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The Poultry Red Mite, *Dermanyssus gallinae*, travels far but not frequently, and takes up permanent residence on farms

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ABSTRACT

Management of *Dermanyssus gallinae*, a cosmopolitan hematophagous mite responsible for damage in layer poultry farming, is hampered by a lack of knowledge of its spatio-temporal population dynamics. Previous studies have shown that the circulation of this pest between farms is of strictly anthropogenic origin, that a mitochondrial haplogroup has been expanding on European farms since the beginning of the 21st century and that its local population growth may be particularly rapid. To refine our understanding of how *D. gallinae* spreads within and among farms, we characterized the genetic structure of mite populations at different spatial scales and sought to identify the main factors interrupting gene flow between poultry houses and between mitochondrial haplogroups. To this end, we selected and validated the first set of nuclear microsatellite markers for *D. gallinae* and sequenced a region of the CO1-encoding mitochondrial gene in a subsample of microsatellite-genotyped mites. We also tested certain conditions required for effective contamination of a poultry house through field experimentation, and conducted a survey of practices during poultry transfers. Our results confirm the role of poultry transport in the dissemination of mite populations, but the frequency of effective contamination after the introduction of contaminated material into poultry houses seems lower than expected. The high persistence of mites on farms, even during periods when poultry houses are empty and cleaned, and the very large number of nodes in the logistic network (large number of companies supplying pullets or transporting animals) undoubtedly explain the very high prevalence on farms. Substantial genetic diversity was measured in farm populations, probably as a result of the mite's known haplodiploid mode of sexual reproduction, coupled with the dense logistic network. The possibility of the occasional occurrence of asexual reproduction in this sexually reproducing mite was also revealed in our analyses, which could explain the extreme aggressiveness of its demographic dynamics under certain conditions.

1. Introduction

The Poultry Red Mite, *Dermanyssus gallinae* (De Geer, 1778) has a considerable impact on poultry health and welfare, causing significant worldwide economic losses in the egg production sector (Sparagano et al., 2014). The recurrent failures of products and practices to manage *D. gallinae* underline the need to improve our understanding of this parasite's population biology (Decru et al., 2020). As this mite lives largely off-host and congregates with conspecifics in the bird nest or in

the interstices of above-ground farm structures (perches, nest boxes and other supports that shelter resting poultry inside the henhouse), it is both difficult to reach and to monitor. Parallel work on population genetic structure and demographic dynamics in multi-generational laboratory experiments have provided some answers, but have also raised many questions (Roy et al., 2021; Zriki et al., 2021; Dupray et al., 2022). For example, a meta-analysis of the mite's genetic structure based on mitochondrial DNA sequences highlighted recurrent gene flow between distant farms (haplotypes shared between countries, even continents),

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despite the fact that wild birds do not play a role in farm contamination (Roy et al. 2021). A strongly divergent mitochondrial haplogroup (haplogroup C on CO1) appears to be rapidly replacing the two initially cosmopolitan haplogroups (A and B), but the extent to which these populations hybridize remains unknown. Does the expansion of haplogroup C reflect a genuine replacement or massive admixture of previous populations? The globalisation of trade, typical of integrated agro-industrial systems of the 20th century, has been shown to contribute strongly to the spread of pathogenic microorganisms in the poultry industry (Li et al., 2021). We assume that it may have the same impact on the spread of blood-feeding mites, leading to secondary contacts between divergent populations. Furthermore, in closed mesocosms (experimental units to reproduce poultry ecosystems under controlled replicated conditions), we found that population growth rates were more rapid than previously described and that the life cycle duration is shorter than previously estimated from *in vitro* experiments (Zriki et al., 2021a,b, Dupray et al., 2022). Our experimental results in the mesocosm system support the idea that a very small inoculum of mites can rapidly populate a poultry house. On the other hand, we have also recorded recurrent cases of mite population growth failure from relatively large inocula, the reasons for which remain to be clarified (Dupray et al., 2022). To maintain sanitary conditions, poultry houses are thoroughly cleaned between flocks (ie, during the empty period). However, we have no information on the efficiency of this practice in terms of eliminating mite infestation.

Integrated genetic epidemiology approaches (*sensu* Tibayrenc, 1998) make it possible to investigate the characteristics of such hematophagous ectoparasites, which is essential for optimizing their management. By cross-referencing the genetic structure of parasite populations with their life history traits, farming practices and the socio-professional organization of production, we can frequently identify the main factors that explain infestation dynamics. For example, considering connections in livestock transport networks and the way in which livestock are introduced onto a farm (or into a barn) can help explain the mixing of parasite populations from different farms, the establishment of parasites in new farms and the spread of resistance to pesticides.

The aim of the present study is to refine our understanding of *D. gallinae* dissemination among farms dedicated to egg production. More specifically, the goal is to identify the main factors favouring the transfer of mites between farms at different spatial scales, estimating the frequency of effective contamination and assessing the effect of the empty period on genetic diversity in this sexually reproducing haplodiploid species (Hutcheson and Oliver, 1988; Oliver, 1966, 1971). In highly connected populations, individuals from different locations are almost as likely to reproduce with each other as those from within their own population. In contrast, individuals from isolated populations are more likely to reproduce with individuals from their own population. The degree of connection between farms, and thus the probability of mite dispersal and gene flow, is likely to vary according to various factors closely linked to poultry transfer routes, including geographical proximity, logistic networks and sanitary practices. Given the small, or non-existent, role of wild birds in mite circulation (Roy et al. 2021), no linear correlation is expected between the spatial distances between farms and the genetic distances between their mite populations. On the other hand, administrative boundaries (between regions or countries) and road infrastructures, in interaction with upstream logistic networks, are likely to determine the rate of gene flow at different spatial scales. For example, farms that share transport companies, pullet suppliers (rearing farms) and/or slaughterhouses, may have higher gene flow due to the potential circulation of mites with transport trucks and crates. Certain practices during transport, in particular poor sanitary practices, may also favour the transfer of mites between farms, for example via the introduction of inert material (e.g. transport crates) from other poultry houses, the lack of use of single-use protective clothing, or the transfer of poultry at night when mites are active.

Our approach here is therefore to identify the factors that interrupt

gene flow at different spatio-temporal scales by investigating 1) the population genetic structure in France and a few nearby countries, starting from the lowest level of spatial organization, 2) the temporal dynamics of allelic frequencies within farms, 3) the relationships between agricultural practices and variation in gene flow, and 4) the reproductive compatibility between mitochondrial haplogroups. We endeavour to treat these four aspects using a hierarchical spatial sampling design, a combination of population genetic analyses based on newly developed nuclear microsatellite markers, a field experiment and farm level surveys to establish both farm practices and the logistic networks used by farmers. We then propose an integrative analysis of these results.

2. Materials and methods

2.1. Biological materials

Dermanyssus gallinae mites were collected from several poultry farms of different types between 2020 and 2023 according to a standardized protocol: at each farm, six to ten randomly selected points in the poultry house were sampled for mites. At each point, mite aggregates from within a 1-m radius of the point were collected into hermetically sealed ziplock bags with a piece of towelling to limit moisture. In total, we analysed samples from 24 farms across five regions of France and three additional countries [Bretagne ($n = 4$), Centre-Val de Loire ($n = 1$), Bourgogne-Franche-Comté ($n = 1$), Auvergne-Rhône-Alpes ($n = 6$), Occitanie ($n = 4$), Belgium ($n = 3$), UK ($n = 4$) and Germany ($n = 1$); Table 1 and Fig. 1]. As the areas sampled in the French regions of Auvergne-Rhône-Alpes, Bourgogne-Franche-Comté and Occitanie correspond to a functional entity in terms of road infrastructure (Rhône and Languedoc arcs), we considered them as a group (South-East France region or S-E France; Fig. 1). As *D. gallinae* is mainly a problem in the egg production industry, we focused on layer farms: the majority of these farms were standard commercial layer farms, with aviary ($n = 6$), cage ($n = 3$), and barn ($n = 11$) type henhouses, of which some were free-range henhouses ($n = 6$) and others organic ($n = 5$) (Table 1). One of these also had pullet houses (POUL6). To refine our understanding of the impact of the socio-economic organization on mite dispersal, we also analysed mites from 4 atypical French farms: a very small layer farm with an alternative housing system (RIV: 600 hens in four self-built wood-frame henhouses run in an agroecological farming system), a farm selling ornamental hens (PDL, a few dozen hens of atypical breeds in a farm also housing horses, dogs and other mammals), a Bresse broiler chicken farm (FR08; a special kind of French traditional broilers) and a backyard henhouse (MAR; 4 hens in a private garden)(Fig. 1).

In order to obtain a hierarchical sample that can represent the connections at small (intra-farm), medium (inter-regional) and large spatial scales (inter-country), we also collected mites from multiple laying hen or pullet houses within certain farms (farms TES, BE03, POUL6). Samples from farm BE03 were distributed between 4 compartments of a single experimental building, divided internally into 8 compartments separated 4 by 4 by a corridor (Fig. 1). The other two farms (TES, POUL6) consisted of separate buildings. In order to assess the persistence of mites in the poultry house and the potential for gene flow between flocks to be interrupted by sanitation measures during the empty period, temporal samples were taken from within the same poultry house during two successive flocks, i.e. before and after the empty period (farms FR01, FR02, POUL6), or during a single flock (farms MAR and UK1).

In order to calibrate our estimate of variations in genetic differentiation of *D. gallinae* by farm pair, we supplemented our sample with mites from wild bird nests sampled in one of the targeted geographical regions. We searched for mites in tit nest boxes located in a site of the S-E France region (Fig. 1; 9.4 km from the PDL farm and 33.5 km from the MAR backyard henhouse). We received the nests in closed ziplock bags from A. Charmantier's ornithology team, and collected the mites

Table 1
Information on the samples collected.

Country	Region / Province	Source farm/bird group	Henhouse sample ID	Farm type	Type of unit (house, nest)	Sampling date	GPS coordinates		
France	Auvergne-Rhône-Alpes (S-E France)	ARD	ARD	Standard layer farm	Free-range (organic)	Jul-21	44.6345793,4.5402421		
		DIN	DIN	Standard layer farm	Free_range	Apr-22	44.3633962,4.6886853		
		FR12	FR12	Standard layer farm	Free_range	Dec-20	46.2731881,5.0600233		
		FRNP01	FRNP01	Standard layer farm	Free_range	Sep-20	45.9287415,4.9528959		
		ROU	ROU	Standard layer farm	Free-range (organic)	Sep-22	44.9088153,5.0037767		
		TES	FR07	Standard layer farm	Free-range (organic)	Nov-20	44.8748795,4.8843407		
			FR10	Standard layer farm	Free-range (organic)	Nov-20	44.8748795,4.8843407		
		Bourgogne Franche-Comté (S-E France)	FR08	FR08	Bresse broiler farm ^o	Free_range	Nov-20	46.7463135,5.3105786	
		Occitanie (S-E France)	SPT	SPT	Standard layer farm	Aviary	Oct-22	43.4823281,2.749844	
			MAR*	MAR / MAR2	Family henhouse ^o	Wooden backyard henhouse	Sep-22/Oct-22	43.892256,3.735707	
			PDL	PDL	Ornamental poultry farm ^a	Wooden backyard henhouse	Aug-21	43.6480123,3.7053329	
			RIV	RIV	Uncommon layer farm ^o	Free-range (organic)	Jul-21	44.0142778,4.0896547	
			Bird nests	MES1241	-	-	Nest of wild tits	May-23	43.660966, 3.665265
				MES22	-	-	Nest of wild tits	May-23	43.659698, 3.66635
				MES330	-	-	Nest of wild tits	May-23	43.658349, 3.677409
				MESR338	-	-	Nest of wild tits	May-23	43.665548, 3.679139
			Brittany	FR01**	FR01IN / FR01	Standard layer farm	Aviary	May-20/May-21	48.2918384,-2.942162
				FR02**	FR02IN / FR02	Standard layer farm	Aviary	May-20/May-22	48.235956,-2.7336711
		FR03		FR03	Standard layer farm	Cage	Dec-20	48.4977408,-3.0433723	
		POUL6**		POUL6D1 / POUL6D1BIS	Standard layer farm	Cage	Mar-22/Aug-22	48.2637975,-3.417136	
				POUL6P1	Standard rearing farm	Cage	Feb-22	48.2637975,-3.417136	
		POUL6P2	Standard rearing farm	Cage	Mar-22	48.2637975,-3.417136			
	Centre-Val de Loire	PG	PG	Standard layer farm	Cage	Mar-21	48.0562657,1.7669118		
Belgium	Province Limbourg	BE01	BE01a	Standard layer farm	Free-range (organic)	May-23	50.7328363,5.1727442		
	Province Antwerpen	BE02	BE02a	Standard layer farm	Aviary	May-23	51.2441855,5.1176986		
		BE03	BE03S1	Standard layer farm	Aviary	Nov-20	51.165938,4.9859685		
			BE03S2	Standard layer farm	Aviary	Nov-20	51.165938,4.9859685		
			BE03S6	Standard layer farm	Aviary	Nov-20	51.165938,4.9859685		
		BE03S7	Standard layer farm	Aviary	Nov-20	51.165938,4.9859685			
UK	South West England	UK1*	UK1 / UK1BIS	Standard layer farm	Free-range	nov-20/fev-21	51.3966498,-2.8512652		
		UK2	UK2	Standard layer farm	Free-range (organic)	Nov-20	50.7241516,-3.7836485		
		UK3	UK03OK	Standard layer farm	Free-range	Jul-21	51.3966498,-2.8512652		
		West Midlands England	UK4	UK04	Standard layer farm	Free-range	Oct-21	52.6523311,-2.7342319	
Germany	Near Dresden	ALL	ALL1	Standard layer farm	Aviary	Apr-23	51.05089,13.73832		

Mite individuals came from 30 henhouses on 24 farms and 4 tit nests. Each row describes one infestation unit, namely a poultry house on a farm or a tit nest. Among the farms, 5 henhouses marked with * were sampled at two different times: * during the same flock ($n = 2$), ** before and after the empty period ($n = 3$). Infestation units in blue indicate a grouping of farms (3 farms) or nesting areas (1 nest group). Atypical farms are indicated by a in the farm type column.

accumulating in the bag corners. After DNA extraction, the mite cuticles were placed between slide and coverslip for morphological identification, following the key of Roy et al. (2009). Out of 17 nests examined, six housed mites whose morphology corresponded to *D. gallinae*. There were sufficient numbers in four nests to include them in population genotyping (ID MESR338, MES1241, MES330, MES22 in Table 1).

A sample represents all the individuals taken from a given henhouse (or a given nest) on a given date. A total of 40 samples were taken from 31 different poultry houses on 24 different farms and 4 tit nests (Table 1).

2.2. Genotyping

2.2.1. DNA extraction

In the laboratory, three to six adult females were randomly selected from each farm subsample bag and 24 females from bags containing tit nests for genotyping, such that DNA was extracted individually from 32

individuals per farm sample and 24 individuals per nest sample. DNA extraction was performed using the REExtract-N-Amp Kit (Sigma-Aldrich, Saint Louis, MO, USA) following the manufacturer's recommendations after a rough crushing with a pipette cone.

2.2.2. Microsatellite markers

Microsatellite markers for *D. gallinae* were developed from the genome published by Burgess et al. (2018). Microsatellite loci with di-, tri-, tetra- and penta-nucleotide repeats were located on the genome using Primer3 (v3) software (Untergasser et al., 2012). Among the 150 bp to 220 bp repeat regions, 69 loci were selected and specific primer pairs were designed with Primer3 (v3). Twenty-three loci were selected on the basis of PCR product amplification success and specificity. These loci were then tested by multiplex PCR using a selection of extracted mite DNA and specific primers. These PCRs were performed using 1 μ L of DNA matrix extracted according to the above protocol, 1 μ L of 2 μ M primer R solution, 1 μ L of 2 μ M primer F solution coupled to a

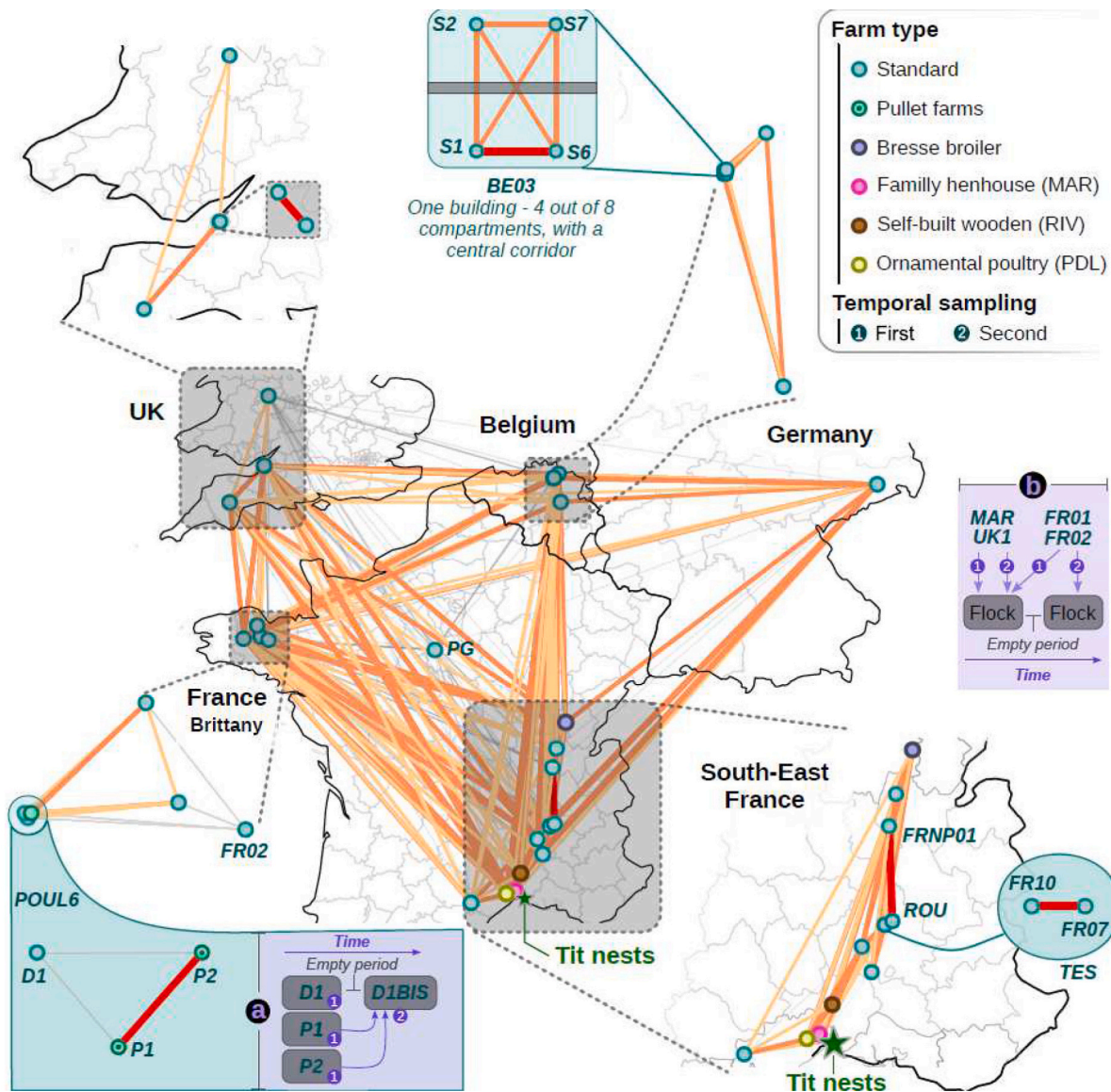


Fig. 1. Location of sampled infestation units and spatial representation of gene flow between inter- and intra-farm sampling of MLG10FARM data. Each circle dot represents a poultry house. The simple blue-grey dots identify the classic commercial layer henhouses (“standard”), the other dots identify the four atypical layer henhouses and the two pullet houses, and the green star indicates the location of the tit nest samples. Zoomed maps connected by grey dotted lines show intra-regional detail; zooms shown in blue areas represent different buildings on the same farm (intra-farm scale). a and b, design of samples taken on 2 occasions in single henhouses (“temporal sampling”). Each line connecting the henhouses represents the F_{ST} value between these samplings as follows: red line, $F_{ST} < 0.05$, dark orange line, $0.05 < F_{ST} < 0.15$, light orange line, $0.15 < F_{ST} < 0.20$, grey line, $F_{ST} > 0.20$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Information on the 12 microsatellite markers multiplexed for the study of *D. gallinae*.

Locus	DNA motif	Sequence of forward primer	Sequence of reverse primer	Fluorochrome	Multiplex ID
DG4-1	tetranucleotide	5'-CCAGCACCCAAGAACAATCG-3'	5'-AGGGCTCCCTTTTCTTCCG-3'	PET	M4a1
DG4-3	tetranucleotide	5'-GCCCATAGCGAAGCAAAGC-3'	5'-ACAACACGTGTCCGAGAGC-3'	6-FAM	M4a1
DG4-5	tetranucleotide	5'-GCCGCTACATGTATGACCT-3'	5'-GCCACTAAGCCATCTTGCTA-3'	NED	M4a1
DG4-18	tetranucleotide	5'-GATCGCTGGTCTGTCTC-3'	5'-AGCTTCATTCGTCGAGCCTT-3'	VIC	M4a1
DG4-6	tetranucleotide	5'-CCCGTTGGACTCAATTGCCT-3'	5'-CTCTGGGCTTTGTCCGGTTC-3'	PET	M4g1
DG3-10	trinucleotide	5'-CGAAACACAACAACCCGCAA-3'	5'-GGAAGGTGAAGACGGGTCAA-3'	6-FAM	M4g1
DG3_18	trinucleotide	5'-CGC-GCT-CCC-TAT-GAA-ATC-CT-3'	5'-ACG-CAG-CTC-ATC-AAC-CIT-CA-3'	NED	M4g1
DG4-19	tetranucleotide	5'-CCCGCTGACACTAGTACACA-3'	5'-ACGATGTCTGTACGCAACGT-3'	VIC	M4g1
DG3-16	trinucleotide	5'-GCTACAAAATTGTACCCTACCC-3'	5'-GAGGACAACGATAACAGCGC-3'	PET	M4g2
DG3-8	trinucleotide	5'-ATACGTGCTTGAATCGACGC-3'	5'-AGCAAAATACCCTCAGATGACGA-3'	6-FAM	M4g2
DG3_9	trinucleotide	5'-TGT-CAA-ACG-GAT-GGC-ATT-GC-3'	5'-CTC-TTC-TTA-TCC-ACC-TTG-GCC-A-3'	NED	M4g2
DG3-2	trinucleotide	5'-GACGACGAATGATGCAGCAC-3'	5'-GGAGCACGTGTCTGTCTGTTA-3'	VIC	M4g2

DG3_18 (multiplex ID M4g1) and DG3_9 (multiplex ID M4g2) were excluded from all analyses and DG4-5 (multiplex ID M4a1) and DG4-18 (multiplex ID M4a1) from analyses involving mites from tit nests due to recurrent failures of amplification in farm mite populations or in mite populations from wild birds, respectively.

fluorochrome, and 7 μL of mix M (QIAGEN® Multiplex PCR Master Mix), for a final volume of 10 μL . DNA amplification in this mix was then carried out with the following program using an Eppendorf Mastercycler thermocycler: initial denaturation for 15 min at 95 °C, followed by 30 cycles consisting of a 30-s denaturation step at 94 °C, a 90-s annealing step at 56 °C and a 60-s elongation step at 72 °C. The PCR program ended with a 30-min terminal elongation at 60 °C. Amplification was verified by electrophoresis of PCR products on 2% agarose gels with Ethidium Bromide staining. Each PCR product was then diluted to 1/300, 1/100 or 1/10 depending on the intensity of its band on the gel, and 3 μL of this diluted solution was blended with 15 μL of Formamide (Applied Biosystems - ThermoFisherScientific) containing standard single stranded DNA fragments labelled with the fluorophore LIZ™ (500 LIZ™ GeneScan™, Applied Biosystems - ThermoFisherScientific). The amplicons contained in each of these prepared solutions were characterized with a capillary fragment analyser (ABI 3500xL Sequencer, Life Technologies).

Using information on the read quality, polymorphism and homozygous/heterozygous status of the markers using GeneMapper (Software5, ©2012 Applied Biosystems) a new selection of markers was made. These markers were assembled into multiplexes using four different fluorochrome colours (PET, 6-FAM, NED or VIC) and were tested on a subsample of 8 individuals from different farms. In the end, 12 microsatellite markers were selected, run in 3 multiplexes of four markers each (see Table 2).

Genotyping of the extracted field-sampled individuals was then carried out by amplifying the 12 selected markers as follows: The reaction solution contained 1 μL of DNA extract, 1 μL of multiplex mix containing each primer coupled to a different fluorochrome (PET, 6-FAM, NED or VIC) where the concentration of each primer was 2 μM , and 8 μL of mix M (QIAGEN® Multiplex PCR Master Mix) in a final volume of 10 μL of multiplex PCR. DNA amplification and amplicon analysis were then carried out as described above. The alleles of each individual for each locus were then assigned using GeneMapper (Software5, ©2012 Applied Biosystems). The assignment was verified visually by two experimenters. A few markers were subsequently excluded from the analyses due to amplification defects in a number of individuals (see Results). Individuals with missing information for one or more of the retained markers were discarded. The set of alleles for each individual at all the loci retained for analysis constitutes their multilocus genotype (MLG).

2.2.3. Mitochondrial DNA sequencing

To determine the extent of admixture between the mitochondrial haplogroups delineated in previous studies and the status of the haplogroup C expansion (replacement or admixture), we sequenced a 685-bp fragment of the CO1 region in a subsample of mites from 8 farms for which we obtained full MLGs (5 farms from France, 2 from Belgium, 1 from the UK). To ensure that genotypes from tit nests were appropriate for calibrating differentiation between farm populations (outgroup status), we sequenced the same region in mites from these nests. PCR, electrophoresis and Sanger sequencing were performed as in Roy et al. (2021), using primers CO1LCF (5'-GAAAGAGGAGCAGGCACTGG-3') and RQCO1-R (5'-CCAGTAATACCTCCAATTGTAAT-3').

2.3. Analysis of genotyping data

All analyses were performed in R (R Core Team, 2022), using the RStudio interface (Version 2023.03.0; RStudio Team (2020)).

2.3.1. Validation of microsatellite markers

The general characteristics of each microsatellite marker, such as the number of alleles per locus, the observed heterozygosity H_o , the observed genetic diversity H_s and the fixation index F_{IS} ($F_{IS} = 1 - H_o/H_s$) were calculated using the whole dataset according to Nei (1987) using the “HierFstat” R package (v05–11; Goudet, 2005). To ensure the

independence of the different markers, linkage disequilibrium between each pair was calculated using the “Genepop” package (v1.2.2; Rousset, 2008). The significance of the F_{IS} estimate for each locus and each group of individuals (farm sample) was also calculated by computing a G test on contingency tables for all pairs of loci using the same package. This information was used to evaluate any potential problems with the markers (ie, deviations from Hardy-Weinberg expectations) or with the defined spatial scale of a population (ie, a Wahlund effect). We also checked for the presence of repeated MLGs that could indicate clonal reproduction.

2.3.2. Gene flow among farm samples

To assess genetic differentiation between pairs of samples, estimates of the pairwise F_{ST} were calculated using the “diveRsity” R package (v1.9.90; Keenan et al., 2013). Their significance was estimated using the diffCalc function (number of bootstraps = 1000): F_{ST} estimates were considered significant if the 95% confidence intervals did not include 0. To visualize the degree of gene flow among samples, sample locations were mapped (“rnatuarearth” R package (v1.0.1; Massicotte and South, 2023) using their geographical coordinates and were connected by links associated with the following differentiation thresholds adapted from Wright (1984) and Balloux and Lugon-Moulin (2002): $F_{ST} < 0.05$ = weak differentiation / strong gene flow, $0.05 < F_{ST} < 0.15$ = moderate differentiation / moderate gene flow, $0.15 < F_{ST} < 0.20$ = strong differentiation / weak gene flow and $F_{ST} \geq 0.20$ = very strong differentiation / almost no gene flow.

2.3.3. Sample clustering

In order to describe the genetic structure of the sampled populations without considering the geographic location a priori, an individual-centred Bayesian clustering approach applying Markov Chain Monte Carlo (MCMC) estimation was carried out using STRUCTURE (Version 2.3, Pritchard et al., 2000) called up in RStudio using the “path_to_structure” function (Supplementary material S1). These analyses were carried out on spatial sampling only (second samples from poultry houses that were sampled twice were excluded), i.e. on 30 different farm samples (30 different poultry houses from 24 different farms). The STRUCTURE analysis was carried out with the following parameters: 10 repetitions for each K value tested, with K ranging from 1 to 35, a burn-in of 100,000 and a total number of iterations of 100,000. All other parameters were left with default options. In order to refine our understanding of the high-level structure of our sample, we carried out a complementary analysis integrating mites from tit nests, where each nest represented a sample. The optimal number of clusters was determined using the Delta-K method of Evanno et al. (2005).

In order to consolidate our analysis of population structure, we also conducted a discriminant principal component analysis (DAPC) (non-parametric multivariate method) using the “adeget” R package (v2.1.10; Jombart, 2008) on the same individuals as above. Given that STRUCTURE clusters are based on the assumption of Hardy-Weinberg equilibrium, which may not hold for species like *D. gallinae*, the robustness of the chosen K from STRUCTURE was checked by comparing it with the results of the DAPC.

2.3.4. Mitochondrial DNA sequence analysis

We assigned sequenced individuals to mitochondrial haplogroups on the basis of the phylogenetic topology obtained after alignment with the homologous sequences of Roy et al. (2021) and phylogenetic reconstruction of haplotypes as in Roy et al. (2021). Sequence alignments were performed with the Clustal omega algorithm in Seaview (Gouy et al., 2010), the individualized haplotype file was made with DNAsp (Rozas et al., 2017) and phylogenetic trees were edited in Mega X (Kumar et al., 2018). Phylogenetic analysis was performed from this file using the Maximum Likelihood method and General Time Reversible model in Seaview.

2.3.5. Correspondence between microsatellite MLGs and mitochondrial haplogroups

To determine whether reproductive incompatibility exists between A-B and C haplogroups, and to assess introgression, we investigated possible segregation between nuclear genotypes (microsatellites) and mitochondrial haplogroups by constructing a minimum spanning network (MSN) of microsatellite MLGs and mapping Bayesian clusters and mitochondrial haplogroups onto it. This MSN was constructed based on the pairwise genetic dissimilarity distance matrix between MLGs calculated using the R package “poppr” v2.9.2 (Kamvar et al., 2014) for individuals from whom we obtained both the MLG genotype and the CO1 haplotype. To broaden the scope of this analysis, we also included the CO1 information of other individuals from a previous study (Roy et al. 2021): FR01 ($n = 20$), FR02 ($n = 20$), DIN ($n = 24$), FRNP01 ($n = 14$), SPT ($n = 16$), BE01 ($n = 18$), BE02 ($n = 16$).

2.4. Survey of the upstream logistic network

To refine our understanding of the effect of the socio-economic organization on the gene flow of *D. gallinae* at different spatial scales, a survey was carried out among poultry technicians and farmers on the layer farms where the mite samples were collected. The aim of this survey was (1°) to determine the extent to which shared transport companies, pullet suppliers (rearing farms) and/or slaughterhouses could explain gene flow between farms (and conversely, if the use of different service providers in farm pairs explains interrupted gene flows) and, (2°) to identify handling practices likely to favour (or prevent) effective contamination. To this end, a questionnaire was drawn up (Supplementary material S2A) and interviews were conducted either by phone or the questionnaire was sent by e-mail to the farmer or technician (a single person interviewed per farm). Responses were entered by the interviewer (CP) or directly by the respondent on a Word document if sent by email. In order to comply with “General Data Protection Regulation” (GDPR) rules, each person was informed of the context of the study, the intended use of the data and gave their consent to take part in the survey. Data were completely anonymised (interviewees, companies quoted in response). A table compiling all the anonymized information is available in Supplementary material S2B. Road distances between rearing farms and layer farms, and between slaughterhouses and layer farms, were calculated using GPS coordinates and the fastest route suggested by Google Maps.

2.5. Field monitoring of pullets and poultry transfer experiment

In order to test our working hypothesis concerning the role of trucks and transport crates as vectors of *D. gallinae*, we conducted a specific study in the Brittany region (France) from January 22nd, 2022 to August 2nd, 2022, as follows:

- 1-. We selected five rearing farms suspected of being infested with *D. gallinae* and assessed the level of infestation in one or two henhouses per farm during the 2 months before the end of flock (between January and May 2022). We used 24 traps per house; 12 water traps as in Mul et al. (2017) and 12 cardboard traps as in Nordenfors and Chirico (2001) in order to maximize the chance of detecting a few mites. The traps were distributed systematically throughout the building and were collected and replaced every month. The mites captured in each trap were counted.
- 2-. For positive henhouses, we sampled truck floors and transport crates for mites during the transfer of new flocks to the receiving layer farm (i.e., just before loading pullets from the rearing henhouse and just after unloading them at the layer henhouse). The protocol involved blindly rubbing pieces of paper towel on surfaces measuring approximately 15×15 cm in the trucks. In each truck and at each time (before loading / after unloading the pullets), 10 pieces of paper towel were individually rubbed at 10

random points on the trailer floor and 10 pieces of paper towel at 10 random points on the transport crates. Immediately after rubbing, each piece of paper towel was inserted into a hermetically sealed ziplock bag. The contents of each bag were then examined in the laboratory, using a stereomicroscope and the total number of mites on the paper towel was counted, distinguishing between young (white larvae and protonymphs) and later stages (salt-and-pepper colored deutonymphs and adults).

In order to cross-reference these data with the genetic structure of *D. gallinae* populations, additional systematic sampling was then carried out following the protocol described above (§2.1) in cases where positive traps were found: we included samples from the source pullet henhouses (once each) and from the receiving layer henhouses (twice: once from the previous flock and once from the new flock, respectively sampled in March and August 2022) in the genetic study (see the Biological material section and Table 1).

3. Results

3.1. Microsatellite markers in *D. gallinae*

In line with the validation criteria set at the start of the study, primer pairs for the twelve selected markers produced good-quality amplicons in all well-preserved mites from farms. However, two markers (DG3_18 and DG3_9) showed recurrent amplification failures in samples from farms and two markers (DG4_5 and DG4_18) showed recurrent amplification failures in samples from tit nests, probably due to mutations in the primer-binding regions. We therefore discarded the first two markers (DG3_18 and DG3_9) for the analysis of farm populations and these four markers (DG3_18, DG3_9, DG4_5 and DG4_18) for the analysis of all populations (tits + farms) (see legend of Table 2). We then created two separate datasets: one grouping the 10-marker MLGs of individuals from farms only (MLG10FARM) and one grouping the 8-marker MLGs of all

Table 3
Diversity and allele frequency of microsatellite markers in analysed datasets.

A.					
Locus	Number of alleles	Range	Ho	Hs	F _{IS}
DG4-1	17	[109–252]	0.3850	0.3953	0.0261
DG4-3	11	[158–227]	0.4269	0.4439	0.0382
DG4-6	44	[152–268]	0.6754	0.7109	0.0499
DG3-10	54	[103–283]	0.6817	0.7584	0.1011
DG4-19	14	[151–223]	0.5629	0.5621	–0.0014
DG3-16	29	[176–294]	0.6466	0.6959	0.0709
DG3-8	55	[129–431]	0.7962	0.8740	0.0890
DG3-2	36	[239–427]	0.6358	0.7526	0.1552
All locus	/	/	0.6013	0.6491	0.0737
B.					
Locus	Number of alleles	Range	Ho	Hs	F _{IS}
DG4-1	9	[124–154]	0.3725	0.3722	–0.0010
DG4-3	11	[170–206]	0.4353	0.4567	0.0469
DG4-5	22	[68–242]	0.1967	0.1990	0.0116
DG4-18	13	[198–258]	0.6108	0.6321	0.0337
DG4-6	39	[152–268]	0.6799	0.7130	0.0465
DG3-10	52	[121–283]	0.7332	0.7770	0.0564
DG4-19	12	[151–223]	0.5851	0.5770	–0.0141
DG3-16	24	[194–269]	0.7090	0.7298	0.0285
DG3-8	52	[129–431]	0.7925	0.8840	0.1034
DG3-2	29	[288–427]	0.6262	0.7528	0.1682
All locus	/	/	0.5741	0.6093	0.0578

A. Eight markers in the MLG8ALL dataset (934 individuals/39 samples). B. Ten markers in the MLG10FARM dataset (845 individuals/35 samples). Ho, mean observed heterozygosity within populations; Hs, mean gene diversity within populations; F_{IS} = inbreeding coefficient.

individuals (MLG8ALL). Of 1120 individuals from farms and 96 individuals from tit nests analysed in the laboratory, we obtained complete genotypes for 845 and 74 respectively, with approximately 24 individuals per sample on average.

The number of alleles, H_o , H_s and F_{IS} for the MLG8ALL dataset is shown in Table 3 A. The average number of alleles per marker was 32.50 ± 17.61 (range 11–55). The MLG10FARM dataset with the two additional markers showed values of the same order of magnitude (Table 3B). With MLG10FARM, the observed heterozygosity H_o for each of these ten loci averaged 0.574 (between 0.197 and 0.7925), the genetic diversity H_s averaged 0.6 (between 0.199 and 0.884) and the inbreeding coefficient or fixation index F_{IS} averaged 0.05, i.e. close to 0. There was no significant linkage disequilibrium present in the overall dataset, and significant tests were found in only 6 of the 35 samples, and between different marker pairs on almost every occasion (Supplementary material S3A). Significant estimates of F_{IS} were found for a few markers in a few samples, all positive, but no overall pattern for any one marker or population was found (significant F_{IS} estimates per marker were 10 in number, ranging between 0.14 and 0.77 over the 35 populations; Supplementary material S3B). Whilst H_o , H_s and F_{IS} values were similar between the two datasets, the number of alleles was higher in the MLG8ALL dataset than in the MLG10FARM dataset for the 10 shared loci except DG4–3 (same number), due to 32 alleles specific to tit nests (present in nests and absent from farms) (Table 4).

No repeated MLGs were observed, in any farm mites. However, two pairs of two individuals from a tit nest (MES330) were identical; only one copy of these MLGs was kept for the analyses.

3.2. Genetic structure of populations

3.2.1. Average number of alleles per locus according to geographical scale

The average number of alleles per marker at different spatial scales in the MLG8ALL is displayed in Table 4. Within French regions, it was equivalent to within UK and Belgium (around 18.00), but was well below the overall value for France (28.62 ± 16.43). Poultry houses contained only a fraction of the alleles of their region, with 8.29 ± 2.31 alleles per marker. Interestingly the farm scale showed intermediate average numbers of alleles per marker between henhouses and the region. For example, the henhouse BE03, with 4 isolated compartments in a single building, had an average number of alleles per marker very close to that of the regional scale. In the tit nests, the average numbers of alleles per marker and per nest was equivalent to the lowest values of the poultry houses.

Table 4
Average number of alleles per locus at different spatial scales in MLG8ALL.

Spatial scale	Unit under scrutiny	Average number of alleles per locus \pm sd
Total	All samples	32.50 ± 17.61
	All Farms	28.50 ± 17.70
Country (multi-farm countries)	Farms in France ($n = 16$)	28.62 ± 16.43
	Farms in Belgium ($n = 3$)	18.75 ± 13.32
	Farms in UK ($n = 3$)	18.12 ± 10.99
Regions (multi-farm regions)	Farms in Auvergne-Rhône-Alpes ($n = 7$)	17.75 ± 11.16
	Farms in Brittany ($n = 4$)	17.50 ± 11.5
	Farms in Occitanie ($n = 4$)	18.63 ± 12.37
Infestation groups (multi-house/nest units)	Farm BE03 (Belgium; $n = 4$)	15.00 ± 10.76
	Farm POUL6 (France, Occitanie; $n = 3$)	12.38 ± 8.94
	Farm TES (France, Auvergne-Rhône-Alpes; $n = 2$)	10.00 ± 9.04
	Tits ($n = 4$)	8.62 ± 5.97
Infestation units (poultry houses / tit nests)	Poultry houses ($n = 24$)	8.29 ± 2.31
	Tit nests ($n = 4$)	5.84 ± 2.36

3.2.2. Pairwise F_{ST} on microsatellite data

According to the pairwise F_{ST} obtained with MLG10FARM and with MLG8ALL, all sampling units (henhouses, nests) were significantly genetically differentiated (F_{ST} significant), with the exception of two pullet houses of the same farm: POUL6P1/POUL6P2. None of the populations sampled in the same poultry house at different times showed any significant differentiation, either for samples taken during a single flock (MAR/MAR2 & UK1/UK1BIS) or, surprisingly, for samples taken between two successive flocks separated by an empty period (FR01IN/FR01, FR02IN/FR02, POUL6D1/POUL6D1BIS). Not only were F_{ST} estimates before/after the empty period not significantly different from 0, but H_s values were maintained at similar levels over time (Supplementary material S3C).

Differentiation by farm pair was highly heterogeneous at all scales in our sample (within farms, between farms 10 km to 1270 km apart, within and between 5 regions and 4 countries). Within farms, three pairs of poultry houses out of ten pairs had F_{ST} values < 0.05 , indicating the highest amount of gene flow, as expected (Fig. 1). However, moderate to high F_{ST} values in the other seven pairs show that gene flow may be considerably impeded within farms. F_{ST} values < 0.05 were also found between farms (one pair of farms from S-E France (ROU/FRNP01) and one from the UK (UK1/UK03)) (Fig. 1). Within each region, differentiation by farm pair covered ranges of similar magnitude as inter-regional pairs. However, poultry farm pairs located within Auvergne-Rhône-Alpes (AURA, the most represented region in our sample), were slightly less differentiated than inter-region pairs including AURA (mean AURA-AURA $F_{ST} = 0.169 \pm 0.069$; mean AURA-other $F_{ST} = 0.212 \pm 0.0886$; Wilcoxon test: $W = 4052, p = 0.026$).

Although no linear isolation by distance was found in our sample and a high degree of variance characterized the F_{ST} by distance class, the F_{ST} values between poultry houses located on the same farm were significantly lower than for pairs involving two different farms (Fig. 2). Three standard commercial layer farms were strongly differentiated from all the others (pairwise F_{ST} with all other farms > 0.20 , except those in the same country for the latter): PG in Centre-Val de Loire, FR02 in Brittany and UK04 in the UK. On the contrary, unexpectedly, none of the four atypical farms showed any marked differentiation from commercial layer farms (F_{ST} of pairs containing PDL, MAR, RIV and other farms other than the three above ranged from 0.029 to 0.434).

With the MLG8ALL dataset, the various values were distributed in approximately the same way between farms, including the F_{ST} values per pair of samples (Supplementary material S3D). The F_{ST} values for poultry houses ranged from 0 (–0.015) to 0.541 and for nest/poultry house from 0.261 to 0.616. This confirms the relevance of our threshold of $F_{ST} > 0.20$ for considering gene flow between two populations to be low or close to zero since the bird nests were sampled right in the middle of a farm sampling area. Interestingly, the pairs of tit nests were all significantly differentiated from each other, with moderate F_{ST} values, even though they were located in the same geographical area and max. 1.5 km apart from each other.

3.2.3. Clustering on microsatellite data

Given that in the case of weak structure, there is a high risk of obtaining $K = 2$ as an artefact with Evanno's Delta-K method (Janes et al., 2017), we worked on the MLG8ALL dataset for the choice of the optimal number of Ks. Tit mites should provide sufficiently strong structure to avoid the artefactual $K = 2$. Delta-K evolution showed a major peak at $K = 3$ and several secondary peaks (Fig. 3A). With $K = 3$, Structure outputs were stable between replicates, systematically separating genotypes from tit nests from genotypes from farms. Within our set of farms, two clusters co-exist (including cases of mixing within the same farm). We therefore retained $K = 3$ as the optimum number of clusters to distinguish the first-level structure.

A second-level structure appeared with $K = 25$ (Fig. 3A). This value is in line with the lowest value of the Bayesian information criterion (BIC) in the outputs of the DAPC performed on MLG8ALL ($K = 25$; Fig. 3A) and

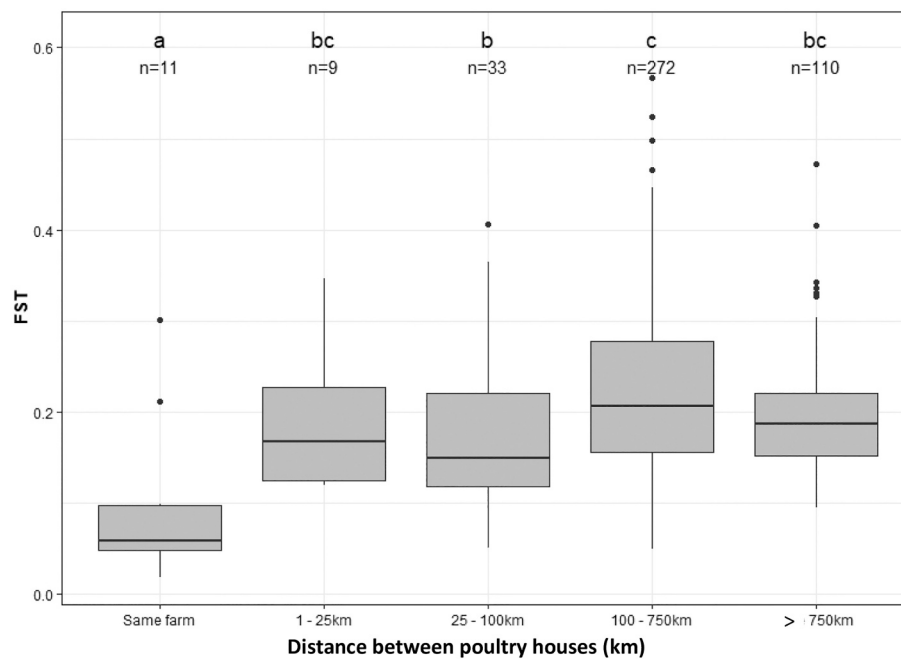


Fig. 2. Relationship between genetic differentiation within farm infestation units (poultry houses) and geographical distance using MLG10FARM spatial data (749 individuals/30 samples). Value of pairwise F_{ST} as a function of distance between samples from: the same farm but different poultry houses (= Same farm), or different farms at different distances. Letters show the significance of pairwise Wilcoxon tests (a letter common to two distance classes indicates a non-significant difference, the absence of a common letter indicates a significant difference).

close to those on MLG10FARM ($K = 21$; not shown). The number 25 is equal to the number of farms included in the analysis, plus one tit colony (four nests). This is consistent with the F_{ST} values, which indicate systematic differentiation between farms. We therefore decided to retain the number of sampling units (24 farms for MLG10FARM and 24 farms +1 for the tit colony with MLG8ALL) as the optimal second-level K . The internal structure of farm sampling with $K = 25$ on MLG8ALL is congruent with the structure obtained with $K = 24$ on MLG10FARM (Fig. 3B).

In the second-level structure with MLG10FARM, the fragmented assignment of some individuals to different clusters suggests a marked admixture in some poultry houses. However, for a large proportion, individuals show a high probability (>0.75) of assignment to a single cluster (Fig. 3B and C). The patterns are consistent with patterns of pairwise F_{ST} as well as DAPC outputs.

The MSN is clearly split in two, with a long, tapering branch grouping all MLG genotypes from tit nests (in dark green on Fig. 4) and a reticulated central group that includes genotypes from various farms (Fig. 4A). Despite a mixture of genotypes from >15 farms in the central area of the network, structuring by farm is apparent and particularly for mites from the two most differentiated farms (PG and FR02) and those from two of the 4 atypical farms (RIV and MAR). The FR02 branch is composed exclusively of hybrid nuclear MLG genotypes between cluster 2 and cluster 3 (Fig. 3B and 4B).

3.2.4. Correspondence between nuclear genotypes and mitochondrial haplogroups

Among the 127 farm mites successfully sequenced for the mt-CO1 (we did not obtain a clear sequence for 37 of the 164 sequenced mites), 11 haplotypes already present in the Roy et al. (2021) database and 25 new haplotypes were recorded (510-bp fragments; accession number in Supplementary materials S4). In individuals from tit nests, 6 new haplotypes were found among the 88 individuals sequenced. The phylogenetic tree shows a very marked isolation of mite populations from tit nests and from poultry farms, even though some farms were located very close to where the tit nests were sampled (PDL, MAR and

RIV; Fig. 1) (Supplementary materials S4). These haplotypes appear as sister group to *D. gallinae* s.s. and *D. gallinae* L1, probably reflecting advanced speciation. This confirms the relevance of using these samples as a reference for calibrating the maximum reproductive isolation in our dataset.

The large number of individuals assigned to both cluster 2 and cluster 3 ($q < 0.6$; Fig. 3B) and the lack of haplogroup segregation between individuals assigned with $q > 0.75$ to either of the two clusters (Fig. 4C) clearly show that there is no reproductive incompatibility between haplogroup C and the others. Considering that haplogroups A and B form a monophyletic group (Roy et al. 2021), the distribution of individuals between the two major haplogroups AB and C was examined based on individuals assigned with $q > 0.75$ to either of the two clusters. Although segregation is incomplete, haplogroup AB appears to be more frequently associated with cluster 3 and haplogroup C with cluster 2 ($\chi^2 = 11.208$, $df = 1$, p -value = 0.0008).

Based on the sequences obtained from other individuals in the FR01, FR02, DIN, FRNP01, SPT, BE01 and BE02 samples (listed in A; Roy et al. 2021), the first five henhouses (Brittany and S-E France) contain exclusively haplogroup C individuals, while BE01 and BE02 (Belgium) contain haplogroup B individuals mixed with haplogroup C (22B + 1C and 3B + 13C resp.). The MLG genotypes obtained from the first five poultry farms form the peripheral branches of the MSN, whereas those from BE01 and BE02 lie at the heart of the reticulated zone, intimately mixed with genotypes from other farms carrying haplogroups A or B among others.

3.3. Logistic network and poultry transfer practices

Of the 24 farms in the study, we obtained responses to the questionnaire for 17 layer farms (15 French farms, 1 Belgian farm and 1 English farm).

The French farms surveyed used at least 13 different external service providers for poultry transport, including 8 for pullet introduction (Table 5). At the beginning of a flock, three transport companies (TRA2, TRA3 and TRA4) were each shared by eight, two and four henhouses

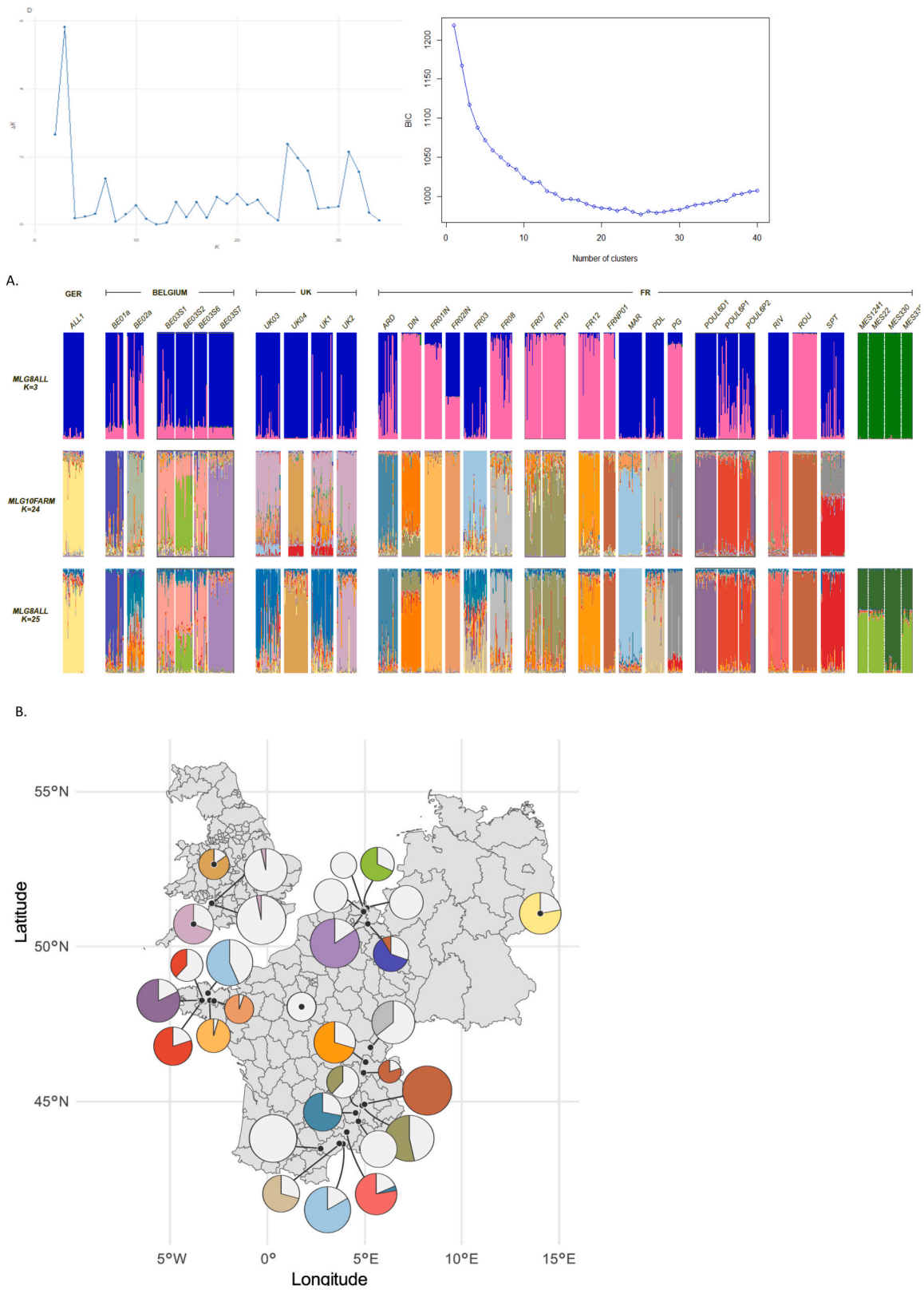


Fig. 3. Genetic structure of *D. gallinae* populations from MLG8ALL and MLG10FARM spatial data (different infestation units) inferred using STRUCTURE. Only the first sample was included in the analysis in the case of poultry houses sampled at two different times. **A.** Optimal number of clusters: *left*, evolution of Delta-K from STRUCTURE outputs (MLG8ALL; Evanno plot), *right*, Evolution of the BIC criterion (MG8ALL). A higher deltaK and a lower BIC value indicate a more likely number of clusters (or gene pools). **B.** Q plot from multi-locus microsatellite genotype STRUCTURE analysis for MLG8ALL with $K = 3$ and $K = 25$ and MLG10FARM with $K = 24$. A vertical line represents an individual, and the proportion of its assignment to each cluster is represented by the colored segments. **C.** Mapping of genotypes assigned with $q > 0.75$ to any of the 24 clusters with MLG10FARM. One pie-chart, distribution of individual genotypes per henhouse. The light grey parts of the pie charts represent hybrid genotypes.

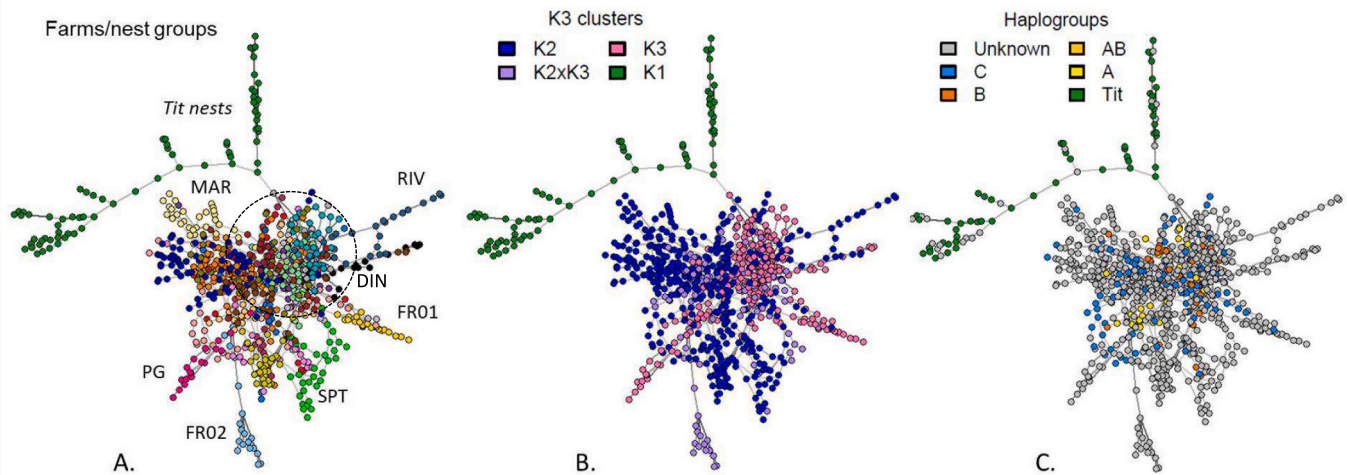


Fig. 4. MLG network (Minimum Spanning Network). Circles = microsatellite MLG genotypes colored according to (A) infestation groups (farm/tit group), (B) clusters inferred by STRUCTURE clusters with $K=3$ (microsatellites) and (C) their respective CO1 haplogroups. The color code is as follows: all graphs: dark green (tit nests; STRUCTURE = $q > 0.9$ for cluster 1); A. One color = one farm. Branches found in single farms, outside the strongly reticulated zone, were labelled with the farm ID. The light blue, light green, yellow and black peripheral branches group together genotypes from the FRO2, SPT, FRO1 and DIN populations respectively. The strongly cross-linked area within the dotted circle contains genotypes from populations BE01 and BE02, among others.; B. blue, $q > 0.75$ for nuclear cluster 2; pink, $q > 0.75$ for nuclear cluster 3; purple, $0.25 < q < 0.75$ for both cluster 2 and cluster 3; C. yellow, CO1 haplogroup A, orange, CO1 haplogroup B, light blue, CO1 haplogroup C, grey, unsequenced individual. Sequences obtained from other individuals in samples FRO1, FRO2, DIN, FRNP01, SPT, BE01 and BE02 (listed in A; Roy et al. 2021), all belong to haplogroup C for the first 5 (peripheral branches) whereas BE01 and BE02 (reticulated zone) contain haplogroup B mixed with haplogroup C (22B + 1C and 3B + 13C resp.). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 5

External service providers used by farmers during the layer flocks studied (from our survey).

ID	Transport company ID	Rearing farm ID	Slaughter-house ID	Dist. rear.	Dist. slaught.	Same company as usual?
FR08	REAR1 / SLA1	REAR1	SLA1	77	14	yes / no
FR12	TRA2 / ?	REAR2	SLA2	110	?	yes / no
FR10	TRA2 / TRA2	?	?	?	?	yes / yes
FR07	TRA2 / SLA12	?	?	?	?	yes / yes
DIN	TRA2 / TRA2	REAR3	?	298	?	no / no
PDL	Own vehicle/ NA*	REAR4 + REAR15	?	223 and 935	?	no / yes
ROU	TRA2 / SLA8	REAR5	?	31	?	no / no
SPT	TRA4 / TRA2	REAR6	?	974	?	yes / yes
FR01	TRA3 / TRA13 + SLA3	REAR7	SLA3	19	46	yes / yes
FR02	TRA3 / TRA14	?	SLA4	?	27	yes / yes
FR03	TRA7 / TRA13	REAR14	SLA3	66	59	yes / yes
PG	TRA2 / SLA5	REAR9 + REAR16	SLA5	361 and 295	ca. 400	no / no
POUL6D1bis	TRA4 / ?	POUL6 (own) + ?	SLA4	?	49	? / ?
POUL6P1	TRA4 / ?	NA	NA	NA	NA	no / no
POUL6P2	TRA4 / ?	NA	NA	NA	NA	no / no
BE03	TRA6 / ?	REAR10	SLA7	150	43	no / no
ARD	TRA2 / TRA2	REAR11	SLA8	215	109	no / no
FRNP01	TRA2 / SLA5	REAR12	SLA5	61	734	no / no
RIV	? / Own vehicle	?	SLA10	?	37	no / yes
UK03	TRA8 / SLA11	REAR13	SLA11	302	330	? / ?

The various companies have been coded in such a way as to make it possible to identify cases of inter-farm sharing of service providers while maintaining anonymity. Identifiers for external service providers beginning with TRA- are road transport companies, those beginning with REAR- are rearing farms and those beginning with SLA- are slaughterhouses. Note that in some cases, rearing farm or slaughterhouse vehicles are used during poultry transfers. Dist. rear., distance between the rearing farm and the layer farm under scrutiny (km); Dist. slaught., distance between the layer farm under scrutiny and the slaughterhouse (km).

n / n, start / end of flock.

?, unknown (no answer).

* Not relevant to slaughter as poultry sold live.

respectively (Table 5). The TRA2 company was used by 6 farms (1–2 henhouses per farm) from the S-E France region and one from the Centre-Val-de-Loire region, TRA3 by two farms from Brittany and TRA4 by one farm from the S-E France region and one from Brittany. For the removal of spent hens, three companies (one of the previous three, TRA2, and two others: TRA13 and SLA5) were shared by two farms, and all the others were different from each other and from the companies used for the introduction of pullets. The companies transporting the poultry were either or general transport companies ($N = 7$), or

specialised live poultry transport companies ($N = 1$), or the rearing farm supplying the pullets (1 out of 18 flocks with response in France), or the slaughterhouse receiving the spent hens (7 out of 13). In one case, one general transport company was hauling the trailer of the slaughterhouse. Three of the four atypical farms used their own vehicles several times. Of the 16 farms with response to these questions, 11 indicated that they generally use a variety of companies, so the current flock is just one example of many.

Concerning rearing farms (responses obtained for 13 French flocks, 1

UK and 1 Belgian flock), all farms used different companies to purchase pullets (Table 5). In our sample, the average distance from rearing farm to layer farm was 246.8 ± 270.1 km (between 18.9 and 974 km). Pullets often came from regions other than the layer farm and three layer flocks were made up of pullets from two different rearing farms.

The destination slaughterhouse for the spent hens was not always known, as some farmers sell their spent hens to the transport company, which in turn sells them to the slaughterhouse. Of the 13 flocks with responses, 10 different slaughterhouses were contacted (Table 5). The average farm-to-slaughterhouse distance for these 13 flocks was 247.84 ± 351.7 km (between 13.6 and 1124 km). Some of the slaughterhouses commonly used were located in countries other than the layer farm country.

With regard to loading and unloading practices between trucks and henhouses, 94% of respondents indicated that transport crates were brought into the building when pullets were introduced at the start of the flock (15/16 respondents) and 69% when spent hens were removed at the end of the flock (11/16 respondents). The time of day (day or night) when the hens were transported varied from farm to farm: at the start of the flock, 7 farms out of 16 reported that the transfer of hens took place either during the day or at night, 7 only during the day and 2 only at night. At the end of the flock, 9 farms out of 16 carried out transport at night, 4 only during the day and 3 either during the day or at night. Among the 44% and 19% of farms with variable practices, the choice of time of day was the result of compromises concerning animal welfare during transport (outside temperature vs. benefit of quietness for hens in the dark during handling, etc.). The change of clothing between barns during poultry transfers was not systematic (46% (6/13 respondents) at the start of a flock; 38% (5/13 respondents) at the end of a flock). Some farmers reported that it was not possible for them to check whether hygiene and safety rules were always respected by employees, and in particular by service teams. However, on all French farms where samples were taken in several different poultry houses (TES, UK1, BE03), farmers indicated that they systematically changed clothes when moving from one poultry house to another (Supplementary material S2B).

3.4. Practical field monitoring of mites from pullets

Of the 5 rearing farms investigated, we detected *D. gallinae* in only one (POUL6). Given the strength of our monitoring protocol, it's unlikely that the 4 negative farms harboured *D. gallinae* mites. As a reminder, our farm sample was small, but deliberately biased in favour of infested farms. Monitoring carried out with the same protocol on 4 rearing farms in Belgium did not reveal any *D. gallinae* infestation either. Interestingly, the only positive rearing henhouse (POUL6P1) was highly infested. As POUL6P1 was intended to supply hens to the POUL6D1 egg-laying house at the same time as POUL6P2 (on the same farm) along with a third external henhouse (not accessible for this study), we subsequently also diagnosed the second rearing henhouse POUL6P2 on the same farm. POUL6P2 also turned out to be highly infested.

During the night of 22/03/22, pullets from POUL6P1 and POUL6P2 were transferred to the laying henhouse on the same farm, POUL6D1. Mite sampled using paper towel were confirmed as *D. gallinae*. Before loading the pullets, a small number of towel samples contained a few mites (Fig. 5). Note: the farmer himself had made the decision not to have the trucks cleaned as he was transferring hens within his own farm. After unloading, the number of mites on the paper towel samples increased substantially (see Fig. 5).

According to microsatellite analyses of *D. gallinae* populations, the value of F_{ST} between the two rearing henhouses was insignificant (95% CI encompassing 0), whereas it was moderate to strongly significant between each of the two rearing henhouses and the layer flock composed of these two pullet flocks (F_{ST} POUL6P1 vs POUL6D1bis = 0.157 with a 95% CI [0.078,0.239], F_{ST} POUL6P2 vs POUL6D1bis = 0.087 with a 95% CI [0.026,0.161]). No significant differentiation was found between the populations sampled at the end of the previous flock in the

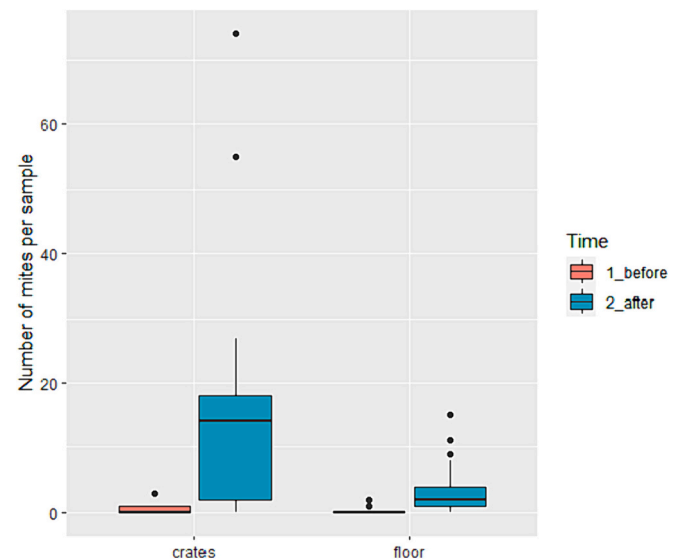


Fig. 5. Mite count during pullet transfer from pullet houses POUL6P1 and POUL6P2 to layer henhouse POUL6D1 (field experiment). Mites were collected by rubbing 10 towel pieces in the crates and 10 on the trailer floor of 2 trucks per pullet house, before pullet loading (1-before) and after unloading into the layer henhouse (2-after)(each box = mites from 20 towel pieces).

layer house (06/03/222) and the new flock composed of the two pullet flocks after 5 months of development (02/08/2022) (unsignificant F_{ST}). Structure analysis with $K = 3$ (MLG8ALL) assigned all individuals from the previous flock to cluster 2 whereas individuals from both pullet houses appeared as hybrids of clusters 2 and 3. With $K = 25$, these hybrids form a cluster specific to the two pullet henhouses and individuals from the previous layer flock were assigned to a cluster of their own, to which 6 individuals from the POULP2 pullet house were also assigned.

4. Discussion

Our study presents an original integrated approach to the spatio-temporal population dynamics of a non-model organism, by crossing different tools and scales. We have developed a set of microsatellite markers useful for studying the genetic epidemiology of *D. gallinae*, responsible for damage in a major agri-food sector. The marked isolation between farms and tit nests in the microsatellite analyses, consistent with DNA sequence analyses and previous studies, confirms the relevance of these markers. The measured genetic diversity and significant F_{ST} values between all farms and tit nests indicate that we have achieved good resolution with these markers to refine our understanding of the epidemiology of *D. gallinae*.

4.1. Gene flow at different spatial scales

The general organization of gene flow in space was very heterogeneous and did not allow us to identify any clear factor reducing gene flow among the factors studied: inter-farm geographical distance, administrative boundaries (country, region, farm), or singularity in use of service providers (i.e, transport companies, pullet suppliers, slaughterhouses). The lack of isolation by distance between farms is in line with previous results demonstrating that wild birds were not involved in the spread of *D. gallinae* among poultry farms (Øines and Brännström, 2011; Roy and Buronfosse, 2011). The marked genetic isolation of mites from tit nests located close to infested farms is further confirmation that the observed gene flow is human-associated.

For pairs of farms, using different service providers (different transporters, different rearing farms and different slaughterhouse) was not associated with an interruption of gene flow in our study, as

moderate to high gene flow was ubiquitous despite the large number of farm pairs that did not share any service provider. The layer farms were all supplied with pullets from different rearing farms, and most used different slaughterhouses. Only the transport companies allowed us to observe cases where service providers might be associated with gene flow: the significantly lower differentiation (higher gene flow) within the Auvergne-Rhône-Alpes region compared to other regions/countries may be explained by the 7 farms (8 henhouses) in the region that used the same transport company to bring in the pullets at the start of flocks. However, contrary to expectations, the three atypical farms that repeatedly used their own vehicles for poultry transportation (RIV, MAR, PDL) did not have highly differentiated mite populations (Fig. 1 and Table 4). All this means that we do not have a complete picture of the logistical network in which *D. gallinae* travels.

One important factor interrupting gene flow seems to be the limited autonomous dispersal of mites between poultry houses within farms. Genetic structure was evident between almost all poultry houses within farms and two intra-farm pairs even showed high F_{ST} values (in line with previous intra-farm observations based on DNA sequences; Roy and Buronfosse, 2011). The significant differentiation found between 4 isolated compartments in a single building on farm BE03 rules out autonomous mite movements as the main driver for inter-house spread, again in line with previous observations (Roy et al., 2009b; Wood, 1917). The within-farm transfer of mites (among poultry houses and between poultry houses and trucks) therefore obviously results from human activities.

The heterogeneous mixture of strongly and weakly differentiated populations across spatial scales is consistent with a predominantly human-mediated spread, as noted in other anthropophilic species such as cockroaches (Vargo et al., 2014) and bed-bugs (Fountain et al., 2014). The highest average gene flow occurred between poultry houses within farms (Fig. 2) and a structure by farm can be distinguished in the MSN (Fig. 4A): the probability of transfer by any medium entering and leaving poultry houses (personnel, equipment) is necessarily higher within a farm than between farms. Personnel come and go daily from one poultry house to another within a farm, while poultry transfers only take place at the beginning and end of a flock. However, the substantial heterogeneity of pairwise differentiation among poultry houses within farms indicates that mite dispersal associated with the coming and going of personnel and equipment is far from systematic.

4.2. No interruption of gene flow by the empty period

There was no detected differentiation between mite populations sampled before and after the empty period in two different types of poultry houses (aviary, cage) indicating that cleaning and disinfection do not have the expected effect of drastically reducing infestations by *D. gallinae*. We detected no reduction in genetic diversity, nor any differentiation whatsoever between mite populations occupying a poultry house at the end of a flock and populations in the same house during the first half of the new flock. Bearing in mind that a comparable result had previously been obtained on a barn farm (a third type of poultry house) using mitochondrial DNA sequences (Roy and Buronfosse, 2011), the ineffectiveness of the empty period cannot be readily explained by the type of structure. This result is consistent with the differentiation between poultry houses noted above: once the poultry house is contaminated, the *D. gallinae* population seems to settle in durably. That said, we have no information on how a *D. gallinae* inoculum behaves when it arrives in an uninfested poultry house. As sanitation practices vary throughout the world, our results cannot necessarily be extrapolated to all cases.

4.3. No detected effect of transfer practices on gene flow

Our pullet transfer field experiment showed that the introduction of crates and trucks carrying large numbers of mites does not necessarily

result in the effective contamination of a poultry house. Although this case represents an extreme situation, likely rare in real life, it still provides important information. In the course of this experiment, we measured massive infestations of the pullets when they were transferred to the laying henhouse, and established the effective transfer of large numbers of mites onto the transport crates which were introduced into the layer henhouse during peak mite activity (night-time). Surprisingly, we found no significant differentiation between the mites of this new layer flock and the previous one, indicating that the introduction of highly contaminated material into a poultry house may not constitute an effective contamination event. This result was surprising because the introduction of the mites took place at the very beginning of the flock, just after the intense cleaning actions that should have drastically reduced the previous mite population. Either the introduced mites were unable to develop (due to competition with resident mites or some unidentified constraint that prevented the mites from moving from the crates into the house), or the established population was so large that the inoculum was simply diluted. The second hypothesis could possibly explain why pairwise differentiation between the two pullet houses and the resulting layer flock POUL6D1bis turned out to be slightly lower (from strong to moderate/low) than between the same pullet houses and the previous layer flock POUL6D1 (resulting layer flock: $F_{ST} \text{ POUL6P1 vs POUL6D1bis} = 0.157$ with a 95% CI [0.078,0.239], $F_{ST} \text{ POUL6P2 vs POUL6D1bis} = 0.087$ [0.026,0.161]; previous layer flock: $F_{ST} \text{ POUL6P1 vs POUL6D1} = 0.301$ [0.272,0.329], $F_{ST} \text{ POUL6P2 vs POUL6D1} = 0.212$ [0.172,0.261]). This raises the question of the size of the remaining mite population following cleaning and what happens when a small inoculum of mites arrives in a henhouse devoid of mites.

Our survey did not identify any practices clearly associated with interrupted gene flow among those considered: equipment kept outside the henhouse during poultry transfers and diurnal transfers were not associated with reduced gene flow, in line with our experimental results. Finally, with regard to changing clothing, we did not receive sufficient responses to obtain a reasonable overview of this practice.

4.4. Mito-nuclear hybridization with advanced introgression of haplogroup C

Correspondence between microsatellite data and mitochondrial haplotypes allow us to reject the hypothesis of reproductive incompatibility between haplogroup C and the other two worldwide farm haplogroups (A and B, which are closely related to each other). The expansion of haplogroup C does not represent the replacement of one species by another. Our results are consistent with well-advanced reciprocal introgression following hybridization between two well-differentiated populations (incipient species) established on the basis of DNA sequences (Roy et al. 2021). Indeed, the strongly reticulated central part of the nuclear MSN shows a two-cluster structure despite intense sexual recombination; mitochondrial haplogroups were widely scattered in this part of the network and we did not detect any signs of excess heterozygosity in the studied populations. This clearly reflects a history of multiple, but not necessarily frequent, contaminations by populations isolated within farms and suggests that once established, these populations are large and highly stable (ie, not heavily affected by drift). This may contrast the situation in natural populations, such as in tit nests, where average numbers of alleles per marker tended to be much lower.

It is nonetheless surprising to still detect traces of segregation between haplogroup C and the microsatellite clusters, given the very large size of the populations per henhouse and the number of generations that have elapsed since this secondary contact over 12 years ago in France (Roy et al. 2021; >1 generation per week for 12 years, i.e. >576 generations). However, in a haplo-diploid species like *D. gallinae*, female hybrids are expected to appear quickly (first-generation crossing), whereas backcrossing is required to see hybrid males appear (Lohse and Ross, 2015). As mitochondrial material is transmitted maternally,

mitochondrial introgression is likely to be faster than nuclear introgression. Alongside other possible reasons, this may explain the persistence of first-level nuclear structuring ($K = 3$ in MLG8ALL) in the highly reticulated zone in the MSN while mitochondrial haplogroups were scattered. This hypothesis is also consistent with the recurrent observation on farms of heterozygous females for nuclear sequence alleles whose depth of divergence is equivalent to the divergence between sibling species in the genus *Dermanyssus* (>700 bp intron of the gene encoding tropomyosin; Roy and Buronfosse, 2011). Interestingly, in both the present study and our previous one (Roy and Buronfosse, 2011), despite high overall average numbers of alleles per marker, only one first-level nuclear cluster was detected in mites collected from wild avifauna (one colony of tits in the present study; one colony of Common Starlings and one colony of European rollers in Roy and Buronfosse, 2011), compared with several (two and three respectively) for mites on farms (including on single farms). The substantially higher nucleotide diversity within *D. gallinae* s.s. than in sister species in both mitochondrial and nuclear regions, as well as some discordances between mitochondrial and nuclear phylogenetic topologies of *Dermanyssus*, have already been interpreted as indicative of an evolutionary history punctuated by hybridizations in this anthropophilic species (Roy et al. 2021): on farms, hybridization between incipient species whose speciation began in different parts of the world could have resulted from their secondary contact through the logistic networks of the poultry industry. Further exploration of mito-nuclear introgressions in *D. gallinae* would advance our understanding of the evolution of haplodiploidy in general, as few studies outside Hymenoptera have been conducted on this subject (Blackmon et al., 2015). At the microevolutionary scale, it could also help to understand farm contamination processes, but would require a consequent sampling and analysis design to disentangle the effects of incomplete lineage sorting from introgression (Twyford and Ennos, 2012).

One explanation of the apparent replacement of mitochondrial haplogroup AB by mitochondrial haplogroup C could be the infection of the latter by *Wolbachia* which causes cytoplasmic incompatibility that blocks the development of hybrids (see Werren et al., 2008). However, this seems unlikely since the MSN contains haplogroup C branches associated with each of the nuclear clusters. The phenotypes induced by unidirectional cytoplasmic incompatibility due to *Wolbachia* may nonetheless be insufficient to maintain genetic divergence (Werren et al., 2008) and *Wolbachia* DNA sequences have been detected in some populations of *D. gallinae* (Hubert et al., 2017). The success of haplogroup C could also be due to its ability to mix sexual and asexual reproduction. While the genetic structure of *D. gallinae* populations developing on farms largely involves sexual reproduction, the organization of the MSN into tapering branches suggests some lineage-type evolution in populations. The close association of the peripheral branches with haplogroup C in comparison with the reticulated zone of the MSN with mainly haplogroup A-B individuals suggests that the recently-expanding haplogroup C could have brought competence for asexual reproduction (Fig. 4b, C). For example, the tapering branch of FR02 in the MSN could result from recent hybridization between the two first-level nuclear clusters, followed by recurrent events of asexual reproduction (see lineage-like shape grouping only cluster 2-cluster 3 nuclear hybrid MLG genotypes and only mitochondrial haplogroup C in Fig. 4B, C). The presence of two repeated MLG genotypes in tit nests could be a result of such partial clonality (although they could also be twins or simply share MLGs by chance). *Wolbachia*-induced parthenogenesis has already been recorded in an haplodiploid mite genus (Weeks and Breeuwer, 2001). However, since even rare occurrence of sexual recombination substantially attenuates deviations from the Hardy-Weinberg equilibrium of asexual reproduction, it is very difficult to assess the existence of partial clonality (Stoeckel et al., 2021). A specific study should be carried out to determine whether, and under what conditions, asexual reproduction can occur in *D. gallinae*. The presence of this mode of reproduction would have important consequences for

D. gallinae colonisation potential.

4.5. Understanding the dispersal dynamics of *D. gallinae*

In order to better reconcile our results, let us compare them with two other anthropophilic wingless haematophagous arthropods that have been studied using microsatellite markers: the Northern Fowl Mite *Ornithonyssus sylviarum* (McCulloch et al., 2020) and the bedbug *Cimex lectularius* (Saenz et al., 2012, Akhoundi et al., 2015; Fountain et al., 2014). The comparison with *Ornithonyssus sylviarum* may seem more relevant than with *C. lectularius* as it is closely related to *D. gallinae* and also feeds on poultry. However, unlike *D. gallinae* and *C. lectularius*, *O. sylviarum* remains on the host for a long period of time and does not survive host deprivation for more than 3 weeks (Kirkwood, 1963; Polanco et al., 2011). Although the two mite species are both haplo-diploid, *O. sylviarum* females are able to lay eggs without mating and produce offspring from the mother's mating with her son hatched from an unfertilized egg (McCulloch and Owen, 2012), whereas *D. gallinae* (and *C. lectularius*) females need to mate before laying eggs whether fertilised or unfertilised (Oliver Jr., 1971).

Studies of both *Ornithonyssus sylviarum* and *Cimex lectularius* show striking similarities with *D. gallinae*: an absence of isolation by distance between infestation units and significant population differentiation at all levels. Such a pattern is typical of animals with human-assisted transportation, as mentioned above (Vargo et al., 2014). An additional point in common between *O. sylviarum* and *D. gallinae* is the marked persistence of populations after the empty period in the poultry house. Despite these similarities, we note two major differences between *D. gallinae* and the other two species:

- (1) The average number of alleles per marker is significantly higher in *D. gallinae* (17.5–18.5 alleles per marker within a region) than in *O. sylviarum* and in *C. lectularius* (1–3 alleles per marker within a region). Furthermore, wild bird mites contain fewer alleles per locus in *D. gallinae*, whereas the opposite is true in *O. sylviarum* (McCulloch and Owen, 2012),
- (2) The recurrent occurrence of hybrids inferred by Structure between geographically distant farms in *D. gallinae* contrasts with hybrids being found only within single farms/hotels for the other two species (McCulloch et al. 2020, Akhoundi et al., 2015).

The frequent extinction-recolonization events demonstrated for *C. lectularius* in human dwellings or the recurrent bottlenecks suggested in *O. sylviarum* on farms and attributed to insecticide treatments (Fountain et al. 2015, McCulloch et al. 2020) seem much rarer in *D. gallinae*, which durably establishes large, genetically diverse populations in poultry houses despite cleaning and treatments. *O. sylviarum* presents an intermediate picture on farms, with high persistence but low allelic diversity: as this species stays for long periods on the host and has to feed quite often, the population may undergo significant bottlenecks as a result of control treatments and starvation (McCulloch et al. 2020). As the adult female *O. sylviarum* does not need to mate before founding a new population, population persistence may result from a few surviving female nymphs scattered around the henhouse (McCulloch et al. 2020). In contrast, as *D. gallinae* females must mate before laying eggs, persistence undoubtedly requires the maintenance of mite aggregates in henhouses to ensure a sufficient mating probability. Indeed, a very small number of mated *D. gallinae* females can be sufficient to produce heavy infestations within a few months (LR, pers. obs.). However, we have also observed considerable variation in population growth after 5 to 7 weeks in mesocosms from controlled inoculums of 25 to 200 live adult females (Zriki et al., 2021; Dupray et al., 2022). Overall, the genetic structure of *D. gallinae* in poultry farms is consistent with a metapopulation essentially made up of source patches (namely poultry houses), with almost no sinks, a situation particularly favourable to persistence (Arino et al., 2019).

At the henhouse (patch) level, effective contamination should result from the 4-step process of invasion described by [Williamson and Fitter \(1996\)](#), namely (1) introduction of contaminated material into the henhouse (importation step), (2) transfer of mites from the transport material to the equipment inside the henhouse (introduction step), (3) first reproduction cycles in the henhouse (establishment step) and (4) exponential growth (invasion step). Our results do not allow us to estimate the frequency of effective contamination, but farmers' testimonies indicate that new henhouses are generally free of *D. gallinae* for a few years before becoming definitively infested (LR, pers. comm.). Our field experiment shows that an importation step, even with a large inoculum, may not result in effective contamination in an already infested poultry house. The probability of effective contamination of an uninfested henhouse is likely much higher: an uninfested henhouse can receive inoculums from various sources, either at the start of a laying flock that contains pullets from different farms, or at the time of withdrawal of spent hens from slaughterhouse cages that pass through different farms (both cases are frequent, at least in France, [Roy and Buronfosse, 2011](#)). Furthermore, only two of the contacted farmers (not included in the study) were found to have successfully kept their farms free of *D. gallinae* for >10 years each by bypassing the integrated poultry logistical network: both prevented the invasion process before step 1, by producing their own pullets and using their own vehicles and cages to transfer spent hens or by refusing any batch of contaminated or doubtful pullet flocks before entrance into the house (LR, pers. comm.). We do not know how readily steps 2, 3 and 4 can be interrupted, but our previous experimental data in mesocosms ([Dupray et al., 2022](#); [Zriki et al., 2021](#)) suggest that the probability of interrupting the last two steps is very low.

At the metapopulation level, the considerable diversity of service providers and of distances covered by trucks in our study indicates that our sample is scattered throughout a large and complex network. The system likely has "small-world" properties sensu [Bertelsmeier and Keller \(2018\)](#), a condition particularly conducive to biological invasion (any node in the network can be reached from any other node in a few steps). In the study by [Floerl et al. \(2009\)](#), the most active nodes in the network were 75% more likely to be infected by an invader than the quietest nodes, regardless of the level of connectivity of the sources of the invasion. The multi-actor, sprawling organization of the logistic network upstream of egg production has been shown to create conditions particularly conducive to the global spread of microorganisms ([Li et al., 2021](#)). The history of hybridisation between well-differentiated populations due to secondary contacts in trucks may have reinforced the invasive potential of *D. gallinae* through heterosis ([Schierenbeck and Ellstrand, 2009](#); [Drake, 2006](#); [Lavergne and Molofsky, 2007](#)), not to mention that possible asexual reproduction events could further stimulate the invasion process.

5. Conclusions and perspectives

Populations of *D. gallinae* found on European layer farms circulate within a vast and complex logistical network, which extends far beyond the stakeholders contacted as part of this study. Human activity seems to break down geographical barriers even more in this species than in *O. sylviarum* and *C. lectularius*, two hematophagous species known for their potential for human-assisted propagation. Highly frequented hubs probably explain the numerous pairs of poultry houses with little or moderate *D. gallinae* differentiation, despite an apparently low probability of new contamination. Although it is difficult to include all these hubs in a single study, identifying them and implementing measures could help to improve prophylaxis.

The general pattern of infestations in European commercial layer farms seems to be based on a single effective contamination per henhouse that then persists over time. Secondary contacts likely to generate hybrid populations may occur when pullets are introduced or spent hens are removed from poultry houses. Investigating haplogroup C expansion and studying the effects of hybridization in highly differentiated

populations could help better understand the potential for *D. gallinae* on-farm establishment and invasion while enriching fundamental knowledge. Determining whether, and under which conditions, *D. gallinae* is able to asexually reproduce would also be an important issue in the management of this pest.

In the process of henhouse contamination, successful completion of the post-importation steps will depend in particular on whether the house is already infested or not. Preventing the importation step as soon as the henhouse is built might be an effective strategy for avoiding contamination, but requires the farmer to operate rigorously and independently of the major integrators (e.g., carrying out several activities in parallel to overcome periods without egg production following refusal of a batch of contaminated pullets). Crossing the empirical testimony of the most diverse farmers with models of logistical mite dissemination networks and stakeholder constraints, within a co-construction framework, would certainly contribute to progress in the prophylaxis of red mite infestations.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used DeepL Pro in order to construct their text in good quality English while maintaining good accuracy by producing the first draft in the native language of the main writers. More precisely, the first draft was produced in French by LB and LR, then translated into English using DeepL Pro and revised in full by LR before submission for proofreading to all the co-authors. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRedit authorship contribution statement

L. Boulanger: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **C. Planchon:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **A. Taudière:** Writing – review & editing, Visualization, Software, Methodology, Formal analysis, Data curation. **K.D. McCoy:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **S. T.G. Burgess:** Writing – review & editing, Methodology, Investigation. **A.J. Nisbet:** Writing – review & editing, Methodology, Investigation. **K. Bartley:** Writing – review & editing, Methodology. **P. Galliot:** Methodology, Investigation. **P. Creach:** Methodology, Investigation. **N. Sleenckx:** Project administration, Funding acquisition. **Lise Roy:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors have no conflict of interest to declare.

Data availability statement

Data are available on Zenodo repositories:

Microsatellite data (S1): <https://doi.org/10.5281/zenodo.10512249>

Survey data (S2): <https://doi.org/10.5281/zenodo.10512318>

CO1 Genbank acc. Numbers (S4): <https://doi.org/10.5281/zenodo.10495528>

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Appendix A. Supplementary materials

Supplementary materials S1, S2, S3 and S4 can be found online at <https://doi.org/10.1016/j.meegid.2024.105584>, with links to four Zenodo repositories.

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