinelibrary.com]

hybrids (see also Pusch, Seifert, et al., 2006). Nuclear and mitochondrial DNA revealed the presence of two genetically different clusters, but those clusters were non concordant and non-spatially explicit. Nest densities and the presence of queens in the nests were impacted by the number of adjacent forest patches within 400 m of the focal patch. Finally, the proportion of queenless nests was also impacted by both patch size and shape.

In this study we exhaustively sampled all forest patches over an area of 100 km^2 to catch fine-scale occupancy patterns and more finely characterize the spatial occurrence of *T. crassispinus* and it sibling *T. nylanderi* in their hybrid zone in the Franconian Alb. As previously observed, *T. nylanderi*, which is widely distributed in Western Europe, occurred in relatively few patches compared to the eastern species *T. crassispinus* (Pusch, Seifert, et al., 2006). We found consistent occupancy pattern in the three previously studied patches (Patches 1–3 in the present study and in Pusch, Heinze, & Foitzik, 2006).

Within *T. crassispinus*, the first genetic cluster identified by microsatellite genotypes was found in most of the study area while the second cluster was represented by few individuals from four patches (Patches 4, 5, 22, 29; Table S1). Among these patches, only one had individuals with high *Q*-values (Patch 4). This suggests that this forest patch might have a population of *T. crassispinus* genetically different from all other patches of the study area. This pattern might result from a local introduction of *T. crassispinus* from another population, for example, due to the use of acorns from other regions as supplementary food for pigs (Wealleans, 2013), the introduction of garden waste (see e.g., Buckham-Bonnett & Robinson, 2017), the displacement of litter during the construction of the adjacent highway, or other forestry activities. Wind dispersal might also have facilitated the spread of this genetic cluster in the western forest patches (e.g., Pusch, Heinze, & Foitzik, 2006). As winds from southwest to northeast are predominant in the studied area (*Windfinder statistics*, 2022), winged ants originating in the *T. crassispinus* population from Patch 4 could have been drifted away by wind into the patches further east.

Nest densities were higher in the eastern part of the study area than in the western part. This might reflect that the sampling was conducted close to the edge of the species distribution, suggesting that *T. crassispinus* must compete with *T. nylanderi* further west. This could alternatively relate to both the wind direction (see above), preventing dispersion along an east–west axis, and to the presence of the Hohenfels natural area in the east, which could act as a source area for propagule pressure of *T. crassispinus*. Nest densities were also positively impacted by the number of adjacent forest patches

TABLE 1 Test results and parameter estimates of the three models exploring how landscape affects nest density, queen number, and genetic diversity of the ant *Temnothorax crassispinus* in the Franconian Jura.

		Estimate	Standard error	Adj. *SE*	*z* value	*Pr*(>|*z*|)	2.5%	97.5%
Nest density	(Intercept)	−304.100	84.200	86.720	3.507	0.001*	−474.040	−134.115
	Longitude	26.170	7.243	7.459	3.509	<0.001*	11.556	40.794
	Nbneig400	0.386	0.186	0.192	2.017	0.044*	0.011	0.762
	Connect1	−0.001	0.003	0.003	0.433	0.665	−0.010	0.004
Queen presence	(Intercept)	1.004	1.045	1.079	0.930	0.352	−1.111	3.119
	Size	−1.259e−06	5.896e−07	6.105e−07	2.062	0.039*	−2.456e−06	−6.229e−08
	Nbneig400	0.7603	0.279	0.289	2.635	0.008*	0.195	1.326
	Shape	−4.020	1.485	1.537	2.616	0.009*	−7.032	−1.008
	Connect1	−0.004	0.005	0.005	0.846	0.398	−0.016	0.178
	Latitude	−0.060	0.193	0.197	0.306	0.760	−1.034	0.342
Genetic diversity	(Intercept)	1.073	1.353	1.386	0.774	0.439	−1.644	3.790
	Connect1	−0.000	0.000	0.000	0.484	0.628	−0.000	7.026e−05
	Nbneig200	0.001	0.004	0.004	0.271	0.786	−0.008	0.022
	Nbneig400	0.001	0.004	0.004	0.268	0.789	−0.007	0.020
	Longitude	−0.021	0.116	0.119	0.180	0.857	−0.780	0.410

Note: Longitude, latitude: coordinates of the samples; NBneig200/400: Number of neighboring forest patches within 200 or 400 m of the focal patch; Connect1: Distance to the closest forest patch; Area/shape: Size (m^2) and shape (area/perimeter) of the forest patch. Significance codes: * = <0.05.

surrounding a patch within 400 meters. The lower number of nests found in isolated patches corroborates with previous results in the ants *Formica lugubris* and *F. aquilonia*, in which fragmentation led to isolation of subpopulations, decreasing the total number of nests in both species (Mäki-Petäys et al., 2005). In contrast, in a study by Braschler and Baur (2003), nest density and forager abundance were higher in experimentally fragmented grassland plots than in large control plots.

In any case, a reduction of population size is expected to result in a loss of genetic diversity and increased inbreeding. Surprisingly, we did not find a correlation between connectivity and genetic diversity, probably because size reduction and the time span since fragmentation were too small to lead to major changes in the heterozygosity of *T. crassispinus*. Furthermore, while fragment isolation may strongly affect genetic diversity in insects with poor dispersal ability (Driscoll & Weir, 2005; Tscharntke et al., 2002), the studied species disperses during nuptial flights and might therefore be able to maintain gene flow even between remote forest patches. A similar absence of genetic structure at comparable spatial scale has previously been found in two other species of the same genus: *Temnothorax nigriceps* (Cordonnier et al., 2022) and *T. nylanderi* (Khimoun et al., 2020).

The presence of queen(s) in colonies (including both fertile, mated reproductives and dealate, unmated female sexuals) was also positively impacted by the number of adjacent forest patches within 400 m. This might be explained by a lower queen replacement in isolated patches due to the lack of recruitment of new queens from other patches, and/or a lower competition for empty nesting sites, as suggested by the decrease in nest densities in these same isolated patches. Workers from queenless nests contribute considerably to the production of males in a population (Giehr, Senninger, et al., 2020; Giehr, Wallner, et al., 2020) and might therefore help to offset detrimental genetic effects of fragmentation in isolated patches by promoting genetic exchange within and between patches. To a lesser extent, the proportion of queenright colonies was also positively impacted by the size and shape of the forest patches (with more queenright colonies in small, round patches). However, given the low effect sizes, these last results must be interpreted with some caution. A study at a finer spatial scale might be needed to understand how spatial distribution and social structure of *T. crassispinus* within the forest patches is organized and to determine if, for example, an edge effect impacts the occurrence of the species. The studied sites are characterized by numerous forest paths and clearings, which all might affect microclimate within the patch. Furthermore, not all types of wood are equally suitable for nesting: *T. crassispinus* prefers pine and oak, the distribution of which in the forest patch might be more influential on spatial patterns than, for example, the distance to the forest edge (see e.g., Debuse

et al., 2007; Didham, 1997; Golden & Crist, 2000). Finally, *T. crassispinus* is seasonally polydomous, that is, individual colonies may temporarily inhabit multiple nest sites during the reproductive period and move together for hibernation (Giehr, Senninger, et al., 2020; Giehr, Wallner, et al., 2020; Strätz & Heinze, 2004; Tichá & Štys, 2002). Areas sampled later in the year might therefore show a higher percentage of queenless nests than areas sampled in early spring. However, seasonal polydomy does not explain our result: the correlation coefficient between collecting date and queen presence is 0.42.

To conclude, *T. crassispinus* is only partly impacted by habitat fragmentation, likely because its high dispersal abilities allow it to overcome detrimental genetic consequences. However, the propagule pressure could be reduced in the more isolated forest patches given the lower densities of nests evidenced in this study and the lower proportion of queenright nests likely resulting from these low densities. Even if the observed changes in ant nest density were relatively small, they may have a large impact on ant services. In the study of Braschler and Baur (2003) for instance, subtle changes in nest density translated into strong differences in the number of ant foragers observed on baits and the number of ants tending aphids. Finally, we strongly suggest further investigation regarding the drivers of occurrence at a finer spatial scale to feature the role of edge effects on the occurrence of this species. How the studied factors (e.g., nest densities) translate into species interaction patterns, such as the frequency of heterospecific colony fusion (Pusch, Meindl, & Heinze, 2006) is also of main importance.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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