Fera

Genetic analysis of Asian hornet samples from Ireland 2025

Determining nest relatedness and genetic origins for the 2025 Asian hornet incursion in Ireland to predict risk and inform future management actions

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1. Introduction

Fera Science were commissioned by the National Biodiversity Centre to deliver microsatellite genotyping of Asian hornet (*Vespa velutina nigrithorax*) samples from Ireland. These samples were from three hornet nests found in Cork, Cobh and Belfast in 2025, the first year that hornet nests had been found in Ireland.

The aims of the analysis were:

- **Aim 1:** To understand the origin of the Irish nests, namely whether they derive from the secondary invasive population present in Europe, or whether they are independent arrivals from elsewhere (likely Asia).
- **Aim 2:** To understand the relatedness of any recovered individual 'lone' hornets from Cork, Cobh or Belfast to the recovered nests, i.e. to understand whether all the lone hornets found locally derive from the nearby nest; if they do not, this is a strong indication that these individuals are from a different nest(s) that were not detected locally.
- **Aim 3:** To ensure that the genetic data is appropriately stored to freely inform future research on this species either in Ireland or internationally.
- **Aim 4:** To understand whether the nests were destroyed before they had moved into the reproductive phase, i.e. whether the queens of the nests had started laying haploid drones and at which life stage.
- **Aim 5:** To understand if any other genetic effects were present in the nest, such as: diploid (sterile) males; triploid individuals; the presence of worker laying. Diploid drones are the likely result of an error in the sex-determining mechanism, made more common by low population genetic diversity and inbreeding.

2. Materials and Methods

2.1. Samples

Sample hornets were taken from three nests in Ireland, at Cork (destroyed 4/09/2025), Cobh (destroyed 9/09/2025) and Belfast (destroyed 18/10/2025). Samples were taken across the different life stages (eggs, larvae, pupae, teneral adults and adults) and sexes present in the nest. 'Lone' hornets captured near the nests were also collected at Cork and Cobh.

Hornets from Cobh and Cork were received at Fera frozen in ethanol, while hornets from Belfast were received in ethanol at room temperature. Eggs were received frozen without ethanol. All samples were frozen at -25^{o}C upon receipt. The samples received are given in Table 1 below.

Table 1. Summary of hornet samples sent to Fera for analysis, giving the number in each category. Some samples were sexed based on morphology, shown in brackets after the number of samples.

	Nest samples					Lone adults
	Eggs	Larvae	Pupae	Teneral adults	Adults	
Cork	10	10	10 (F), 1 (M)	10 (F)	11 (F)	7
Cobh	10	14	13 (F), 1 (M)	9 (F), 1 (M)	2 (F)	7
Belfast	12	10	3 (F), 4 (M), 3	5 (F), 5 (M)	8 (F), 8 (M)	0

2.2. Molecular methods

A detailed description of the methods (DNA extraction, PCR cycling conditions, genotyping) can be found in Budge *et al* (2017) and Jones *et al* (2020). In brief, for each individual sampled, DNA was extracted from a hind leg (adult hornets) or from a portion of tissue (larvae or pupae) using a QIAGEN DNeasy Blood and Tissue Kit. The extracted DNA was amplified by PCR for 15 microsatellite loci in three multiplexed reactions. Fragment analysis was undertaken on a 3130 ABI Fragment Analyser via a commercial provider (DNASeq, Dundee). The raw data were scored into alleles using GeneiousPrime 2019.2.1 using preset bins and manually checked by eye, giving the multilocus genotype for each individual. These are provided as a separate excel file.

Controls and QC: DNA extraction batches included blank controls, which were carried through PCR and fragment analysis to monitor for cross contamination. Positive controls were included in the PCR and fragment analysis steps to control for methodological failure. Around 10% of samples were repeated to ensure the genotyping error rates were acceptably low. Two GB samples from previous studies were re-run to ensure there had been no 'drift' in the allele scores and ensure comparability with previous data.

2.3. Analysis

2.3.1. Origin of Irish individuals (Aim 1)

The alleles recovered in the genotyping data from the Irish hornets in 2025 were compared to the alleles recovered in Great Britain (Jones *et al* 2020), and France and South Asia (Arca *et al* 2015). The presence of fully overlapping alleles with the previous UK datasets and alleles from Arca *et al* (2015) from France were treated as evidence of a secondary incursion from continental Europe. The presence of multiple alleles not previously reported in Europe (or found exclusively in SE Asia samples from Arca *et al* 2015) would have been taken as evidence of suspect non-European Asian hornet origin.

2.3.2. Ploidy and identification of true drones (Aim 4)

Any individuals homozygous for all microsatellite loci were considered to be haploid (drones). Individuals with any heterozygous markers were considered to be diploid (or triploid, should that rare case arise). Any individual which was identified as a male based on morphology but was a diploid was identified as a diploid male.

Provided no evidence of worker laying was found (see below), the presence of haploid males was taken as evidence that the nest was entering its reproductive phase. The presence of haploid males at the teneral adult/ adult stage was considered high risk of reproductive individuals having been released into the environment.

2.3.3. Determining the nest parental genotypes: multi-mating and worker-laid individuals (Aim 5)

The genotypes of the offspring within each nest were used to manually infer the maternal and paternal genotypes of the queen and drone(s) that formed the nests, applying the rules of Mendelian genetics. This analysis gave the minimum number of fathers that contributed to the nest and provided the nest parental genotypes for other analyses.

Where haploid individuals were found in a nest, the presence of worker-laid individuals was assessed using the inferred queen and drone genotypes; if the haploid had alleles only found in the drone father and not the mother, it was taken as confirmation of worker-laying (haploids are produced by unfertilised eggs, and therefore have no father) .

2.3.4. Allocation of lone individuals to a nest and identification of unallocated hornets (Aim 2)

Using the inferred parental genotypes identified from the nests (i.e., the parent queen and drone genotypes), we identified whether lone hornets were likely to be offspring of the nearby destroyed nests. This was done manually using allele exclusion and inferences from Mendelian genetics (Jones and Ardren 2003).

3. Results

Data quality was acceptable with a low number of failed samples, no contamination in the blank controls, and no genotyping errors across the repeats. One marker (R4-100) genotyped erratically (the fragment analysis plots were unclear) in samples from the Belfast nest and was excluded. This did not affect the outcomes of the analysis,

3.1. Origin of Irish individuals (Aim 1)

The alleles found across the Irish individuals were all previously found in the UK and European population, consistent with them deriving from this invasive population. It is highly likely the nests derive from this population.

3.2. Ploidy, multi-mating, worker laid individuals and lone hornets (Aims 2, 4, 5)

3.2.1. Cork City

A summary of the samples genotyped is given in Table 2 below.

Table 2. Summary of hornets from the Cork nest, giving the number of samples that failed to genotype, number of haploid individuals and number of diploid individuals.

	Samples	Fails	Haploid	Diploid
Eggs	10	2	8	0
Larvae	10	0	3	7
Pupae (F)	10	0	0	10
Pupa (M?)	1	0	0	1
Teneral adults (F)	10	0	0	10
Adults in nest (F)	11	0	0	11
Lone adults (F)	7	0	0	7

Ploidy and worker laying

Haploids (drones) were found at the egg and larval stages only (Table 2, Figure 1). All the eggs and 1/3 of the larvae were haploids (Figure 1). Based on their genotypes, some or all of these haploids were laid by the workers rather than the queen as they had alleles found in the in inferred paternal genotype, absent from the maternal queen genotype. The remainder of the samples were diploid, including one pupa that was tentatively identified as a male based on morphology

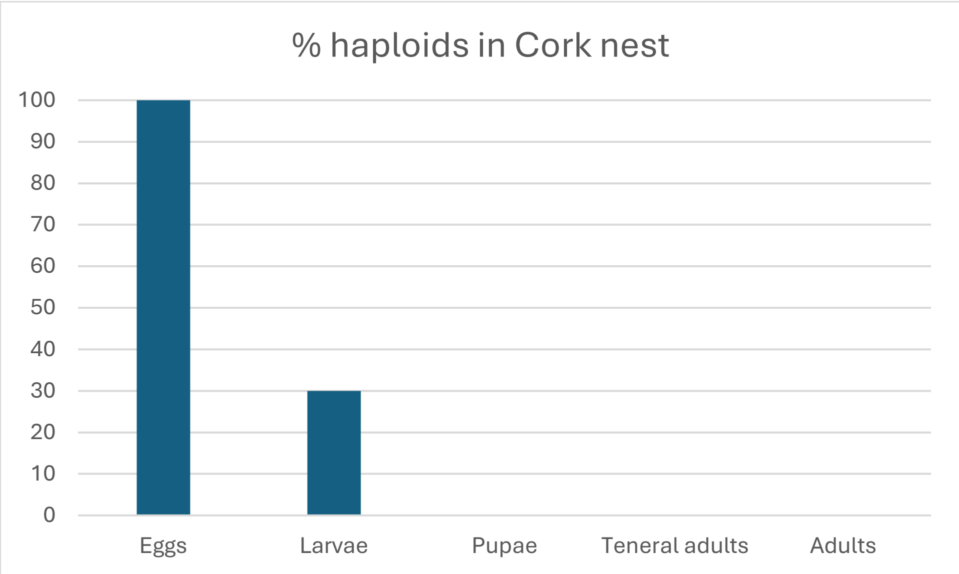

Figure 1. Proportion of the sampled hornets from the Cork nest that were haploid.

Multi-mating

All samples were consistent with being the offspring of one queen and one drone. The queen was not among the hornets genotyped.

Lone hornets

All lone hornets had genotypes consistent with coming from the Cork nest and were highly likely to come from this nest.

3.2.2. Cobh

A summary of the samples genotyped is given in Table 3 below. A relatively high percentage (50%) of the eggs failed to genotype, which is not uncommon given the difficulties sampling them and the low amounts of DNA they contain.

Table 3. Summary of hornets from the Cobh nest, giving the number of samples that failed to genotype, number of haploid individuals and number of diploid individuals.

	Samples	Failed	Haploid	Diploid
Eggs	10	5	5	0
Larvae	14	0	14	0
Pupae (F)	13	0	0	13
Pupae (M)	1	0	1	0
Teneral adults (F)	9	0	0	9
Teneral adults (M)	1	0	1	0
Adults in nest (F)	2	0	0	2

<u>Ploidy and worker laying</u>

Haploids (drones) were found at the egg, larval, pupal and teneral adult life stages (Table 3, Figure 2). All the successfully genotyped eggs and larvae were haploids, while a smaller proportion of the pupae and teneral adults were haploid (Figure 2; note that this sampling may have been biased as samples were selected according to morphological sex). Based on their genotypes, some or all of these haploids were laid by workers rather than the queen.

The remainder of the samples were diploid.

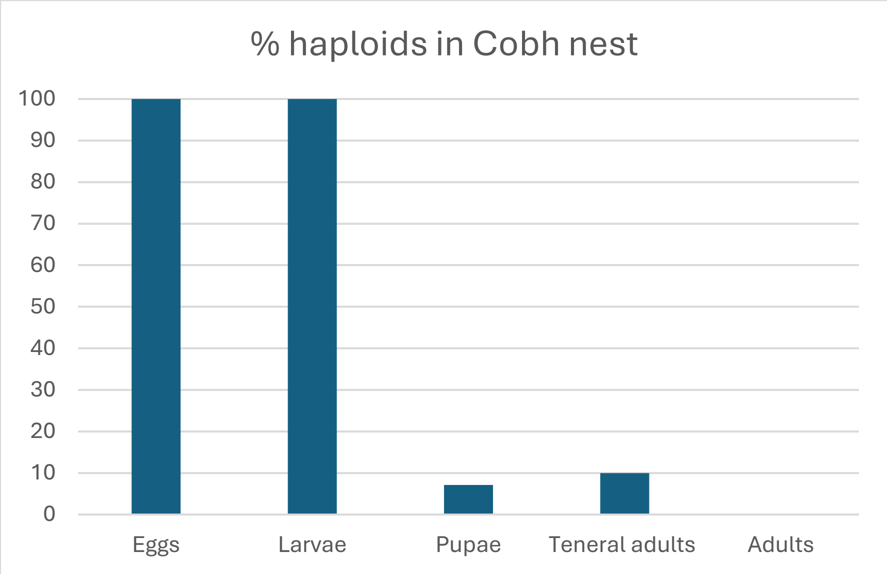

Figure 2. Proportion of the sampled hornets from the Cobh nest that were haploid.

<u>Multi-mating</u>

All samples were consistent with being the offspring of one queen and three or more drones. The queen was not among the hornets genotyped.

<u>Lone hornets</u>

All lone hornets had genotypes consistent with coming from the Cobh nest. It is highly unlikely they were from a different nest.

3.2.3. Belfast

A summary of the samples genotyped is given in Table 4 below.

Table 4 Summary of hornets from the Belfast nest, giving the number of samples that failed to genotype, number of haploid individuals and number of diploid individuals. Known diploid males are shown in bold type.

	Samples	Failed	Haploid	Diploid
Eggs	12	2	8	2
Larvae	10	0	9	1
Pupae (unsexed)	3	0	2	1
Pupae (F)	3	0	0	3
Pupae (M)	4	0	**4**	0
Teneral adult (F)	5	0	0	5
Teneral adult (M)	5	0	**2**	3
Adults in nest (F)	8	0	0	8
Adults in nest (M)	8	0	**3**	5

Ploidy and diploid drones

Haploids (drones) were found at all life stages (Table 4, Figure 3). A greater proportion of the eggs and larvae were haploids, while a smaller proportion of the pupae and teneral adults were haploid (Figure 3). Note that this sampling may have been biased as samples were selected according to morphological sex. There was no evidence of worker laying.

Around half of the samples identified as morphological males were diploid, and were therefore 'diploid drones' laid by the queen with the intention that they develop as workers rather than drones.

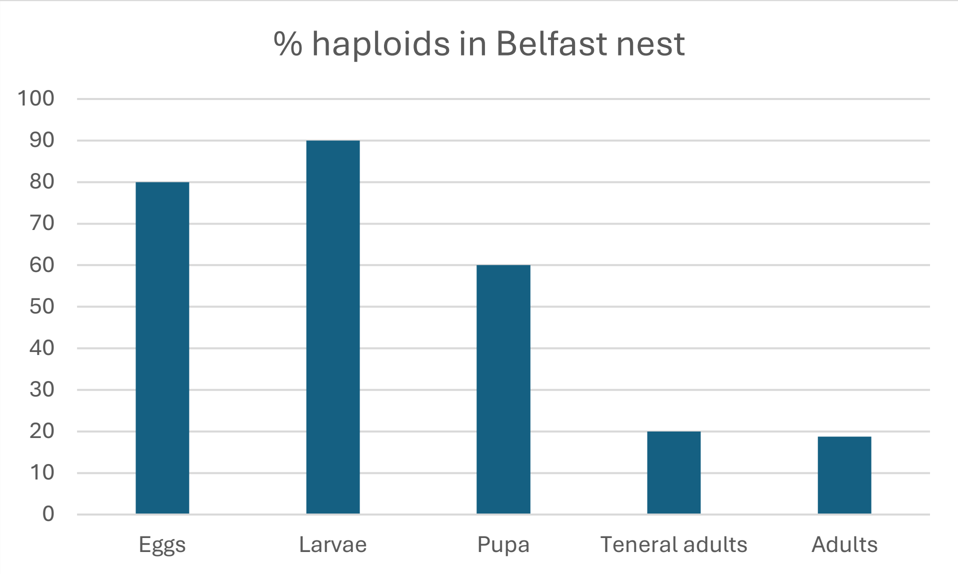

Figure 3. Proportion of the sampled hornets from the Belfast nest that were haploid.

<u>Multi-mating</u>

All samples were consistent with being the offspring of one queen and one drone. The queen was not among the hornets genotyped.

<u>Lone hornets</u>

There were no lone hornets.

4. Discussion

4.1. Origin of Irish individuals (Aim 1)

All hornets sampled were highly likely to derive from the European Asian hornet population.

4.2. Lone hornets and possible presence of further nests (Aim 2)

All lone hornets recovered (near the Cork and Cobh nests) were highly likely to derive from the nest they were caught near to. There was no evidence of additional nearby nests.

4.3. Potential for nest reproduction (Aims 4 and 5)

4.3.1. Cork

The 'reproductive status' of an Asian hornet nest can be assessed by whether the queen has changed from producing workers, typically in spring to autumn, to producing drones and then gynes in autumn, using the presence of haploids as a marker for drone production (Monceau, Bonnard and Thiery, 2014). Although the Cork nest had haploids (drones) at the egg and larval stages, some or all of these were laid by the workers. Worker laying likely occurs when the queen has died or has become unwell, and the workers begin to lay eggs. As they have not mated, the workers are only able to lay haploid eggs and cannot produce gynes; a nest with only worker-laying can therefore release the next generation of drones but cannot release the next generation of gynes. In the Cork nest, the queen had laid at least 70% of the larvae (which were diploid), but may no longer have been laying the eggs, which were all haploid. She may have died just before or during the time period that the larvae were being laid, and the workers subsequently began laying.

It is highly unlikely the nest had produced adult drones or gynes.

4.3.2. Cobh

Similar to the Cork nest, the Cobh nest had haploid individuals at multiple life stages but also strong evidence of worker laying. From the genetic data it is unfortunately not possible to be confident which haploids were queen-laid. Figure 2 shows that all the eggs and larvae were haploid, as were a small proportion of the pupae and teneral adults. The queen could have been laying alongside the workers when the teneral adults and pupae were being laid. The queen may have died by the time the eggs and larvae were being laid or could have started small levels of drone production in the time that the teneral adults and pupa were being laid. In either scenario, it is likely that the nest had produced some adult drones (either laid by the queen or workers), and may have produced some adult gynes.

If gynes have been produced, this is likely to be a low number. This, coupled to the relatively small size of the nest, the fact that worker laying was taking place, and the low rate of queen overwintering survival (extrapolated from other Vespidae: Archer 1984), means the expected risk of more than a small number of mated, overwintered queens can be considered very low.

4.3.3. Belfast

Unlike the other two nests, the Belfast nest had no evidence of worker laying. Morphological males were identified in the nest at the pupal, teneral adult and adult stages but around half of these were ‘diploid males’ that had been laid by the queen with the intention that they develop as workers (or gynes). It is theoretically likely these diploid males make the nest less productive. The nest had produced drones at all life stages, with a peak proportion of drones at the larval stage (Figure 3). This may represent the point at which the queen swapped from producing drones to producing gynes.

The nest had produced adult drones and was likely to have started producing gynes, a small proportion of which may have reached adulthood. This is consistent with the relatively late date of destruction of this nest (18/10/2025).

5. References

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6. Acknowledgements

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