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Semipermeable species boundaries create opportunities for gene flow and adaptive potential

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Abstract

Hybridisation and gene flow can have both deleterious and adaptive consequences for natural populations and species. To better understand the extent of hybridisation in nature and the balance between its beneficial and deleterious outcomes in a changing environment, information on naturally hybridising nonmodel organisms is needed. This requires the characterisation of the structure and extent of natural hybrid zones. Here, we study natural populations of five keystone mound-building wood ant species in the *Formica rufa* group across Finland. No genomic studies across the species group exist, and the extent of hybridisation and genomic differentiation in sympatry is unknown. Combining genome-wide and morphological data, we demonstrate more extensive hybridisation than was previously detected between all five species in Finland. Specifically, we reveal a mosaic hybrid zone between *Formica aquilonia*, *F. rufa* and *F. polyctena*, comprising further generation hybrid populations. Despite this, we find that *F. rufa*, *F. aquilonia*, *F. lugubris* and *F. pratensis* form distinct gene pools in Finland. We also find that hybrids occupy warmer microhabitats than the nonadmixed populations of cold-adapted *F. aquilonia*, and suggest that warm winters and springs, in particular, may benefit hybrids over *F. aquilonia*, the most abundant *F. rufa* group species in Finland. In summary, our results indicate that extensive hybridisation may create adaptive potential that could promote wood ant persistence in a changing climate. Additionally, they highlight the potentially significant ecological and evolutionary consequences of extensive mosaic hybrid zones, within which independent hybrid populations face an array of ecological and intrinsic selection pressures.

KEYWORDS

adaptation, *Formica* wood ants, hybridisation, Hymenoptera, mosaic hybrid zone, speciation

1 | INTRODUCTION

Hybridisation and gene flow between closely related species are common in nature and predicted to increase because of human activities and climatic warming (Chunco, 2014; Mallet, 2005; Rieseberg, 2009; Scheffers et al., 2016), as changing environmental conditions induce range shifts and connections between previously allopatric populations. This may, over longer evolutionary time scales, either collapse species barriers (Owens & Samuk, 2019) or reinforce them (Lemmon & Juenger, 2017; Pfennig, 2016). For some time, the species barriers can also remain semipermeable, a situation where the taxa remain distinct in their natural habitat despite gene flow between them.

Hybridisation and gene flow have both positive and negative effects for fitness. Complete reproductive isolation is not necessarily optimal (Barton, 2020): increased genetic diversity through hybridisation may fuel adaptation (Kulmuni et al., 2023; Torda & Quigley, 2021), and introgression has repeatedly underlied adaptation into new niches (De-Kayne et al., 2022; Kagawa & Takimoto, 2018; Meier et al., 2017). However, populations can also suffer from hybridisation due to deleterious fitness effects (Ålund et al., 2013) and mortality (Ellison et al., 2008), arising from the inviability of offspring or hybrid breakdown in later generations. Revealing the extent and consequences of hybridisation in natural populations is needed to clarify the fate and adaptive potential of hybrid populations in the face of changing environment, like warming climate.

The geographical extent and type of hybrid zone greatly influence the evolutionary and ecological impacts of hybridisation: In contrast to more commonly studied, geographically restricted clinal zones (Barton & Hewitt, 1985), some taxa exhibit mosaic hybrid zones. These may be as large as the species' sympatric area. In mosaic zones (Arnold, 1997), hybridisation events are typically independent, and recently admixed populations form a spatial mosaic with nonadmixed and backcrossed populations of at least two species (Arnold, 1997; Harrison, 1986; Rand & Harrison, 1989). Under the mosaic zone model, the species are assumed to be adapted to different environments, and their hybrids may either have lower fitness (due to intrinsic selection) or be favoured (extrinsic, environment-dependent selection) (Arnold, 1997). Mosaic hybrid zones have been reported, for example for various plant species (Abbott & Brennan, 2014; Rieseberg et al., 1999) and molluscs (Fraïsse et al., 2014). Since mosaic zones can be large, and selection can act independently in different hybrid populations, they may result in more heterogeneous patterns and geographically widespread effects than those in clinal hybrid zones. Furthermore, independently evolving hybrid populations allow predictability in the outcomes of hybridisation to be studied (Nouhaud, Martin, et al., 2022).

Hybridisation is commonly investigated between two species. However, genomic data have facilitated the detection of multispecies hybridisation in many taxa, including white oaks (Reutimann et al., 2020), birds (Grant & Grant, 2020; Natola et al., 2022; Ottenburghs, 2019), fish (Banerjee et al., 2022), and butterflies (Heliconius Genome Consortium, 2012). Multispecies hybridisation is likely to be more frequent in sympatric groups with a low degree of reproductive isolation, and one species may act as a 'conduit' of gene flow between two otherwise isolated species (Grant & Grant, 2020).

Wood ants of the *Formica rufa* species group (Hymenoptera, Formicidae) represent ideal models of hybridisation and understanding its role in adaptation: they have diverged recently, within the past 500,000 years (Goropashnaya et al., 2004, 2012; Portinha et al., 2022), have a wide sympatric range in Eurasia including Finland and have different climatic adaptations (Table 1 and Martin-Roy et al., 2021). Furthermore, there are premises for the formation of mosaic hybrid zones: morphological and genetic marker findings indicate extensive hybridisation across the *F. rufa* group within their sympatric Eurasian ranges (Seifert, 2018, 2021; Seifert et al., 2010). Albeit reproductive isolation is not complete within the group, both pre- and postzygotic isolation, including intrinsic and environmentally dependent selection, have been detected (Kulmuni et al., 2010; Kulmuni & Pamilo, 2014; Seifert, 2018). Wood ant hybridisation has resulted in male-biased mortality during development, but also female heterosis and potential for thermal adaptation (Kulmuni & Pamilo, 2014; Martin-Roy et al., 2021). Instability in the barriers of gene flow has been reported previously (Kulmuni et al., 2020) in this system, suggesting that the balance between the costs and benefits of hybridisation may vary across space and time. Understanding hybridisation in the *F. rufa* group is important since these ants are keystone species in boreal and mountain forests across Eurasia (Stockan & Robinson, 2016; Trigos-Peral et al., 2021). They have a significant role in the forest ecosystem: they build stable nest mounds (Seifert, 2018), and populations of polygynous (i.e. multiple queens per nest) species in particular can reach densities of hundreds of nests per km^2, and impact ecosystem characteristics, ranging from nutrient cycling below ground to aphid farms in the forest canopy. In

TABLE 1 Characteristics of the study species.

	Polygynous, polydomous, limited dispersal	Mainly monogynous, monodomous, high dispersal
Northern Temperate to cold climates	*F. aquilonia* 56.3°N to 71°N	*F. lugubris* 55.5°N to 71°N
Southern Mainly temperate climates (From subtropical to cold)	*F. polyctena* 42°N to 61°N (Putatively in Finland to 63°N)	*F. rufa* 40.5°N to 64.8°N
		F. pratensis 37°N to 63.9°N

Note: Continuous ranges (Seifert, 2018) and corresponding climatic zones (European Environment Agency, 2009) in Europe. Social strategies as in Finland. The colors shown here are used throughout the manuscript to represent the different species.

Finland, at least five previously described species of the *F. rufa* group have been reported to occur in sympatry and to be ecologically similar enough for potential hybridisation: *Formica rufa* (Linnaeus, 1761), *F. polyctena* (Förster, 1850), *F. lugubris* (Zetterstedt, 1838), *F. pratensis* (Retzius, 1783) and *F. aquilonia* (Yarrow, 1955), which is the most common of these species in Finland (Punttila & Kilpeläinen, 2009). Multiple hybrid populations of *F. aquilonia* and *F. polyctena* have been genetically characterised within a small region in southern Finland (Beresford et al., 2017). In addition, morphological and allozyme data support several other observations of *F. aquilonia* and *F. polyctena* hybridisation, mainly in southern Finland (Pamilo & Kulmuni, 2022; Seifert, 2021; Sorvari, 2022), and indicate *F. aquilonia* and *F. lugubris* hybridisation in northern Finland (Pamilo & Kulmuni, 2022). Since many species pairs within the group hybridise, this raises the possibility of previously undetected multispecies hybridisation. However, outside *F. aquilonia* and *F. polyctena* (Nouhaud, Martin, et al., 2022; Portinha et al., 2022), no genomic studies on the *F. rufa* group exist, and consequently also no information on the congruence between morphological and genomic data is available.

Under a changing climate, semipermeable species boundaries and hybridisation between differentially adapted species may provide an opportunity to reshuffle adaptations and help species to cope with environmental shifts. Hybridisation between the *F. rufa* group species may have potential for this, since the five species targetted here differ in their life history characteristics and habitat preferences (Seifert, 2018) and, based on their Eurasian distributions, are expected to be adapted to different climatic conditions (Table 1). We refer to northern *F. aquilonia* as cold-adapted and southern *F. polyctena* as warm-adapted, since in laboratory conditions *F. aquilonia* tolerates cold and *F. polyctena* survives better in hotter conditions compared to *F. aquilonia* (Martin-Roy et al., 2021). Similarly to *F. aquilonia*, *F. lugubris* has a boreo-alpine distribution and is likely cold-adapted. In contrast, *F. rufa* and *F. pratensis* live at lower altitudes, have a more southern distribution (similar to *F. polyctena*), and are assumed to be warm-adapted (Table 1) (Seifert, 2018).

Since successful wood ant hibernation, reproduction and brood development are temperature-dependent, climatic conditions that affect within-nest temperatures are critical for their fitness and survival throughout the year (Frouz, 2000; Frouz & Finer, 2007; Kadochová & Frouz, 2014). However, the ants can enhance their fitness by active nest thermoregulation from early spring until autumn (Horstmann & Schmid, 1986; Kadochová & Frouz, 2014). Active regulation is paused during hibernation, but recovers in the spring, when the species start to reproduce at different but overlapping times (Seifert, 2018), with warmth-dependent brood production and development continuing until summer. In the winter, high hibernation temperatures may increase ant metabolism, leading in contrast to reduced lipid reserves, and thus reduced fitness and increased mortality, as has been shown in the laboratory for cold-adapted *F. aquilonia* (Sorvari et al., 2011). Specifically, workers need these body fat reserves in early spring for nest heating (Seifert, 2018), and queens for successful sexual reproduction (Sorvari et al., 2011). Understanding the extent and consequences of hybridisation between wood ant species with different climatic and life history characteristics is important for understanding their survival. This is especially relevant near the polar regions, such as in Finland, where climate warming is faster than near the equator and may lead to pronounced extreme weather phenomena (Masson-Delmotte et al., 2021). Specifically, hybridisation between warm- and cold-adapted species could provide a fitness advantage for the hybrids when climate fluctuates. Indeed, an earlier laboratory study indicates that natural hybrids of *F. aquilonia* and *F. polyctena* can tolerate warmer temperatures than *F. aquilonia* (Martin-Roy et al., 2021), yet whether this tolerance is reflected in the microclimatic distribution of natural hybrid populations remains unknown.

This study has two aims. First, we ask to which extent *F. polyctena* and *F. aquilonia* hybridise across Finland, and if there are any signs of hybridisation across the five species within the group. We expect such hybridisation based on the prevalence of *F. polyctena* and *F. aquilonia* hybridisation in southern Finland, as well as the morphological indications of hybridisation across the *F. rufa* group in their sympatric ranges. If morphologically distinguishable groups (i.e. the currently identified *F. rufa* group species) cannot be grouped based on genome-wide data, this suggests that the morphotypes are part of a larger panmictic gene pool. On the contrary, if the sympatrically occurring *F. rufa* group species are genomically distinct, it suggests that barriers to gene flow are sufficient to keep the gene pools separate even in sympatry. If the latter is true, we aim to genomically characterise the hybrids, ask how abundant the hybrid populations are, and how they are distributed geographically. This will reveal potential hybrid zones, as well as their ecological and evolutionary impacts. Second, we ask whether specific environmental factors (temperature or precipitation) that are crucial for nest thermoregulation correlate with the occurrences of *F. aquilonia* × *F. polyctena* hybrids. Based on earlier laboratory studies, we hypothesise that these hybrids will occur in warmer and drier habitats than non-admixed cold-adapted *F. aquilonia* in Finland. Through the genetic and climatic characterisation of the hybrid populations, we discuss the balance of adaptive and deleterious outcomes: the potential of semipermeable species boundaries and hybridisation to contribute to species' adaptive potential, and thus to persistence of wood ants in a changing climate.

2 | MATERIAL AND METHODS

2.1 | Sampling for genomic analysis

We asked whether five sympatrically occuring wood ant species show signs of hybridisation and whether they remain genomically distinct in sympatry. We also aimed to characterise the potential hybrid populations in terms of their geographical abundance, heterozygosity and the presence of F1 versus later stage hybrids. To do this, we used genomic data from five *F. rufa* group species: *F. aquilonia*, *F. polyctena*, *F. rufa*, *F. lugubris* and *F. pratensis* sampled at 69 locations throughout Finland or in Russia, near the Finnish border. We refer to all these as Finnish samples, due to the geographic proximity of the one Russian sample. These locations represent different populations (minimum distance between populations within a species was at least six kilometres, except for two *F. aquilonia* populations 2.6 km) and one individual per location was sequenced. We complemented these with 20 already published genomes from 16 different populations in Finland, Scotland and Switzerland (Portinha et al., 2022), including both admixed and nonadmixed *F. aquilonia* and *F. polyctena* individuals to provide a broader view and to verify our interpretations on the sympatric samples. One Finnish *F. aquilonia* population is represented in both datasets (see Table S1 for more information about the samples including location, sampling year and morphological data). All ant samples were stored in ethanol before DNA extraction. Altogether the genomic data hence include 89 individuals collected at 84 populations.

We aimed at covering the known distribution of each species in Finland (Stockan & Robinson, 2016), and the samples were chosen to be as evenly distributed across each species' distribution in Finland as possible. To characterise the extent of hybridisation between *F. polyctena* and *F. aquilonia* across Finland, we included more populations for these species (and all available putative admixed populations). The majority of the Finnish samples originate from the National Forest Inventory (NFI years 2005–2008; Punttila & Kilpeläinen, 2009), supplemented by samples from individual researchers. We chose the samples based on initial morphological evaluation aiming at eight or more populations for each species (one individual per population). However, we carried out a more detailed morphological investigation using Numeric Morphology-Based Alpha-Taxonomy (NUMOBAT, see below) after choosing the samples.

2.2 | Morphological data

We carried out state-of-the-art morphological species identification with NUMOBAT (Seifert, 2021) using three to six individuals from each nest sampled for genomic data. Since both the genomic analysis and morphological identification required the use of whole individuals, we used different individuals from each nest for these separate purposes, which is justified by the fact that wood ant nests are genetically homogeneous (Kulmuni et al., 2020; Nouhaud, Martin, et al., 2022; Pamilo, 1982). We measured altogether 17 morphological characteristics (See Table S2 for a description of the characteristics, and Table S3 for primary morphological data), producing species assignment probabilities at the nest level. The morphological characteristics measured and their recording is described in detail in Seifert (2021).

All 77 locations with NUMOBAT data (69 nests collected for this study and eight nests analysed in Portinha et al., 2022, see Table 2 and Table S1), were run in a Linear Discriminant Analysis (LDA). Of these, the 73 Finnish samples were run as wild-cards (i.e. without imposing a species hypothesis), and the four non-Finnish samples were run with previously allocated species hypotheses. We analysed these 77 study samples together with a reference sample of 1681 workers of 399 Palaearctic nest samples with pre-established species hypotheses. The 1681 reference individuals of the five target species originate from previously published work (Seifert, 2021) and do not show any phenotypical indications of a hybrid identity. There was only a small amount of reference data for *F. lugubris* and *F. pratensis* for six of the morphological characteristics. Hence, we ran first all samples in a 5-class LDA using 11 morphological characteristics for which sufficient reference data was available (see also Table S2). This led to a fixation of posterior probabilities for samples assigned as *F. lugubris* or *F. pratensis*. Next, we ran all samples that were not classified as either *F. lugubris* or *F. pratensis* as wild-cards in a 3-class LDA with reference data considering all available 17 characteristics. The resulting posterior probabilities were then adjusted such that the sum of probabilities of all five species was equal to 1.0000, as follows:

$$a = p(F.\ lugubris) + p(F.\ pratensis)\ \text{from the 1st step}$$

$$b = 1 - a$$

$$F.\ aquilonia, F.\ polyctena, \text{and } F.\ rufa: p(\text{final}) = b \times p(\text{2nd step})$$

$$F.\ lugubris, F.\ pratensis: p(\text{final}) = p(\text{1st step})$$

To study the concordance of morphological and genomic species assignments, we computed species means and standard deviations of the respective LDA values for all nonadmixed individuals. For the individuals admixed between *F. aquilonia* and the *F. polyctena*/*F. rufa* clade, we calculated the Pearson's correlation between the ADMIXTURE ($K=5$, see below) and LDA values of *F. aquilonia*, and for the admixed *F. lugubris* individuals, the respective *F. lugubris* values. The lack of variance in ADMIXTURE values prevented testing this correlation for the subset of nonadmixed individuals used in ADMIXTURE.

TABLE 2 Number of individuals with genomic data used in this study.

	Finnish samples	Swiss samples	Scottish samples
Genomes from Portinha et al. (2022)	8 (NUM = 4)	9 (NUM = 3)	3 (NUM = 1)
Samples sequenced for this study	69 (NUM = 69)	-	-

Note: Number of individuals with morphological NUMOBAT data available = 'NUM'.

2.3 | Genomic data

For the genomic characterisation of our study species and individual samples, as well as documenting the extent of hybridisation within the *F. rufa* group in sympatry, we studied nuclear admixture and mito-nuclear mismatch. To achieve this, we performed whole-genome sequencing for our 69 new samples and analysed these along with the 20 previously sequenced genomes (Portinha et al., 2022).

2.3.1 | DNA extraction and sequencing

We extracted DNA with a Qiagen DNeasy extraction kit using a single diploid worker ant per nest and population, according to the manufacturer protocol for insects. DNA libraries were constructed and sequenced at the Biomedicum Functional Genomics Unit (FuGU), with NEBNext Ultra II FS DNA library preparation and Illumina NovaSeq S4: 2 × 150 bp (1 lane) sequencing.

2.3.2 | Variant identification

Raw Illumina reads and adapter sequences were trimmed using TRIMMOMATIC (v0.39; Bolger et al., 2014) before mapping against a Finnish *F. aquilonia* × *F. polyctena* hybrid reference genome (Nouhaud, Beresford, et al., 2022) using BWA MEM (v0.7.17; Li, 2013). Duplicates were removed using PICARD TOOLS (v2.21.4; http://broadinstitute.github.io/picard). Average read depth was 16.0×, as computed across individuals with MOSDEPTH (v0.3.3; Pedersen & Quinlan, 2018) after duplicate removal (Table S4).

Nuclear genomic data

Single nucleotide variants (SNPs) for the nuclear genome were called with the FREEBAYES software (v1.3.1, -k option was used for disabling population priors; Garrison & Marth, 2012) and the VCF was normalised using VT (v2015.11.10; Tan et al., 2015). Sites located at less than two base pairs from indels were excluded, as were sites supported by only Forward or Reverse reads, using BCFTOOLS (v1.10; Danecek et al., 2021). Multinucleotide variants were decomposed with the vcfallelicprimitives command from VCFLIB (v1.0.1; Garrison et al., 2022). The following steps were computed with BCFTOOLS. Only biallelic SNPs with quality equal or higher than 30 were kept. Sites were then filtered based on individual sequencing depth: individual genotypes that had more than twice the mean depth of the individual in question were set as missing. Genotyping errors that would occur, for example due to misaligned reads were removed by excluding sites that displayed heterozygote excess after pooling all samples ($p < .01$, VCFTOOLS v0.1.16; Danecek et al., 2011). Subsequently, individual genotypes with quality <30 and depth of coverage <8 were coded as missing data. Sites with more than 10% missing data over all samples were discarded. Finally, singletons were removed (minor allele count <2) with VCFTOOLS (v0.1.16).

After these steps, 1,829,565 SNPs from 89 individuals with sequencing depths of at least 8× remained. All the genetic analyses (detailed below) were performed by subsampling 9770 genome-wide SNPs. These were obtained by thinning the final dataset with 20 kb distances to minimise linkage disequilibrium, as well as by excluding chromosome number three (social chromosome with reduced recombination due to supergene inversions; Brelsford et al., 2020) and shorter contigs with no known genomic location.

Mitochondrial genomic data

Single nucleotide variant calling for the mitochondrial genome was done with FREEBAYES and a frequency-based approach. The SNP filtering followed the same pipeline that was used for the nuclear genome except that the steps after the retention of biallelic SNPs with a quality greater than or equal to 30 were not applied to the mitochondrial data. This resulted in 248 biallelic SNPs across the 89 individuals. Individual FASTA files were created using vcf-consensus (VCFTOOLS v0.1.16) and aligned with MAFFT (v7.505; Katoh & Standley, 2013).

We utilised two lines of evidence to examine genome-wide differentiation and to identify admixed individuals and the extent of introgression in our study species: we investigated individual nuclear genomic ancestry proportions with ADMIXTURE, and studied mito-nuclear mismatches. These results were supported by information from the phylogenetic network and principal component analyses (PCA). We used the genomic analyses in combination with the morphological identification from NUMOBAT, which enabled the identification of the samples at the species level.

2.3.3 | Admixture analysis

We performed ADMIXTURE (Alexander et al., 2009) with the 9770 nuclear SNP data to assess the extent of hybridisation in the *F. rufa* group in sympatry, to reveal whether the samples group genomically by species, and to detect nuclear genomic signs of hybridisation. All five species were included, but we aimed at balanced sample sizes of nonadmixed individuals (from six to eight individuals per species) and thus randomly selected six *F. aquilonia* individuals (see Table S1), since unequal sample sizes could yield biased clustering results (Toyama et al., 2020). For ADMIXTURE, the number of clusters K was chosen to range from $K = 2$ to 5, with prior knowledge of sampling from five morphologically identified species. Random selection of six *F. aquilonia* individuals was repeated multiple times to confirm that certain individuals did not drive the results. The most likely value of K was chosen based on cross-validation error (CV, prediction of withheld data; Alexander & Lange, 2011).

2.3.4 | Mitotype network

To detect hybridisation via mitonuclear mismatches, we characterised the mitotypes of our samples using the mitochondrial SNP data.

All sequenced samples were used. Samples of the same species are expected to have mitochondrial haplotypes that cluster together. Mitonuclear mismatches, that is cases in which an individual's mitochondrial and nuclear genomes originate from different species, are indications of hybridisation and introgression. A mitochondrial median-joining network was created with POPART (Leigh & Bryant, 2015).

2.3.5 | Phylogenetic network analysis

Using the nuclear genomic SNP data from all sequenced samples, we constructed a phylogenetic network to support the other genomic analyses, especially assessing whether the species are genomically distinct. In a phylogenetic network, individuals assigned to the same species should form clusters irrespective of their geographical origin. In contrast, if samples from different species cluster together, alternating with each other, this indicates eco- or morphotype-level difference rather than genomically distinct species. If no clear clusters exist at all, this indicates extensive gene flow between morphologically differentiated samples. If an individual is located in between species' clusters, and does not cluster with other samples, this is a putative signal of hybridisation and introgression.

For the phylogenetic network, we computed pairwise genetic distance between all pairs of samples using the script distMat.py from github.com/simonhmartin/genomics_general. A phylogenetic network was computed from the distance matrix with SplitsTree (version 4.17.1; Huson & Bryant, 2006), using the NeighbourNet approach (Bryant & Moulton, 2004) and default parameters.

2.3.6 | PCA

To further visualise the species' genetic structure, genomic distinction, and hybridisation, we ran a principal component analysis (PCA) using the nuclear genomic SNP data from all sequenced samples with PLINK (v2.00a3; Purcell et al., 2007). As in a phylogenetic network, genomically diverged species are expected to form separate clusters in ordination space.

2.3.7 | Observed heterozygosity

To characterise the hybrids, we also studied the observed heterozygosity of our Finnish samples (*N* = 77, see Table S1). Specifically, we tested whether admixed individuals between *F. aquilonia* and the *F. polyctena*/*F. rufa* clade, whose adaptive potential we were interested in studying due to the prevalence of their hybridisation, harbour elevated heterozygosity in comparison with nonadmixed individuals of these species. Higher heterozygosity in hybrids can promote adaptation to changing climatic conditions. Calculation of the per individual observed heterozygosity was performed with VCFTOOLS (v0.1.16). We used a Wilcoxon Rank Sum test to test for a difference in observed heterozygosity between hybrid individuals (*N* = 14) and nonadmixed individuals of *F. aquilonia* (*N* = 39) or *F. polyctena*/*F. rufa* clade (*N* = 6).

2.3.8 | Ancestry heterozygosity and hybrid index

Finally, we computed both ancestry heterozygosity and the hybrid index (HI) for our Finnish samples that were shown to be admixed between *F. aquilonia* and the *F. polyctena*/*F. rufa* clades, to provide more information on the admixed populations: in combination, these measures are informative about the stage of the hybrids (F1 vs. backcrosses) and independence of the hybrid populations. For this purpose, from our set of 9770 nuclear SNPs we identified markers (Ancestry Informative Markers, AIMs) that displayed an allele frequency difference ≥80% between all nonadmixed *F. aquilonia* individuals and nonadmixed *F. polyctena*/*F. rufa* clade individuals. We allowed max. 25% missing data per site, calculated for both nonadmixed *F. aquilonia* and *F. polyctena*/*F. rufa* clade individuals separately. We thus recovered 305 Ancestry-Informative Markers (AIMs) that were used to compute HI and ancestry heterozygosity. For these analyses, we excluded two admixed samples with more than 50% missing data for the AIMs (see Table S1).

2.4 | Climate data

2.4.1 | Ambient temperature and precipitation

To address our second aim of assessing whether temperature or precipitation correlate with the occurrences of hybrids between *F. aquilonia* and *F. polyctena*, and specifically, if the hybrids occur in warmer and drier habitats than cold-adapted *F. aquilonia*, we analysed climate data at Finnish nest locations. We compared nonadmixed *F. aquilonia* populations with populations that were admixed between *F. aquilonia* and the *F. polyctena*/*F. rufa* clade. To restrict the comparison to the sympatric range, all nests north of the northernmost *F. rufa* locality (in the absence of nonadmixed *F. polyctena* populations, and since *F. rufa* was revealed to be involved in the *F. aquilonia* × *F. polyctena* hybridisation) at 63.36°N latitude, were excluded from this analysis, leaving a total of 33 nest sites, one per population (see Table S1). We generated climatic time series for each of these nests extending back 1, 5, and 20 years from the nest-specific field sampling date. The time series data were extracted from digital rasters of daily mean air temperature and daily precipitation sum (sourced from the Finnish Meteorological Institute ClimGrid gridded daily climatology at a 10-km spatial resolution; Aalto et al., 2016) based on the nest site coordinates.

For each nest and year in the time series, we calculated annual winter (hibernation period) and spring (reproductive period) mean daily temperatures and precipitation from the daily values. The winter hibernation season was defined as the months of December to February and the spring reproductive season as April and May. To represent the warmest conditions experienced in winter at each site, we used the upper quantile of winter temperatures and the number of days when temperature reached over 0°C. To represent the coolest conditions experienced in spring, we used the lower quartile of spring temperatures and the number of frost days. These values were averaged over each of seasons and time periods and used in subsequent regression modelling.

To test whether *F. aquilonia* and hybrid nest sites diverged climatically within their sympatric range, we fitted Bayesian logistic regression models using the R package *brms* (Bürkner, 2017) with nest status (hybrid vs. *F. aquilonia*) as the response variable. We fitted 10 separate models, each with either one of the climatic covariates, or site latitude or longitude, as the explanatory variable. We did not include multiple explanatory variables in a single model due to high multicollinearity between the climatic variables and the coordinates, and the relatively low sample size. We normalised the explanatory variables to mean zero and unit variance, and used an informative normal(0,2) prior distribution for the model parameters. We fitted the models using four chains each with 4000 iterations and discarded the first 2000 iterations as warm up resulting in a total of 8000 posterior samples. The convergence and mixing of the fitting algorithm was checked with the Rhat and Effective Sample Size values. The models were ranked according to their expected log pointwise predictive density from leave-one-out cross-validation using the *loo* R package (Vehtari et al., 2017). We also checked whether the exclusion of three nests that are spatial and climatic outliers, due to their location in the Åland islands in the Baltic Sea, altered the model results.

2.4.2 | Within-nest temperature

Lastly, we studied whether ambient temperature affects within-nest temperatures as experienced by the ants, and hence whether our regional climatic modelling results are likely to have biological relevance. For this purpose, we tested whether within-nest temperatures correlated with ambient temperatures and whether they differed between hybrid and *F. aquilonia* nests. Three of the five ant populations used in these analyses (for which internal nest temperature data were available) were same, and two different from those used in the above morphological, genomic, and ambient climatic analyses (see Table S1).

In the years 2020 and 2021, we deployed Hobo data loggers to record within-nest temperatures hourly in 42 ant nests located within three admixed and two *F. aquilonia* populations in southern Finland (Figure S1). In total, four populations were sampled in 2020 and three populations in 2021; two of these were sampled in both years. In most cases, data logging ran continuously from 1st January until 31st July. However, data logging in one of the 2021 populations ('Svanvik') ran during May–July only. Daily mean within-nest temperatures per nest were calculated from the hourly values. Daily mean ambient temperatures for the corresponding dates were then extracted for each ant population based on the mean coordinates of nests within that population from the ClimGrid gridded daily climatology rasters at a 1-km spatial resolution (Aalto et al., 2016).

We modelled the mean daily within-nest temperatures of these nests in selected winter (January, February), spring (March–May) and summer (June, July) months as a function of nest status (hybrid vs. *F. aquilonia*), site ambient temperature and sampling year (2020 vs. 2021) using the R package *brms* (Bürkner, 2017). We compared a simple additive model structure (base model) with one including an interaction between nest status and ambient temperature (interaction model). Nest identity was included as a random effect. In the current analysis we have no data on nest volumes but based on field observations the studied *F. aquilonia* and hybrid nests have a similar size above ground. We fitted the models using four chains each with 4000 iterations and discarded the first 2000 iterations as warm up resulting in a total of 8000 posterior samples. The convergence and mixing of the fitting algorithm was checked with the Rhat and Effective Sample Size values. The fits of the two models were compared for each month according to their expected log pointwise predictive density from leave-one-out cross-validation.

3 | RESULTS

3.1 | Extensive hybridisation between *F. aquilonia* and the clade of *F. polyctena* and *F. rufa*, as well as admixed *F. lugubris* populations

Our study reveals extensive hybridisation and admixture in *F. rufa* group wood ants, especially between sympatric *F. aquilonia*, and the clade of *F. rufa* and *F. polyctena*. The admixed samples always include ancestry from the northern species, *F. aquilonia*, and from one or both southern species, *F. polyctena* and/or *F. rufa* (Figure 1a). However, disentangling the relative contributions of *F. polyctena* and *F. rufa* to the hybrids is challenging, likely due to their low genomic divergence. The relative contributions of *F. polyctena* and *F. rufa* to the hybrids varied depending on which nonadmixed *F. aquilonia* individuals were chosen for the ADMIXTURE analysis. Admixed populations between *F. aquilonia* and the *F. polyctena*/*F. rufa* clade (or 'Admixed 1') are located in southern Finland, up to a latitude of 62.6°N (Figure 1b). Moreover, all but three ('Att', 'Jar', '105') of these 14 admixed populations have their mitochondria from the southern species. In addition, one individual ('44') at 65.5°N latitude with a *F. aquilonia*-like nuclear genome showed a mitotype of a southern species (*F. polyctena*/*F. rufa* clade), suggesting admixture. Furthermore, three *F. lugubris* populations ('Admixed 2') show indications of admixture with likely contributions from *F. aquilonia*, *F. pratensis* and the *F. polyctena*/*F. rufa* clade. One of these hybrid populations is located in southern Finland (latitude ca. 60.2°N), and two are in eastern Finland (latitude ca. 62.7°N). In summary, southern Finland represents a mosaic hybrid zone between multiple species, where nonadmixed populations are interspersed with hybrid populations.

There was no significant correlation between the ADMIXTURE and morphological LDA values in admixed individuals, showing that the relationship of the genotype and phenotype was not linear

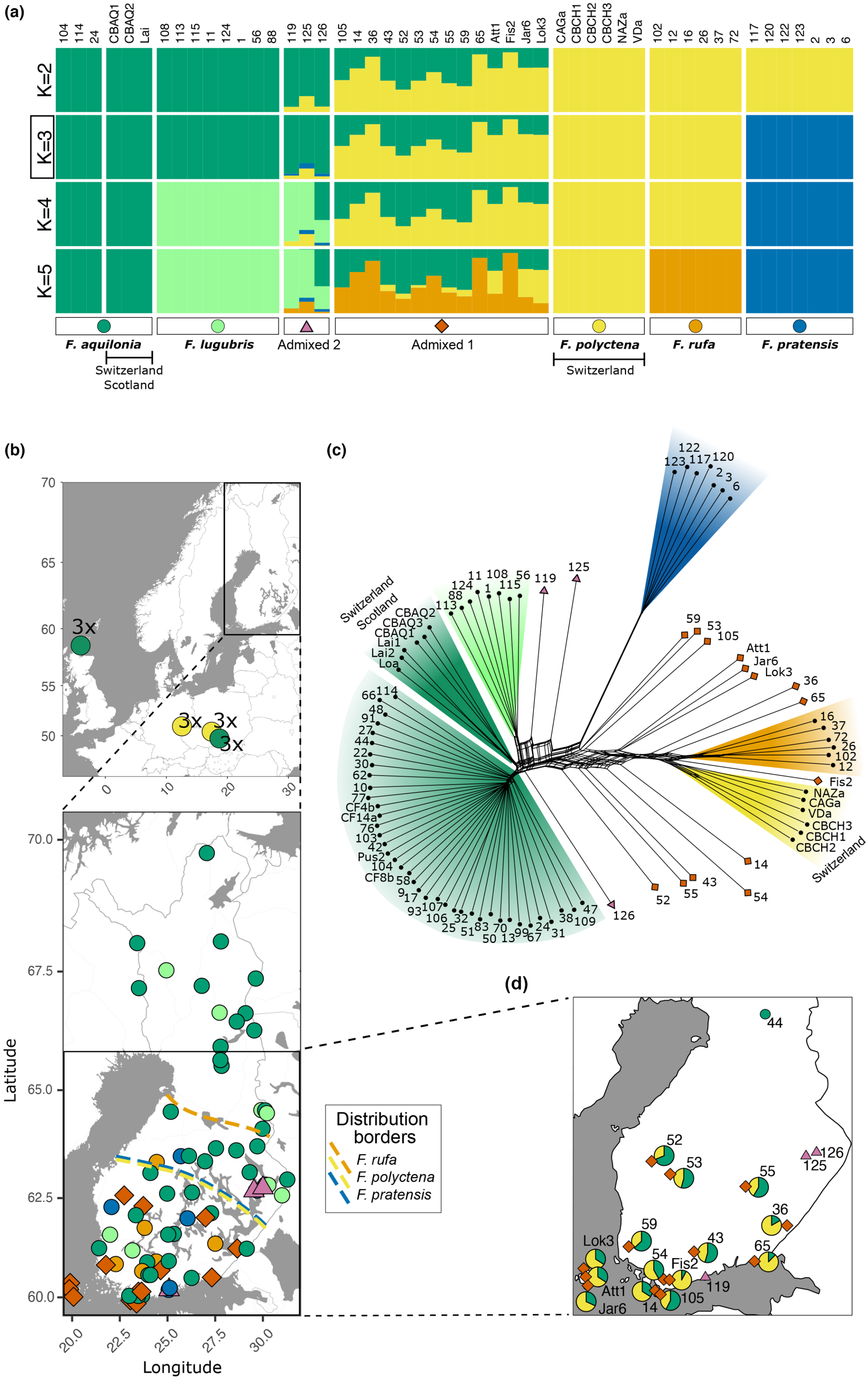
(a)
104 114 24 CBAQ1 CBAQ2 Lai 108 113 115 11 124 1 56 88 119 125 126 105 14 36 43 52 53 54 55 59 65 Att1 Fis2 Jar6 Lok3 CAGa CBCH1 CBCH2 CBCH3 NAZa VDa 102 12 16 26 37 72 117 120 122 123 2 3 6
K=2
K=3
K=4
K=5
F. aquilonia
Switzerland
Scotland
F. lugubris
Admixed 2
Admixed 1
F. polyctena
Switzerland
F. rufa
F. pratensis
(b)
70
65
60
55
50
3x
3x
3x
3x
0
10
20
30
70.0
67.5
65.0
62.5
60.0
Latitude
20.0
22.5
25.0
27.5
30.0
Longitude
Distribution borders
F. rufa
F. polyctena
F. pratensis
(c)
122 123 117 120 2 3 6
125 119
11 108 56 124 1 115 88 113
Switzerland
Scotland
CBAQ2 CBAQ3 CBAQ1 Lai1 Lai2 Loa
59 53 105
Att1 Jar6 Lok3
36 65
16 37 72 26 102 12
Fis2
NAZa CAGa VDa CBCH3 CBCH1 CBCH2
Switzerland
66 114 48 91 27 44 22 30 62 10 77 CF4b CF14a 76 103 42 Pus2 104 CF8b 58 9 17 93 107 106 25 32 51 83 70 50 13 99 67 24 31 38 109 47
126
14 43 55 52 54
(d)
44
52 53 55 126 125 36 59 43 65 Lok3 54 Fis2 119 Att1 Jar6 14 105

FIGURE 1 (a) ADMIXTURE analysis on nuclear SNP data recovers five species and admixture involving all species. Coloured legend bars indicate nonadmixed samples, while admixed individuals are divided into two groups based on the hybridising species. $K=3$ has the smallest cross-validation error. Depending on which *F. aquilonia* samples are chosen for the analysis, the order at which a distinction between sister species (*F. aquilonia* vs. *F. lugubris*, and *F. polyctena* vs. *F. rufa*) is seen within the clades varies, as does the relative contribution of *F. rufa* and *F. polyctena* in hybrids. (b) The upper map presents sampling locations from Scotland (3 × *F. aquilonia*), eastern Switzerland (3 × *F. aquilonia* and 3 × *F. polyctena*) and western Switzerland (3 × *F. polyctena*). The lower map shows sampling for the genomic and morphological data in Finland (one sample is located at the Russian side of Finland's eastern border). Colours and symbols are as in panel (a). The northern distributional borders are shown for species that do not inhabit the whole country, as in Stockan and Robinson (2016). (c) Phylogenetic network based on absolute genetic pairwise distances computed over nuclear SNPs supports the results of the ADMIXTURE analysis. Note that while the nuclear genome of sample 44 indicates nonadmixed *F. aquilonia* ancestry, it has mitochondria from the *F. polyctena*/*F. rufa* clade. (d) Sampling locations with individual IDs for all samples that show admixture or mito-nuclear discordance, and ADMIXTURE proportions ($K=3$) for the admixed samples of 'Admixed 1' group, i.e., individuals admixed between *F. aquilonia* and the *F. polyctena*/*F. rufa* clade. As in panel (a), $K=3$, green indicates the *F. aquilonia*/*F. lugubris* clade and yellow the *F. polyctena*/*F. rufa* clade.

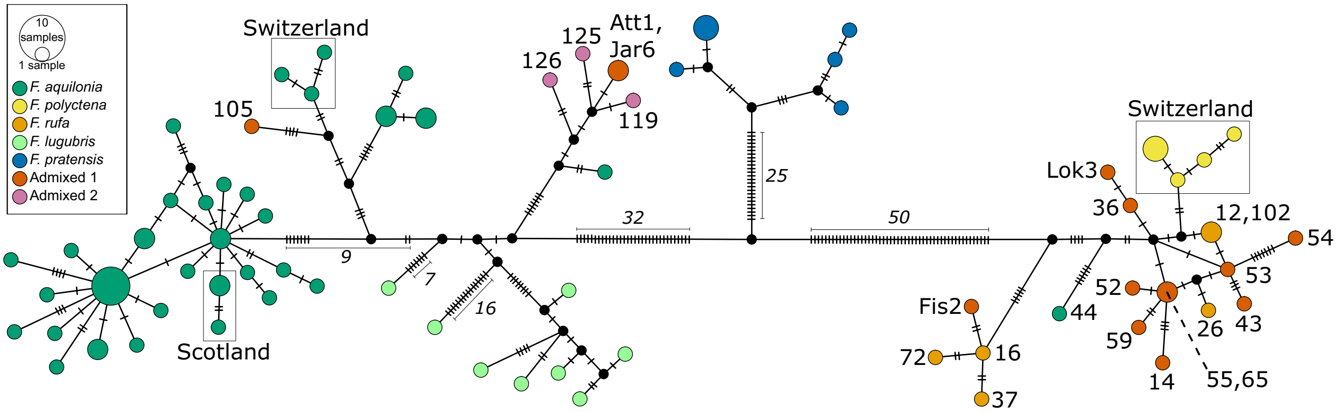

FIGURE 2 A mitochondrial haplotype network based on 248 SNPs and all sequenced samples. The circles represent individual haplotypes. The colours indicate nuclear genomic ancestry. The circle sizes indicate the number of identical haplotypes sampled. The dashes represent the number of mutations between the haplotypes, and italicised numbers help illustrate species differences. Red circles ('Admixed 1') = admixed individuals between *F. aquilonia* and *F. polyctena*/*F. rufa* clades, pink circles ('Admixed 2') = admixed *F. lugubris* individuals. For clarification, selected individual IDs are given. The mitotypes cluster by species, although the difference between *F. polyctena* and *F. rufa* is relatively small given their distant sampling locations. Eleven out of 14 admixed individuals between *F. aquilonia* and the *F. polyctena*/*F. rufa* clade have mitochondria from the latter southern species.

TABLE 3 NUMOBAT LDA probabilities of assigning nonadmixed samples to the species in which they belong to according to genomic data.

	F. aquilonia (N = 39)	*F. polyctena* (N = 2)	*F. rufa* (N = 6)	*F. lugubris* (N = 8)	*F. pratensis* (N = 7)
Mean (SD)	0.93 (0.20)	1.00 (0.00)	0.99 (0.20)	1.00 (0.00)	0.77 (0.30)

Note: We present here data for all nonadmixed nests for which morphological data is available. These probabilities indicate good congruence of genomic data and morphological features.

(Pearson's correlation of *F. aquilonia* ancestry and respective LDA probability in 'Admixed 1' group: $r(10)=.36$, $p=.250$; correlation of *F. lugubris* ancestry and respective LDA probability in 'Admixed 2' group: $r(1)=.96$, $p=.175$).

3.2 | Wood ant species maintain their genomic distinctiveness despite hybridisation

Despite the incomplete reproductive isolation and extensive hybridisation in sympatric *F. rufa* group wood ants we found support for four nonadmixed *F. rufa* group species (*F. aquilonia*, *F. lugubris*, *F. rufa*, and *F. pratensis*) in our Finland-wide sample, indicating genomically distinct species rather than a panmictic gene pool. This result is based on ADMIXTURE, PCA, mitochondrial haplotypes, and the phylogenetic network, detailed below (Figures 1 and 2, for PCA see Figure S2). All Finnish *F. polyctena* samples were admixed. When including nonadmixed *F. polyctena* from outside Finland, both the genomic data and the morphological analysis identified all five studied species. The comparison of ADMIXTURE and NUMOBAT LDA results for nonadmixed individuals showed a good congruence of genomic data and morphological features. This indicates that nonadmixed samples can be confidently identified based on morphology, even though the admixed samples cannot (Table 3, Table S5).

Formica pratensis was genomically most distinct from all the others and formed a well-separated genepool in both nuclear and mitochondrial analyses (Figures 1a,c and 2, Figure S2A). None of the seven *F. pratensis* populations showed signs of admixture. Furthermore, eight nonadmixed *F. lugubris* populations were identified across Finland (Figures 1a,c and 2), and their mitochondria similarly clustered together. *Formica aquilonia* also separated well from all other species in terms of both nuclear and mitochondrial DNA. Altogether 38 of the *F. aquilonia* populations were found to be nonadmixed and to occur across Finland, with relatively low differentiation between mitotypes, considering the great number of samples and wide sampling area. Six nonadmixed *F. rufa* populations formed a cluster next to the sister species *F. polyctena* sampled from Switzerland (Figures 1a,c and 2). Nonadmixed *F. polyctena*, in contrast, is rare in Finland: no nonadmixed *F. polyctena* populations were identified in our study. Since we do not have *F. polyctena* and *F. rufa* from the same location (all nonadmixed *F. polyctena* samples included here are from Switzerland) we cannot exclude the hypothesis that when sympatric they do not form distinct gene pools.

3.3 | Hybrid populations are not transient and the hybrids have higher heterozygosity than the nonadmixed individuals

We were interested in the amount of genetic variation as a proxy for adaptive potential especially in the case of individuals admixed between *F. aquilonia* and the *F. polyctena*/*F. rufa* clade ('Admixed 1'), since they are abundant in the landscape. These populations had significantly higher observed overall heterozygosity than either sympatric nonadmixed *F. aquilonia* (Wilcoxon Rank Sum test, $z = 4.92$, $p < .001$) or *F. rufa* ($z = 3.38$, $p < .001$) individuals (Figure 3). Furthermore, Ancestry Informative Markers (AIMs) indicate that these were late-generation hybrids, since heterozygosity based on AIMs was well below 0.5, suggesting hybridisation leads to persistent hybrid populations. Furthermore, hybrid individuals from different populations vary in ancestry, supporting the idea that the hybrid populations evolve independently of one another (Figure 4).

3.4 | Hybrids occur at sites with warmer temperatures and less spring precipitation than *F. aquilonia* populations

Our results indicate that the hybrid populations between *F. aquilonia* and the *F. polyctena*/*F. rufa* clade ('Admixed 1') occur in locations with warmer temperatures both during the ant hibernation (December–February) and reproduction (April–May) periods. The effects of temperature correlated with hybrid occurrence more strongly when warmer extremes both in winter (upper quartile, number of nonfrost days) and spring (lower quartile, number of frost days) were used instead of the average temperatures (Figure 5). Especially, the winter results were consistent over measurement periods of 1, 5 and 20 years (see also Figure S3). Spring precipitation had a strong negative effect on hybrid occurrences over the 20-year period (but not the 1- and 5-year periods), suggesting that hybrid nest locations tend to receive less precipitation in the spring than *F. aquilonia* locations. Latitude and longitude had slight negative effects, reflecting fewer hybrid nest occurrences in the north and the east of the sympatric area than in the south and the west. Winter precipitation did not have any consistent effects on the probability of hybrids occurrence with our

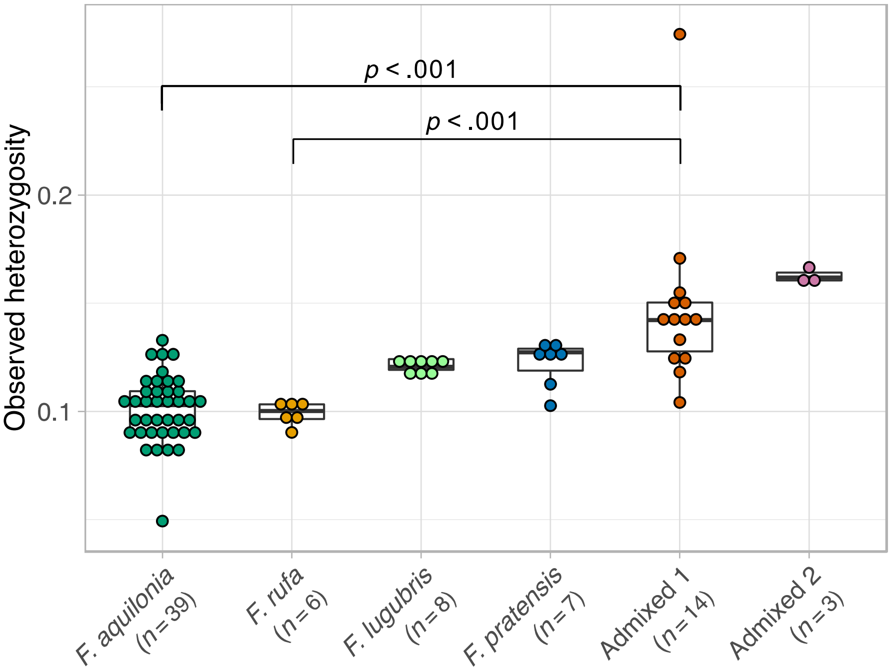

FIGURE 3 Within all Finnish samples, individuals admixed between *F. aquilonia* and the *F. polyctena*/*F. rufa* clade show more heterozygosity than nonadmixed individuals. *p*-values for Wilcoxon Rank Sum tests are shown. We use here nuclear genomic SNP data, and each dot indicates an individual. 'Admixed 1' consists of admixed individuals between *F. aquilonia* and *F. polyctena*/*F. rufa* clade. 'Admixed 2' are admixed *F. lugubris* individuals. Observed heterozygosity medians, first and third quartiles (hinges), and largest and smallest values no further than 1.5 × inter-quartile range from the hinges (whiskers) are shown.

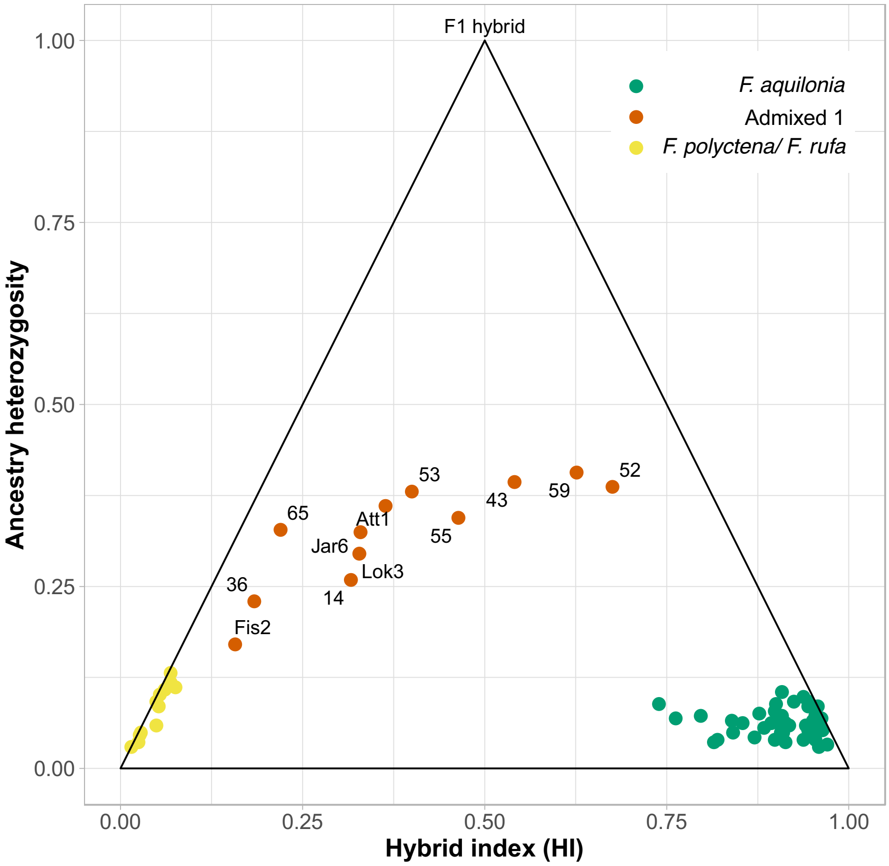

FIGURE 4 Admixed individuals between *F. aquilonia* and the *F. polyctena*/*F. rufa* clade show <0.5 ancestry heterozygosity and variable hybrid indices (HI), indicating they are further generation hybrids. These results are based on 305 genome-wide Ancestry Informative Markers (AIMs) acquired using all sequenced nonadmixed *F. aquilonia* ($N=45$), *F. polyctena* ($N=6$), and *F. rufa* ($N=6$) individuals. The admixed individuals ('Admixed 1', $N=12$) each represent a separate population.

dataset. The estimated effects were weaker but showed a similar trend when the three Åland island nests were removed. Crucially, among others the 20-year period results on winter upper quartile temperatures and spring precipitation still retained significance. The absolute values of these effect estimates are highly correlated with the difference in the expected log pointwise predictive density (elpd-diff) calculated with loo, which was used to rank the models according to their predictive performance (Figure S4).

3.5 | Ambient temperature impacts within-nest temperature and hybrid nests are warmer than *F. aquilonia* nests

Monitoring of three hybrid and two *F. aquilonia* populations shows that ambient temperature has a linear effect on within-nest temperatures in most months (Table 4, Table S6). Furthermore, even when controlling for the ambient temperature and inter-year effects, hybrid nests were internally warmer, on average, than *F. aquilonia* nests. This result is consistent in models with and without an interaction between nest status and ambient temperature (Table 4, Table S6) from January to March and in June. In addition, during January and April–May, hybrid nests warmed up more efficiently with respect to increases in ambient temperatures (see interaction model, Table 4). In June, the hybrid nests tended to warm up more slowly than the cooler *F. aquilonia* nests (Table 4).

4 | DISCUSSION

Hybridisation in natural populations is widespread and has increased, mediated by human activities and a warming climate (Chunco, 2014; Mallet, 2005; Rieseberg, 2009; Scheffers et al., 2016). Consequently, there is a topical need for knowledge on the extent and consequences of hybridisation and gene flow in natural populations. We report the first genomic study of five *F. rufa* group wood ant species and reveal geographically extensive hybridisation between these diverged species within Finland. We find that all the studied members of the *F. rufa* group hybridise with at least one other *F. rufa* group species in Finland. *Formica aquilonia*, *F. polyctena* and *F. rufa* form a mosaic hybrid zone in southern Finland with hybrid and nonadmixed populations scattered across the landscape. In addition, in three populations from southern and eastern Finland *F. lugubris* shows hybridisation with *F. aquilonia* and other *F. rufa* group species. We also

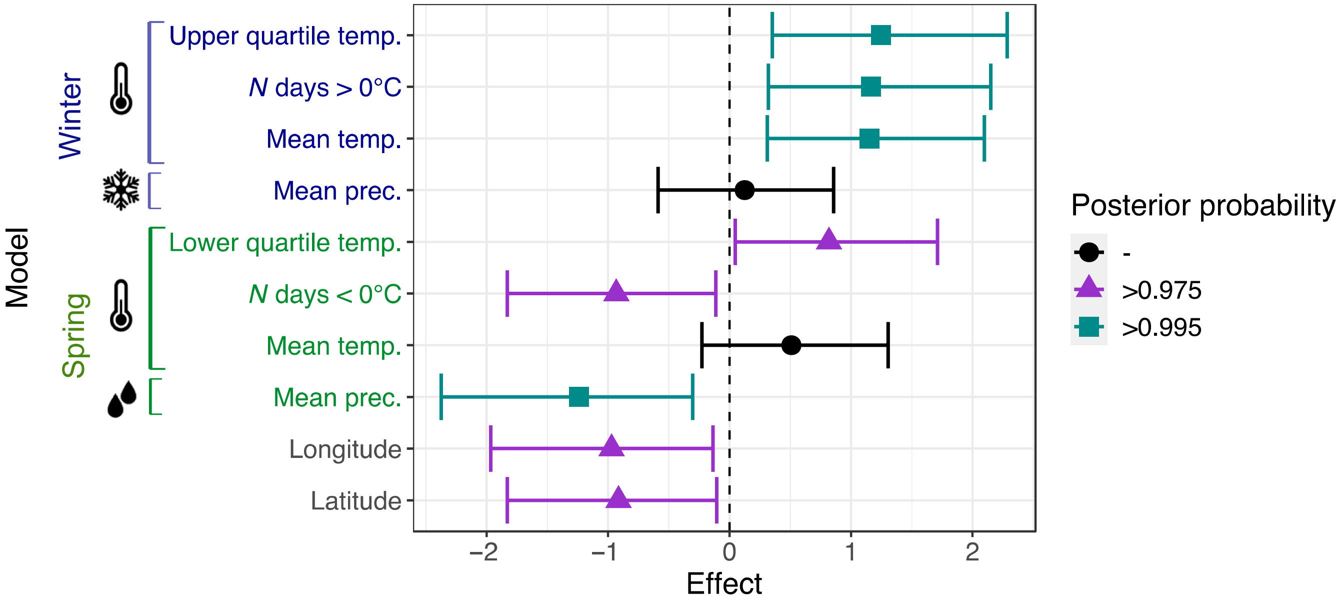

FIGURE 5 Bayesian logistic regression using climatic data from a total of 33 sympatric *F. aquilonia* populations and populations admixed between *F. aquilonia* and the *F. polyctena*/*F. rufa* clade ('Admixed 1') shows that they occur in different microclimates. Hybrid nest locations are warmer both in the winter and in the spring, and receive less precipitation in the spring than *F. aquilonia* nest locations. The climate data spans 20 years backward from the nest sampling year. Covariates that significantly correlate with hybrid occurrence have nonoverlapping credibility intervals (95% credibility intervals are given) with the midline (zero). The winter hibernation season ('Winter') = December–February; spring reproductive season ('Spring') = April–May. Upper quartile temp. = upper quartile of winter temperatures, *N* days >0°C = number of >0°C winter days, Mean temp. = annual mean winter or spring temperature, Mean prec. = annual mean winter or spring precipitation, Lower quartile temp. = lower quartile of spring temperatures, *N* days <0°C = number of spring frost days.

show that all Finnish *F. polyctena* populations sampled in this study are admixed. Within the mosaic zone, admixed populations of *F. aquilonia* and *F. polyctena*/*F. rufa* clade tend to occur in habitats with higher overwintering and reproductive season temperatures and less spring precipitation than nonadmixed populations of cold-adapted *F. aquilonia*. We further show that internal nest temperatures of these hybrids are warmer than those of *F. aquilonia*, suggesting that hybrids and *F. aquilonia* occupy different microclimatic niches. These hybrid populations persist, are abundant in the landscape, and have a higher genetic diversity than the nonadmixed populations. Hence, depending on the balance of ecological and intrinsic selection pressures, they may have potential to adapt to a warmer climate better than the nonadmixed populations, especially in comparison with the most abundant *F. rufa* group species in Finland, the cold-adapted *F. aquilonia*.

Extensive hybridisation, as detected here, is likely to impact the ecology and evolution of wood ants. Moreover, the semipermeable species boundaries may allow the genetic basis of e.g. climate adaptation (Martin-Roy et al., 2021) of wood ant species to be combined in novel ways in the hybrids. Previous work on thermal tolerance shows that hybrids survive better than *F. aquilonia* when heat-challenged (Martin-Roy et al., 2021) and that *F. aquilonia* suffers from warm winters (Sorvari et al., 2011). Together with the results from this study, this indicates that rising winter and spring temperatures may favour wood ant hybrids over *F. aquilonia*. In general, our results add important information on the extent of hybridisation in natural populations. The high number of established, potentially independently evolving hybrid populations raise the possibility that the wood ants may cope in changing environments through hybridisation. Wood ants illustrate how mosaic hybrid zones can have important evolutionary consequences with both ecological and intrinsic selection impacting spatially dispersed independent hybrid populations.

4.1 | Genomic distinctness in sympatry, and mosaic hybrid zone with broad implications between differentially adapted *F. rufa* group species

Despite our results on extensive hybridisation and the finding that all the studied species hybridise with one or more other species in sympatry, we detected both whole-genome and mitochondrial divergence among most of them in Finland. Specifically, four out of the five studied species—*F. aquilonia*, *F. lugubris*, *F. rufa* and *F. pratensis*—were found in nonadmixed populations in Finland. Our current dataset, in which all the *F. polyctena* populations are admixed in sympatry, does not allow testing whether *F. polyctena* and *F. rufa* represent different species or ecotypes of the same species. The mosaic hybrid zone that we detected (ca 60°N to 63°N) greatly expands the known area and extent of wood ant hybridisation in Finland (Beresford et al., 2017; Pamilo & Kulmuni, 2022; Seifert, 2021). We report 10 new populations that are admixed between *F. aquilonia* and the *F. polyctena*/*F. rufa* clade, bringing the total within this dataset, comprising both published and unpublished genomes, to 14. The hybrid zone is likely not restricted to

TABLE 4 The relationship of within-nest and ambient temperature in *F. aquilonia* populations and populations that are admixed between *F. aquilonia* and the *F. polyctena*/*F. rufa* clade.

	Intercept	Y2021	Hybrid	ExtT	Hybrid:ExtT	SD (nest)
Jan	**3.65* (3.16–4.15)**	**−1.59* (−1.84 to −1.34)**	**0.83* (0.18 to 1.45)**	0.01 (−0.01 to 0.04)	**0.08* (0.05 to 0.12)**	**0.96* (0.76–1.22)**
Feb	**2.95* (2.26–3.66)**	**−3.36 (−3.71 to −3.03)**	**1.14* (0.25 to 2.00)**	**0.04* (0.01 to 0.06)**	0.03 (−0.01 to 0.07)	**1.33* (1.05–1.70)**
March	**2.87* (1.98–3.77)**	**−3.05 (−3.38 to −2.71)**	**1.62* (0.46 to 2.79)**	0.05 (−0.00 to 0.10)	0.05 (−0.02 to 0.12)	**1.93* (1.53–2.46)**
Apr	**4.40* (2.83–5.98)**	**−2.38 (−3.05 to −1.71)**	0.84 (−1.09 to 2.80)	**0.47* (0.32 to 0.61)**	**0.40* (0.22 to 0.59)**	**3.17* (2.54–3.99)**
May	**6.54* (4.51–8.66)**	**−1.94 (−2.61 to −1.28)**	1.86 (−0.54 to 4.19)	**0.79* (0.70 to 0.88)**	**0.20* (0.09 to 0.31)**	**4.49* (3.69–5.44)**
June	**7.80* (5.85–9.65)**	−0.21 (−0.57 to 0.13)	**6.68* (4.39 to 8.95)**	**0.85* (0.79 to 0.90)**	**−0.35 (−0.42 to −0.29)**	**3.79* (3.06–4.74)**
July	**14.65* (12.77–16.59)**	**−3.17* (−3.59 to −2.74)**	−0.02 (−2.15 to 2.19)	**0.52* (0.46 to 0.59)**	0.07 (−0.01 to 0.15)	**3.50* (2.87–4.28)**

Note: Interaction model: Bayesian linear mixed model results summary from five separate populations, of which two combine data from both years 2020 and 2021. Absolute effects (in degrees Celsius) and their 95% credible intervals (CI) are given. * (in boldface) means that the 95% CI of the estimated effect size does not include 0.

Abbreviations: ExtT, Ambient temperature; ExtT:Hybrid, Interaction between hybrid and ExtT; Hybrid, Nest is hybrid (*F. aquilonia* is the reference); SD (nest), Standard deviation of nest random effect; Y2021, Year 2021 (2020 is the reference).

our study area, but may continue across the species' sympatric Eurasian area, as morphological evidence suggests (Seifert, 2021).

The implications and outcomes of mosaic hybrid zones may differ from those of clinal zones. This arises from three features of mosaic zones: (i) their patchy nature, (ii) the balance of environmentally dependent and independent selection and (iii) the potential for independent evolution of the hybrid populations when dispersal is limited. All these apply in the *F. rufa* group. First, the differential thermal adaptations (Martin-Roy et al., 2021) and north–south distributions (Stockan & Robinson, 2016) of the species are reflected in the patchy distribution of the hybrids and the locally abundant nonadmixed *F. aquilonia* populations. That is, the occurrences of nonadmixed populations and the hybrids are not explained only by a geographic cline as in a clinal hybrid zone model (Arnold, 1997; Barton & Hewitt, 1985), but also by local conditions within each patch within the broad hybrid zone as expected according to the mosaic hybrid zone model (Arnold, 1997).

Second, in contrast to clinal zones, mosaic zones typically comprise a mixture of environmentally dependent and independent selection pressures, the strength of which can vary among patches (Arnold, 1997). Clinal hybrid zones, in contrast, are usually assumed to be arrayed linearly along a habitat gradient (Arnold, 1997). The association that we observed between hybrids and warmer microclimatic conditions aligns with earlier evidence of thermally dependent selection (Martin-Roy et al., 2021) that may favour hybrids. This could counteract hybrid breakdown due to intrinsic incompatibilities (Kulmuni et al., 2010; Kulmuni & Pamilo, 2014) and reduced hatching rates (Beresford, 2021) which have been detected previously in wood ant hybrids (Kulmuni et al., 2010; Kulmuni & Pamilo, 2014). Third, variation in *F. aquilonia* ancestry and ancestry heterozygosity, combined with the limited dispersal capabilities of supercolonial wood ants, suggest that hybrid populations may have emerged from independent admixture events. This finding is consistent with previous research on three closely located populations (Nouhaud, Martin, et al., 2022). The mosaic of ancestry proportions across the hybrid zone contrasts with the spatial gradients typical of clinal hybrid zones (Barton & Hewitt, 1985). Together with relatively low ancestry heterozygosity, indicative of later generation or backcrossed hybrids, this attests to long-lived hybrid populations that are not under overwhelmingly negative selection. In summary, the evolutionary outcomes of mosaic hybrid zones may differ from those of clinal zones. This is because hybrid populations may evolve independently and be shaped in various ways by both ecological and intrinsic selection pressures and interactions with the nonadmixed populations leading to a diversity of hybrid lineages and phenotypes (Harrison, 1986; Rand & Harrison, 1989).

Which selection pressures drive the genomic and species composition of the mosaic zone is an interesting question for future studies. They may be, for example, affected by the degree of forest fragmentation, since in Central Europe *F. polyctena* and *F. rufa* hybrids are more prevalent in small fragments than large forests (Seifert et al., 2010). Furthermore, the interplay of ecological factors such as temperature, habitat preferences, differences in mating times (Martin-Roy et al., 2021; Seifert, 2018; Stockan & Robinson, 2016), and intrinsic selection on genetic incompatibilities (Kulmuni et al., 2010; Kulmuni & Pamilo, 2014) makes predicting the evolutionary dynamics of such a mosaic challenging. Our unique setting of more than 10 independent hybrid populations will provide an unrivalled opportunity to study the dynamics and outcomes of hybridisation in natural populations.

We predict that the northern border of the hybrid zone, that is the northern range limit of *F. rufa* (and *F. polyctena*), is likely to shift and extend northwards in the future following a warming climate, as has been observed in other taxa (Antão et al., 2022). Global warming is markedly faster in Fennoscandia than the global average (Masson-Delmotte et al., 2021), yet biodiversity responses may be complex and not necessarily follow directly climatic changes (Waldock et al., 2018).

4.2 | Hybridisation patterns are consistent with the mating strategies of the different species

Our findings are among the few that pertain to a mosaic hybrid zone involving multiple species. We add a new system to the groups of taxa exhibiting natural mosaic hybrid zones (Abbott & Brennan, 2014; Fraïsse et al., 2014; Rieseberg et al., 1999) and multispecies hybridisation (Banerjee et al., 2022; Grant & Grant, 2020; Heliconius Genome Consortium, 2012; Natola et al., 2022; Ottenburghs, 2019; Reutimann et al., 2020).

The majority of the mosaic zone hybrid populations are of the *F. polyctena*/*F. rufa* clade mitochondrial type, which suggests that hybridisation has mainly occurred between *F. polyctena* or *F. rufa* females and *F. aquilonia* males, consistent with previous modelling work (Portinha et al., 2022). This pattern could be due to the life history characteristics of the species and/or selection. The simplest explanation is that *F. polyctena* or *F. rufa* queens are forced to mate with *F. aquilonia* males due to a lack of conspecifics, because of differences in their abundance in Finland (Seifert, 2018), similarly to swordtail fish three-way hybridisation in Mexico (Banerjee et al., 2022). A complementary explanation is that since highly supercolonial *F. aquilonia* mates on top of or close to its nest (Seifert, 2018), workers protect the queens from nonconspecifics, as has been observed in other ant species (Eyer et al., 2023; Staab & Kleineidam, 2014). However, *F. aquilonia* males could disperse further as reported for polygynous *F. lugubris* (Gyllenstrand & Seppä, 2003), which would explain the existence of hybrids. On the contrary, monogyne *F. rufa* queens mate further from nests, are not guarded by the workers, and thus may mate more easily with nonconspecifics. Alternatively, the mitochondria of warm-adapted species may be advantageous in environments occupied by hybrid populations (including possibly outcompeting *F. aquilonia*) or mitonuclear incompatibilities may occur when the mitochondria come from *F. aquilonia* but the nuclear genome from *F. polyctena* or *F. rufa*. These possibilities remain to be addressed in future studies. The directionality of introgression will also be an interesting study question, as unidirectional gene flow from *F. aquilonia* to *F. polyctena* throughout their speciation history has been inferred previously (Portinha et al., 2022).

Our interpretation of clear genomic differentiation between *F. rufa* and *F. polyctena* should be treated with some caution, as all our nonadmixed *F. polyctena* populations are from Switzerland. Therefore, we cannot exclude the possibility that sister species *F. rufa* and *F. polyctena* have such a weak reproductive isolation and substantial gene flow that they cluster genomically when in sympatry, or resemble different ecotypes of the same species.

4.3 | Genomic data are essential in detecting admixture and reveal the rarity of nonadmixed *F. polyctena* in Finland

Despite previous morphological evidence, the lack of nonadmixed *F. polyctena* within our Finnish samples highlights the importance of integrating genetic data when studying admixture in species that are challenging to distinguish morphologically (Rellstab et al., 2016; Seifert, 2009). The original Finnish National Forest Inventory morphological investigations suggested the presence of *F. polyctena* within Finnish samples (Punttila & Kilpeläinen, 2009) as did other morphology-based studies (Härkönen & Sorvari, 2014; Sorvari, 2006, 2022; Vepsäläinen & Pisarski, 1982), but it is possible that nonadmixed *F. polyctena* does not exist in Finland.

We show that NUMOBAT morphological analysis can accurately discriminate nonadmixed wood ants at the species level. However, in hybrids, the genotype–phenotype relationship is not linear. We demonstrate that a reasonable identification of hybrids and their ancestries by means of NUMOBAT is not possible in the context of multispecies hybridisation. Morphological indication has proven to be a good tool if there is generally a small frequency of hybridisation in the material, if the species are sufficiently different and if there is a true F1 sample (and not different stages of backcrossing involved). Given these preconditions, good examples for morphological identification of hybrids in ant worker samples are those of *Camponotus herculeanus* × *C. ligniperda* (Seifert, 2019a), *Lasius emarginatus* × *L. platythorax* (Seifert, 2019b) and *Myrmica scabrinodis* × *M. vandeli* (Yazdi et al., 2012) or, in the case of gyne samples, *Lasius umbratus* × *L. merdionalis* (Seifert, 2006).

Nonlinearity of genotype and phenotype can also arise from transgressive segregation: admixed individuals do not necessarily show intermediate phenotypes but can even exceed phenotypic values of the nonadmixed populations due to, for example overdominance, epistasis or complementary action of genes with additive effects (Benowicz et al., 2020). This transgressive segregation has been demonstrated in a variety of taxa including conifers (Benowicz et al., 2020) and flowering plants (Rieseberg et al., 2003; Rubini Pisano et al., 2019), but has also been detected in multiple hymenopterans (Gadau et al., 2019; Linksvayer et al., 2009).

4.4 | Hybrids occur in warmer and drier habitats and have elevated heterozygosity compared to *F. aquilonia* suggesting they have opportunities to adapt to warming climate

We show that within the mosaic hybrid zone the admixed populations live in habitats with higher overwintering and reproductive season temperatures than those of nonadmixed cold-adapted *F. aquilonia*. Further, we show that ambient and within-nest temperatures are correlated in wood ants, and that hybrid nests tend to be internally warmer than *F. aquilonia* nests.

We cannot rule out nonadaptive explanations for the warmer hybrid habitats, arising e.g. from dispersal or species distribution patterns—for instance, if more hybridisation occurs close to the southern species' populations. This is because our modelling of the population niches does not directly test differences in fitness between admixed and nonadmixed populations. However, the association between hybrids and warmer habitats in our results could arise via selection: In the winter, cold-adapted species may suffer more from warm temperatures due to increased body fat use and a higher metabolic rate. This could lead to decreased survival, as has been demonstrated in *F. aquilonia* (Sorvari et al., 2011), or hamper the ants in heating their nests in early spring (Seifert, 2018). Thus, hybrid populations could outcompete *F. aquilonia* in warmer habitats, as indicated by the better heat tolerance of hybrids in the laboratory, which suggests a connection between temperature and ant fitness (Martin-Roy et al., 2021). On the other hand, cold winters may reduce hybrid survival, since the studied populations are located at the northern range limits of both *F. polyctena* and *F. rufa*. In a laboratory experiment, *F. aquilonia* × *F. polyctena* hybrids were found to be less cold-tolerant than nonadmixed *F. aquilonia* (Martin-Roy et al., 2021). Both these alternatives align with the hypothesis of better adaptation to warm temperatures in hybrids than in *F. aquilonia*. For the spring reproductive season, our results on both warmer hybrid habitats as well as hybrid nests being internally warmer and warming up more quickly than *F. aquilonia* nests align with the adaptive hypothesis as well. The springtime is when new males and queens are produced by the queen(s), and as such is a crucial time window for the long-term maintenance of the colony. However, wood ants can actively control their within-nest temperatures, and during springtime the within-nest temperatures deviate most significantly from ambient temperatures (Frouz & Finer, 2007). This may make ambient spring temperatures less crucial for ant survival than winter temperatures. One nonadaptive explanation for warmer hybrid nests would be differences in colony sizes, which in turn affect colony thermoregulation (Kadochová et al., 2019). We were unable to control for colony size here, but based on field observations this does not markedly differ between our hybrid and *F. aquilonia* populations. Overall, these results suggest that ambient temperature fluctuations might affect the wood ants' hibernation and reproductive success. It will be of interest in future studies to determine whether and to what extent thermal differences detected between *F. aquilonia* and hybrid nests are caused by either differences in the worker population size, ambient temperature, relative sun exposure, nest structure, and/or ant activity patterns.

The longer term adaptive potential of the hybrids in a changing environment is further supported by our finding of more heterozygosity in hybrids compared to the nonadmixed individuals. Genetic diversity arising from hybridisation may fuel adaptation (Torda & Quigley, 2021) even in the presence of hybrid incompatibilities (Kulmuni et al., 2023) and could provide means to adapt to new environmental challenges (Grant & Grant, 2019; Seehausen, 2013).

5 | CONCLUSIONS

We used combined genomic and morphological approaches to analyse species divergence and hybridisation in natural populations of extensively hybridising (Seifert, 2021), evolutionarily young (Goropashnaya et al., 2004, 2012) boreal keystone ant species. This first genomic study on five *F. rufa* group wood ant species suggests that the species barriers are semipermeable, since it shows that despite the gene flow they form their own gene pools. However, we cannot exclude the possibility that *F. rufa* and *F. polyctena* are two different ecotypes rather than species. We further reveal a mosaic hybrid zone in southern Finland, composed of three interbreeding wood ant species with differing climatic preferences. Our results align with other recent studies proposing that hybridisation is more prevalent in natural populations than previously thought. Moreover, they support a hypothesis that the wood ant hybrids may harbour adaptive potential (Martin-Roy et al., 2021) in the face of an increasingly warm northern climate (Masson-Delmotte et al., 2021), which affects species' distributions and requires adaptation to new conditions (Antão et al., 2022). This is because we show that the hybrid populations are abundant in the landscape, are presumably long-lived, have increased genetic diversity, and tend to live in warmer habitats than the sympatric nonadmixed *F. aquilonia* populations. Since the majority of our samples were collected over 15 years ago, repeated sampling of the same and novel locations could provide insights into the evolution of the mosaic hybrid zone and pinpoint the role of climate as a potential driver of its evolution. Future research is needed to examine the relative roles and patterns of barriers to gene flow between the wood ant species, as well as patterns of introgression, and the consequences of hybridisation for the wood ants and the surrounding ecosystem.

AUTHOR CONTRIBUTIONS

Original idea - IS, JK. Conceptualisation - IS, PP, HH, JK. Methodology and Investigation - IS, PN, BS, RS, MJ, JS. Funding acquisition - IS, JK. Project administration - IS, JK. Study material and resources - BS, PP, JK. Supervision - HH, JK. Visualisation – IS, JS. Writing - original draft - IS, MJ, JS, HH, JK. Writing - review & editing - IS, PN, PP, BS, RS, MJ, JS, HH, JK. All authors approved the manuscript before submission.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

OPEN RESEARCH BADGES

This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://doi.org/10.5061/dryad.4f4qrfjhf.

DATA AVAILABILITY STATEMENT

Genetic data (filtered nuclear and mitochondrial SNP datasets used in this study), temperature and precipitation data (broadscale climatic data on temperature and precipitation, and smaller-scale within-nest and ambient temperature data), and related scripts have been archived in Dryad at https://doi.org/10.5061/dryad.4f4qrfjhf. Morphological data is available in the Supplementary tables. Related sample metadata can be found in our Supplementary tables, including georeferences in decimal degrees, sampling year, and unique sample IDs that can be matched to both the deposited genetic and climatic data.

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

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