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From Microsatellites to SNPs: Improving hybridization rate estimation in non-invasive monitoring

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Abstract

The transition from traditional microsatellites to single nucleotide polymorphisms (SNPs) markers is a major objective in modern DNA-based wildlife monitoring. However, empirical comparisons evaluating their relative performance on degraded, non-invasively collected DNA remain scarce. Using the European wildcat (*Felis silvestris*) as a biological model for hybridization with the domestic cat (*Felis catus*), we directly compared results obtained with a traditional 16-microsatellite panel to those obtained with a customized 96-SNP array on the exact same set of 133 wild individuals sampled via non-invasive hair traps. Our results reveal profound methodological divergences in resolution and assignment of hybrid category. While microsatellites indicated an homogeneous wild population, suggesting limited sensitivity for detecting advanced backcrosses, SNPs revealed greater genetic heterogeneity, assigning several individuals to first-generation hybrid or backcross classes. These misclassifications could explain why the overall hybridization rate appeared nearly three times higher with SNPs, a metric also affected by strict assignment thresholds. Furthermore, when considering individuals assigned as pure wildcats, microsatellite data indicated a higher inbreeding coefficient (Fis = 0.28) than SNPs data. These findings offer important insights for molecular ecology. While microsatellites remain cost-effective for assessing broad-scale population genetic structure, this study demonstrates how marker choice can influence hybridization diagnostics and estimates of genetic diversity, underscoring the need for careful calibration when assessing introgression in non-invasive samples.

1. Introduction

The accurate detection of hybridization, alongside population genetic structure and individual multilocus heterozygosity, is a fundamental component of wildlife management and conservation biology (Pérez-González et al., 2023; Puckett & Eggert, 2016; Sunde et al., 2020). These genetic studies reveal underlying biodiversity patterns and provide insights into evolutionary drivers—such as gene flow, genetic drift, selection, and hybridization—thereby supporting conservation decision-making, population delineation, and the identification of hybrids and dispersers (Puckett & Eggert, 2016; Sunde et al., 2020). Discovered in the 1980s, microsatellites quickly became the dominant tool for non-invasive wildlife monitoring due to their high allelic richness and cost-effectiveness for individual identification using a small number of loci (< 20–25) (Beaumariage et al., 2025; Hodel et al., 2017; Kelkar et al., 2010; Osborne et al., 2023; Pigneur et al., 2019).

However, relying on a small number of highly variable markers can poses limitations when assessing complex evolutionary processes such as hybridization, and necessitates a broader sampling effort to reliably compute allele frequencies and overall genetic diversity (e.g., Hale et al., 2012). High mutation rates can lead to homoplasy, null alleles, and complex mutation models that complicate data interpretation, while poor laboratory standardization hinders data sharing across research groups (Fischer et al., 2017; Funk et al., 2012; Haasl & Payseur, 2011; Nussberger et al., 2013; Putman & Carbone, 2014; Queirós et al., 2015; Selkoe & Toonen, 2006; Sunde et al., 2020; Valk et al., 2009).

Consequently, sequencing advances have driven a shift toward SNPs (Davey et al., 2011; Fischer et al., 2017; Kraus et al., 2015; Luikart et al., 2003; Seeb et al., 2011). Although biallelic SNPs exhibit lower individual diversity than microsatellites—requiring larger panels (> 50 SNPs vs. ~20 microsatellites) for equivalent statistical power (Malenfant et al., 2015; Morin et al., 2004; Santure et al., 2010; Zimmerman et al., 2020)—their genome-wide representation reduces interlocus variance (Kraus et al., 2015; Ruibal et al., 2010; Schlötterer, 2004; Smitz et al., 2016; Zimmerman et al., 2020). SNPs compensate for lower per-locus information through abundance, uniform genomic distribution, and low homoplasy, enabling broad evolutionary resolution (Camacho-Sanchez et al., 2020; Coates et al., 2009; Morin et al., 2004). While microsatellites may better track recent bottlenecks, SNPs secure robust broader-scale inferences and universal laboratory standardization (Camacho-Sanchez et al., 2020; DeFaveri et al., 2013; Haasl & Payseur, 2011; Morin et al., 2004).

Beyond structure, SNPs are powerful for detecting hybridization and domestic introgression, a critical conservation challenge complicating species delimitation (Barraclough & Humphreys, 2015; Chan et al., 2019; Harrison & Larson, 2014; Hirashiki et al., 2021; McFarlane & Pemberton, 2019; Miralles et al., 2024; Moran et al., 2021). While microsatellites were historically primary for hybrid recognition, high allelic richness and homoplasy can reduce diagnostic power and increase uninformative allele sharing (Allendorf et al., 2001; Nittinger et al., 2007; Nussberger et al., 2013; Randi et al., 2001; Väli et al., 2010; Verardi et al., 2006). Conversely, biallelic SNPs foster diagnostic allele frequencies. Furthermore, SNP assays target short sequences (< 100 bp) independently of fragment length, facilitating multiplexing with fragmented DNA from faeces or hair (Carroll et al., 2018; Morin & Mccarthy, 2007; Russello et al., 2015; Shafer et al., 2015; von Thaden et al., 2020).

Despite the diversity of genomic methods, robust empirical comparisons between microsatellites and SNPs remain scarce in the literature (Liu et al., 2005; McMahon et al., 2014; Shafer et al., 2015; Zimmerman et al., 2020). Existing comparative studies, primarily in plants, fish, and a few mammals, often rely on simulated genotypes or use disparate analytical frameworks (Camacho-Sanchez et al., 2020; Elbers et al., 2017; McFarlane & Pemberton, 2019; Puckett & Eggert, 2016; Smitz et al., 2018; Stacy et al., 2025; Sunde et al., 2020; Szatmári et al., 2021; Tokarska et al., 2009). Most notably, empirical comparative data specifically addressing hybridization analyses remain rare (Camacho-Sanchez et al., 2020; Elbers et al., 2017; Schlötterer, 2004). While comparisons using real-world tissue data across various taxa demonstrate that SNPs generally outperform microsatellites in resolving hybrid inconsistencies, direct parallel methodological comparisons on degraded, non-invasively sampled individuals remain an important unfulfilled requirement for molecular ecology (Miralles et al., 2024; Parejo et al., 2018; Szatmári et al., 2021; Väli et al., 2010).

To bridge this fundamental gap, our study presents a direct methodological comparison of microsatellite and a SNP panel dedicated to study hybridization processes. In this context, we used the European wildcat (*Felis silvestris silvestris*), a highly vagile species threatened by anthropogenic hybridization with the domestic cat (*Felis silvestris catus*), as a biological model to empirically test this technological transition. As a mobile apex carnivore capable of extensive long-distance dispersal, the European wildcat

serves as an ideal biological model for evaluating hybridization across contiguous landscapes. Detecting this introgression is notoriously difficult: morphological distinction fails beyond the first hybrid generation (F1) (Chan et al., 2006; Daniels et al., 1998; Gaubert et al., 2005), and wildcat monitoring relies heavily on non-invasive samples (e.g., lure stick hair traps) that yield degraded nuclear DNA (Barbosa et al., 2013; Bengtsson et al., 2012; Goossens et al., 1998; Henry et al., 2011; Heurich et al., 2012; Nussberger et al., 2014; Steyer et al., 2013). Because first-generation backcrosses share approximately 75% of their genome with the recurrent parental species, the highly polymorphic microsatellites historically used often fail to reliably detect advanced introgression originating several generations ago (Hertwig et al., 2009; Nussberger et al., 2013, 2014; Say et al., 2012; Vähä & Primmer, 2006). To address this, customized SNP chips and optimized panels have recently been developed to accurately assign F1, F2, and advanced backcrosses from single non-invasive hairs (Martin et al., 2025; Nussberger et al., 2013, 2014; von Thaden et al., 2020).

Our study presents a direct methodological comparison of a traditional microsatellite panel and a customized SNP array dedicated to studying hybridization processes in cats, using the exact same set of wild individuals. More specifically, we aim to: (1) Genetically identify European wildcats, domestic cats, and their admixed progeny, and assess genetic differentiation using a natural non-invasive dataset; (2) Compare individual clustering outcomes; and (3) Evaluate the concordance between the two marker types across common metrics of genetic diversity. Ultimately, this parallel analysis tests the comparative utility of these markers for the genetic assessment of vagile species, providing critical insights into the conservation implications of transitioning from traditional microsatellites to targeted genomic methodologies.

2. Materials and methods

2.1. Study area and non-invasive sampling strategy

To ensure representative nationwide coverage across Luxembourg and minimize spatial autocorrelation, a stratified random sampling approach was applied to select 72 forested squares (≥ 10 ha forest each) to cover the country's main forest types (Titeux et al., 2009). Each 1km^2 square contained three sites equipped with an olfactory hair trap (rugged wooden stick treated with 10 ml of valerian extract, *Tinctura Valerianae* 70% 1:5 API; Caelo) along game trails, and an associated camera trap positioned 1–4 m away to record mammal activity (Beutel et al., 2017; Jerosch et al., 2017; Nussberger et al., 2014; Steyer et al., 2018). The data used for this comparative study were sampled from January to May in 2017 and 2018 across 44 distinct squares (20 squares in 2017 and 24 squares in 2018). Sticks and lures were replaced every two weeks, and up to three hair samples per site visit were preserved in alcohol and graded qualitatively (1–3 for hair quantity; 4 for the presence of a piece of skin attached with the hairs).

2.2. DNA extraction and individual identification

Genomic DNA was extracted from 169 hair samples using the QIAamp DNA Mini Kit (QIAGEN, 2023), with negative controls included at all steps. The dataset was supplemented with two reference hair samples of

known pedigree, including morphologically certified pure wildcat and known domestic cat. The final dataset was filtered to retain 141 high-quality samples representing 133 distinct individuals, defined by the successful amplification of at least 15 microsatellite alleles and < 30% missing SNP data. Specifically, this screening procedure systematically excluded samples exhibiting multi-individual contamination—identified via excess alleles at microsatellite loci or unstable, outlier assignment patterns in genomic frameworks—ensuring that only single-source profiles common to both marker systems were retained for comparative evaluation. To prevent pseudo-replication biases in downstream diversity and clustering analyses, a single consensus genotype per individual was retained. Individual identification was performed via SNP-based matching using a custom Python script with a mismatch threshold of six loci; samples matching at > 88 of the 96 SNPs were considered identical (von Thaden et al., 2020), allowing direct comparative evaluation across markers on the exact same biological baseline.

2.3. Microsatellite genotyping

Samples were genotyped at 16 microsatellite markers (including one sex identifier) (Hertwig et al., 2009; Menotti-Raymond et al., 1999) using the Qiagen Multiplex PCR kit in a 10µL volume across four multiplex mixes. Fluorescently labelled primers (VIC, FAM, PET, NED; Applied Biosystems) were analyzed on an Applied Biosystems 3730xl Genetic Analyser, and alleles were scored using GENEMAPPER 4.0. To mitigate allelic dropout and null allele errors from low-quality DNA, a multi-tube approach was strictly applied with three independent replicates: homozygotes required identical scoring across all three replicates, and heterozygotes required identical combinations in at least two.

2.4. SNP genotyping

Samples were concurrently genotyped using a specialized 96-SNP panel optimized for degraded DNA (Nussberger et al., 2014), including positive controls in each batch. For downstream analyses, the dataset was split: hybridization assessments utilized a diagnostic subset of 52 Fst-outlier SNPs (excluding individual ID and sex-linked markers), while population structure and diversity analyses utilized the full 96-SNP panel.

2.5. Hybridization and admixture analysis

Individual multi-locus genotypes were assigned to hybrid classes (pure parentals, F1, F2, and backcrosses) using NewHybrids V1.1 (Anderson & Thompson, 2002) with predefined reference parental genotypes, running an MCMC algorithm for 100,000 burn-in iterations and 200,000 sweeps (von Thaden et al., 2020). Assignment required a strict posterior threshold of q > 0.90. The population-level hybridization rate was calculated as the number of identified hybrids divided by all assigned individuals. These classifications were validated via Principal Component Analysis (PCA) to visualize multivariate genetic clustering in R 4.2.1 using tidyverse and adegenet (Jombart, 2008; R Core Team, 2013; Wickham et al., 2019). Pairwise genetic differentiation (G’st)—which measures genetic differentiation between subpopulations by reflecting the reduction in heterozygosity due to genetic drift or restricted gene flow—between hybrid classes was quantified using diveRsity (Keenan et al., 2013) and visualized through Multidimensional Scaling (MDS) using tidyverse.

2.6. Population structure and genetic diversity

Analyses were restricted to pure wildcats (q > 0.90 based on SNP data; N = 111) using the full 94 autosomal SNPs and 15 microsatellites. Genetic diversity metrics (Ar, Ho, He, Fis) and the standardized fixation index (G'st) were computed with 1,000 bootstrap replicates in diveRsity (Keenan et al., 2013). Spatial structure was inferred using STRUCTURE 2.3.4 (Pritchard et al., 2000) under an admixture model with correlated allele frequencies and no spatial a priori. Independent runs were evaluated for K = 1 to K = 10 (10 replicates per K; 100,000 burn-in; 2,000,000 MCMC sweeps). The optimal K was determined using the Evanno et al. (2005), Puechmaille (2016), and Pritchard et al. (2000) methods, and individual admixture proportions were summarized using CLUMPAK (Kopelman et al., 2015).

3. Results

3.1. Hybridization and admixture analysis

3.1.1. Probabilistic assignment and hybrid rates

A total of 133 unique individuals were analysed using the Bayesian clustering algorithm implemented in NewHybrids (Fig. 1). Based on the microsatellite dataset, 123 individuals were assigned as pure European wildcats (*Felis silvestris silvestris*), four as domestic cats (*Felis silvestris catus*), and three as second-generation hybrids (F2). Three samples failed to reach the strict 90% posterior probability threshold for any category and were classified as "undetermined". In contrast, the SNP panel identified 111 pure wildcats, five domestic cats, one first-generation hybrid (F1), six wildcat backcrosses (Backcrosses F. *silvestris*), and one domestic backcross (Backcross *F. catus*), leaving nine samples undetermined.

To evaluate introgression at the population level, comparative hybrid rates were calculated. The microsatellite dataset yielded a hybrid rate of 2.31% (3 hybrids out of 130 assigned individuals), whereas the SNP panel detected a higher hybridization rate of 6.45% (8 hybrids out of 124 assigned individuals).

Individual assignment discrepancies between the two marker types were notable. Nine individuals classified as pure wildcats by microsatellites were deemed undetermined by SNPs. Conversely, two individuals classified as undetermined by microsatellites were confidently assigned as pure wildcats by SNPs. Furthermore, SNPs revealed introgression missed by the traditional markers: six microsatellite-assigned wildcats were reclassified as wildcat backcrosses by SNPs, one microsatellite-assigned domestic cat was reclassified as a domestic backcross, and one undetermined individual was identified as an F1 hybrid. Lastly, the three F2 hybrids identified by microsatellites were re-assigned by the SNP panel as one pure wildcat and two domestic cats.

3.1.2. Geometric distribution vs. probabilistic assignment

To confront the probabilistic assignments obtained via NewHybrids with the geometric distribution of the genetic variance, a Principal Component Analysis (PCA) was conducted for each marker type.

The microsatellite PCA (Fig. 2A) exhibited a generally weak geometric signal. The primary axis of variation (Axis 1) explained only 6.1% of the total variance, indicating that the separation between wild and domestic lineages is not the overwhelmingly dominant signal in this dataset. Wildcats formed a relatively compact cluster compared with domestic cats, which displayed greater dispersion in ordination space. It should be noted that the PCA geometric overlap between F2 hybrids and wildcats highlights the limited diagnostic resolution of microsatellite markers for advanced hybrid generations, compared to the probabilistic assignment provided by NewHybrids. This pattern is consistent with the reduced power of microsatellite datasets to distinguish advanced backcrosses and later-generation hybrids, a limitation that has motivated the increasing use of SNP panels in wildcat hybridization studies.

In contrast, the SNP PCA (Fig. 2B) delivered a clear signal. Axis 1 captured an exceptionally high 31.2% of the variance, confirming that species divergence is the dominant factor driving the genetic structure. The PCA revealed a distinct gap between the compact wildcat cluster (left, in green) and the domestic cat cluster (right, in orange). The geometric positioning of the hybrids validated the NewHybrids assignments: the F1 hybrid was located precisely in the intermediate space between the parental clusters (reflecting a ~ 50% genomic contribution from both lineages). Similarly, wildcat backcrosses clustered tightly near the pure wildcats but were slightly pulled toward the domestic continuum, while the domestic backcross was positioned closer to the *catus* group.

3.1.3. Genetic differentiation between hybrid classes

To further quantify the divergence among the assigned categories (q > 0.90), pairwise genetic differentiation (G'st) was calculated and visually projected using Multidimensional Scaling (MDS).

For the microsatellite dataset (Table 1; Fig. 3), pairwise comparisons yielded high and logically structured G'st values. Differentiation was strongest between pure wildcats and pure domestic cats (Mean G'st = 0.511; 95% CI: 0.431–0.614). Comparisons involving F2 hybrids showed comparatively lower differentiation against both wildcats (Mean G'st = 0.336; 95% CI: 0.208–0.490) and domestic cats (Mean G'st = 0.334; 95% CI: 0.137–0.570), although the confidence intervals overlapped. The associated MDS (Fig. 3) visually captured this dynamic across Dimension 1, plotting a clear biological gradation from domestic cats to hybrids, and finally to pure wildcats.

Table 1
Pairwise genetic differentiation (G'st) between assignment categories based on microsatellite data.

Comparison	Mean	Lower 95% confidence interval	Upper 95% confidence interval
F. silvestris vs F. catus	0.511	0.4311	0.6141
F. silvestris vs. F2	0.3363	0.2085	0.4901
F. catus vs. F2	0.3346	0.137	0.5708

For the SNP dataset (Table 2; Fig. 4), the G'st values demonstrated a similar biological logic but with higher differentiation metrics, particularly between the pure parental lineages. The differentiation between

wildcats and domestic cats reached a mean G'st of 0.766 (95% CI: 0.737–0.812). This substantial increase compared to the microsatellite dataset confirms that the targeted SNP panel successfully maximizes the detection of genetic divergence between the two pure strains. Expectedly, differentiation was reduced between related categories (e.g., *F. silvestris* vs. F1: 0.079; *F. silvestris* vs. Backross *F. silvestris*: 0.074). The negative pairwise G'st values observed in certain comparisons involving admixed individuals (e.g., Backcross *F. catus* vs. F1) likely reflect mathematical artifacts stemming from the extremely limited sample size within these specific, rare hybrid categories (N = 1 for both the F1 and Backcross *F. catus* groups). Consequently, to ensure accurate interpretation, these artefactual negative estimates were masked as non-applicable (NA) in Table 2. Overall, the SNP-based MDS (Fig. 4) and the elevated G'st values confirm that SNPs possess a superior statistical power to highlight and quantify the genetic boundaries between pure and introgressed categories.

Table 2
Pairwise genetic differentiation (G'st) between assignment categories based on SNP data. *Note: Mathematical artifact due to small sample size (N = 1) in rare hybrid categories.

Comparison	Mean	Lower 95% confidence interval	Upper 95% confidence interval
F. silvestris vs. F. catus	0.7669	0.7375	0.8125
F. silvestris vs. Backcross F. catus	0.5677	0.5493	0.5848
F. silvestris vs. F1	0.0797	0.06	0.0983
F. silvestris vs. Backcross F. silvestris	0.0748	0.0274	0.1594
F. catus vs. Backcross F. catus	NA*	NA*	NA*
F. catus vs. F1	NA*	NA*	NA*
F. catus vs. Backcross F. silvestris	0.6138	0.5545	0.688
Backcross F. catus vs. F1	NA*	NA*	NA*
Backcross F. catus vs. Backcross F. silvestris	0.3547	0.2923	0.4416
F1 vs. Backcross F. silvestris	NA*	NA*	NA*

3.2. Population structure and genetic diversity of pure wildcats

3.2.1. Dataset filtering

To accurately compare traditional genetic diversity metrics and population clustering between the two marker systems, downstream analyses were restricted to pure European wildcats. Because the SNP panel possesses superior diagnostic power for detecting introgression (as demonstrated in Section 3.1), we relied on the SNP-based NewHybrids assignments ($q > 0.90$) to define the baseline population. This

filtering yielded a robust subset of 111 pure wildcat individuals, which were subsequently analysed using both the 15 microsatellites and the full 94-SNP panel (excluding sex markers).

3.2.2. Genetic clustering and population structure

Bayesian clustering was performed to evaluate the capacity of both marker types to detect fine-scale spatial structuration within the study area.

Using the microsatellite dataset, the optimal number of clusters (K) varied depending on the evaluation metric: Puechmaille's method suggested K = 2, while Evanno's Delta K and Pritchard's methods indicated K = 5 (ln P(D) = 93.97). However, visual inspection of the admixture bar plots (Fig. 5) revealed that the vast majority of individuals exhibited admixed profiles ($q < 0.90$), failing to assign to any distinct genetic subgroup. Consequently, no clear or biologically meaningful spatial substructure could be identified using microsatellites.

Conversely, the SNP dataset consistently identified K = 3 as the optimal clustering solution across all three evaluation methods (ln P(D) = 397.29). Interestingly, at K = 2, the SNPs produced a spatial division similar to the microsatellites but with notably higher assignment probabilities for the respective clusters (Fig. 6).

3.2.3. Genetic diversity metrics

To accurately estimate baseline genetic diversity and mitigate the confounding effects of introgression, indices were calculated exclusively for the subset of pure European wildcats (N = 111) using the diveRsity package in R (Keenan et al., 2013). The results revealed striking differences in the diversity landscapes captured by the two marker systems.

For the microsatellite dataset (Table 3), the population exhibited a moderate level of polymorphism, yielding a total of 108 distinct alleles across the 15 amplified loci and an average allelic richness (Ar) of 6.67 alleles per locus. However, a pronounced heterozygote deficit was observed: the observed heterozygosity (Ho = 0.50) was substantially lower than the expected heterozygosity (He = 0.69). Consequently, the inbreeding coefficient was highly positive and significant (Fis = 0.2784; 95% CI: 0.2290–0.3253), indicating a strong deviation from Hardy-Weinberg equilibrium.

Table 3
Genetic diversity indices for pure European wildcats. N=number of individuals; A=total number of alleles; %=percentage of total alleles observed; Ar=allelic richness; Ho=observed heterozygosity; He=expected heterozygosity; HWE = fit to Hardy-Weinberg equilibrium (p-value); Fis=inbreeding coefficient; Fis_Low=lower bound of the 95% confidence interval for Fis; Fis_High=upper bound of the 95% confidence interval for Fis.

Panel	N	A	%	Ar	Ho	He	HWE	Fis	Fis_Low	Fis_High
Microsatellites	111	108	100	6.67	0.5	0.69	0	0.2784	0.229	0.3253
SNPs	111	188	100	2	0.21	0.22	0	0.0242	-0.0201	0.0682

In contrast, the diversity metrics derived from the SNP dataset (Table 3) depicted a fundamentally different genetic diversity. Because the 94 SNPs are strictly biallelic markers, the dataset comprised a total of 188 distinct alleles, and the allelic richness (Ar) was logically fixed at 2.0. Crucially, the observed heterozygosity (Ho = 0.21) was nearly identical to the expected heterozygosity (He = 0.22). As a direct result, the inbreeding coefficient was low and statistically non-significant, with its confidence interval encompassing zero (Fis = 0.0242; 95% CI: -0.0201 to 0.0682).

4. Discussion

The parallel analysis of European wildcat (*Felis silvestris*) populations using 16 microsatellites and a 96-SNP array revealed key empirical outcomes with direct ecological implications. First, SNPs identified advanced backcrosses that were classified as pure wildcats by microsatellites and clearly separated them in multivariate space. Ecologically, these results indicate that microsatellites markers may underestimate the extent of domestic cat introgression, potentially leading to an overestimation of the genetic integrity of wildcat populations and an underdetection of hybridization processes. Second, diversity metrics yielded a biological contradiction: microsatellites suggested a more inbred population, whereas SNPs indicated near-zero inbreeding. Because genomic SNPs are more robust to the DNA degradation artifacts typical of non-invasive sampling, they could provide more accurate ecological diagnosis. These outcomes show that capturing different properties of genetic variation can fundamentally influence conservation diagnoses derived from identical non-invasive samples, (Pérez-González et al., 2023; Szatmári et al., 2021; Zimmerman et al., 2020). Nevertheless, we must acknowledge the limitation of our 2-year study using only 44 different sampling points to draw definitive conclusions on the population genetic of the wildcat in Luxembourg. Although our interpretation is supported by the available data, caution is still required regarding the broader and definitive implications of these findings for the genetic structure of the wildcat population. Further studies based on larger datasets and longer time series will be necessary to confirm these initial results.

4.1. Detection of hybridization

Accurately detecting advanced introgression requires diagnostic loci because backcrosses share extensive genomic portions with parental species, challenging non-diagnostic markers like microsatellites (Vähä & Primmer, 2006). Our findings support the consensus that standard microsatellites lack the resolution to identify introgressed individuals beyond F1 generations (Austin et al., 2011; Hertwig et al., 2009; Kingston & Gwilliam, 2007; Nussberger et al., 2013; Oliveira et al., 2008a; Say et al., 2012). Specifically, seven backcrosses identified by SNPs were misclassified as pure wildcats by microsatellites, aligning with simulations where traditional markers misattribute up to 20% of backcrosses (Oliveira et al., 2008b). This enhanced sensitivity stems from our SNP array's design, which targets diagnostic Fst-outlier loci maximizing divergence between wildcats and domestic cats. Consequently, biallelic SNPs—characterized by low homoplasy and differentiated allele frequencies—secure superior genome-wide ancestry estimation and backcross detection (Miralles et al., 2024; Nussberger et al., 2013; Parejo et al., 2018; von Thaden et al., 2020).

The misclassifications could explain why the overall hybridization rate appeared nearly three times higher with SNPs, a metric also affected by strict assignment thresholds. Indeed, these discrepancies operated bidirectionally, as three individuals classified as hybrids by microsatellites were left "undetermined" by the SNP panel due to missing data. This reflects differing detection thresholds rather than a unilateral failure, a phenomenon recorded across various taxa (Miralles et al., 2024; Parejo et al., 2018; Väli et al., 2010). Consequently, variations in historical hybridization metrics should be interpreted with caution, as they might partially reflect the differing sensitivities of the methodological frameworks utilized rather than solely biological shifts (Steyer et al., 2018). Harmonizing historical microsatellite data and contemporary SNP series involves establishing a shared dual-genotyping reference baseline, modelling score regressions, adjusting assignment thresholds, correcting historical biases, unifying the longitudinal dataset and validating the resulting demographic trends against ecological field realities. Ultimately, a "naive" set of microsatellites captures less diagnostic variance than a targeted SNP array optimized for introgression (Nussberger et al., 2013; Väli et al., 2010), where lower per-locus information is compensated for by deploying a greater volume of highly adapted markers (Smitz et al., 2018; von Thaden et al., 2020).

In multivariate space, the SNP-based PCA resolved clear population boundaries, positioning the F1 intermediately and backcrosses along the periphery of their recurrent parental clusters, matching patterns in mustelids (Szatmári et al., 2021). Conversely, the microsatellite PCA exhibited substantial overlap with the pure wildcat cluster. This indicates that introgression was not the primary variance captured, aligning with other studies where microsatellites similarly lacked the spatial resolution to separate distinct genetic groups (Karlsson et al., 2007; Puckett & Eggert, 2016; Rašić et al., 2014). The high allelic diversity of microsatellites likely obscure weaker admixture signals. This can influence conservation strategies by suggesting managers to falsely diagnose an admixed population as ecologically purer. Despite small sample sizes for rare categories, the precise geometric positioning in the SNP space validates the diagnostic sensitivity required for effective conservation management.

Furthermore, when managing multi-individual sample contamination at a single trap, both marker systems successfully detect mixed samples—ultimately leading to their exclusion from downstream analyses—albeit through different mechanisms. While microsatellites intuitively flag cross-contamination by displaying more than two alleles at a locus, SNPs typically fail sequencing quality controls or emerge as outliers in frameworks like NewHybrids, displaying weak and unstable assignment probabilities rather than generating an erroneous hybrid signal. Given the sensitivity of genomic methods, transitioning to SNPs dictates rigorous field protocols. Hairs collected from different parts of the same lure stick must be systematically stored in separate tubes to minimize the risk of artificially pooling DNA from multiple individuals. Additionally, coupling hair traps with camera traps provides significant added value, as photographic evidence of multiple cats visiting the same site can proactively flag high-risk samples before costly genotyping.

4.2. Population structure and genetic diversity of pure wildcats

Within the pure wildcat cluster, spatial sub-structuring remained ambiguous. Bayesian clustering via STRUCTURE yielded inconsistent optimal K values and high admixture for microsatellites, showing split individual assignment q-values. This reflects a well-documented limitation: microsatellites often struggle to isolate weak spatial boundaries, whereas high-density SNPs provide the sharper statistical resolution needed to map fine-scale patterns in mobile species (Camacho-Sanchez et al., 2020; Stacy et al., 2025; Sunde et al., 2020), which is valuable for species with large home ranges where boundaries are difficult to delineate (Lah et al., 2016; Puckett & Eggert, 2016; Zimmerman et al., 2020).

These clustering results benefit from a nuanced interpretation. This specific SNP array was optimized for inter-specific hybrid identification using diagnostic loci rather than intra-specific demographic structuring, unlike other genomic panels (e.g., von Thaden et al., 2020). This highlights that the marker choice is an essential element of the study pipeline, depending on the questions of future monitoring programs. Marker selection and genomic coverage are more critical than the specific clustering algorithms used; while both tools can yield comparable structural conclusions in highly divergent lineages (Coates et al., 2009; Sunde et al., 2020), SNPs can provide cleaner separation at fine regional scales (Smitz et al., 2018).

Comparing genetic diversity indices across these marker types requires accounting for their distinct mutational histories (Morin et al., 2004). Highly polymorphic microsatellites maintain high numbers of alleles per locus, whereas biallelic SNPs are mathematically limited (Ar = 2). Evaluating diversity metrics like expected heterozygosity or differentiation G’st requires a larger absolute number of SNP loci to balance the per-locus information content, which reduces overall statistical variance (Morin et al., 2004; Stacy et al., 2025; Sunde et al., 2020; Waples, 1998). Addressing this theoretical benchmark, recent simulations by Mariette et al. (2002) demonstrate that four to ten times more biallelic markers are strictly necessary to reliably match multi-allelic estimates of genome-wide variation.

The most notable discrepancy in our study lies in the inbreeding coefficient estimates. While some authors report high concordance between markers for diversity metrics (Zimmerman et al., 2020), others have found allelic richness to be uncorrelated (Camacho-Sanchez et al., 2020; Elbers et al., 2017) or noted that SNPs show no correlation with microsatellite Fis values (Pérez-González et al., 2023). In our context, the high microsatellite Fis (0.28) could theoretically suggest a biological inbreeding risk or a hidden Wahlund effect. However, the near-zero Fis (0.02) derived from the SNP dataset suggests a more technical explanation.

Non-invasive hair sampling yields degraded, low-concentration DNA. In length-dependent microsatellite genotyping, this degradation can cause allelic dropout—where larger alleles fail to amplify—artificially inflating homozygous genotypes and driving a falsely elevated Fis. Conversely, short-amplicon SNP assays (< 100 bp) bypass fragment-length biases, providing robust genetic profiles (Carroll et al., 2018; von Thaden et al., 2020).

4.3. Conservation implications

Wildlife management increasingly leverages genomic resources to refine biodiversity assessments and map population structure (Stacy et al., 2025; von Thaden et al., 2020). Transitioning long-term monitoring

programs from legacy microsatellites to high-throughput platforms is a growing priority (Osborne et al., 2023). Paralleling the long-term monitoring of wolverines in Scandinavia (Stacy et al., 2025), transitioning to targeted SNP panels provides increased power to evaluate gene flow and introgression. For active conservation management, we join Parejo et al. (2018) in recommending SNP-based tests to maintain genetic variation and monitor hybridization.

However, from a long-term monitoring perspective, a holistic view is essential. The primary strength of wildlife monitoring lies in the temporal consistency of the dataset, and a sudden marker transition risks breaking the continuity of a time series. To mitigate this transition, we advocate maintaining a period of overlap between microsatellite and SNP approaches rather than prematurely abandoning traditional markers (Morin et al., 2004). Because microsatellite- and SNP-based estimates are often correlated but not directly equivalent—microsatellites frequently yielding larger absolute values due to higher variance, limited loci, and ascertainment bias (Fischer et al., 2017)—dual genotyping of a subset of samples combined with long-term calibration across designated reference sites offers a powerful calibration opportunity. Such intercalibration enables the derivation of conversion factors for key parameters, by establishing a shared reference baseline during the overlap period, modelling score regressions, adjusting assignment thresholds and harmonizing historical datasets to build a continuous, longitudinally robust temporal trend.

The choice between these methods must remain guided by the specific research question, alongside logistical and financial constraints (Elbers et al., 2017). The performance of these markers is strongly dictated by the scale of the panel; while large genomic panels (~ 3000 loci) systematically outperform traditional markers, microsatellites often exhibit comparable or greater statistical power when compared to very low SNP numbers (Fischer et al., 2017), and assertions favouring SNPs can sometimes be overemphasized depending on the analytical framework (Camacho-Sanchez et al., 2020).

To guide future conservation-oriented monitoring, we propose a framework for marker selection: microsatellites remain viable for broad population genetics status assessments, mapping macro-population patterns (Pérez-González et al., 2023), identifying first-generation (F1) hybrids when parental lineages are well-delineated (Szatmári et al., 2021), and assessing small or sporadic sample sizes where the rigid 96-well batch processing requirements of high-throughput SNP arrays can be logistically or financially restrictive (Schlötterer, 2004; Zimmerman et al., 2020). Additionally, their high allelic richness remains an advantage for identifying multi-individual sample contamination at a single trap. Targeted SNP panels should be prioritized for tracking multi-generational introgression, conducting high-throughput screening of severely degraded non-invasive samples (Schlötterer, 2004; von Thaden et al., 2020), studies of population connectivity, fine-scale genetic structuring, reducing amplification-driven heterozygosity artifacts, and establishing universal laboratory standardization across multi-agency research groups (DeFaveri et al., 2013).

Looking forward, the methodological horizon is expanding. Genotyping traditional microsatellites via next-generation sequencing (NGS) is emerging as a valuable intermediate solution to standardize cross-laboratory data and increase precision. Concurrently, low-coverage whole-genome sequencing (WGS)

represents a high-resolution frontier, though its high computational demands must be weighed against the practical needs of routine monitoring. Ultimately, researchers must continue to empirically evaluate marker suitability (Liu et al., 2005) and rigorously assess the accuracy of chosen panels against their specific biological objectives (Guichoux et al., 2011; Morin et al., 2004; Tokarska et al., 2009; von Thaden et al., 2020) to ensure management decisions remain scientifically sound.

Declarations

Declaration of Competing Interest

The authors declare that they have no known competing financial interests.

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Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Anaïs Beaumariage, Xavier Mestdagh, Lionel L'Hoste, Youri Martin, Nicolas Titeux, Marc Moes and Johan Michaux. The first draft of the manuscript was written by Anaïs Beaumariage and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability

The datasets generated during the current study are available in the Figshare repository https://doi.org/10.6084/m9.figshare.33415228

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Figures

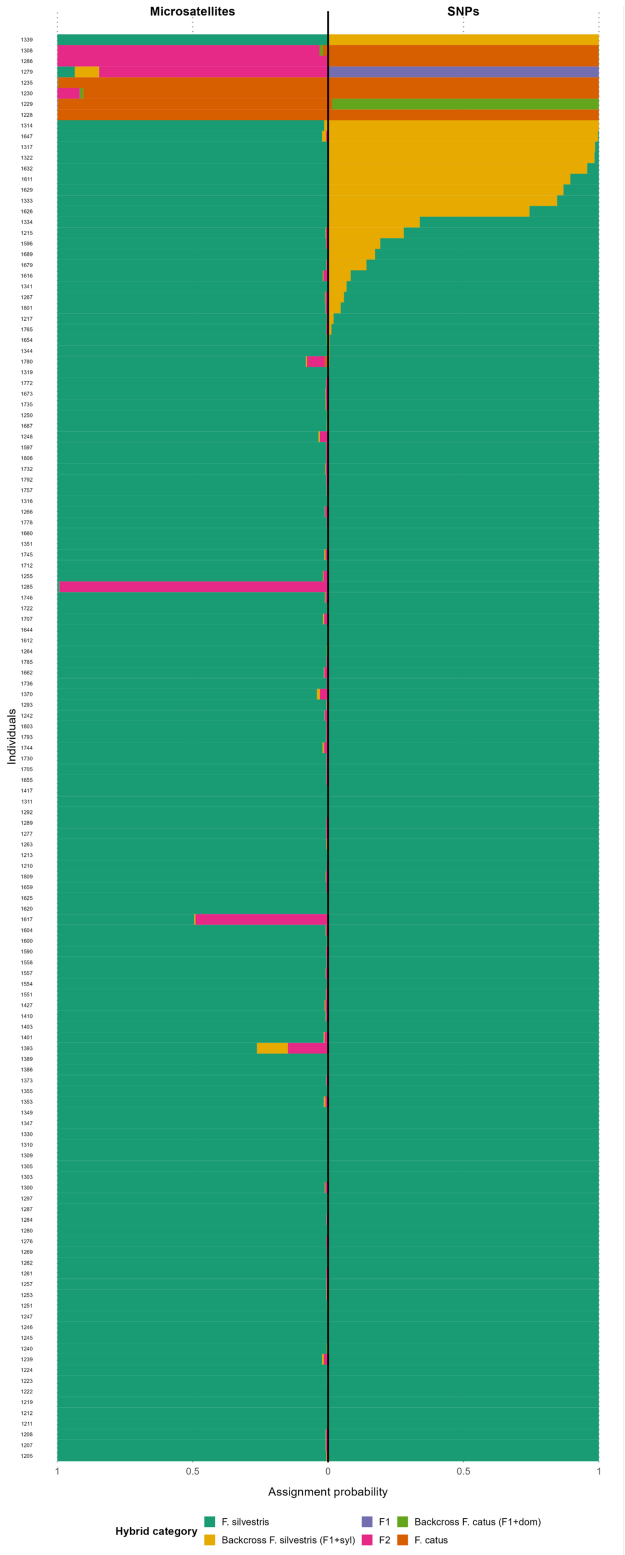


Figure 1

Assignment probabilities of sampled individuals to distinct hybrid and parental classes based on NewHybrids analysis. Each vertical bar represents a single individual, with colours indicating the posterior probability (q) of belonging to a specific genotypic category. The upper panel displays results derived from microsatellite markers, and the low panel displays results from the SNP array

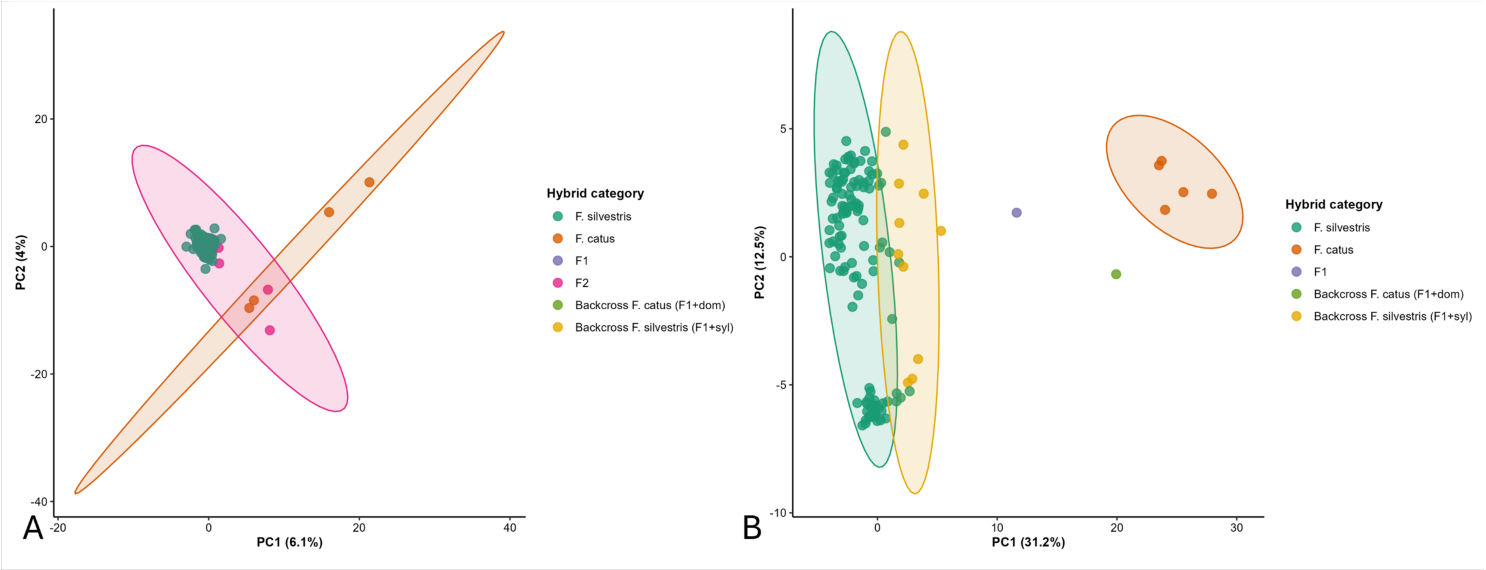


Figure 2

Principal Component Analysis (PCA) based on (a) microsatellite genotypes and (b) SNP genotypes). Individuals are coloured according to their assignment class determined by NewHybrids (q>0.90)

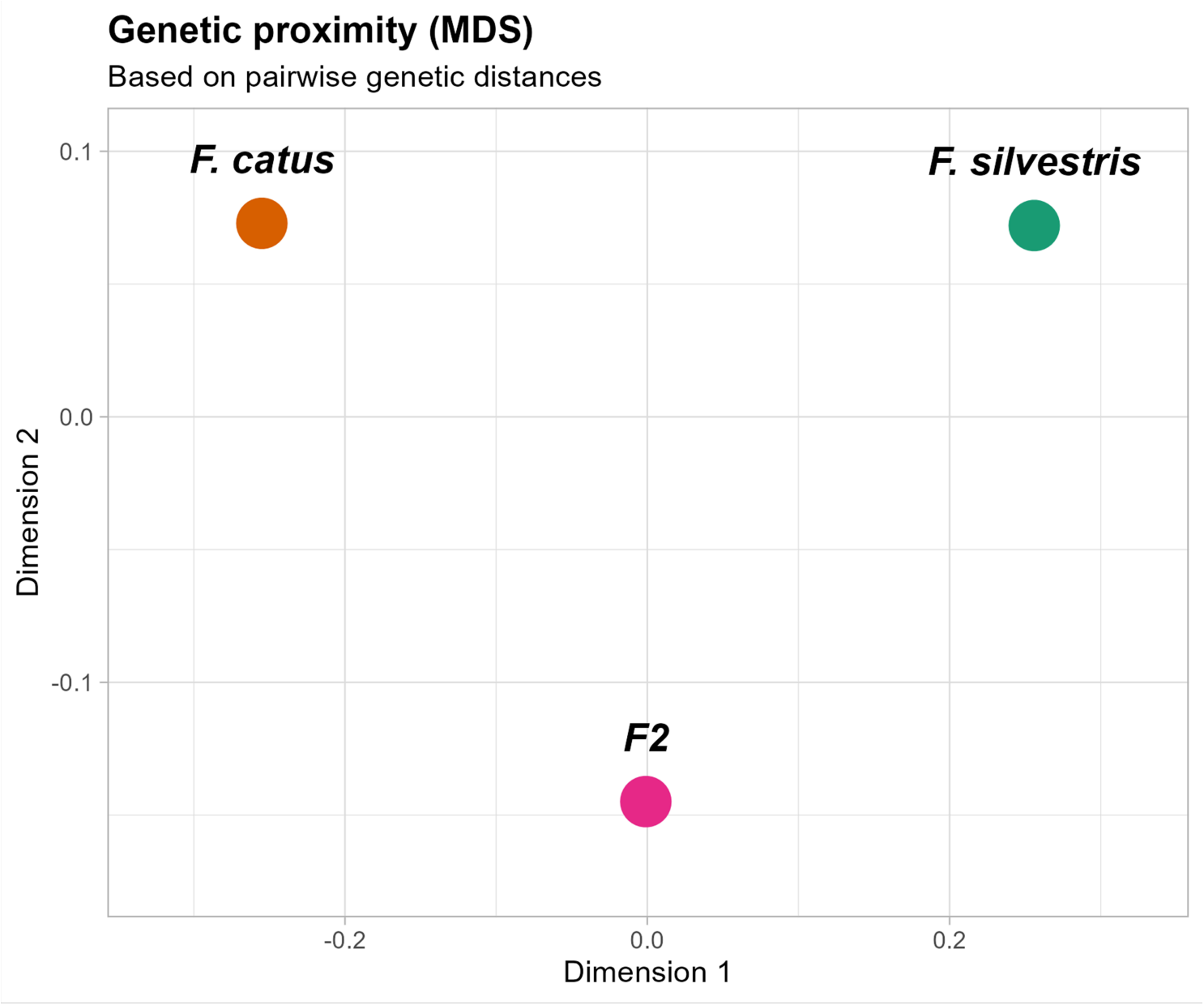


Figure 3

Multidimensional Scaling (MDS) plot of pairwise G'st values calculated from microsatellite data

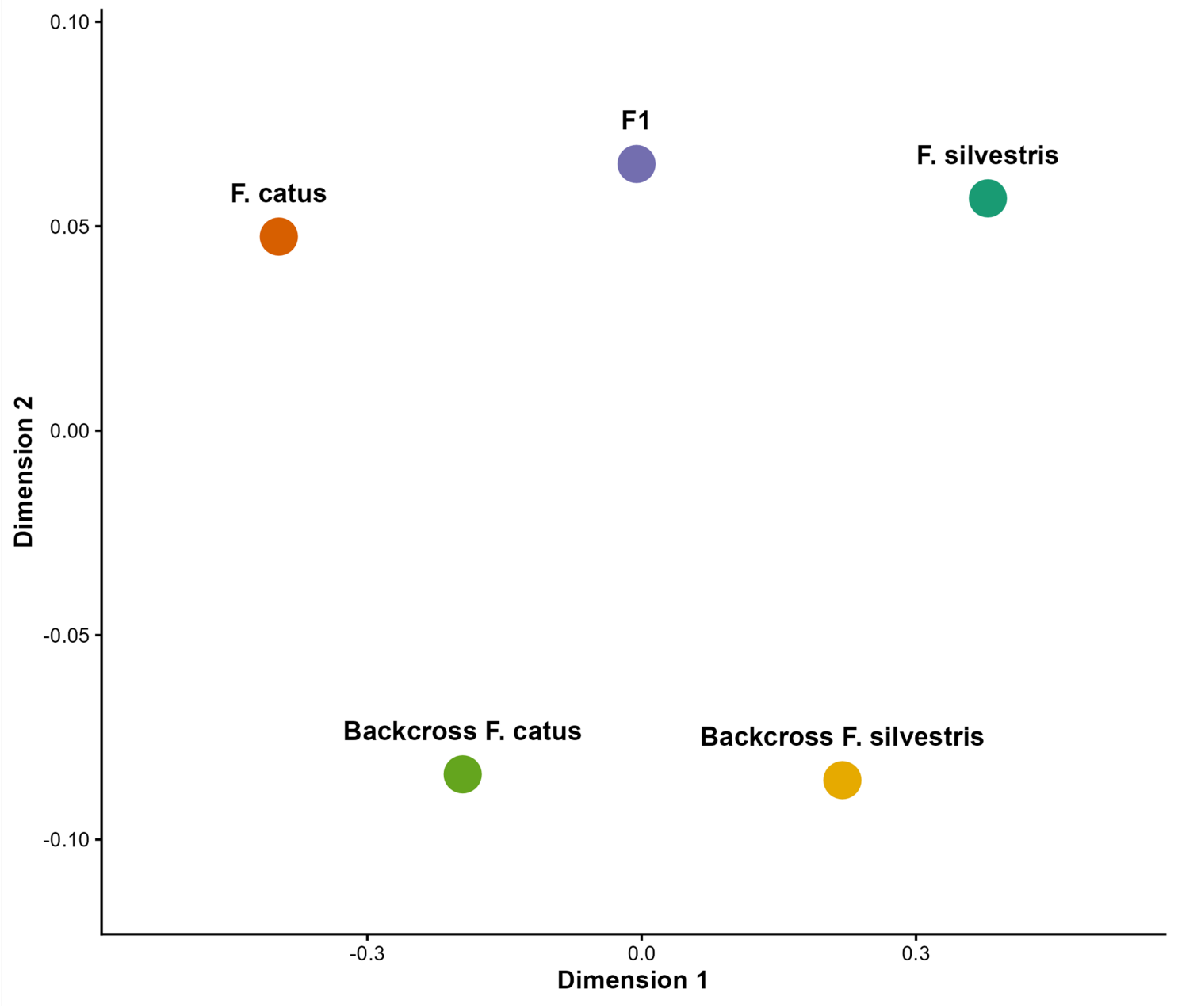


Figure 4

Multidimensional Scaling (MDS) plot of pairwise G'st values calculated from SNP data

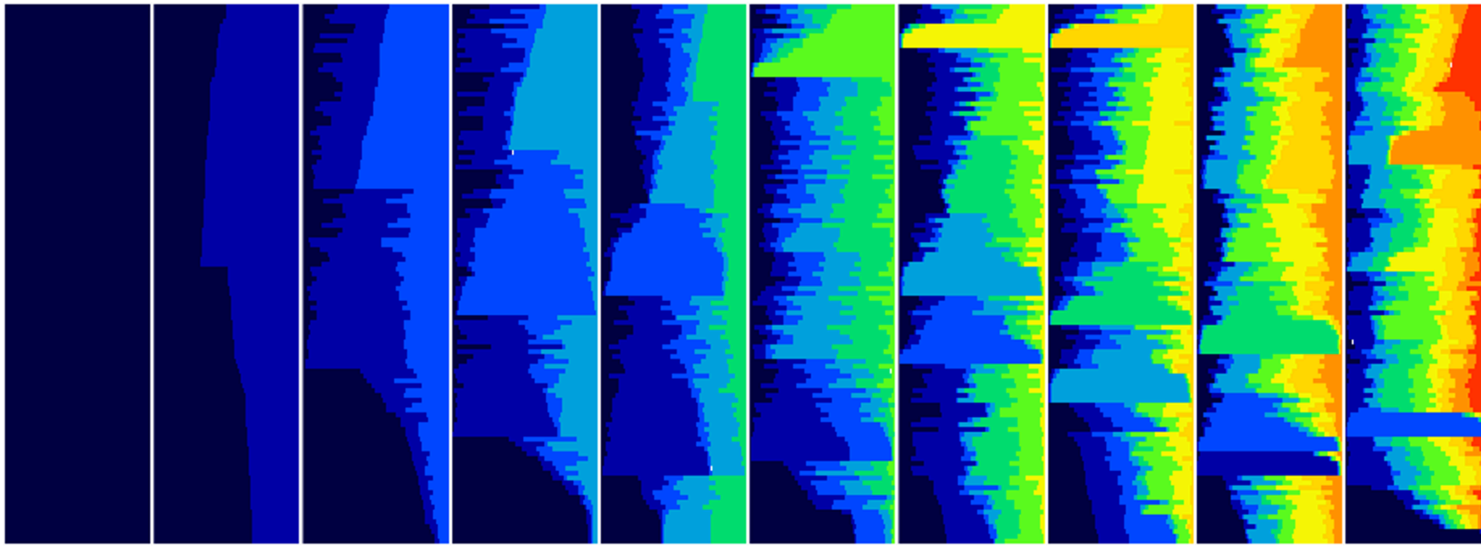


Figure 5

Bayesian clustering analysis (STRUCTURE) of 111 pure wildcats based on microsatellite loci. Each bar represents a single individual, and colours indicate the probability of membership (q) to genetic clusters for values of K ranging from 1 to 10

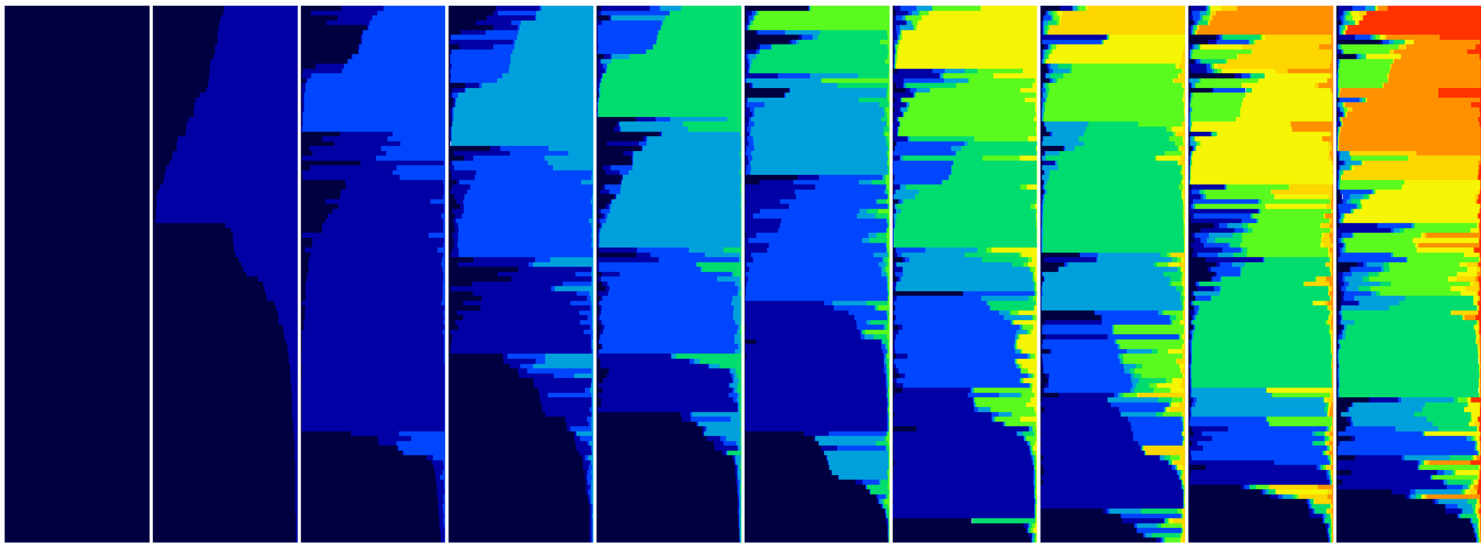


Figure 6

Bayesian clustering analysis (STRUCTURE) of 111 pure wildcats based on the 96-SNP panel. Each bar represents a single individual, and colours indicate the probability of membership (q) to genetic clusters for values of K ranging from 1 to 10