IBIS
BOU international journal of avian science
*Ibis* (2022) doi: 10.1111/ibi.13087

# Cryptic hybridization between Common (*Apus apus*) and Pallid (*A. pallidus*) Swifts

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Artificial structures, and particularly in urban settings, attract species showing similar ecological niches and provide nest-sites for cavity-breeding species. It is, however, unknown whether this proximity creates opportunities for hybridization and gene flow across related species. We investigated whether two colonial species, the Common Swift *Apus apus* and the Pallid Swift *Apus pallidus*, are experiencing gene flow by genotyping individuals that breed in sympatry in the town of Bastia (Corsica, France). We compared them with individuals sampled in colonies where a single species is breeding, in the Mediterranean region and in Switzerland. Our results provided evidence of gene flow between the two species and showed that introgression was not limited to sympatric urban colonies. Gene flow was asymmetrical, with more Pallid Swifts than Common Swifts showing evidence of mixed ancestry. Several individuals were assessed as late-generation hybrids, suggesting that introgression between the two species was associated with their range expansion since the Last Glacial Maximum. However, we also identified individuals that exhibit the characteristics of recent-generation hybrids, particularly in Bastia. This result suggests that hybridization between the two species is an ongoing and underestimated phenomenon, with a single observation of a mixed pair in the literature, and may be favoured by close proximity in urban colonies.

**Keywords:** Apodidae, colonial breeding, genetic markers, hybrid zone, introgression.

Reproductive isolation between closely related species is generally achieved when geographically isolated populations gradually increase their genetic differences through drift and selection (Harrison 1993, Price 2008). This isolation can, however, partially fail when populations come into secondary contact and experience hybridization and gene flow in sympatry (Rhymer & Simberloff 1996, Rheindt & Edwards 2011, Joseph 2018). A large number of situations might contribute to secondary contact between closely related species. In the Northern Hemisphere, range expansion following demographic increase after the Last Glacial Maximum (LGM, 24.0–15.0 ka) (Frenzel 1992) created secondary contact zones between populations that evolved independently in glacial refugia, and has led to many cases of introgression between closely related species

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(for instance flycatchers *Ficedula*, Sætre *et al.* 2001, Qvarnström *et al.* 2010; gulls *Larus*, Sonsthagen *et al.* 2012; crows *Corvus*, Vijay *et al.* 2016). On insular systems such as islands, dispersal and new island colonization have created secondary contacts and sometimes hybrid zones (honeyeaters, Sardell & Uy 2016; weavers, Warren *et al.* 2012). At smaller spatial scales and over more recent timeframes, range displacement resulting from local habitat changes has also occasionally led to hybridization between ecologically distinct species. These changes in species distribution enabling secondary contact can be induced by: habitat dynamics and biome succession (e.g. bluebirds *Sialia* in North America, Duckworth & Semenov 2017); the consequences of climatic modifications (i.e. warming for chickadees *Poecile*, Taylor *et al.* 2014, and kingbirds *Tyrannus*, Worm *et al.* 2019); changes in land use (for instance on Chatham Island, where deforestation led to an increase of the Red-crowned Parakeet *Cyanoramphus novaezelandiae chathamensis* and subsequent introgression with the rare Chatham Parakeet *Cyanoramphus forbesi*, Chan *et al.* 2006); or a combination of both climate and disturbance, as in the case of two species of rails (*Rallus*) in North America (Coster *et al.* 2018). In an urban environment, local range expansion has been facilitated by the creation of anthropogenic nesting sites, in particular for cavity-nesting birds (Budinski *et al.* 2010, Tella *et al.* 2014, Tomasevic & Marzluff 2017), but it is unknown whether the availability of artificial nests enabling closer spatial proximity of closely related species can act as a facilitator for hybridization and gene flow.

In that context, we investigated the potential for introgression between two species of colonial birds, the Common Swift *Apus apus* and the Pallid Swift *Apus pallidus*, for which a single mix-pair has been documented (Oberli *et al.* 2013). The Common Swift has a large breeding range in the Palaearctic, extending from Western Europe and North Africa to China. The Pallid Swift has a scattered distribution across the Southwest Palaearctic, from Portugal to Turkey, and in North Africa and the Middle East, from Mauritania to Iran. The whole breeding range of the Pallid Swift is embedded within that of the Common Swift, and at many locations both species breed in close proximity (Cramp 1985). Common and Pallid Swifts are sister species in the phylogenetic tree of Apodidae inferred by Päckert *et al.* (2012). Pellegrino *et al.* (2017) estimated the time of divergence between the two species to 1.8–2.1 million years ago. Based on their current ecological preferences, Pellegrino *et al.* (2017) suggested that the two taxa probably underwent a phase of allopatric differentiation during glacial periods, when Pallid Swifts, adapted to warmer conditions, restricted their breeding grounds to southern refugia in the Palaearctic, whereas Common Swifts persisted in other refugia (probably East Siberia). Warming conditions after the LGM allowed for the expansion of both species in Europe, and in particular of Pallid Swifts in the northern parts of the Mediterranean.

However, a more recent phenomenon has also probably played a role in the distribution of swifts: the use of artificial structures as breeding sites. Ancestral breeding sites, mostly tree cavities and cliffs, are still used by Common Swifts, although the number of breeders in buildings is considerably higher, particularly around the Mediterranean (Keller *et al.* 2020). Pallid Swift colonies are frequently found on natural sites (mostly cliffs), with nesting in buildings quite widespread, particularly in the northern part of their range (Chantler & Driessens 2000). The earliest evidence in the literature of swifts nesting in artificial structures dates to the 15th century (Ferri 2018), although it is likely that swifts took advantage of such structures much earlier: for instance Common Swifts were observed nesting on thatched roofs in England (White 1947). Pallid Swifts have experienced a recent increase in several towns, for instance in Nice (C. Frelin pers. comm.) and Bastia (Thibault *et al.* 2022) in France, and in Sofia in Bulgaria (Antonov & Atanasova 2002). Although the two species are known to form mixed colonies at the same natural sites (i.e. cliffs; Brichetti *et al.* 1988, Avellà & Muñoz 1997), sympatry predominantly occurs within urban regions where both species breed in buildings. Because they share the same preferred urban breeding sites, the proximity of the two species has probably increased compared with the time when they only bred on natural sites.

Morphological differences are slight between the two species and identification can be difficult (Chantler & Driessens 2000). Briefly, Pallid Swifts are best described as bulkier, browner and with greater scaling on the plumage than Common Swifts, and have a dark eye-patch contrasting with pale forehead and lores, and a larger pale throat-

patch. The outer primaries of Pallid Swifts are blacker than the rest of the wing, whereas the wing is more uniform in Common Swifts. In most Pallid Swifts the 'saddle' (i.e. a contrasting pattern on the back and rump) is more pronounced than in Common Swifts. Calls are usually disyllabic in Pallid Swifts and shorter than the typical high-pitched *shree* of Common Swifts. In hand, the longest primary is usually P9 in Pallid Swifts, whereas P10 (outermost) and P9 are of equal length in Common Swifts, and the tail is more deeply forked in Common than in Pallid Swifts (Glutz Von Blotzheim & Baeur 1980, Boano *et al.* 2015, Demongin 2016).

In this study, we focused on a Mediterranean urban site, the town of Bastia in Corsica, where the two species breed in close proximity. On the island, the two species of swifts are common breeders, in addition to the Alpine Swift *Tachymarptis melba* that breeds mainly in rocky sites located inland and on islets (Thibault & Bonaccorsi 1999). The Common Swift is distributed in most villages and towns of Corsica, the largest numbers being observed in Ajaccio and Bastia. Breeding sites in forests, in tree holes, have also been regularly documented (Thibault *et al.* 2020). The Pallid Swift breeds in crevices of rocks along the seacoast and on islets, as well as on cliffs at higher elevation inland. Urban colonies are scarce, with the notable exception of Bastia, where the species has maintained a breeding population since the 1930s (Mayaud 1936, Mouillard 1938).

We used the Common and Pallid Swifts, which experienced contact after their post-glacial expansion and recently enlarged their range to urban settings, to test whether anthropogenically induced sympatry has led to gene flow and, if so, to determine to what extent gene flow is occurring. In this context, we aim to identify the occurrence of recent (i.e. F1) or late-generation (backcross) hybrids in our data set. If hybridization has occurred over the long term, linked to post-glacial expansion, backcrossing would be expected (see for instance Slager *et al.* 2020), whereas if hybridization is a recent phenomenon due to the enhanced proximity of breeding colonies in urban areas we would expect to see a greater proportion of F1 hybrids. We evaluated the introgression between the Common and Pallid Swifts using the genotypes (mitochondrial DNA and nuclear microsatellite markers) of individuals of both species at several localities throughout their range, with a focus on the sympatric zone in the town of Bastia.

## METHODS

### Sampling and DNA extraction

A total of 488 individuals (Common Swifts $n = 380$; Pallid Swifts $n = 108$) from four localities in Europe were included in this study (Fig. 1). Sampling focused on the town of Bastia (Corsica Island, France), where the two species are breeding. This sampling was complemented with individuals from the Cerbicale Islands (southern Corsica), where Pallid Swifts breed in isolation, and the town of Nîmes (Gard, France), which holds only colonies of Common Swifts. Sampling was conducted in Bastia between 2014 and 2019 within the town's limits and in neighbouring localities (Supporting Information Table S1; see Thibault *et al.* 2022 for the distribution and characteristics of the nesting colonies). In Bastia, the two species breed in close proximity, sometimes in the same building (Fig. 2). The Common Swift is dominant in the old town, whereas the Pallid Swift has colonized many new urban constructions in the suburbs (J.-C. Thibault pers. obs., Thibault *et al.* 2022). Blood and feather samples were collected from birds captured at breeding sites with mist-nets and from rescued juveniles found near the colonies, and additional skin samples were obtained from dried-out swift carcasses collected at breeding sites, for a total of 174 individuals (Common Swift $n = 89$; Pallid Swift $n = 85$). In 2005–2006, G. Gory collected blood samples from individuals captured in Nîmes (nestboxes, $n = 21$ Common Swifts) and Cerbicale Island (natural nests, $n = 23$ Pallid Swifts). The age of the sampled individuals (adult, juvenile or chick) is indicated in Table S1. In addition, we sampled a Common Swift colony located outside the Mediterranean region, in Fribourg (Switzerland), more than 100 km beyond the expected northern edge of the Pallid Swift's range: the only known Pallid colony in Switzerland is 143 km away in Locarno, and the closest colony, Domodossola in Italy, lies 116 km from Fribourg (Lardelli 2014). Sampling was conducted in Fribourg as part of a study on a Common Swift colony located on the building of the Natural History Museum. Adults and juveniles were captured and ringed during yearly visits to nestboxes as part of


**Figure 1.** Sampling locations. (1) Fribourg (Common Swift, $n = 270$). (2) Nîmes (Common Swift, $n = 21$). (3) Bastia (Common Swift, $n = 89$; Pallid Swift, $n = 85$). (4) Cerbicale Islands (Pallid Swift, $n = 23$). The distribution of the Pallid Swift in this area is indicated in orange. The widespread distribution of the Common Swift in the Palaearctic covers the entire map.

the population monitoring. We used this opportunity to collect samples by rubbing standard cotton-buds on the inner cheek surface (buccal swabs, $n = 270$). Additionally, the nuclear dataset for Alpine Swifts ($n = 157$), sampled from two colonies near Bern (Switzerland) and genotyped with the same markers for parentage studies, was used as an outgroup in the cluster analysis. Because most of the sampling was conducted in addition to monitoring or as a consequence of rescue operations, no birds were collected as part of this study.

DNA was extracted from blood, feather or skin samples following the manufacturer's recommendations (DNeasy Blood and Tissue Kit; Qiagen, Valencia, CA, USA), with the addition of dithiothreitol (DTT 1 M, 20 µL) for skin samples. To the buccal swabs, which had been kept dry, 500 µL 100% ethanol was added before DNA extraction. Tubes were centrifuged for 10 min at 14 000 *g* for pellet cells that may have detached from the swabs. Swabs were then dried out at room temperature for 30 min and ethanol was discarded from the tubes. We then pipetted 500 mL of Shorty Buffer (0.2 M Tris-HCl pH 9.0, 0.4 M LiCl, 25 mM EDTA, 1% SDS) into each tube and added the air-dried swab. Samples were then incubated at room temperature for 15 min, shaken vigorously for 5 min and centrifuged for 5 min at 13000 rpm (190 *g*). We transferred 350 µL of the supernatant into a new 1.5-mL tube and an equal volume of isopropanol. Tubes were mixed by inversion (15–20x) and centrifuged for 10 min at 13 000 rpm (190 *g*). The supernatant was discarded and tubes were let open to dry at room temperature for 30 min. DNA was resuspended in 100 µL of water and shaken at room temperature at 900 rpm (90 *g*).

## Mitochondrial DNA (mtDNA) sequencing

We PCR-amplified 657 base pairs (bp) of the Cytochrome C Oxidase subunit I (COI) gene, a

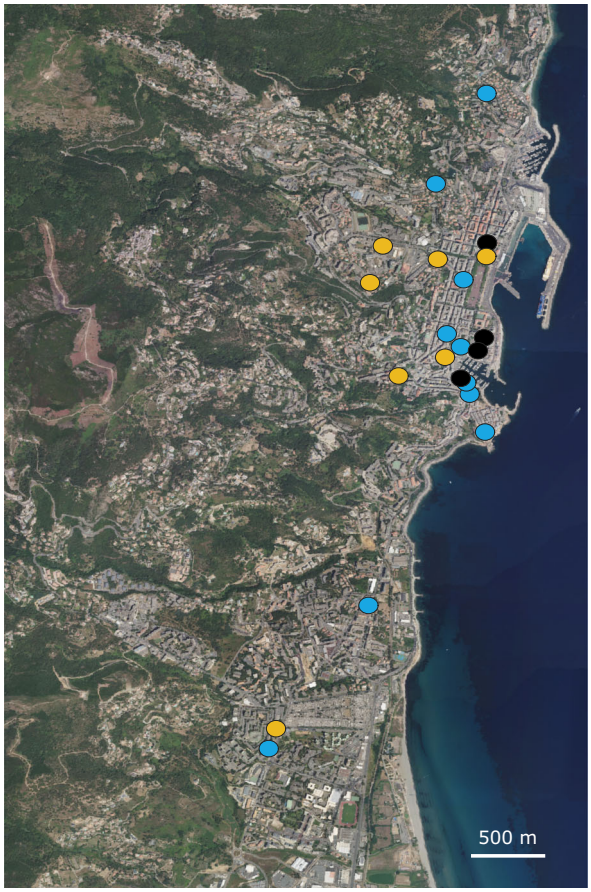


**Figure 2.** Sampling localities in Bastia (city centre). The colonies of Common Swifts (blue) and Pallid Swifts (orange) are often in close proximity. Black dots indicate sampling sites where both species were nesting in the same building (although not considered as mixed colonies, as different parts of the building were used).

mitochondrial marker selected by Pellegrino *et al.* (2017) to test the taxonomic status of Common and Pallid Swifts in Italy. The Alpine Swifts were not included and only a subset of individuals from Fribourg were sequenced (32 individuals). We used the primers BirdF1 (Hebert *et al.* 2004) and Passer-R1 (Lohman *et al.* 2009). PCR-amplifications were performed in 25-μL reactions containing 2 μL of template and 0.4 mM of each primer. The thermocycling procedure started with an initial denaturation of 3 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 40 s at annealing temperature (50 °C) and 40 s at 72 °C for elongation. PCR products were cycle-sequenced in both directions at a contract sequencing facility (Macrogen, Amsterdam, The Netherlands) on an ABI3730 XL automatic DNA sequencer, with the same primers used in PCR. Sequences were checked and aligned using Sequencher 4.8 (Genecodes, Ann Arbor, MI, USA).

## Microsatellite genotyping

In conjunction with monitoring programmes of the Common Swifts and Alpine Swifts in Switzerland, we developed a set of nine microsatellite primers. We first constructed and sequenced libraries enriched in DNA fragments containing microsatellite motifs (one for each species; outsourced to EcoGenics Gmbh, Schlieren, Switzerland) and kept only DNA fragments containing simple tetranucleotide motifs. We then identified sequences showing >95% homology between species ($n = 21$) using the localblast function in BioEdit (Hall 1999) and designed primer pairs targeting amplification products in the ranges 100–150, 150–200 and 200–250 base pairs using primer3 (http://primer3plus.com/cgi-bin/dev/primer3plus.cgi). Readability of the candidate loci was assessed using M13 labelling and fragment analysis on an ABI 3130 (Applied Biosystems, Waltham, MA, USA). The nine most promising polymorphic loci were combined into a single multiplex (Table 1). We also used a CHD-gene fragment to determine the sex of the individuals (Cayuela *et al.* 2019). The 10 molecular markers were PCR-amplified in a single multiplex-PCR. PCRs were set up in a 10-μL reaction containing 1x Type-it Master Mix (Qiagen) or Hot FIREPol Multiplex Mix (Solis BioDyne, Tartu, Estonia), 0.3–0.7 μM of each primer (Table 1) and 2 μL DNA, under the following PCR conditions: 5 min at 95 °C, 35–37 cycles of 30 s at 94 °C, 2 min at 58–60 °C and 45 s at 72 °C; and 15 min at 72 °C. A subgroup of the samples was amplified in independent PCRs to quantify the risk of genotyping errors (Taberlet *et al.* 1996, Miquel *et al.* 2006). PCR fragments were mixed with an internal size standard (Orange Size Standard; MCLAB, San Francisco, CA, USA) and analysed by electrophoresis on a semi-automated DNA sequencer (ABI 3130; ThermoFisher, Waltham, MA, USA) at the University of Fribourg or using a commercial facility (Ecogenics GmbH). We used Genemarker (SoftGenetics, State College, PA, USA) and Geneious (https://www.geneious.com) to determine

**Table 1.** List of amplified markers and estimates of genetic diversity based on nine microsatellite loci. For each locus, we indicate forward and reverse sequences and the final concentration of each primer (μM). For each species, we report the number and the size range of observed alleles ($N_A$ and *Range*), and the levels of expected and observed heterozygosities ($H_E$ and $H_O$). Sample size: *A. apus* $n$ = 380, *A. pallidus* $n$ = 108.

| Locus | Sequences (5′–3′) | Concentration (μM) | Species | $N_A$ | Range | $H_O$ | $H_E$ |
|---|---|---|---|---|---|---|---|
| T05 | F: NED-GCAGAAGGTGTGGATGGAGT | 0.6 | *A. pallidus* | 6 | 144–164 | 0.51 | 0.64 |
| | R: GGTGCTTCCCAACCCTAACA | | *A. apus* | 10 | 136–172 | 0.73 | 0.76 |
| T06 | F: VIC-GGCTTTTATCCTTTGCTACTCGT | 0.4 | *A. pallidus* | 10 | 123–161 | 0.84 | 0.82 |
| | R: CATGGTGATGTGCGTGCTC | | *A. apus* | 16 | 123–169 | 0.75 | 0.78 |
| T08 | F: NED-CACATCTTAAGTGAGTGCTCTGA | 0.5 | *A. pallidus* | 8 | 177–213 | 0.72 | 0.70 |
| | R: TCACTGTCCAAAGGCTCTCA | | *A. apus* | 14 | 161–221 | 0.69 | 0.74 |
| T10 | F: FAM-ACTGATTTTGGGCTTTTCTCTCA | 0.7 | *A. pallidus* | 13 | 222–270 | 0.90 | 0.89 |
| | R: TGAAGTGCTCAAAATCTACCTGT | | *A. apus* | 17 | 218–282 | 0.85 | 0.90 |
| T12 | F: VIC-CTGCAGAAGTGGCAGTTGTT | 0.4 | *A. pallidus* | 11 | 216–256 | 0.83 | 0.87 |
| | R: GCAACACCATCAAACCTCAGT | | *A. apus* | 13 | 200–252 | 0.81 | 0.85 |
| T14 | F: PET-ACATCCCACAGGTAGGTCTT | 0.5 | *A. pallidus* | 13 | 220–268 | 0.82 | 0.83 |
| | R: AGGCTCTGATTCCCGAATGA | | *A. apus* | 24 | 212–280 | 0.80 | 0.90 |
| T15 | F: FAM-AGTGCCCTGATCTGATACTTGT | 0.6 | *A. pallidus* | 8 | 176–212 | 0.84 | 0.76 |
| | R: TCAGCCAATAGTTGTCAAATCCT | | *A. apus* | 9 | 180–212 | 0.69 | 0.70 |
| T16 | F: PET-ACAGAGGTGGTAGGATGTTAGA | 0.5 | *A. pallidus* | 8 | 126–156 | 0.64 | 0.72 |
| | R: TCACCTGATTTGGCTGAATTTTC | | *A. apus* | 13 | 118–156 | 0.69 | 0.86 |
| T17 | F: FAM-AGGGTACTGTGGACATAGAGAT | 0.6 | *A. pallidus* | 13 | 79–145 | 0.73 | 0.73 |
| | R: TGAGCATGGAAACTGAGTTGAG | | *A. apus* | 19 | 79–143 | 0.68 | 0.80 |

the size of the PCR fragments and record the allele combination at each locus.

## Genetic polymorphism

mtDNA sequences were translated into protein sequences using Mega 6 (Kumar *et al.* 2018) to verify the absence of a stop codon that could indicate nuclear copies (Zhang & Hewitt 1996). Haplotype diversity ($H$) and nucleotide diversity ($\pi$) were calculated using DnaSP 6.12.3 (Rozas *et al.* 2017). We constructed a median-joining haplotype network using PopART (Leigh & Bryant 2015). For microsatellite markers, we used the R package Hierfstat (Goudet 2005) to calculate observed heterozygosity, fixation index, and allelic richness and genetic differentiation (Nei's $F_{ST}$) between pairs of populations (bootstrap estimate of confidence interval, 10 000 permutations). The programs MSA 4.05 (Dieringer & Schötterer 2003) and Arlequin 3.11 (Excoffier *et al.* 2005) were used to test for deviation from Hardy–Weinberg genotype frequency equilibrium (10 000 permutations). We estimated the frequency of null alleles and corrected values for genetic differentiation ($F_{ST}$), accounting for the presence of null alleles using FreeNA (Chapuis & Estoup 2007).

## Clustering and hybrid estimation analyses

We first evaluated the number of genetic clusters ($K$) using Structure 2.3.4 (Pritchard *et al.* 2000) under various demographic scenarios. Preliminary simulations showed that models assuming that clusters are demographic and genetic independent units (independent allele frequencies) performed poorly (results not shown). Although expected, this finding confirmed that Common and Pallid Swifts derived from a recent common ancestor and that the study sites were connected by the dispersal of individuals among colonies. All simulations were then run assuming correlated allele frequencies between genetic clusters, with admixture and using *a priori* information from individual location (ADMIXTURE and LOCPRIOR set to True). We conducted 10 simulations for each model for $K$ = 1–10, using a burn-in period of 200 000 and data collected over 500 000 replicates. Runs were analysed with the R package Pophelper (Francis 2017) and we used Structure Harvester 0.6.94 (Earl & vonHoldt 2012) to perform the Evanno method to evaluate the value of $K$ with the highest probability (Evanno *et al.* 2005).

Discriminant analysis of principal components (DAPC function) implemented in Adegenet

(Jombart *et al.* 2010) was applied with prior groups corresponding to the populations sampled, first on the whole dataset, then only within the Mediterranean area. DAPC was conducted with four prior groups and posterior probabilities were estimated for each individual, similarly to the Structure analysis. We designated, within the Mediterranean swifts, individuals as parental Common or parental Pallid if these respective ancestry proportions ($P$) exceeded 0.95: all other individuals were treated as 'mixed ancestry'. According to previous studies on birds, the threshold in hybrid analyses varies between 0.98 (crows, Slager *et al.* 2020) and 0.75 (wagtails, Semenov *et al.* 2017). We choose a 0.95 value that also statistically refers to a 5% error. A lower value would overestimate the number of individuals with mixed origin, whereas a higher value would decrease the number of individuals considered to be parental. The 0.95 threshold, according to our preliminary analyses, allows a good comparison with other cases of hybridization in birds. We calculated the hybrid index ($S$) and the intertaxon heterozygosity ($H$) using the R package HIest (Lynch 1991, Fitzpatrick 2012). F1 hybrids should theoretically have a hybrid index of 0.5 and heterozygosity of 1, whereas F2s and backcrosses show a reduced heterozygosity. We first evaluated our data by generating F1, F2 and backcrosses from the parental pool using the *hybridize* function in Adegenet (Jombart 2008, Jombart & Ahmed 2011) and an *ad hoc R* script (Andriollo *et al.* 2018). Then we used the *HItest* function to evaluate, for each of the mixed ancestry individuals, the most likely class among the six early generation diploid hybrid genotypes (parental 1 and 2, F1, F2, backcross to parental 1, and backcross to parental 2).

## RESULTS

The 250 COI sequences obtained showed no stop codons and aligned well with previous sequences deposited in GenBank, for instance with the sequences obtained by Pellegrino *et al.* (2017), which were identical to some of our haplotypes or differed by a single mutation. New sequences are available under GenBank accession numbers OM966298–OM966312. We observed 10 haplotypes (haplotype diversity $H = 0.348$ and nucleotide diversity $\pi = 0.00099$) in the 148 Common Swifts, and five haplotypes ($H = 0.253$, $\pi = 0.00059$) in the 102 Pallid Swifts we sampled.

Mean pairwise mismatch distributions within species were negative, but not statistically different from 1 (Tajima's $D_{\text{Common}} = -1.484$, Tajima's $D_{\text{Palid}} = -1.293$, both with $P > 0.10$), which is in line with the observed demographic expansion of both species.

The haplotype network revealed two haplogroups that correspond to the two swift species, a result consistent with that obtained by Pellegrino *et al.* (2017) using reduced sampling. However, we also found six individuals identified as Pallid Swifts based on their morphological characters that have mtDNA sequences belonging to the Common Swift haplogroup (indicated with an asterisk on Fig. 3). Five of these individuals were sampled in Bastia, where the two species occur in sympatry, and one individual was sampled on the Cerbicale Islands, where no Common Swifts are breeding. Such discrepancy between morphotype and mtDNA was not observed in any Common Swifts sampled in this study, which all belong to the same haplogroup.

The genetic diversity of the nine microsatellite markers is detailed in Table 1 (the raw allele scores are provided in Supporting Information Table S2). The number of alleles varied from 6 to 24. Deviation from Hardy–Weinberg equilibrium (HWE) ($\alpha = 0.05$) was found in five cases (T08, T10, T14 and T16 for Common Swifts and T16 for Pallid Swifts). Null allele frequency values estimated by FreeNa for these loci and populations were $< 0.2$, suggesting that null alleles were uncommon or rare. Between-species pairwise $F_{ST}$ estimates with or without correction for the presence of null alleles, i.e. the ENA procedure described by Chapuis and Estoup (2007), did not differ significantly (global $F_{ST}$ 0.049, $F_{ST}$ using ENA 0.048), suggesting that further estimation of population structure should not be biased by null alleles. Because we did not detect sub-groups between the two species, we suspected that deviation from HWE might be due to the presence of admixture, as revealed in the following analyses.

We observed no differences in observed heterozygosity between populations and species (Supporting Information Table S3). Levels of $F_{IS}$ and allelic richness were similar in the three species, although on average were larger in Common Swift than in Pallid Swift and Alpine Swift populations. Levels of genetic differentiation (Nei's $F_{ST}$, Nei 1986) between populations within species were not significant except between the two most

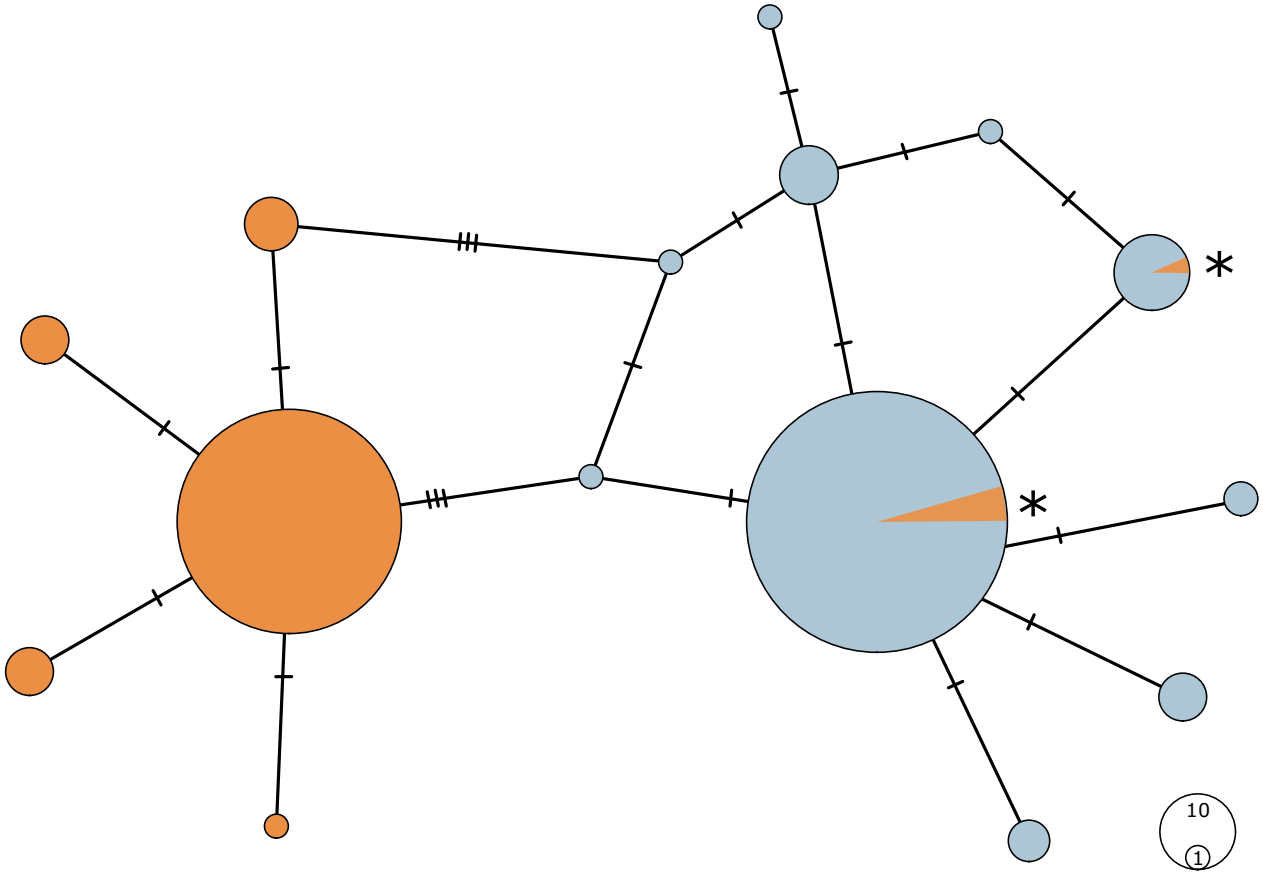


**Figure 3.** Haplotype network for Common Swifts (blue) and Pallid Swifts (orange). The circles are proportional to the number of individuals. Mutations are indicated by dashes. Asterisks show the six individuals identified based on their morphology as Pallid Swifts that have Common Swift haplotypes.

distant Common Swift populations in Fribourg and Bastia, whereas pairwise comparisons between species were significantly larger than zero (Supporting Information Table S4).

In the Structure analyses of the nuclear microsatellite markers, the computation of Evanno's delta *K* indicated a shift in the likelihood at $K = 2$ (Supporting Information Fig. S1), a partition that corresponds to the Alpine Swift for one cluster and the Common plus Pallid Swifts as a second cluster. This result highlights the close genetic ancestry of the Common and Pallid Swifts. Indeed, in the analysis considering the three species ($K = 3$), all Alpine Swifts were recovered with pure ancestry, whereas Common and Pallid Swifts showed trace levels of introgression (Fig. 4; similar results were obtained in an analysis conducted ($K = 2$) with Alpine Swifts excluded). In Fribourg, 5.9% (16/270) of the individuals showed mixed ancestry between Common and Pallid Swifts (i.e. with a $P > 5\%$), even in the absence of Pallid Swift colonies in that area. In Bastia, 13.5% (12/89) of the Common Swifts showed a level of mixed ancestry with Pallid Swift. In Nîmes, where only Common Swifts are known to breed, the proportion of introgressed birds rose to 19% (4/21). Pallid Swifts in Bastia also showed evidence of nuclear introgression in 14.1% (12/85) of the sampled individuals. One individual (PA04) found in a Pallid Swift colony, and for which the morphotype was uncertain (a mummified bird), showed evidence of being a Common Swift from its mtDNA haplogroup and an ancestry proportion of $P = 0.99$. Finally, in the Cerbicale Islands, where only Pallid Swift colonies occur, the proportion of individuals with mixed ancestry reached 30.4% (7/23).

In the DAPC analysis with the three species, most of the information was conveyed by the first

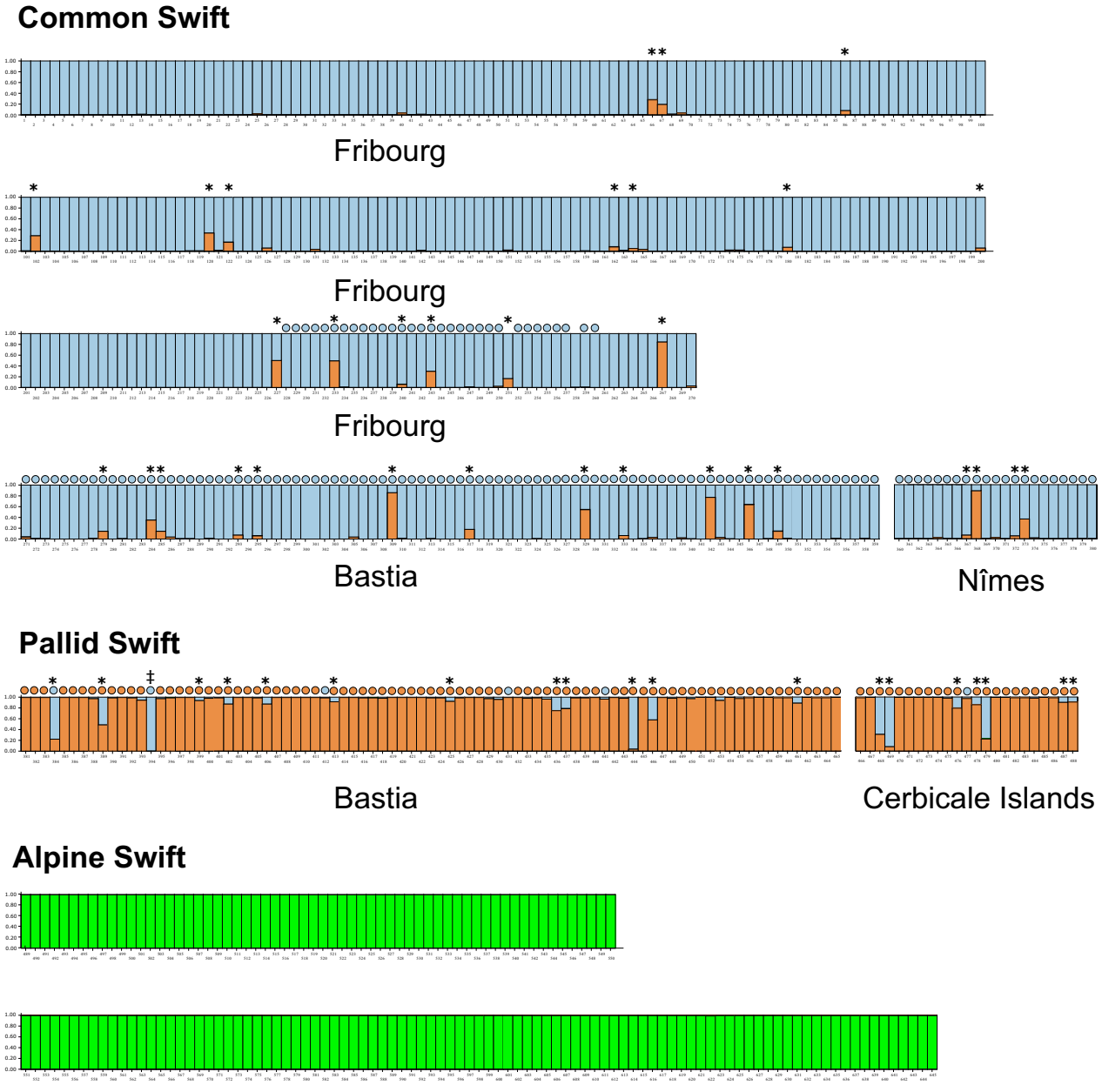


**Figure 4.** Evidence for introgression between Common Swifts (blue) and Pallid Swifts (orange); Alpine Swifts (green) showed no trace of introgression. Bars indicate nuclear markers ancestry proportions between 0 and 1 (Strcuture analysis with $K = 3$). Circles show the mtDNA haplogroups for the Mediterranean individuals and a selection of birds from Fribourg. Asterisks indicate individuals that are introgressed ($P < 0.95$). ‡Indicates the mummified individual PA04 that was identified genetically as a Common Swift.

axis, due to the high level of genetic differentiation between the Alpine and the Common/Pallid Swifts (Supporting Information Fig. S2A). When considering only Pallid and Common Swifts, the relative information of the second axis increased, showing genetic differentiation within Common Swifts, between the Fribourg and Mediterranean populations (Fig. S2B). The Adegenet analysis with four clusters (Fig. 5), focusing on the Mediterranean dataset, showed similar results to the Structure analysis but with a lower estimate of individuals having mixed ancestry (i.e. with a $P > 5\%$) in Bastia: 5.6% (5/89) of Common Swifts and 5.9% (5/85) of Pallid Swifts (excluding the 'Pallid' individual PA04,

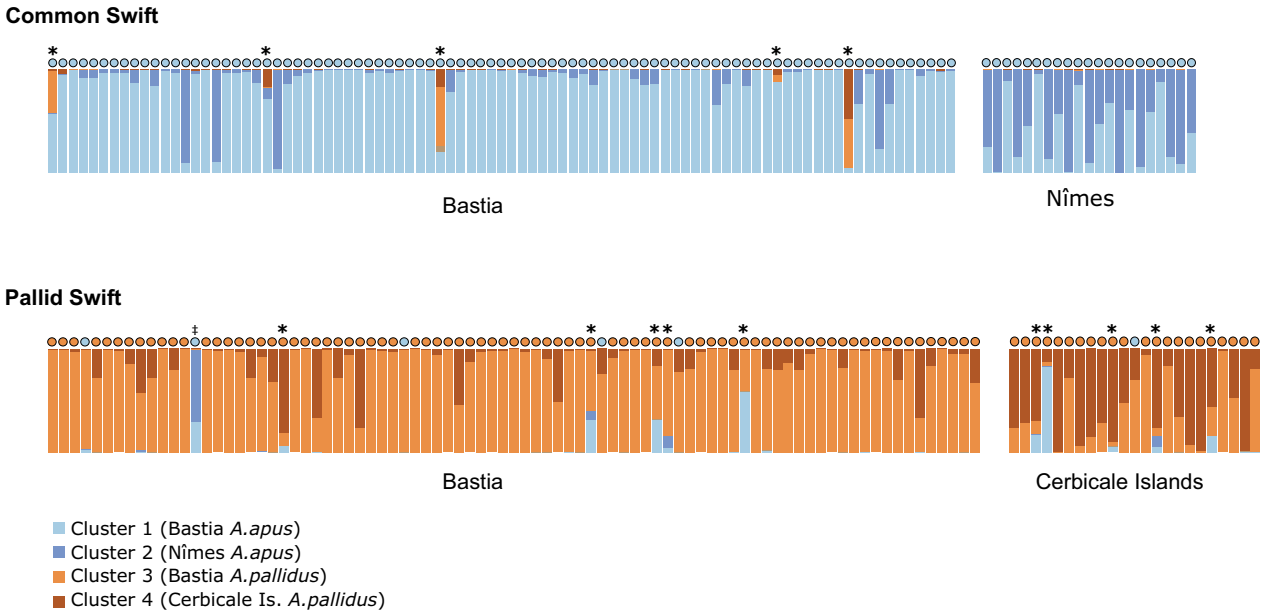


**Figure 5.** Evidence of introgression between Common Swifts (shades of blue) and Pallid Swifts (shades of brown) for the Mediterranean dataset. Bars indicate nuclear marker ancestry proportions (Adegenet analysis with $K = 4$). Circles show the mtDNA haplogroups. Asterisks indicate individuals that showed introgression ($P < 0.95$). ‡Indicates the mummified individual PA04 that was identified genetically as a Common Swift.

which was again genotyped as a Common Swift). For Cerbicale Islands the result was similar to the Structure analysis, with 21.7% (5/23) of the sampled individuals having mixed ancestry. The situation for Nîmes is inconsistent, with no hybrids detected when analysing the data with Adegenet, whereas three individuals are identified as hybrids in the Structure analysis.

When analysing the Mediterranean dataset ($n = 216$), the parental pool (i.e. the individuals having a $P > 95\%$ in the Adegenet analysis) represented 202 individuals, with 15 individuals characterized as mixed ancestry ($P < 95\%$). We simulated F1, F2 and backcrosses from the parental pool, and evaluated for each of the mixed ancestry individuals the most likely class among the six early generation diploid hybrid genotypes (parents, F1, F2 and backcrosses). The results provided in Table 2 indicated that 28.5% (4/14) of the mixed ancestry individuals were F1, all found in Bastia (two adults and one juvenile), with the exception of one individual found sampled in the Cerbicale Islands (one chick). These F1 individuals were predominantly (3/4) morphologically identified as Pallid Swifts. The remaining individuals (adults, juveniles or chicks) were all classified as backcrosses. None presented discrepancies regarding their mtDNA haplotypes, and four of them presented an ancestry proportion $P > 0.95$ in the Structure analysis.

## DISCUSSION

In the present study, we showed that Common and Pallid Swifts are genetically differentiated species due to most individuals being unambiguously assigned to one or the other species based on multi-locus nuclear genotypes and mitochondrial haplotypes. Our result confirms those obtained by Pellegrino *et al.* (2017), though with a larger sample size. However, a proportion of individuals showed evidence of mixed ancestry between Common and Pallid Swifts, thus providing evidence for gene flow between the two species. This introgression seems to be promoted by the occurrence of mixed-species colonies and propagated by unnoticed dispersal of hybrid individuals between monospecific colonies.

### Post-glacial expansion and dynamics of introgression

Common and Pallid Swifts are notably difficult to identify, vocalizations being often the best

**Table 2.** Estimation of the most likely class for the mixed ancestry individuals (Best class). *S* is the hybrid index and *H* the intertaxon heterozygosity. Individual PA34 had considerable missing data and was excluded from these analyses. Chick = non-flying individual sampled in the nest (downy stage); juvenile = feathered individual found outside the nest; adult = 1 year old or older, prospecting or breeding; unknown = mummified individuals.

| Sample | Age | Species | Locality | *S* | *H* | Best class | mtDNA | Structure*P* (*apus/pallidus*) |
|---|---|---|---|---|---|---|---|---|
| AP108 | Juvenile | *A. apus* | Bastia | 0.50 | 1.00 | F1 | *apus* | 0.850/0.149 |
| PA13B | Adult | *A. pallidus* | Bastia | 0.44 | 0.87 | F1 | *pallidus* | 0.124/0.876 |
| PA27 | Adult | *A. pallidus* | Bastia | 0.50 | 1.00 | F1 | *pallidus* | 0.204/0.790 |
| PA86 | Chick | *A. pallidus* | Cerbicale | 0.51 | 0.85 | F1 | *pallidus* | 0.688/0.311 |
| AP01B | Unknown | *A. apus* | Bastia | 0.59 | 0 | backcross | *apus* | 0.957‡/0.042 |
| SP03 | Unknown | *A. apus* | Bastia | 0.56 | 0 | backcross | *apus* | 0.996‡/0.004 |
| AP42B | Adult | *A. apus* | Bastia | 0.38 | 0 | backcross | *apus* | 0.137/0.862 |
| AP77 | Juvenile | *A. apus* | St Florent[a] | 0.28 | 0 | backcross | *apus* | 0.228/0.771 |
| PA19 | Adult | *A. pallidus* | Bastia | 0.37 | 0.37 | backcross | *pallidus* | 0.039/0.961 |
| PA26 | Adult | *A. pallidus* | Bastia | 0.37 | 0 | backcross | *pallidus* | 0.247/0.752 |
| PA87 | Chick | *A. pallidus* | Cerbicale | 0.44 | 0 | backcross | *pallidus* | 0.917/0.082 |
| PA93 | Chick | *A. pallidus* | Cerbicale | 0.26 | 0.51 | backcross | *pallidus* | 0.020/0.980‡ |
| PA97 | Chick | *A. pallidus* | Cerbicale | 0.64 | 0 | backcross | *pallidus* | 0.771/0.228 |
| PA102 | Chick | *A. pallidus* | Cerbicale | 0.25 | 0 | backcross | *pallidus* | 0.019/0.980‡ |
| PA34 | Adult | *A. pallidus* | Bastia | – | – | – | *pallidus* | 0.961/0.038 |

[a]Locality near Bastia. ‡Indicates individuals with $P > 0.95$ in Structure.

criterion (Reyt & Duquet 2020). Hybrids are probably not identifiable based on phenotypic traits. Indeed, all recent and late-generation hybrids were unambiguously assigned to Common or Pallid Swifts in the field, based on plumage patterns (Chantler & Driessens 2000) and/or wing formula (G. Gory pers. obs.). Observers never reported a mixture of phenotypic traits suggestive of a hybrid origin. Genotyping at nuclear markers is thus the only tool available to reliably identify individuals with hybrid origin and to track the dynamics of introgression between the two species.

Our results provided evidence for gene flow between the two taxa and suggested that introgression was not limited to sympatric colonies in urban settings. Most individuals with mixed ancestry were found in Bastia (northern Corsica), where the two species co-occur, but hybrids were also found in the Cerbicale Islands (southern Corsica), where only Pallid Swifts breed at natural sites, and in Fribourg (Switzerland), where only Common Swifts are breeding. Clustering analyses in Nîmes (Gard), where only Common Swift was known to breed, suggested some levels of introgression when considering two genetic clusters (one for each species), but no introgression when accounting for genetic differentiation between localities within species ($K = 5$). This result suggested that even low levels of genetic differentiation between colonies within species, if not implicitly modelled in clustering analyses, may lead to erroneous estimation of individual proportion of mixed ancestry and level of introgression between species. Selecting the most likely number of genetic cluster ($K$ = number of species) would have resulted in overestimating the level of introgression between species in the present study. Based on this observation, we strongly advise carefully describing and interpreting the output of clustering analyses in the light of current knowledge on the biology and ecology of the species, setting the number of genetic clusters from one up to the number of sampling locations, or more if prior knowledge suggests sub-structuring within localities. Alternatively, because the admixture estimations were similar using both Structure and Adegenet for the three remaining populations in Bastia and in the Cerbicale Islands, this contradiction might also result from the lack of samples from the closest Pallid Swift population on the French Riviera. A denser sampling in the area might be required to untangle these alternatives.

Most hybrids in the Mediterranean region showed characteristics of being backcrossed, supporting the idea that introgression between the two species may be associated with their range expansion since the LGM. This hypothesis is supported because individuals with mixed ancestry were also found in western Switzerland. However,

three individuals in Bastia and one from the Cerbicale Islands showed characteristics of recent-generation hybrids (i.e. F1). The Cerbicale Islands are only 10 km from the closest colonies of Common Swifts on the island of Corsica, and our results suggest that swift colonies on Cerbicale are not genetically isolated from other colonies. This finding is not surprising given that the mobility of swifts around colonies can exceed 30 km (Wellbrock *et al.* 2018). Because mixed-pairs have only been observed once in well-monitored breeding colonies (Oberli *et al.* 2013), it is also possible that introgression occurs partly or mainly through extra-pair copulations, thus preventing direct observations, as suggested in the study of a Common Swift colony in Oxford, UK (Martins *et al.* 2002). However, the predominance of F1 hybrids in Bastia can be seen as evidence that hybridization mainly occurs within the town, where the two species live in close proximity. By providing sufficient breeding sites and sustaining high densities of both species, urban areas could be seen as facilitators or catalysts of hybridization, and ultimately introgression between the two species.

## Asymmetrical introgression

We detected higher levels of introgression of Common Swift into Pallid Swift, including six individuals showing Common Swift mtDNA capture, but no Common Swifts with Pallid Swift mtDNA. We collected more than 200 DNA samples of each species in localities where one or both species bred, which allowed us reliably to estimate the genetic diversity in the two species. We can safely exclude that the observed pattern results from a sampling bias. In Europe, the Common Swift meta-population is estimated at 38–65 x $10^6$ mature individuals, i.e. 300 times larger than the Pallid Swift population, estimated at 12.6–21.3 x $10^4$ mature individuals (BirdLife-International 2015). This trend is reversed in North Africa, where the Common Swift is less abundant than the Pallid Swift in natural and urban populations (Isenmann & Moali 2000, Thévenot *et al.* 2003, Isenmann *et al.* 2005). Because of larger population size, Common Swift populations tend to retain higher levels of genetic diversity (allelic richness) compared with Pallid Swift populations (Table S2), thus decreasing the probability of identifying introgression of the rare Pallid alleles into Common Swift, as shown when the two populations in contact have a marked difference in abundance (Cianchi *et al.* 2003). Additionally, differences in productivity between the two species may partly explain expansion of Pallid Swift at local (urban areas) and regional scales, which in turn leads to asymmetrical introgression. Common Swifts usually have a single clutch (Cramp 1985 but see Gory 2009), whereas Pallid Swifts usually lay two clutches in urban sites and one in natural sites (G. Gory pers. obs., Boano 1979, Thibault *et al.* 1987, Finlayson 1992). Expanding populations are more likely to capture genes, and in particular mitochondrial genes, from the local, stable population, than for such introgression to occur in the opposite direction, mainly because of a difference in density at the front of the expansion wave (Currat *et al.* 2008, Toews & Brelsford 2012). Our observation that mtDNA capture by Pallid Swifts was always recovered for individuals that did not show nuclear introgression would be consistent with a recent expansion of this species, if the increase noted in urban areas in the Mediterranean and Eastern Europe reflects real population growth and not an observational bias, as suggested by Keller *et al.* (2020).

A recent expansion could also be linked to differences in dispersal and philopatry between the two species. Most studies on European swift species have recovered high philopatry to the colony in which an individual was born (Lack 1951, Boano *et al.* 1993), although Pallid Swifts showed the lowest levels of mate and nest fidelity, and of survival rate among European Swift species. More pronounced exploratory behaviour in Pallid Swifts may induce the observed differences in survival and philopatry (trend towards lower levels of $F_{IS}$; Table S2). Indeed, Pallid Swifts' aptitude to disperse was observed locally in Bastia where new buildings were colonized more readily by Pallid Swifts than by Common Swifts, with Common Swift colonies dominating buildings in the old town (Thibault *et al.* 2022). Where the two species breed in close proximity, individuals regularly visit colonies of the other species, as indicated by a dead Common Swift found in a Pallid Swift colony on a building (PA04, Figs 4 and 5) as well as at natural sites (Lavezzi Islands, Corsica; Gory 2004–2005). A male Pallid Swift was observed breeding in a Common Swift colony in the Swiss Jura Mountains, 140 km from the nearest Pallid Swift

colony (Oberli *et al.* 2013) and a ringed Pallid Swift of unknown sex was recaptured in two consecutive years in a Common Swift colony in Morocco (Pineau & Giraud-Audine 1979).

Small numbers of Pallid Swifts either isolated or within a group of Common Swifts are probably overlooked by the vast majority of amateur bird watchers because the two species are so hard to separate based on morphology. Indeed, only five observations of Pallid Swifts have been reported outside of the distribution range in Switzerland since 2001, all by experienced ornithologists (see also Reyt & Duquet (2021) for observations in France). Hybrids can hardly be assigned to Common or Pallid Swifts based on morphological traits and are most probably not identifiable in flight and are overlooked in Common Swift colonies. Thus, the presence of individuals showing different levels of mixed ancestry reflects introgression between the two species, probably the consequence of hybrids dispersing cryptically from localities where the two species co-occur. However, the reporting of a mixed pair in the Swiss Jura Mountains (Oberli et al., 2013), outside of the Pallid Swift distribution range, suggests that rare long-range dispersal events may also promote Pallid genome introgression into distant Common Swift populations.

### Species recognition in multispecies colonies

Both species are stable or increasing at urban sites, suggesting that these habitat preferences and behavioural traits may be beneficial, favouring shared defence strategies against predators (Jungwirth *et al.* 2015) and social stimulation (Darling 1938, Waas *et al.* 2005). Yet, Common and Pallid Swifts are genetically differentiated, implying that species isolation is maintained by phenotypic differences between the two sister-species with behavioural or morphological traits acting as pre-zygotic barriers and decreasing the probability of hybridization, and/or post-zygotic barriers decreasing the viability of hybrids (Weber & Strauss 2016). The occurrence of several recent hybrids and backcrosses, adults or juveniles, suggests that post-zygotic barriers, if they occur, are not the primary factor maintaining species isolation. J.-C. Thibault *et al.* (unpubl. data) inferred from a literature search and observations in mixed colonies in Bastia and Nice that both species use similar structures as nesting sites in buildings (e.g. tiles, eaves or roller shutter boxes) and that preferences for nesting structures varied between sites but not between species, suggesting that nesting structures play no major role in species isolation through assortative mating in swifts. Isolation between species from nestling diets, acoustic and foraging behaviour (Cucco *et al.* 1993), breeding biology (Boano & Cucco 1989) or differences in dates of arrival at breeding sites have also been proposed (Päckert *et al.* 2012), although which species return first from winter migration differs among locations (Lardelli 2014, J.-C. Thibault *et al.* unpubl. data). Which traits are involved in species recognition remains unanswered, yet we could speculate that flight calls, a criterion used by ornithologists to differentiate between the two swift species, may play an important role in mate choice. More observations and studies are required to determine whether species isolation could result from a mismatch between species in their timing of migration, breeding period, courtship timing or characteristics of display flights.

## CONCLUSION

Our results revealed a significant level of introgression between Common and Pallid Swifts, a phenomenon that was detectable in a Swiss population of Common Swift located more than 100 km north of the edge of the Pallid Swift distribution range. Clearly, the proximity of both species at urban sites favours hybridization, as shown by the presence of recent hybrids in the town of Bastia. Extending the spatial scale of the study by sampling colonies in urban and natural sites at the southern and northern margins of the species' range will be required to confirm this result. Similarly, European Starling *Sturnus vulgaris* and Spotless Starling *Sturnus unicolor*, two sister species that experienced post-glacial range expansion (Zuccon *et al.* 2008) and breed in sympatry in several towns in the Mediterranean area (Motis 1992), may provide a valuable system to study the consequence of the use of urban sites on the frequency of hybridization and spatial scale of introgression between closely related species.

In Corsica, we are grateful to the people and organizations who facilitated our access to the buildings where swifts were nesting or entrusted chicks that had fallen from the nest or were injured, in particular: Chantal

Belgodere, Stéphane Japet, Cécile Jolin, Arnaud Lebret and Elisabeth Stodd. Antoine Leoncini and Jean-Marc Pons helped us with the captures at the Sainte-Claire wall. Véronique Vancoillie coordinated our project with the town of Bastia. To all of them, we address our warmest thanks. We are grateful to Tommy Andriollo for providing useful R scripts. Comments by Gary Voelker (Associate Editor), Rauri Bowie (Editor) and two anonymous referees greatly improved the manuscript.

## AUTHOR CONTRIBUTIONS

**Alice Cibois:** conceptualization (equal); data collecting (equal); formal analysis (equal); writing – original draft (lead); writing – review & editing (lead). **Michel Beaud:** data collecting (equal); writing – review & editing (supporting). **Francesco Foletti:** data collecting (equal); writing – review & editing (supporting). **Gérard Gory:** data collecting (equal); writing – review & editing (supporting). **Gwenaël Jacob:** data collecting (equal); Formal analysis (equal); writing – original draft (equal); writing – review & editing (equal). **Nathalie Legrand:** data collecting (equal); writing – review & editing (supporting). **Ludovic Lepori:** data collecting (equal); writing – review & editing (supporting). **Christoph Meier:** data collecting (equal); writing – review & editing (supporting). **Antoine Rossi:** data collecting (equal); writing – review & editing (supporting). **Peter Wandeler:** data collecting (equal); writing – review & editing (supporting). **Jean – Claude Thibault:** conceptualization (equal); data collecting (equal); writing – original draft (equal); writing – review & editing (equal).

## CONFLICT OF INTEREST

None.

## PERMITS

Handling and sampling in Bastia were conducted under permits issued by the DREAL de Corse and delivered by the Préfet de la Haute-Corse, numbers 02B/0004/2016/02 (2016–2017) and 2B-2018-06-13-00 (2018–2019). Permits for handling, sampling and banding in the Cerbicale Islands and in Nîmes were issued by the *Centre de Recherches sur la Biologie des Populations d'Oiseaux* (CRBPO, France) (G. Gory pers. programme), and in Fribourg by the Service de la sécurité alimentaire et des affaires vétérinaires du canton Fribourg SAAV, number 2016_22_FR (2016–2018).

## FUNDING

None.

## Data Availability Statement

Raw data are provided in Table S2.

## ETHICAL NOTE

None.

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Received 7 July 2021;
Revision 13 November 2021;
revision accepted 7 May 2022.
Associate Editor: Gary Voelker

## SUPPORTING INFORMATION

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

**Figure S1.** Evanno's Delka *K* plotted over *K*, the number of clusters.

**Figure S2.** Discriminant analysis of principal components (DAPC) with (A) all populations (six clusters) and (B) the Common and Pallid Swift populations (five clusters).

**Table S1.** Sample information

**Table S2.** Allele scores

**Table S3.** Basic summary statistics for the populations of Common Swift (Fribourg, *Bastia_apus* and Nîmes), Pallid Swift (*Bastia_pallidus* and Cerbicale) and Alpine Swift (Baden).

**Table S4.** Levels of population pairwise genetic differentiation (Nei's $F_{ST}$, Nei 1986) were ≤ 0.0125 within species (highlighted in bold), in the range 0.04–0.05 between *Apus apus* and *A. pallidus* and ≥ 0.2 between *Apus* sp. and *Tachymarptis melba*.