



MIGRATION STRATEGY OF THE ORTOLAN BUNTING FINAL REPORT OF THE SCIENTIFIC COMMITTEE

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ABSTRACT

During five years, we conducted a research study to identify the migration flyways of European breeding ortolan buntings (*Emberiza hortulana*). We aimed at determining the numbers and trends of populations using each flyway, their stopover sites and wintering grounds. We also aimed at identifying the origin of ortolans migrating by Les Landes (south-western France), with a special focus on the large Russian population. Thanks to data from light loggers, genetics and stable isotopes analyses, we were able to identify two main migration flyways for European ortolan buntings (eastern and western), while light loggers also suggested the existence of a third flyway (central).

The eastern flyway is used by breeding populations of Russia and of all countries east of a migration divide running from Belarus to Serbia (included). This eastern flyway is thus used by ca. 4,283,250 pairs (range 2,907,250 to 6,310,500 pairs), representing 90% of European ortolan buntings, with a recent (2000-2014) population trend estimated between -10% and -20%. These birds winter on Ethiopian and Eritrean Highlands. The main contributing countries to this flyway are Russia, Turkey, Romania, Bulgaria, and Azerbaijan.

The western flyway is used by birds breeding west of a migration divide in central Europe, and wintering in western Africa (Sierra Leone to southern Mauritania and Mali, mainly in Guinea). It concerns approx. 468,700 breeding pairs (354,622 to 619,479), representing 10% of the European population size. The recent trend of these populations is estimated between -10% and -20% (2000-2014). The main stopover sites of these migrants are located in Spain and northern Morocco, south-west France being a fly-by area along the autumn migration route for a part of them. Indeed, along the western flyway, we can distinguish two different routes. The more western is the breeding population, the more Atlantic is its migration route. 'Eastern' breeders (e.g. almost all Finnish and Baltic ortolans) use a continental/ Mediterranean route, flying over eastern Poland to northern Italy, then following the Mediterranean coast of France and Spain; they do not visit south-west France. 'Western' breeders (e.g. most Swedish, all German and probably all Norwegian ortolans) use an Atlantic route, entering France from Département Nord to Alsace and crossing the Pyrénées at their western end – flying over south-west France.

We estimated that 81,000 breeding pairs (range 46,000 – 116,000) use the Atlantic route, depending on scenarios on proportions of some populations using the continental or Atlantic routes. Among these, approx. 75% come from Poland. The recent trend (2000-2014) of these populations is estimated between -20% and -30%. This means that currently, each year, this breeding population decreases by an average 1,500 pairs (e.g. 3,000 spring mature individuals). Birds breeding in Russia do not use the western flyway, only the eastern.

Finally, an unknown part of populations breeding west of the migration divide use a third, central flyway, reaching Africa from Italy to Greece south to Tunisia or Libya, to further winter in Nigeria. Some individuals from Poland and Finland use this flyway, which might be the normal flyway for populations breeding in Italy, Slovenia, Croatia and Bosnia, also Slovakia, Austria and Hungary. This third flyway is used by some central European birds, including part of the Polish and Finnish populations, hence the real proportion of Polish birds using the Western flyway, thus possibly the Atlantic route, could be lower than proposed in the middle scenario here, hence impacting the global estimate for this flyway and that route.



INTRODUCTION

In the context of the protection status of the Ortolan Bunting, listed in Appendix I of the Birds Directive, and the ongoing traditional hunting of the species during the autumn migration in south-western France, the French Ministry in charge of Environment asked the National Museum of Natural History, helped by the ONCFS (French Hunting & Wildlife National Office) to propose guidelines to conduct research to unravel the migration strategy of the species at the continental scale. These guidelines have been validated by all present stakeholders, including the hunters and the local BirdLife partner.

The main objectives of the research had been listed in the guidelines:

- Identifying the main flyways and wintering grounds of the different European breeding populations.
- Determining the origin, numbers and trends of ortolan buntings migrating over south-west France.
- Identifying the main stopover sites during the autumn and spring migration on each flyway.
- Determine the importance of breeding ortolan bunting populations from Russia and Belarus within the migrants visiting south-west France.

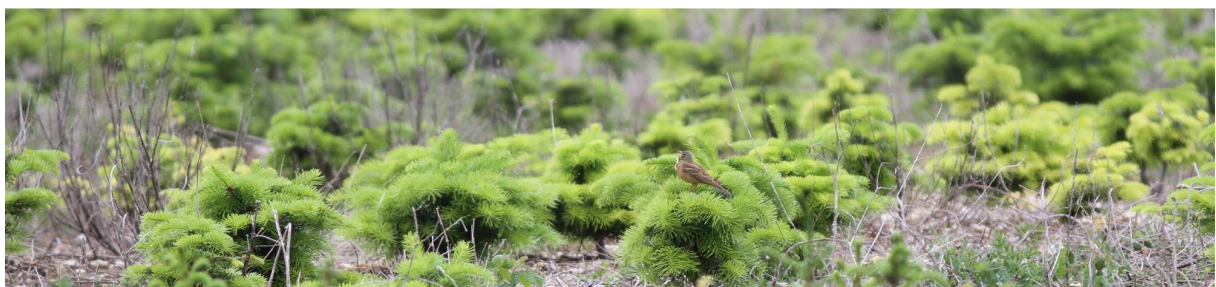
To obtain the necessary results, this study used three main tools: light loggers, stable isotopes and genetic variability. Field work was conducted in various countries on breeding populations (Spain, France, Serbia, Greece, Russia, Belarus, Finland, Sweden, Norway, Estonia, Lithuania, Poland, Germany) or on migration sites (France, Kuwait, Israel).

Light loggers are small electronic devices, weighing less than 0.8g and fixed on the bird's back with light strings. They record the light intensity which if retrieved enables the calculation of the approximate position of the logger given the duration of the day and the time hour of the sun peak, both depending on latitude and longitude for a given year's day.

Stable isotopes are variation of a same atom but with different numbers of neutrons (but same numbers of protons and electrons). The most known are the ^{12}C and ^{14}C used e.g. for dating organic materials. Bird feather have deuterium concentrations (stable isotope of hydrogen) proportional to concentrations of the rainwater, which have a defined continental latitudinal pattern in structure. Studying the deuterium concentration in feathers helps to identify the probabilistic origin areas where the feathers can have grown (in the nest for juveniles or during moult) – in fact, it identifies the geographic areas where the isotopic signatures in the environment are similar to those where the feathers have grown.

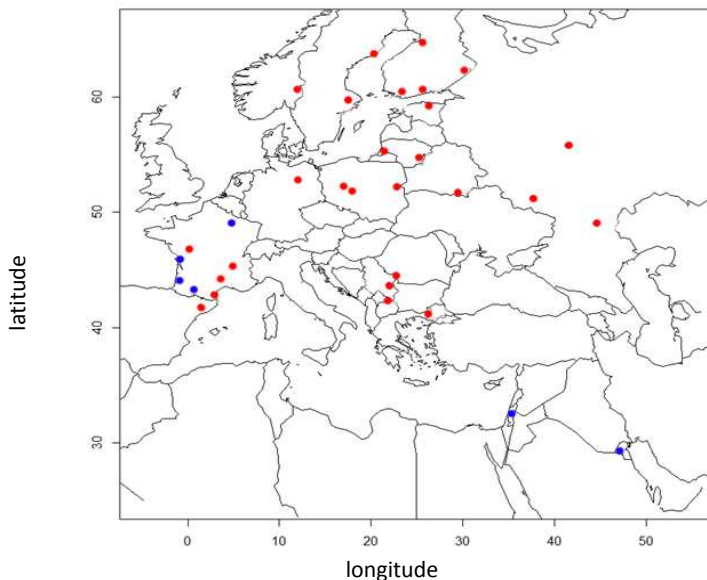
Finally, by mapping the genetic diversity of ortolan breeding populations across the continent, from Spain to eastern European Russia, and genotyping migrants captured and released in south-west France and in Kuwait, we further assigned these migrants to potential breeding areas.

The combination of these three different complementary approaches allowed the clear identification of an eastern flyway and a western flyway, with a migration divide running across central Europe.



I. STUDY SITES, MATERIALS AND SAMPLES

1) Study sites where we operated



By mobilizing colleagues across Europe, we were able to study breeding populations in various countries including Spain, France, Germany, Poland, Serbia, Greece, Belarus, Lithuania, Estonia, Finland, Sweden, Norway and Russia (three sites: Belgorod near Ukraine, Volgograd near Kazakhstan, and Vladimir north-east of Moscow). The following figure maps the sites where ortolan buntings have been sampled: red plots for breeding populations, blue dots for migrants.

2) Feathers we sampled

On each ortolan bunting captured on breeding grounds, we took a few body feathers or scapulars, and one single tail feather. Body coverts are moulted twice each year: once on the breeding grounds before the autumn migration, and once on the wintering grounds before the spring migration (to acquire the full breeding plumage). Hence, body coverts of territorial adults have been moulted on the wintering grounds, hence have an isotopic signatures of the wintering grounds. The tail feather provided DNA material for the genetic analysis, while the feather tip holds the isotopic signatures of where the feather has grown.

Hence, we sampled a single tail feather on the migrant ortolans we captured in autumn, to obtain DNA material (bases of the feather) and the isotopic signature of where the feather has grown in the nest (for first-year birds) or has been moulted before migration (for adults). We collected also body coverts on spring migrants captured in Israel and Kuwait.

3) Loggers we fitted

Light loggers were fitted on territorial males, as they needed to be retrieved to download the recorded light data – and territorial males have a higher probability to come back and hold a territory the next year. We used mainly loggers provided by the society Migrate Technology (UK), called Intigeo P65C2-7 (weight 0.74g), but also loggers from the Swiss Ornithological Institute in Switzerland (SOI-GL05.10, weighing 0.5g). Loggers were fixed with leg loops in UV-proof strings.



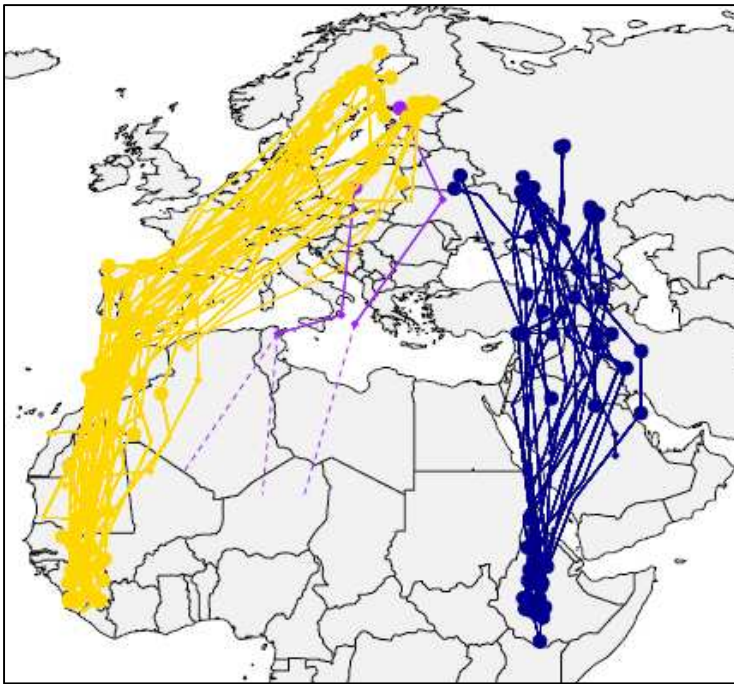
Left: male “white-white”, captured 16 May 2015 north of Volgograd, eastern European Russia; logger visible on its back (photo F. Jiguet). Right: same male recaptured 25 May 2016, logger retrieved (photo Julien Laignel).

II. THREE MIGRATION FLYWAYS

Results from geolocators, genetics and stable isotopes are all congruent to identify two main flyways used by the studied breeding populations, with a third flyway used by two individuals only, though probably used by populations breeding in central /south-central Europe.

1) A clear east/west migration divide revealed by geolocation data

We retrieved 13 Swiss loggers and 13 British loggers which contained data, retrieved in 2014 (1), 2015 (1) and 2016 (24). Our Swedish colleagues further provided data collected by 9 British loggers they retrieved in 2014 and 2015, providing 11 tracks as 2 males were tracked during 1.5 years. Petra Bernardy also shared information on 16 tracked ortolan buntings from Germany in 2014 and 2015.



The adjacent figure presents the autumn and spring tracks obtained with all British and Swiss loggers (partial tracks, in purple), identifying very clearly two main migration flyways (in blue and yellow) used by the studied breeding ortolan buntings: an eastern flyway, concerning all tracked individuals from the three study sites in Russia (Vladimir 1, Belgorod 5, Volgograd 5), and one individual tracked from Belarus. All individuals using the eastern flyway made stopovers in the Caucasus and the Middle East in autumn (from Georgia to northern Syria and northern Iran), while spring stopovers were located further south in the Middle East and north part of the Arabian Peninsula. They all spent the winter on the Ethiopian plateau in Ethiopia and Eritrea. None of these 12 tracked individuals used a western flyway.

The loggers retrieved in the following countries used a western flyway: Spain (2), France (1), Lithuania (1), Poland (1), Norway (1), Sweden (9), Finland (6), Germany (16). Along this flyway, main stopover areas are located in Spain and northern Morocco in autumn. They all winter in West Africa, from Sierra Leone to southern Mauritania, in an area centered around Upper Guinea.

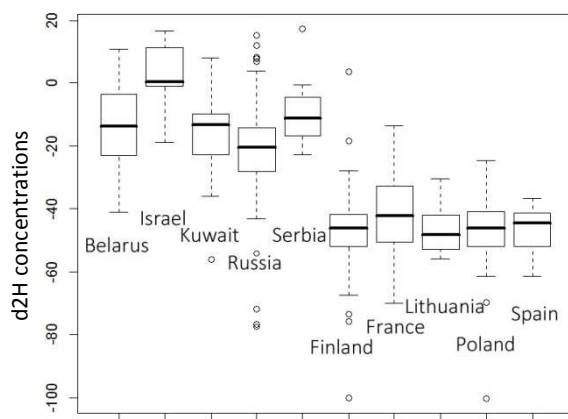
Along the western flyway, two main routes are used in autumn. Birds from eastern populations migrate over eastern Poland, central Europe to northern Italy then follow the French and Spanish Mediterranean coasts to reach Africa. This route was followed by all tracked individuals from Finland using the western flyway, and the Lithuanian individual. From Sweden, about a quarter of the tracks used the Mediterranean route. Birds from more western populations use what we will call an Atlantic route, entering France by the east or the north, and reaching Spain by the western part of the Pyrénées – thus flying over Les Landes. This route concerned most tracks obtained from birds of Sweden (8 out of 11), all individuals tracked from Germany, an unknown but probably small part of birds from Poland (as 1 out of 40 birds equipped with a logger in western Poland in May 2014 was captured by a hunter in Les Landes in September 2014, but the two tracks obtained with geolocators did not use the Atlantic route), but no individuals from Finland or the Baltic states.

Finally, two individuals (one from west Poland, and one from south-west Finland) started to use a third flyway, which we will refer to as 'central', with tracks stopping near Libya when their loggers stopped recording light intensity. They were likely on their way to the known wintering grounds of the species in northern Nigeria (see isotopic assignment to winter range).

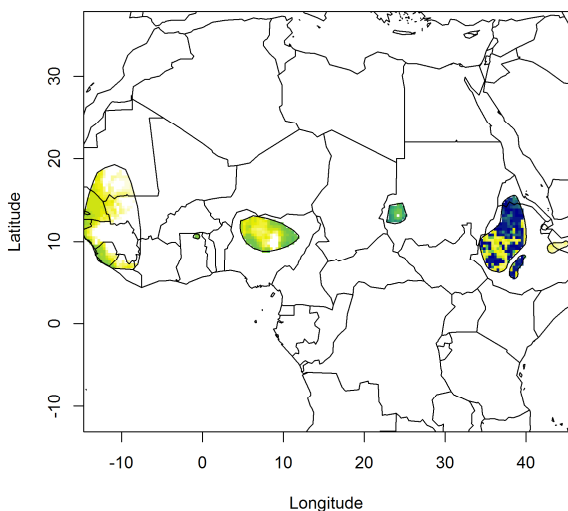
2) The west/east migration divide confirmed by stable isotopes

The analysis of deuterium concentration from body feathers collected on breeding grounds – body coverts moulted on the wintering grounds – revealed two distinct groups of birds.

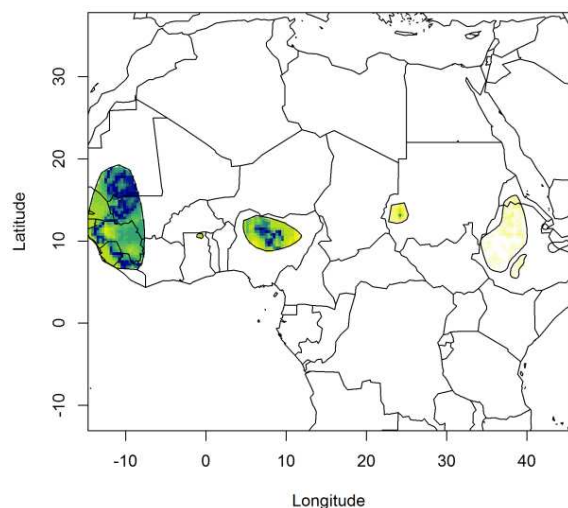
A further assignment to origin, performed using the known winter range of the species in sub-Saharan Africa, allows identifying the areas where these feathers might have grown. The first group corresponds to birds having moulted in eastern Africa (Ethiopia and Eritrea), the second group to birds having moulted in Western Africa or in northern Nigeria. Clearly, birds having moulted in East Africa have used the eastern flyway, birds having moulted in West Africa have used the western flyway. Birds using the central flyway could probably winter in Nigeria, where the isotopic environment is similar to that in West Africa.



Boxplot of average deuterium concentrations in body coverts / scapulars of spring and breeding individuals, by country. Two groups are identified: individuals with high concentrations of deuterium (Belarus, Russia, Serbia, and spring migrants captured in Israel and Kuwait, and individuals with a medium low concentration (Spain, France, Poland, Lithuania, Finland). ANOVA for the two groups, $t=-25.4$, $df=539$, $P<0.0001$



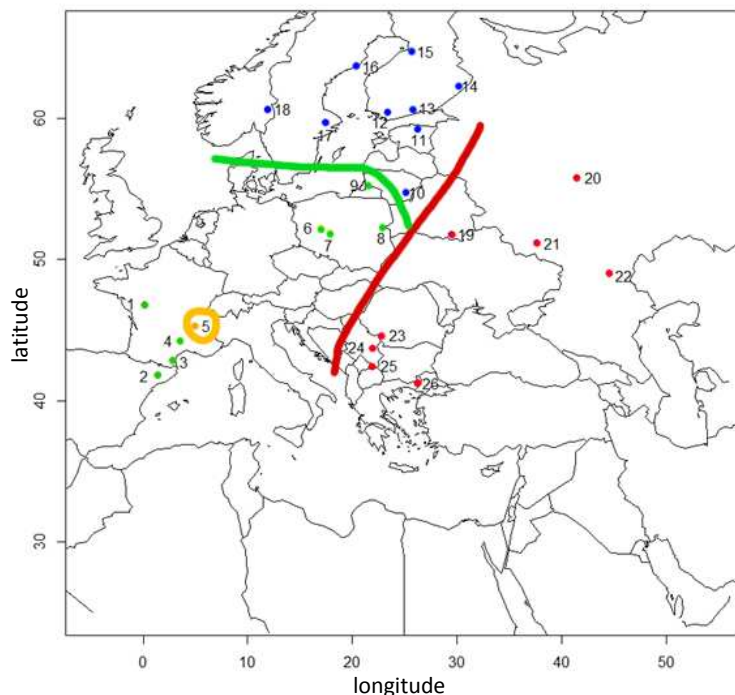
Probabilistic areas where feathers of 297 individuals from Belarus (43 individuals), Russia (three sites, 192 individuals), Serbia (7), and spring migrants from Israel (10) and Kuwait (45) have been moulted during the winter. The highest probabilities are located within the winter range where Russian and Belarus individuals equipped with geolocators have overwintered (Ethiopia/Eritrea). Assignments obtained for only individuals breeding north-east of Moscow (13 individuals) or breeding in Serbia are very similar. For assignment maps by country of origin, see Appendix 2.



Probabilistic areas where feathers of 238 individuals from Finland (139 individuals), Lithuania (21 individuals), Poland (24), France (35) and Spain (19) have been moulted during the winter. The highest probabilities are located within the winter range where individuals equipped with geolocators and breeding in Germany, Norway, Sweden, Finland and Lithuania have overwintered (West Africa), but also in Nigeria. For assignment maps by country of origin, see Appendix 2.

3) The genetic structuration of breeding populations further delineates the migration divide

The genetic variability between the studied breeding populations was low (approx. 1.3%). This variability however allowed identifying two main genetic clusters: one in the North (Scandinavia and the Baltic states), and one in the South (from Spain to Russia); a third very localized cluster groups all individuals sampled in Drôme, France – an unexpected originality which was not due to cross-contamination or errors during DNA extraction, amplification and genotyping. Statistical models further developed to separate four clusters performed also well and further separated the southern cluster in two groups: an eastern group (with breeding populations of Serbia, Greece, Belarus and Russia) and a central/western group (with breeding populations of Spain, France except Drôme, Poland, and western Lithuania). The northern cluster then comprises the breeding populations sampled in Norway, Sweden, Finland, Estonia and eastern Lithuania.



The four clusters identified by the genetic structure of the breeding populations (575 individuals from 26 sites; microsatellites, 21 loci). The red line separates populations attributed to the eastern and western flyways. The green line separates the northern group and the west/central groups of the western flyway. The orange circle locates the Drôme population.

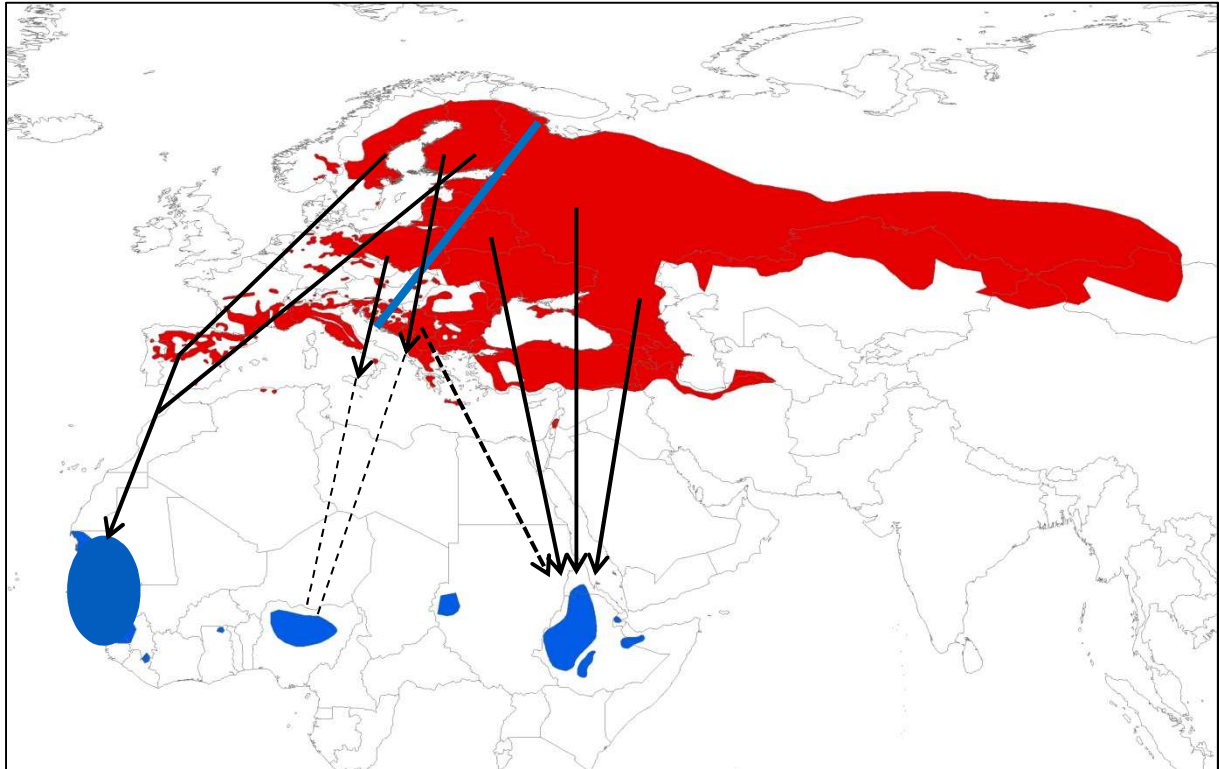
The 'eastern' genetic affinities of populations breeding in Greece and Serbia, and the isotopic assignment of Serbian birds to the wintering area in Ethiopia/Eritrea, further reveal that these populations use the eastern flyway. The red line on the above map therefore illustrates the west/east migration divide of European breeding ortolan buntings.

A ringing recovery in Les Landes, of an ortolan bunting ringed in Russia, is considered anecdotic. This bird had been ringed near Saint Petersburg, a region that has been colonized in the mid-20th century by ortolans originating from Finland (Karelia), but these northern Russian populations have undergone a dramatic decline (as in Finland and Estonia) and the species is now rare or absent from these Russian regions (see Appendix 5). Furthermore, this bird had been ringed at the end of August, during the autumn migration, so its breeding origin can't be known with certainty. Three further ring recoveries from Russia (two from Leningrad, one from Kaliningrad) have been obtained in Italy during the autumn migration, confirming that these former north-western Russian populations (birds ringed in 1971 and recovered in 1971, 1973 and 1973) migrated along the Mediterranean route of the western flyway, as do most birds from Finland nowadays. [Information from S. P Kharitonov fide M.A. Czajkowski].

4) Graphical summary

Three identified flyways for European ortolan buntings:

- Eastern flyway, with various eastern routes converging to the wintering grounds in Ethiopia
- Central flyway, but obtained tracks did not reach the wintering grounds, putatively in Nigeria
- Western flyway, with two different routes, Atlantic and continental/Mediterranean

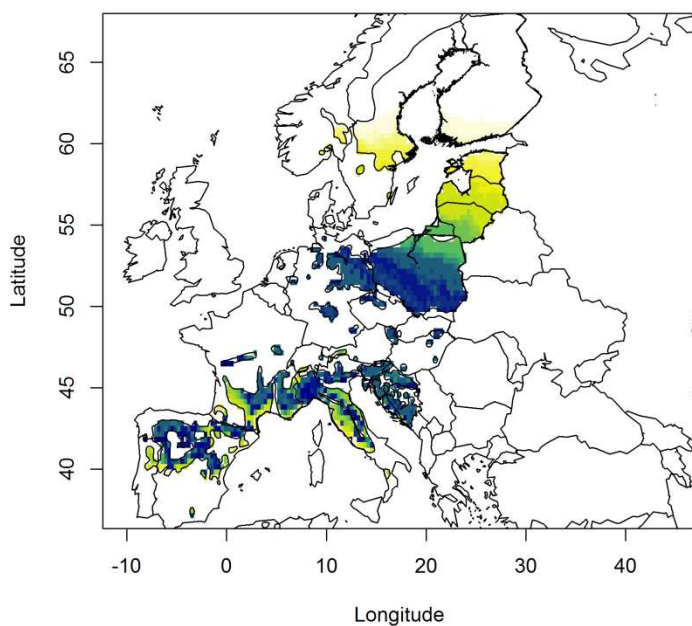


III. ORIGIN OF ORTOLAN BUNTINGS MIGRATING OVER SOUTH-WESTERN FRANCE

During the autumn (end of August and September) of 2012, 2013, 2014 and 2015, CRBPO ringers have been trying to capture migrating ortolan bunting in France along the western flyway. They captured, ringed, sampled (one tail feather for DNA and deuterium analysis) and released 40 individuals, mainly in Landes and Vendée (28), but also in Gironde (5), Haute-Garonne (4), Haute Marne (2), Charente-Maritime (1) and Pas de Calais (1) (see blue dots on Figure page 6).

To this sample, we added 34 first-calendar year birds seized by the police and held at the Alca Torda care center, which were sampled during ringing before being released in the wild. Other seized individuals, either birds of unknown status (decoys or wild) or known decoys (with plumage colour aberrations, or heavy papillomatoses on toes) were not considered in the isotopic analysis, as the deuterium concentration in their feathers is very low, as they do not drink rain water but tap water. The group of seized birds of 'unknown' status is a mix of wild birds and of decoys (with a majority of decoys, according to the distribution histogram of the deuterium concentrations in their tail feathers; see Appendix 2). However, for the genetic assignment, we found no differences in the distribution of the wild (40+33; DNA amplification and genotyping failed for one sample), attested decoys and individuals of 'unknown' status (chi-square test, $\chi^2 = 16.6$, $p > 0.16$), so we mixed all groups to obtain a more general picture (see Appendix 3).

Concerning the stable isotopes, the probabilistic assignment to origin was restricted to the countries with breeding populations using the western flyway, to avoid any misinterpretation of the maps.

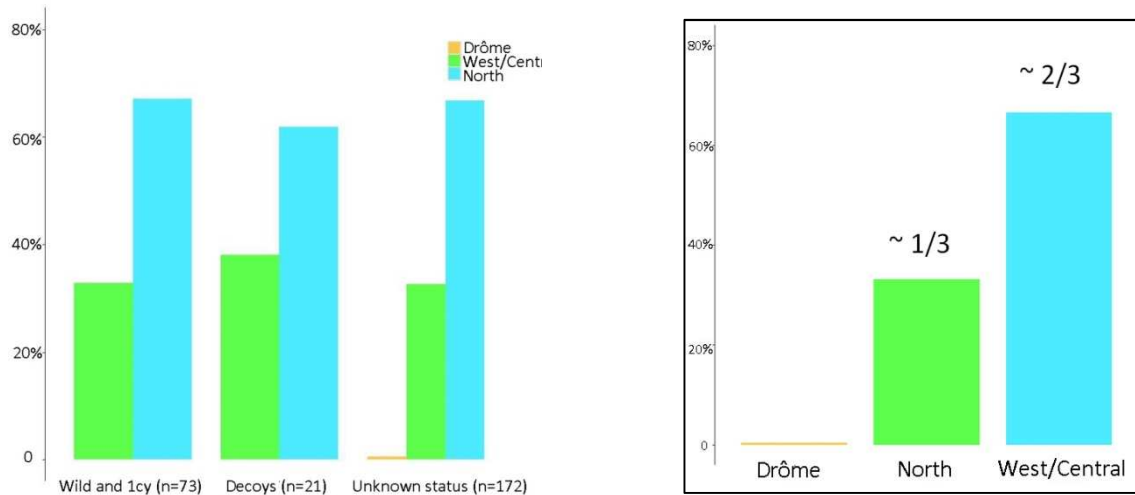


Assignment to origin of 74 migrant ortolan buntings captured in France during autumns 2012-2015. The most probable areas where their feathers have grown are in Poland, Germany and western France. Populations breeding around the Adriatic and in Mediterranean France probably use the Mediterranean route of the western flyway. In comparison, assignments to more northern countries (Scandinavia, Finland and Baltic states) was very much lower in probability, suggesting that these northernmost populations do not constitute a large fraction of our study sample. See Appendix 2 for comparisons of maps obtained for ringed birds only, and for decoys or all birds including decoys and birds of 'unknown' status.

This map means that ortolan bunting feathers grown in area of high probability have similar deuterium concentrations as those of the feathers of migrants captured in France. It does not mean that all ortolan buntings growing feathers in these areas do migrate by France. For example, Spanish ortolan buntings have similar isotopic signatures but do not migrate by France.

The genetic assignment was performed on 266 individuals to the three genetic clusters known to use the western flyway. Globally, one third of the migrants were assigned to the northern cluster (characterizing breeding populations of Scandinavia and the Baltic states), and two thirds to the western/central cluster (characterizing populations breeding mainly in Poland, Germany and France). Only one individual of unknown status was assigned to the Drôme population.

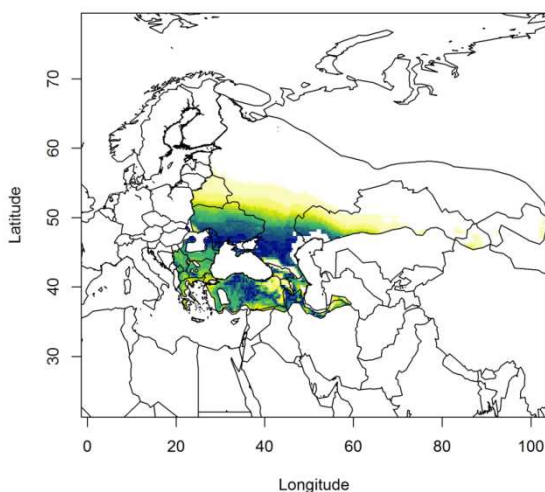
Genetic assignments of migrants captured in France during autumn migrations to genetic clusters. Left: separating birds known to have been wild when they grew their feathers, attested decoys (feathers grown in captivity) and individuals of unknown status (either decoys with feathers grown in captivity, or wild adults having been captured recently by hunters). Right: same genetic assignments when mixing all three groups of birds.



If considering also the eastern cluster in the assignment process, the proportion of individuals assigned to the eastern cluster was equivalent to the proportion of breeding individuals of the western/central cluster assigned to the eastern cluster in a cross-validation performed to validate the clustering process (see Appendix 3 for further details). This means that there is no reason to consider that migrants using the western flyway might come from the eastern cluster – as already confirmed by the geolocators and the isotopes.

IV. ORIGIN OF ORTOLAN BUNTINGS MIGRATING BY THE EASTERN FLYWAY

We performed similar analyses (isotopic assignments and genetic assignments) to samples collected along the eastern flyway in September 2014 (Kuwait, 78 samples - isotopes of breeding grounds and genetics) and in April 2015 (Kuwait, 45 samples, and Israel, 10 samples – genetics).



The results of the probabilistic assignments to origin from deuterium concentration (78 samples collected in Kuwait in autumn 2014, so of birds wearing feathers grown on the breeding grounds) suggest that ortolans using the eastern flyway come mainly from southern Russia, Romania and Turkey, but also Georgia, Azerbaijan, and Ukraine. Migrants from Belarus and most of European Russia (north of 50°N) do not constitute a large part of the migrants using the eastern flyway, because breeding populations are small there. The same is true for far-eastern breeding populations of Kazakhstan and Mongolia, which either are small, or use a different route (e.g. a more eastern route over Oman?).

Genetically, the migrants captured along the eastern flyway (Israel and Kuwait) were assigned mainly to the eastern cluster and to the other two clusters (northern and western/central) in the same proportions as were the test sample in the cross-validation of the assignment procedure (chi-square test, $\chi^2 = 3.36$, $df = 2$, $P = 0.18$).

V. POPULATIONS SIZES AND TRENDS ALONG EASTERN AND WESTERN FLYWAYS

[details of the calculations can be found in ESM1]

1) Eastern flyway

By summing the breeding population sizes from European countries where ortolan buntings use the eastern flyway (those countries coloured in the map above), we obtain a breeding population size of 4,283,250 pairs (2,907,250 to 6,310,500 pairs). The main contributing countries are Russia, Turkey, Romania, Bulgaria, and Azerbaijan. The global European population size is estimated at 4,754,440 breeding pairs (3,262,000 to 6,930,000 pairs), so the eastern flyway is used by 90% of European ortolan buntings.

By comparing the current population estimates to those published earlier, recent average trend of these eastern flyway populations gives a small increase by 10.6%, though comparing the sums of the upper and lower limits of previous and current national population sizes gives a range of -4% to +27%. This eastern population could be either in small decline, or increasing. However, it should be noted that the main trend comes from the Russian population, for which comparison of previous vs current estimates gives +7% (-14% to +33%), while Russian experts generally agree that the population has declined by 15 to 30%. As Russia holds two thirds of this eastern flyway European population, it is wiser to consider that the eastern flyway populations is decreasing, probably by 10 to 20%.

2) Western flyway

Note 1: here we considered that birds from the western side of species range use the western flyway, though there are certainly some populations included here using a central flyway to winter in Nigeria – probably populations from Italy, countries around the Adriatic Sea, Austria and Hungary, and possibly part of the populations breeding in Poland, in other central European countries, in Finland. The values given for the western flyway are therefore highly conservative in the sense that the real population size using the western flyway is lower. It is however possible that some breeders of western Belarus (500-1000 pairs) use either the western or the central flyway, though we have no evidence for this in this study, and it would concern only small numbers not susceptible to change the global picture.

Note 2: we have actualized the breeding population size for Poland, compared to Jiguet *et al.* 2016 (Appendix 5). Two different sources (the recent atlas of breeding birds, and an analysis of densities conducted by Jakub Kosicki) are congruent in estimating the national population size of ortolan bunting in Poland to 154,000 pairs (140,000 to 171,000 pairs). The values published previously of 197,000 to 298,000 are therefore overestimates, as even Polish experts do not understand where this estimate comes from. In our work, we now consider these updated estimates for Poland (in the following calculations). References to be consulted are Kosicki & Chylarecki 2012a, Kosicki & Chylarecki 2012b, Kuczyński & Chylarecki 2012.

Kosicki J.Z., Chylarecki P. (2012a) Habitat selection of the Ortolan bunting *Emberiza hortulana* in Poland: predictions from large-scale habitat elements. *Ecological Research* 27: 347-355.

Kosicki J.Z., Chylarecki P. (2012b) Erratum to: Habitat selection of the Ortolan bunting *Emberiza hortulana* in Poland: predictions from large-scale habitat elements. Ecological Research 27:357.

Kuczyński L., Chylarecki P. (2012) Atlas of Common Breeding Birds in Poland: Distribution, Habitat Preferences and Population Trends. GIOŚ Warszawa.

Note 3: methods to obtain estimates of population trends. We used two ways to calculate average multi-national population trends. First, we calculated the geometric mean of the lower and upper values of a national population size range estimates, and summed these means among countries. We further compared the trend of this summed geometric mean between 2000 and the most recent year considered (generally 2012 or 2014). We did the same for the lower values of the national population size range, and the same for the upper values. Second, we calculated the mean of national trends weighted by national population size (the weights being the geometric means of national upper and lower values, or the lower values, or the upper values). The recent national trends are those reported by each country, which are not a direct comparison of current and previous population sizes, but come from dedicated monitoring of breeding birds, such as breeding bird survey (in most countries; France, Spain, Poland, Finland, ...), or comparison of densities found on sampled sites (Russia, etc.). Trend values have been rounded to the nearest unit.

a) Global western and central flyways

By summing the national population sizes of all countries lying west of the identified migration divide, we obtain an estimate of 468,701 breeding pairs (354,622 to 619,479). This represents ca. 10% of the European breeding population size.

The post-2000 trend of these populations is:

- comparison of geometric means: -10% (-13% for the lower values, -6% for the upper values);
- weighted means of national trends: -18% (-18% for the lower values, -17% for the upper values).

b) Numbers and trends over France

Within the national populations breeding west of the migration divide, we then considered that birds breeding in Portugal, Spain, Italy, Andorra, Slovenia and Croatia do not migrate over France. Therefore, the population size susceptible to be migrating over France is estimated at 194,070 pairs (165,598 to 227,435).

The post-2000 trend of these populations is:

- comparison of geometric means: -32% (-17% for the lower values, -45% for the upper values);
- weighted means of national trends: -25% (-24% for the lower values, -26% for the upper values).

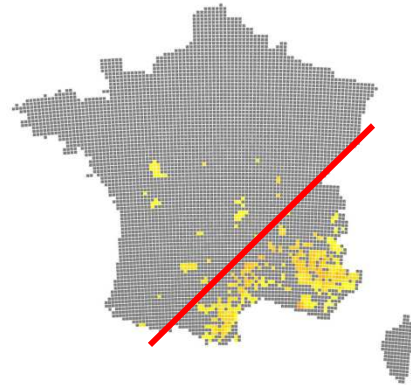
Given the similarity of the estimates obtained with the two methods, we are confident that the global trend of these populations is between -25% and -32% (a decrease by one quarter to one third).

c) Numbers and trends over south-west France

There are 15 countries holding ortolan bunting breeding populations likely to migrate over France. Geolocators have revealed that the large majority of Finnish breeders use a Central European/Mediterranean route, and do not visit south-west France, and part of them even use a central flyway. In Sweden, 3 out of 11 used this Mediterranean route while all 16 German tracks used the Atlantic route, thus over south-west France (Petra Bernardy pers. comm., see Appendix 1). In Poland, one track follows a Mediterranean route, another bird migrated southwards along the third central flyway, while one Polish bird with a logger was caught by a hunter in 2014 in Les Landes. To be able to obtain an estimate of how many birds do visit south-west France during migration, we therefore propose three scenarios, considering that only a part of some national populations use the Atlantic route, while another part uses a Mediterranean route (or even the central flyway). Typically, birds using the Atlantic route enter France between Nord and Alsace departments, while birds using

the Mediterranean flyway enter France – if they do - at the Alps or along the coast from Italy. In all three scenarios, we considered that most of the French breeding population uses the Mediterranean route, while the 100-200 pairs breeding in central and western France (mainly in Poitou-Charentes) use the Atlantic route.

Map explaining how we shared the French breeding population between the Atlantic and the Mediterranean routes of the western flyway. Background map from the recent Atlas of birds of France (Issa N, Muller Y (coord.) 2015. Atlas des Oiseaux de France métropolitaine. Delachaux & Niestlé, Paris, France).



The three scenarios considered that the following proportions of national population sizes were migrating by the Atlantic route:

Scenario 1: Finland and Baltic states (Estonia, Latvia and Lithuania) 0%
 Sweden 60%
 Poland 20%
 Germany, Norway, Czech Republic, Austria, Switzerland 100%
 France 100-200 pairs

This scenario occurs if there is a confirmed central flyway used by birds from Poland, and in normal years, or years of western winds, when Finnish, Baltic and eastern Polish birds all use the Mediterranean route.

In this scenario 1, the population size susceptible to migrate over south-west France is estimated at 46,446 pairs (40,254 to 53,591). The post-2000 trend of these populations is:

- comparison of geometric means: -11% (+8% for the lower values, -26% for the upper values);
- weighted means of national trends: -17% (-17% for the lower values, -16% for the upper values).

Scenario 2: Finland and Baltic states 20%
 Sweden 80%
 Poland 40%
 Germany, Norway, Czech Republic, Austria, Switzerland 100%
 France 100-200 pairs

This scenario considers that ringing recoveries from Finland attest that some Finnish birds do migrate by les Landes, at least in some years, despite the light loggers we retrieved did not confirm this is the usual route. For Sweden, this scenario respects the proportions of the tracks obtained with light loggers, while for Poland it considers that less than half of the breeding birds do use the Atlantic route, which is very conservative given the geolocation data obtained in this study.

In this scenario 2, the population size estimated to migrate over south-west France is 81,325 pairs (70,255 to 94,140).

The post-2000 trend of these populations is:

- comparison of geometric means: -22% (-5% for the lower values, -35% for the upper values);
- weighted means of national trends: -21% (-20% for the lower values, -21% for the upper values).

Scenario 3: Finland and Baltic states 40%
Sweden 100%
Poland 60%
Germany, Norway, Czech Republic, Austria, Switzerland 100%
France 100-200 pairs

This scenario considers that in some exceptional years, strong eastern winds could force most of the western breeding populations to use the Atlantic route.

In this scenario 3, the population size likely to migrate over south-west France is estimated at 116,289 pairs (100,255 to 134,888). The post-2000 trend of these populations is:

- comparison of geometric means: -25% (-10% for the lower values, -38% for the upper values);
- weighted means of national trends: -22% (-22% for the lower values, -23% for the upper values).

We propose to consider the mean estimate of the medium scenario as the average population size, and the mean estimates of the lower and upper scenarios (1 and 3) as the lower and upper values of population sizes. As a consequence, **the population size of ortolan buntings migrating by south-west France is estimated at 81,000 pairs (range 46,000-116,000)**. In our calculations, ~75% of these birds do come from Poland, so Poland is the main country contributing to this route. All three scenarios are congruent in estimating the recent decrease of these breeding populations by one fifth to one quarter (-20% to -30% during the last 14 years).

A further way to estimate the recent trend of the populations migrating over south-west France is to use the genetic assignments (1/3 of Nordic individuals, 2/3 of central/western individuals), and to weight the regional trends of these northern and central/western populations by these proportions. The regional trend for [Finland, Sweden, Norway, Estonia, Latvia and Lithuania] is -53% since 2000 (national trends weighted by national population sizes). The regional trend for [France, Germany, Poland, Czech Republic, Austria] is -19%. Then the average recent trend of the populations migrating over south-west France would be estimated as -30%, which is coherent with the estimates obtained by comparing population sizes.

Indeed, **the recent trend of these populations can be estimated between -20% and -30% (2000-2014)**. This represents an average decrease of nearly 1.4% to 2.1% each year. This means that the population size using the Atlantic route of the western flyway is on average currently decreasing by e.g. 1,500 pairs (3,000 mature adults) each year.

VI - Conclusions

Based on data from light loggers, genetics and stable isotopes analyses, we were able to identify two main migration flyways for European ortolan buntings. The eastern flyway drains the breeding populations of Russia, and of all countries east of a migration divide running from Belarus to Serbia (included). This eastern flyway is thus used by ca. 4,283,250 pairs (range 2,907,250 to 6,310,500 pairs), which winter on the high plateaus of Ethiopia and Eritrea. The main contributing countries are Russia, Turkey, Romania, Bulgaria, and Azerbaijan. As the global European population size is estimated at 4,754,440 breeding pairs (3,262,000 to 6,930,000 pairs), the eastern flyway is used by 90% of European ortolan buntings, for which recent trends are estimated between -10% and -20% (period 2000-2014). The western flyway drains birds breeding west of the migration divide, and wintering in western Africa (Sierra Leone to southern Mauretania and Mali, mainly in Guinea). It concerns 468,701 breeding pairs (354,622 to 619,479), representing 10% of the European breeding ortolan buntings. The recent trend of these populations is estimated between -10% and -20% (2000-2014). The main stopover sites of these migrants are located in Spain and northern Morocco, France being a fly-by area along the migration route. Along the western flyway, we can distinguish two distinct routes. The more western is the breeding population, the more Atlantic is its migration route. 'Eastern' breeders (e.g. almost all Finnish and Baltic ortolans) use a continental/ Mediterranean route, flying over eastern Poland to northern Italy, then following the Mediterranean coast; they do not visit south-west France. 'Western' breeders (e.g. most Swedish, all German and probably all Norwegian ortolan buntings) use an Atlantic route, entering France from Nord to Alsace and crossing the Pyrénées at their western end. We estimated that 81,000 breeding pairs (range 46,000 – 116,000) use the Atlantic route and thus migrate over south-west France. The recent trend (2000-2014) of these populations is estimated between -20% and -30%. This means that each year, this population decreases by e.g. 1500 pairs (at least 3000 individuals).

Birds breeding in Russia do not use the western flyway, only the eastern. There is a ringing record of a bird ringed in western Russia near Saint Petersburg, and recovered in Les Landes by a hunter, but this bird came from a region that was colonized by ortolans from Finland and Karelia – thus from populations using the western flyway – but almost disappeared in the last decades – a similar huge decrease as occurred in adjacent Finland and Baltic states.

An unknown part of the populations breeding west of the migration divide use a central flyway, reaching Africa from Italy to Greece south to Tunisia or Libya, to further winter probably in Nigeria. Some individuals from Poland and Finland use this flyway, which might be the normal flyway for populations breeding in Italy, Slovenia, Croatia and Bosnia, also Slovakia, Austria and Hungary.

APPENDIX 1 – LIGHT LOGGERS’ REPORT

[Data on location estimates are available in ESM2]

[Most generalities on light loggers copied from Migrate Technology website]

I - METHODS

1) Materials

Geolocators are miniature archival light level loggers (also known as GLS tracking or geologgers) for tracking birds. These geolocation loggers record ambient sunlight over very long periods. From this recorded information, the areas that the animal visited can be determined, because at a given date, the duration of the daylight, and the time of the meridian are latitude- and longitude-dependent, respectively. Light level geolocation is applicable whenever there are periods of night and day. Due to the rate of change of day length with respect to latitude, latitude accuracy decreases near the equator. Also, due to the lack of variation in day length with respect to latitude close to equinox, uncertainty is large at this time. Geolocators are not online realtime tracking devices; in order to see the data, tags must be retrieved to download the data archive. This necessitates animal recapture and, thus, site fidelity at wintering, breeding or stopover locations. Recapturing animals in order to download the data recorded enables tags to be made much smaller than a radio, cellphone or satellite technology. Also, archival light level geolocators can be made far smaller than GPS loggers or those that have local remote download capability. The accuracy of light level geolocation is appreciably less than GPS but it is enough to provide detailed timing and movement data as well as to identify important stopover areas and connectivity relating to bird migration.

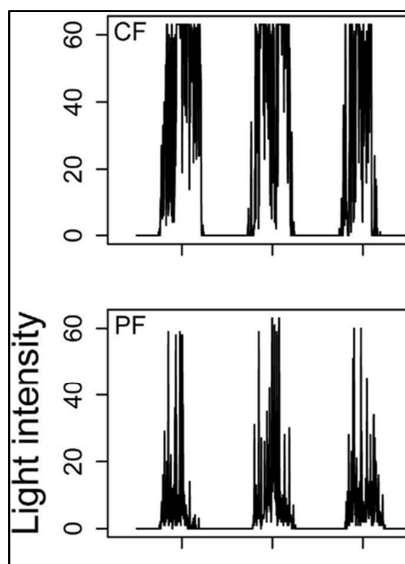


Figure 1 (left). Two examples of light pattern during three days, with zigzags during the day, as a consequence of shading caused by habitat and bird behaviour.

Figure2 (right). In 2015, we used INTIGEO P65C2-7 light loggers from Migrate Technology (0.74g).



In the first years of the program (2013-2014), we deployed SOI-GDL1.0 from the Swiss Ornithological Institute, mounted on the birds’ backs using a leg-loop silicon harness. The device including harness weighed 1.2 g, corresponding to 5-6% of adult body weight. We discovered in spring 2014 that most returning birds had lost their logger, probably because they cut the silicon harness with their bill. At that time, we had retrieved only one logger from Norway (with data of a few winter locations), one

from Sweden (no data recorded) and one from France (worked one month, April 2015). In spring 2015, we fitted SOI-GDL 3.0 (Swiss Ornithological Institute, 0.65g without harness) and INTIGEO P65C2-7 (Migrate Technology, 0.74g without harness). We used UV-proof strings to fix the loggers on bird's back as leg-loop harness (strings sold by the BTO as repair strings for mist-nets). This new harness proved not to be removed by the ortolan buntings, and in spring 2016 we retrieved 24 loggers which recorded data (by country, first Intigeo, second SOI loggers): Belarus (1-0), Russia (8-3), Lithuania (1-0), Finland (3-4), Poland (0-2), Spain (0-2).



Male Ortolan Bunting with a SOI-GDL 1.0 light logger from the Swiss Ornithological Institute. Spain, May 2014, photo José Luis Copete



Loggers are attached to the bird with leg loops. Male Ortolan, Spain, May 2014, photo José Luis Copete



Male Ortolan Bunting with an Intigeo P65C2-7 logger. Russia, May 2015, photo Frédéric Jiguet.

Furthermore, colleagues from Sweden had successfully deployed the same Intigeo loggers and already published results of their tracking study (Selstam G, Sondell J, Olsson P. 2015. Wintering area and migration routes for Ortolan Buntings from Sweden determined with light-geologgers. *Ornis Svecica* 25:3–14). They provided us with the raw data of tracks of 9 individuals, corresponding to 11 migration tracks (two individuals tracked during two migration cycles). These data were re-analyzed with similar parameters as the other data.

Finally, Petra Bernardy from Germany contacted us to inform she had tracked successfully 16 German-breeding ortolans, which all migrated over south-western France (e.g. probably Les Landes) to winter in West Africa (with autumn stopovers in western Spain).

2) Methods for analyzing the data

We retrieved thirteen INTIGEO loggers (Migrate Technology, Cambridge, UK) in spring 2016: one from Belarus, one from Lithuania, three from Finland, and eight from Russia (from three sites: one in Vladimir, two in Volgograd, five in Belgorod). INTIGEO loggers sample light every minute but only the maximum light value in a ten minute interval is recorded. All loggers retrieved contained complete data for the period carried. We also retrieved fourteen SOI loggers (Swiss Ornithological Institute, Sempach, Switzerland): one from Belarus, two from Poland, two from Spain, four from Russia and five from Finland). SOI loggers record light intensity every five minutes, increasing data variability. Three SOI loggers failed within a couple of weeks and therefore yielded no usable data. The eleven remaining loggers recorded until early August to early October 2015, so only partial migration tracks could be exploited.

We used the Geolight 2.0 package in R to analyze all loggers using a threshold method (Lisovski and Hahn 2012). Twilight times were identified using a light threshold level of two in R for the INTIGEO loggers and a threshold of three in the program SolarEvent Editor for the SOI loggers. Occasional glitches (likely lightning events) were removed manually. We applied a Loess filter to the twilight events to remove extreme outliers. Outliers were defined as twilight events for which the residual from the local polynomial regression line of sunset and sunrise time exceeded four times the interquartile range for the INTIGEO loggers, and 1.5 times for the SOI loggers.

We sectioned the data into stationary periods, where the individual is likely to remain at a particular site, and into movement periods. We used the changelight function of the Geolight package that examines sunrise and sunset time series and uses a changepoint model to identify multiple changepoints within the data. Settings included minimum length of residency period of three days, probability thresholds for sunrise and sunset of 0.03. We also applied the sun elevation angle (SEA) of -6° corresponding to civil twilight to calculate latitudes and longitudes based on the twilight events. A changepoint analysis was applied on latitude and longitude time series using the cpt.mean function of the changepoint v2.2.2 package in R with the PELT method and a penalty of 200. We built consensus stationary and movement periods based on these three analyses and identified breeding and wintering periods.

Longitudes are estimated using the noon and midnight time from the geolocator. Latitudes are inferred from the day length and require the estimation of the calibration SEA to describe the relation between day length and latitude. For periods where the bird is at a known position, i.e. during breeding, we used the GPS coordinates to estimate the median SEA. For the rest of the year, we split the INTIGEO loggers into two groups corresponding to the eastern and western flyways (longitudinal split), as determined when visualizing the tracks calculated with the -6° SEA. We used the Hill-Ekstrom calibration to infer SEA for each wintering period and for each logger. We then calculated the median winter SEA for each logger and finally the median winter SEA over all loggers within a flyway. These SEAs were -5.5° for the eastern flyway and -6° for the western flyway and were applied to all loggers within a flyway and to every period but the breeding ones.

For each period, we estimated the mode, corresponding to the centre of density, and the 90% quantile for latitude and longitude. We decided to include the periods around equinoxes even if inference of latitude can be highly imprecise as in many cases the use of the mode minimized the impact of equinox while it provided useable data.

The latitude of some periods had to be manually adjusted. Hence for movement periods, the mode did not necessarily make sense and the latitude was adjusted to fit the general route between stationary periods. When the effect of equinox was strong, resulting in high latitudinal variation, we calculated the latitudinal mode when latitude varied little. In few cases, no latitude could be estimated for the period due to the equinox and the position was therefore estimated according to positions before and after the event and to information from other loggers. In other cases, the mode was located in the sea and latitude was adjusted to the nearest coastline.

The data from the SOI loggers were of poor quality and each logger had to be analyzed separately. We could not determine the calibration SEA from the GPS coordinates nor from Hill-Ekstrom method as implemented in GeoLight. We therefore tested several SEAs for each logger to select the one that positioned the mode of the breeding period the closest to the latitude determined by GPS. We applied the same SEA to other periods of the same logger. The latitudes of some periods were manually adjusted as described previously.

Tracks for each logger were mapped in R using the maps package.

Lisovski, S. and Hahn, S. (2012), GeoLight – processing and analysing light-based geolocator data in R. *Methods Ecol Evol*, 3: 1055–1059.

II - RESULTS FROM INTIGEO LOGGERS

Including loggers from Sweden, we were able to map 24 tracks for 22 individuals, which clearly identified two main migration flyways (Fig. 1): an eastern flyway, and a western flyway.

All nine individuals from Russia (three different sites) and the individual from Belarus migrated to the south or south-east, with some stopovers from the Caucasus to northern Syria (mainly in eastern Turkey), to further winter on the highlands of Ethiopia and in adjacent Eritrea. In the spring, the stopover sites were located more southward, in the Middle East, so soon after the crossing of the Arabian desert (see panels of Figure 2).

The other 13 individuals from Finland, Lithuania and Sweden used a western flyway, with main stopover sites in Spain and northern Morocco, and a wintering range spreading from Sierra Leone to southern Mauretania. The spring migration is more direct, rapid and Mediterranean. In the autumn, two different routes are taken by these birds, to pass the Pyrénées to the East or to the West. All tracks from Finland and the track from Lithuania concern individuals that flew over eastern Poland, central Europe (Switzerland to northern Italy) then followed the Mediterranean coast of Spain to reach Africa. We call this route the continental/Mediterranean route of the western flyway. 3 of the 11 Swedish tracks show the same pattern, but none of 16 German tracks do so (fide Petra Bernardy). The other Swedish 8 tracks follow what we call an Atlantic route, entering France by its north-eastern border, then migrating over Les Landes to cross the Pyrénées on their Atlantic side. Given that pattern, with eastern breeders (Finland and Baltic states, few from Sweden) migrating by the continental route and western breeders (most from Sweden, all from Germany) migrating by the Atlantic route, it is fair to believe that Norwegian breeders follow this route too. Only one Norwegian bird was tracked with a SOI logger early in the program, but the device only provided a stationary winter area – in Guinea.

Fig. 1. 24 tracks obtained from the 22 Intigeo light loggers, dividing well into two flyways. Yellow dots are the capture-recapture sites of the birds, blue dots are stopover sites, and purple dots are stationary sites during the winter period. Black triangles are turning point where the direction of the movement changed. Both autumn and spring tracks are shown here

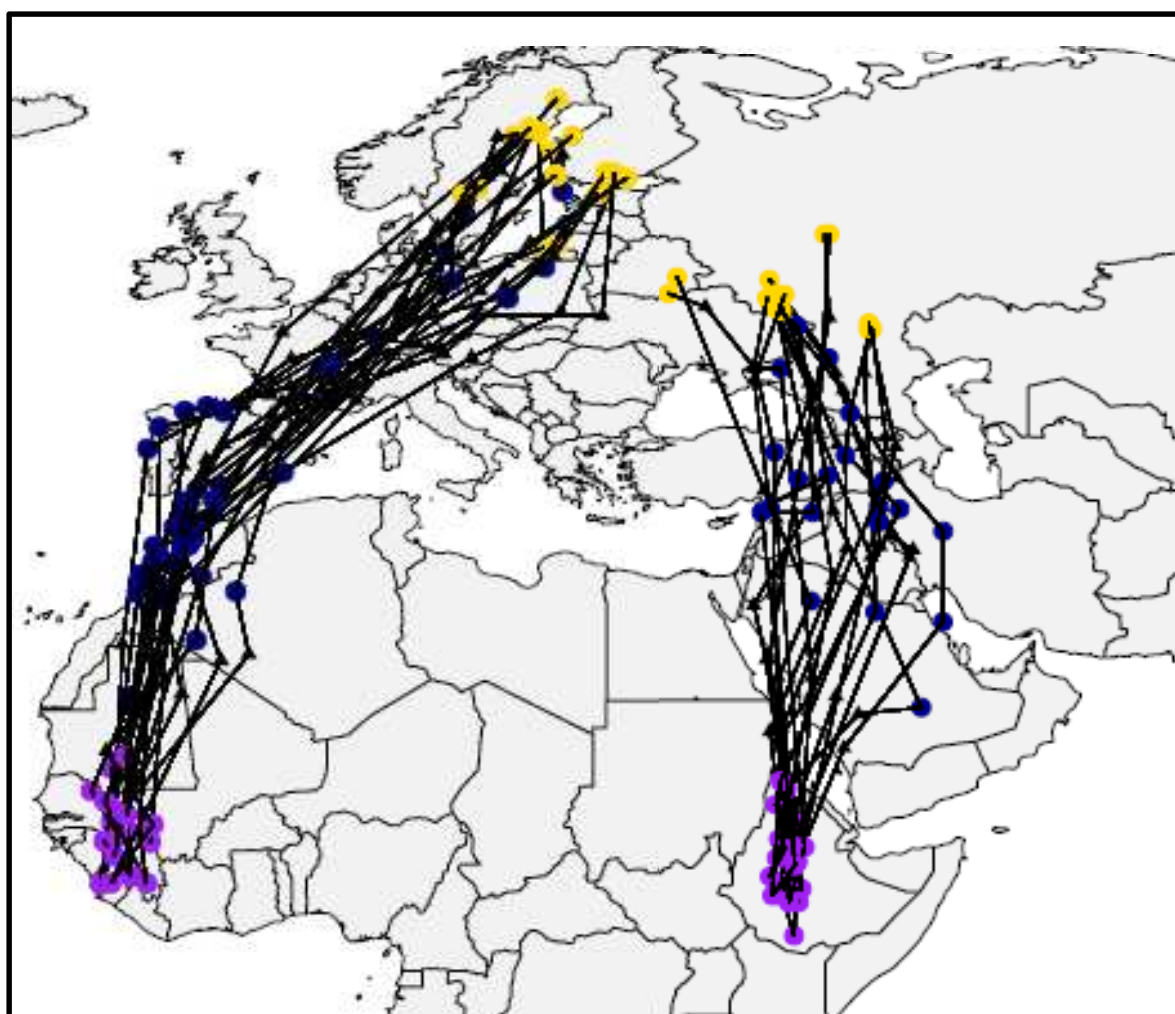


Figure 2. Details of the tracks obtained for individuals using the eastern flyway – one colour by individual. Upper left: autumn tracks. Upper right: spring tracks. Lower left: winter stationary areas, with 90 quantiles. Lower right: zoom on Ethiopia and Eritrea to show the winter stationary sites. Note that some birds used multiple wintering sites.

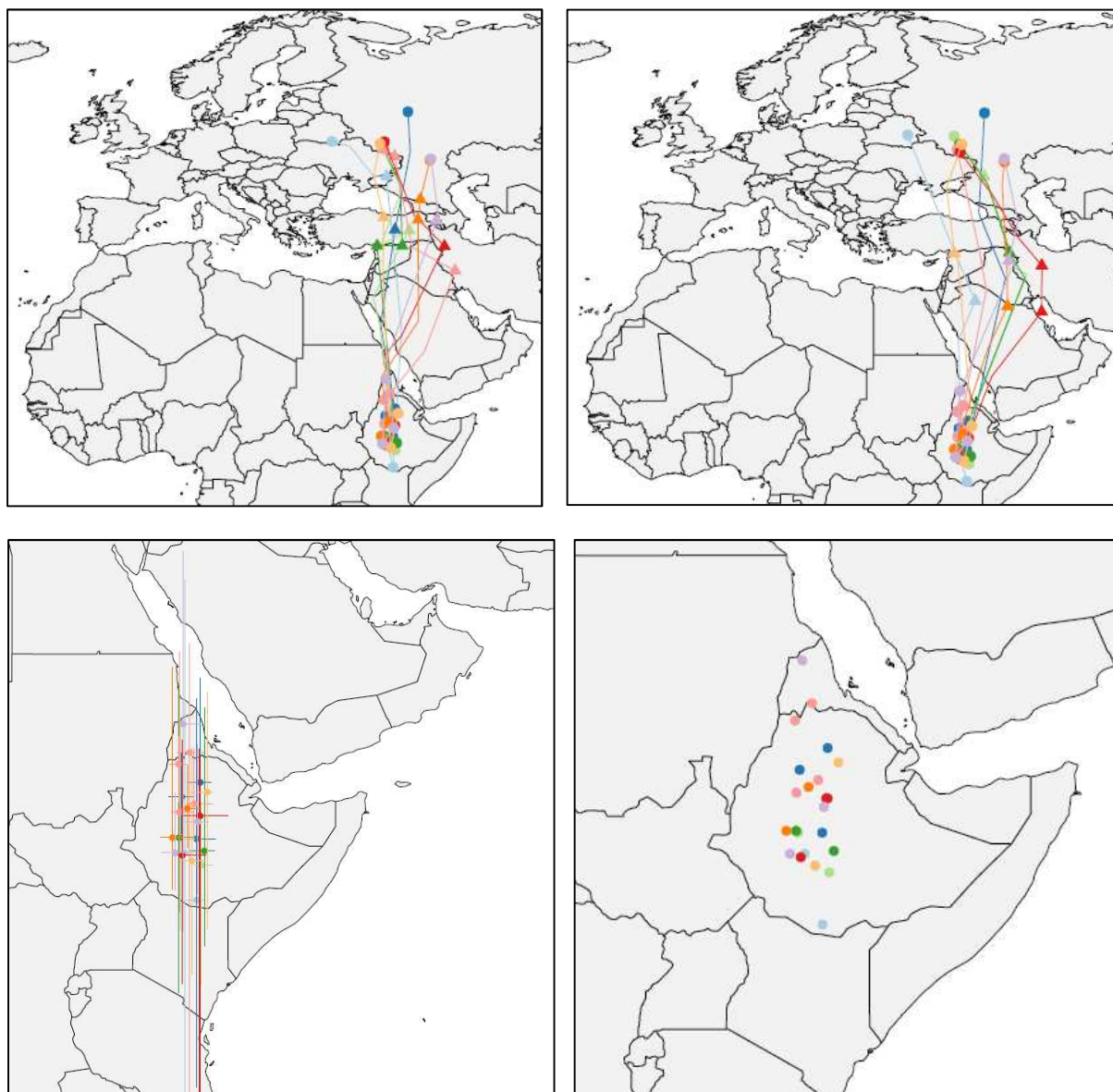


Figure 3. Details of the autumn (left) and spring (right) tracks obtained for individuals using the western flyway – one colour by individual. The autumn Atlantic route (used by Swedish individuals) and the autumn Mediterranean route (used by Finnish individuals) are clearly visible here. Triangles represent stopover sites; in north-western Iberia for the Atlantic route, in southern Spain for the Mediterranean route. In the spring, the route seems to be Mediterranean, though stopovers are in Morocco and southern Spain with no further staging areas in Europe, so the Mediterranean route is only suspected.

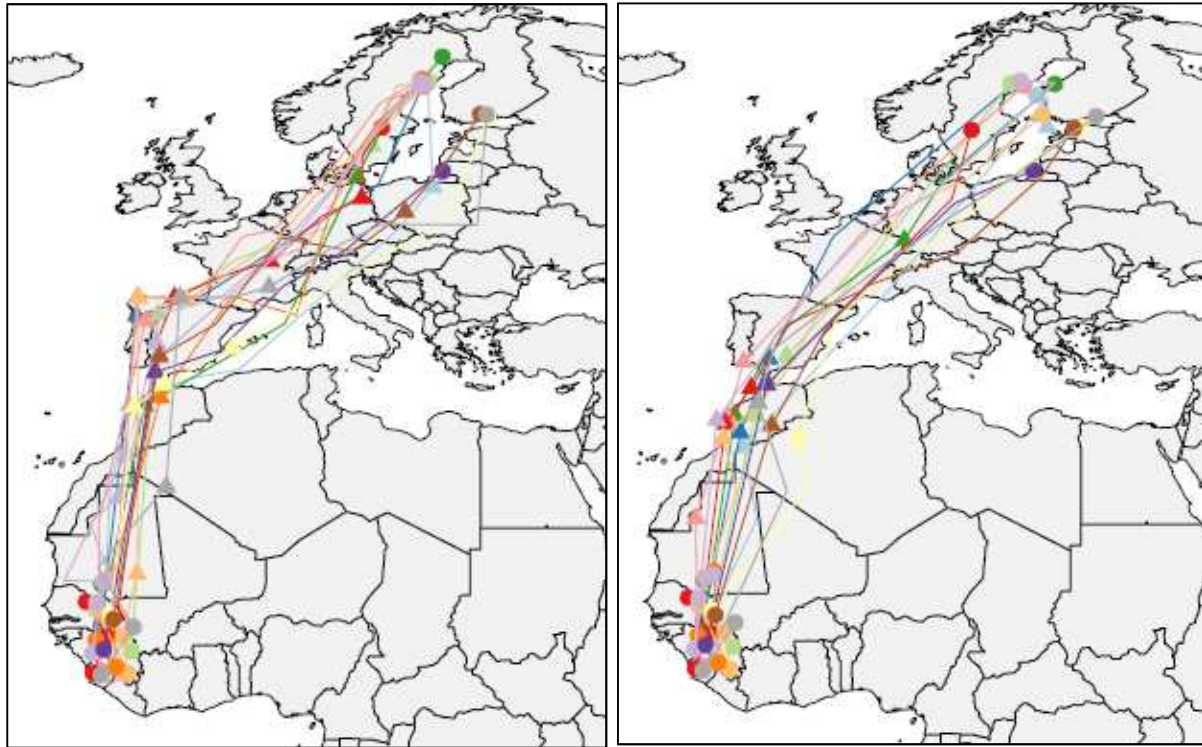
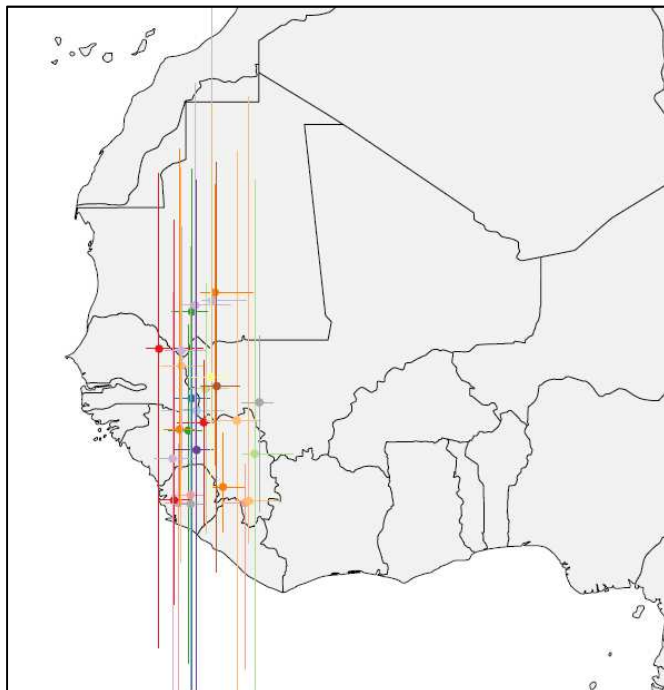
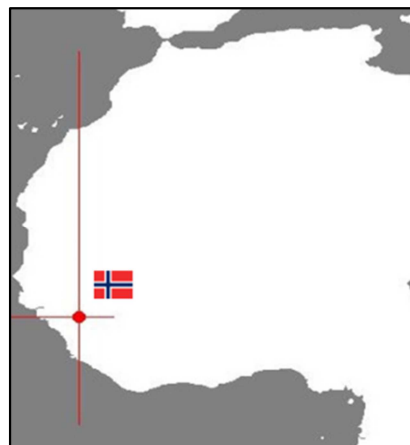


Figure 4. Winter stationary areas (with 90 quantiles) of individuals using the western flyway. Note that some birds used multiple wintering sites.



The insert below is the winter (2012-2013) stationary location (and 90 quantiles) of a Norwegian breeding male equipped with a SOI logger in May 2012. Unfortunately, only the winter location could be estimated from the stored data.



III- RESULTS FROM SOI LOGGERS

The SOI loggers are lighter and did not work as well as the INTIGEO, so we gathered only partial data from them, preventing to identify entire tracks from breeding grounds to wintering grounds. However, most of them recorded data during the start of the migration until the Equinox, so that we are able to present the starts of a few tracks for birds breeding in Spain, Russia, Finland and Poland. For Russia and Finland, we are also able to compare tracks obtained with INTIGEO and SOI, to verify that they are similar in direction. We also obtained part of a spring track of a French breeding male, ringed in Drôme, which obviously wintered in West Africa.

Note: SOI loggers record the instantaneous light intensity every five minutes, while the INTIGEO loggers record every ten minutes the maximum light intensity measured during the interval. A consequence is that the data provided by SOI loggers are more variable and often more difficult to interpret. They also had a shorter lifespan, recording light during one to four months – hence the tracks are shorter and generally stop before the Equinox. For this reason, we decided not to present of global picture of the tracks obtained from SOI loggers, but a country by country (even logger by logger) approach, to better appreciate the short tracks they recorded.

1) One SOI logger from France

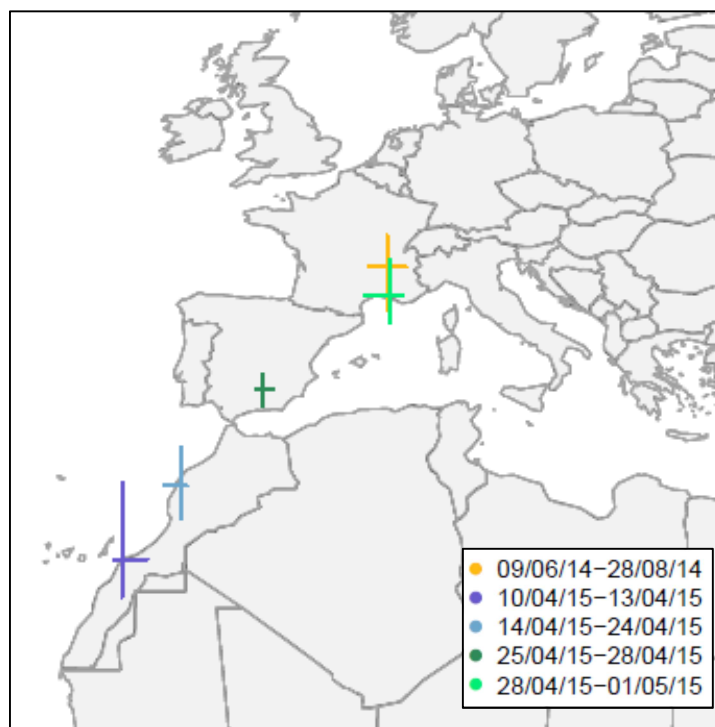
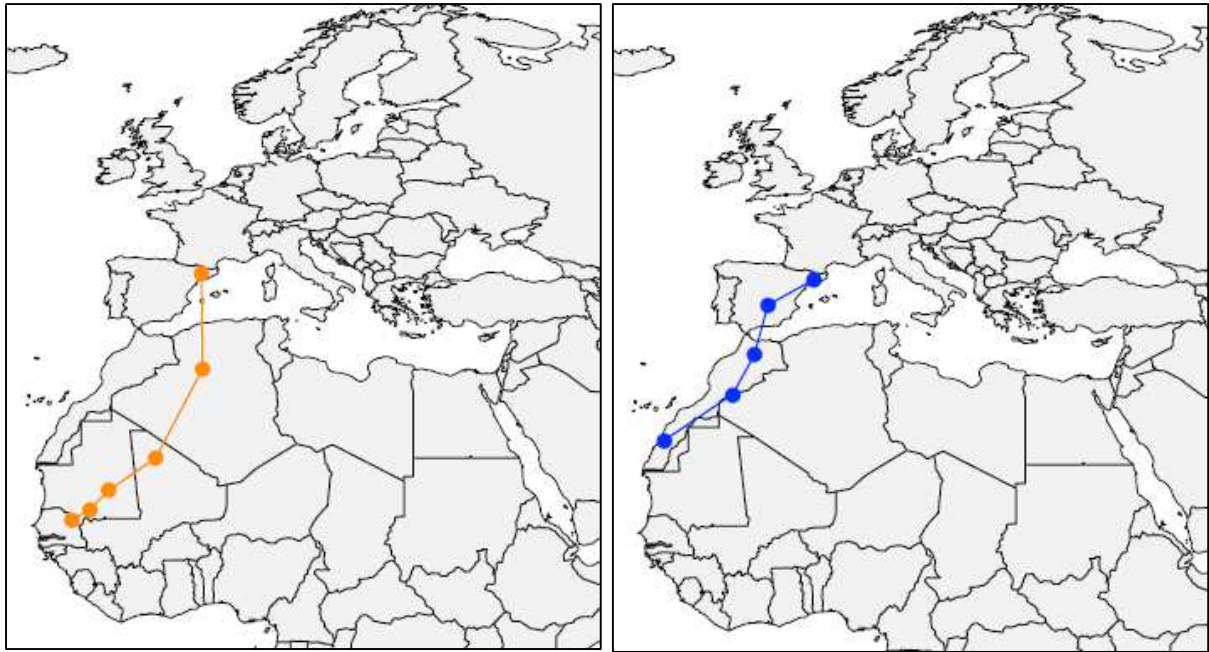


Figure 5. This breeding male was fitted with a logger in May 2014 in Drôme, and recaptured in May 2015. The logger did function only in April 2015 during the spring migration. This bird migrated along the Atlantic coast of Morocco, making stopover near Tarfaya, Essaouira, then near Malaga in Spain and around Bouches-du-Rhône before returning to his breeding site in Drôme. Stopover durations were between 3 and 10 days. This individual clearly used the western flyway.

2) Two SOI loggers from Spain (Catalonia)

Figure 6. Two of the ten males equipped in Catalonia provided data, and as expected migrated in a south-west direction to probably reach their winter quarters in West Africa, as testified by the isotopic signatures of Spanish body feathers.



3) Two SOI loggers from Poland

Figure 7. Concerning the analysis of the SOI tracks, ending close to the equinox, Felix Liechti (from the Swiss Ornithological Institute, providing the SOI loggers), commented: "Longitudes are very reliable, while latitudes are much less, because small errors in day length close to equinox cause huge latitudinal errors. Longitudes vary if the sun-angle for the first light in the morning differs from the corresponding angle in the evening. It is very unlikely that all these angles would have such a huge bias of at least 80 minutes." These arguments confirm that one individual from Poland (and one individual from Finland) moved southwards from its breeding grounds, despite we can't be sure it reached Libya already at the end of August. The direction taken by this individual should probably end in the wintering range of the species in northern Nigeria.

The second Polish bird migrated over central Europe and used the continental Mediterranean route.

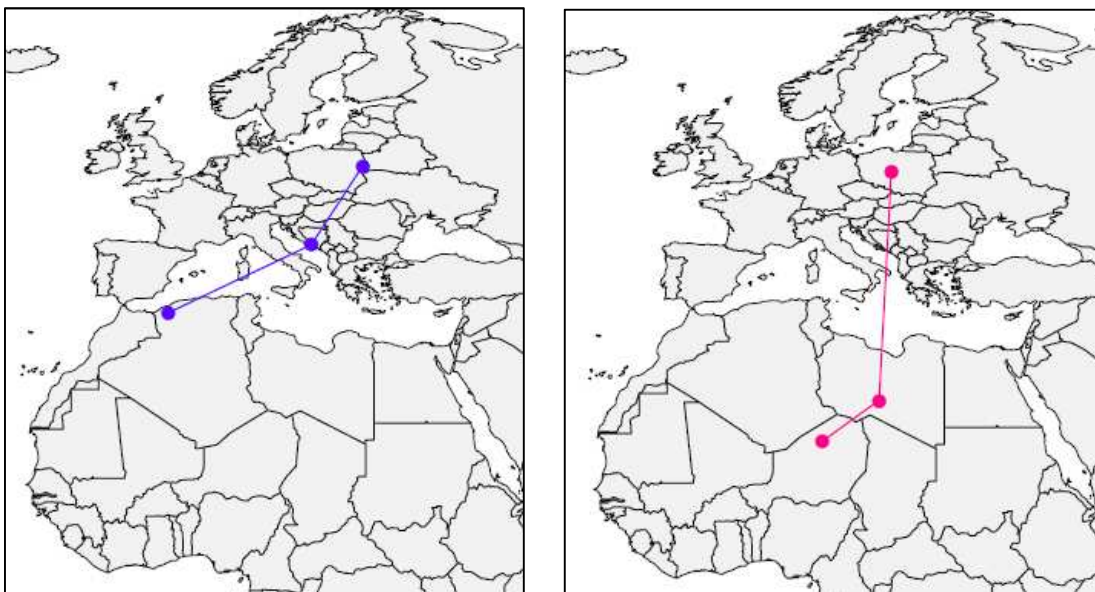
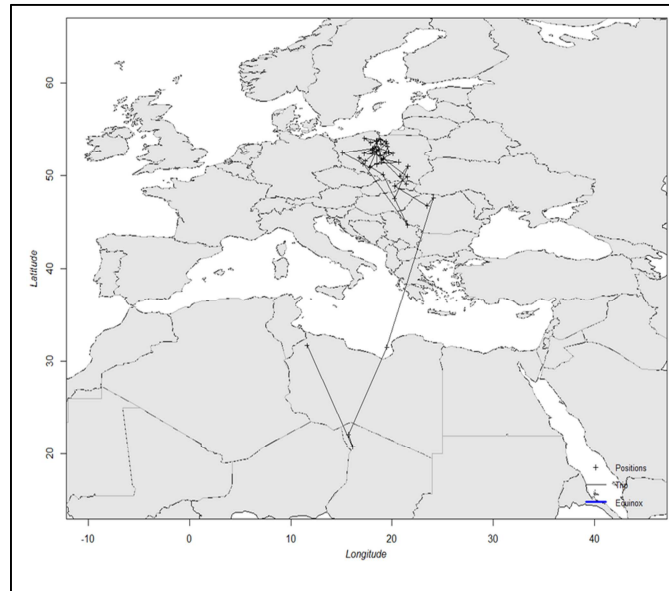


Figure 8. The above maps have been built by Caroline Moussy at MNHN, and we asked Felix Liechti (Swiss Ornithological Institute, who provided the SOI loggers and analyze routinely their data) to also independently analyze the same data to confirm or not the southwards direction taken by this Polish bird equipped with SOI logger 17KV. Below is the map he produced from the same data, confirming that direction.



To be complete, it should be noted that one male fitted with a SOI logger in May 2014 in western Poland was captured by a hunter in Les Landes in early September 2014. The bird was released with its logger. This confirms that some birds from Poland use the Atlantic route of the western flyway, despite the two tracks we obtained did not. It is likely that western populations use the Atlantic route of the western flyway, while eastern populations might use the Mediterranean route of the western flyway, or the central flyway.

4) Four SOI loggers from Finland

Figure 9. The INTIGEO loggers from Finland indicated that all tracks followed the continental Mediterranean route of the western flyway. Three of the SOI did or probably did (one too short to be sure) the same, but the fourth individual took a southwards direction, as did one bird from Poland. From Felix Liechti's comments, we can be confident that this individual did not use the western flyway, and took the direction of (most probably) the central or of the eastern flyway.

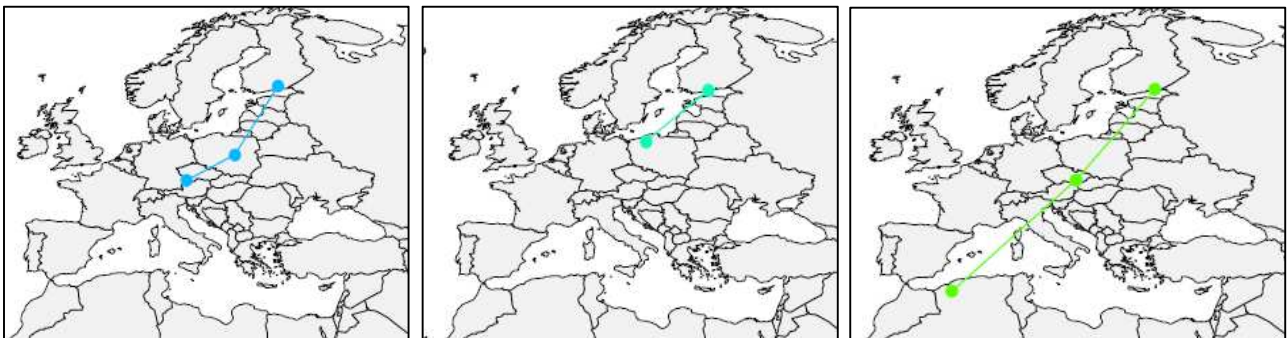


Figure 10. Below is the analysis of the fourth individual as done by Felix Liechti (Swiss Ornithological Institute), confirming the southward direction taken by this individual. See his comments below the figure to testify the reliability of the central flyway (the eastern flyway running east of the Black Sea).

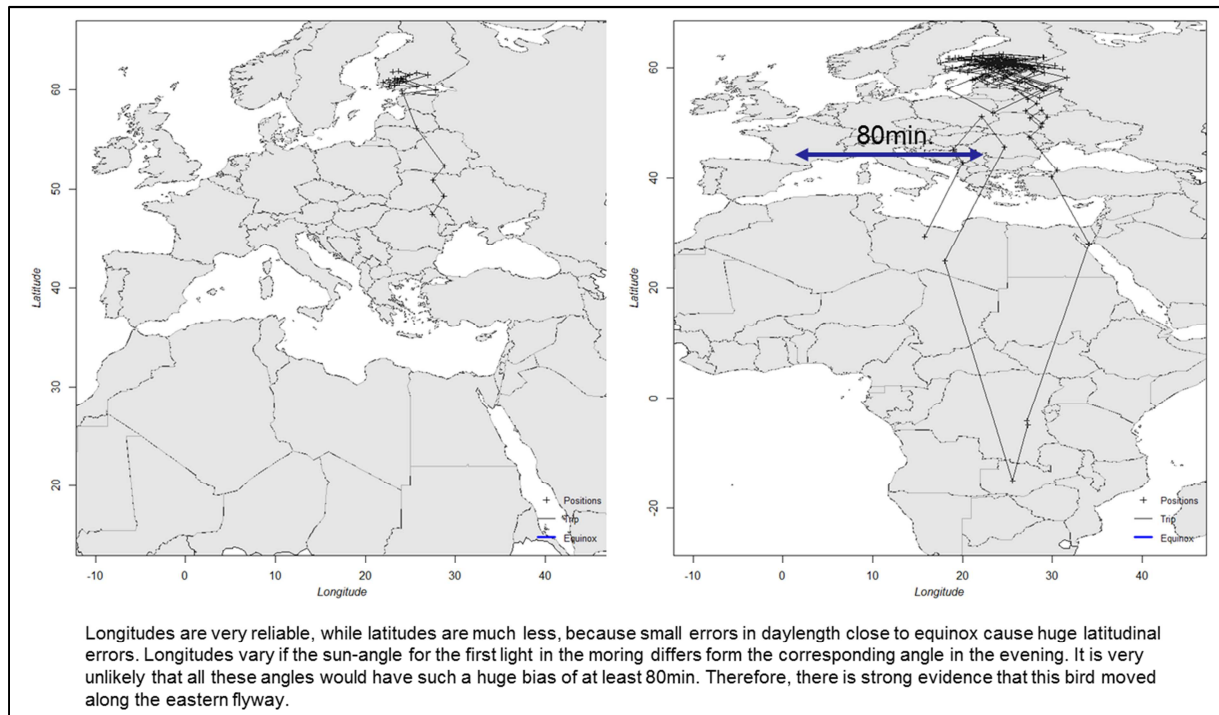
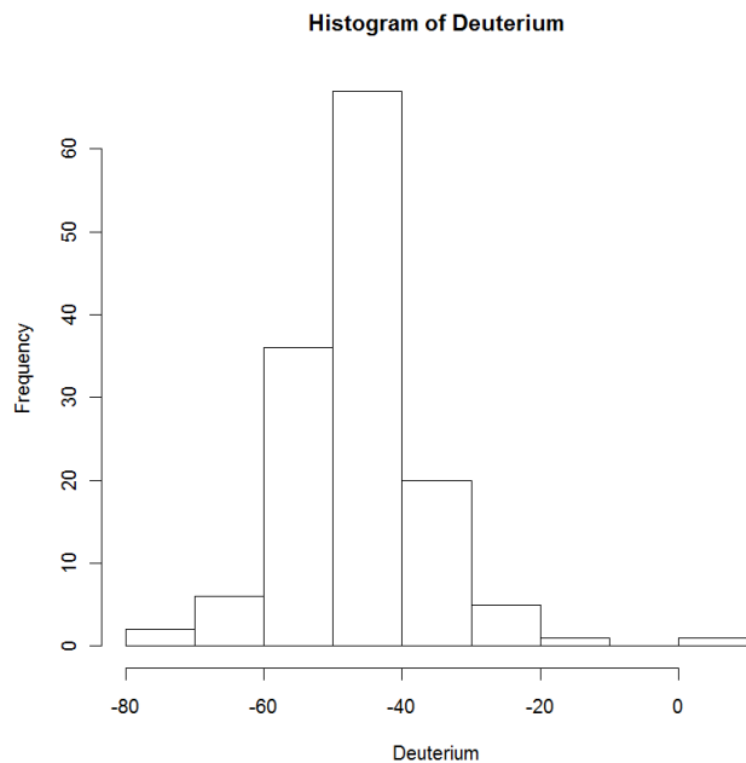
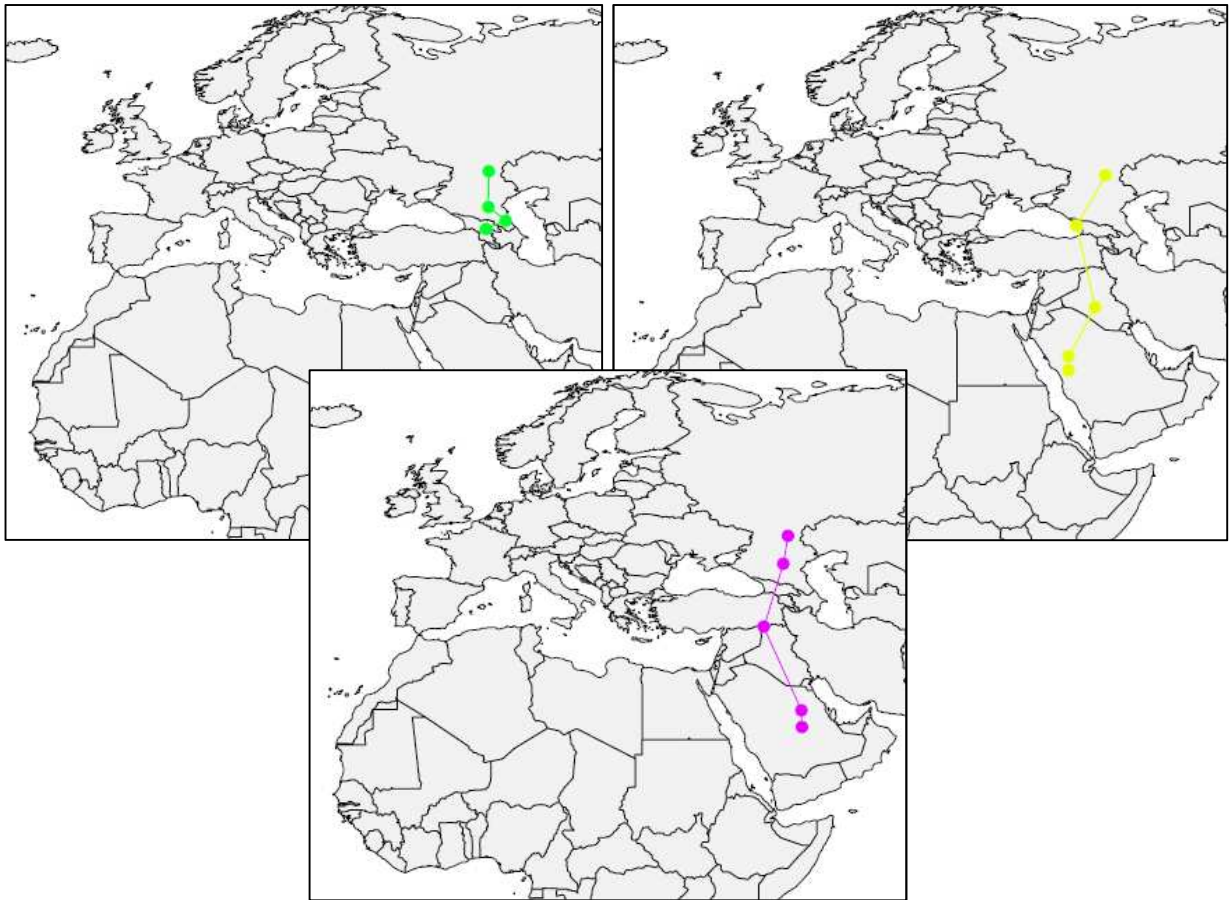


Figure 11. By further looking at the histogram of the deuterium concentrations in body feathers of Finnish breeders (moulted in the winter quarters), it seems obvious that only a very few individuals might winter in East Africa (deuterium values between -20 to positive). As the isotopic environments are similar in the West African and Nigerian winter range of the species (see Stable isotopes' report), it seems likely that part of the population breeding in Finland do winter in northern Nigeria.



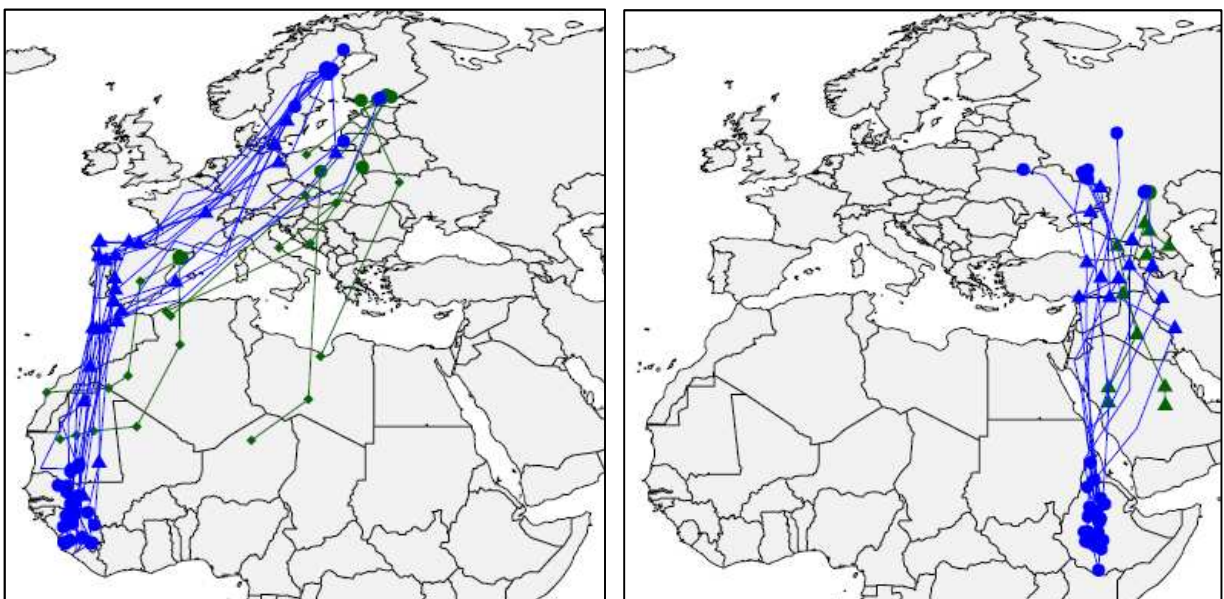
5) Three SOI loggers from Russia

Figure 12. These three birds took the same direction as did the other individuals equipped with INTIGEO loggers.



6) Visual comparisons of tracks from SOI and INTIGEO loggers

Figure 13. Autumn tracks obtained with INTIGEO loggers (blue) and SOI loggers (green) for ortolan buntings breeding in Finland (left) and in Russia (right).



RUSSIA. Globally, for the Russian birds, short tracks obtained from SOI loggers are very similar to the start of the complete tracks obtained with INTIGEO loggers.

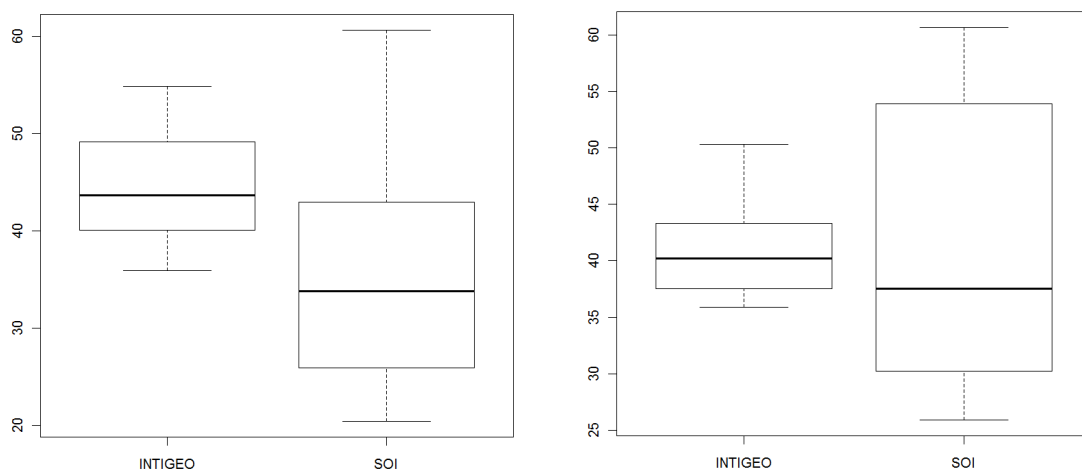
FINLAND. The same is true for Finnish bird, except for one individual which used the central flyway and probably reached Libya by early September, as did one male from Poland (see above).

7) Central flyway and latitudinal bias

As indicated above, two individuals migrated along a central flyway, but their tracks were obtained with SOI loggers stopping their recording before the equinox, at a period when estimating the precise latitude of the locations is difficult and subject to bias. If the longitudes of these two tracks can't be challenged, we are not sure that these two birds reached Libya by late August or early September (last locations 30 August for the Finnish bird, 6 September for the Polish bird). At the same dates (28 August – 8 September), birds tracked with INTIGEO loggers were located at higher latitudes than birds tracked with SOI loggers (all tracks: $t=-7.8$, $df=32$, $P=0.02$; see Figure 14, left). However, if considering only birds originating from countries where we have both SOI and INTIGEO loggers' data, i.e. Russia and Finland, there is no more difference, but a higher variability for SOI loggers ($t=-0.4$, $df=15$, $P=0.93$; Figure 14, right).

As a consequence, we can doubt that these two birds were already in Libya by early September. If they were in fact located at similar latitudes than birds wearing INTIGEO loggers, e.g. at 40°N on average, they were more probably in Italy and in Greece.

Figure 14. Boxplot comparing the latitudes where ortolan buntings tracked with Intigeo or SOI loggers were located between 28 August and 8 September (one location per individual per year). Left panel: all tracked individuals. Right panel: only breeders from Finland and Russia.



IV – NOTE ON GERMAN ORTOLANS

Short note written by Petra BERNARDY

Migration routes, staging- and wintering areas of Ortolan Bunting *Emberiza hortulana* from Germany determined with light-geologgers

The study was conducted in the stronghold of Ortolan Buntings in the eastern part of Lower Saxony in Germany. The study area is located in the county of Lüchow-Dannenberg with a total number of territory sites of about 1.500 males.

The geologgers were produced by Biotrack Ltd., Wareham, Dorset. The tags weighed < 1 g and were attached using a flexible leg-loop harness consisting of an elastic cord. For analyzing light intensity data based on the threshold method we used GeoLight, an R package.

The represented bird (Figure 15) passed through the county of les Landes, made stopovers on the Iberian Peninsula and in Mauritania, and the wintering area was identified in the northern part of Sierra Leone.

Figure 15. Migration routes 2014-2015 for one German Ortolan Bunting.



V – RINGING RECOVERIES

[Foreign ring recoveries of the French CRBPO database available in ESM3]

We know that the origin and numbers of ortolan buntings ringed across Europe and further recovered in France is highly biased by heterogeneous ringing effort on breeding populations, and heterogeneous recapture efforts in France (concentrated in Les Landes). However, it is interesting to present here a summary of these ringing recoveries, the data for which are available as an Excel file joint to the present report. Mapping these ringing recoveries indicate that most ortolans captured in Les Landes originated from western breeding populations.

Norway provided 8 recoveries of birds all ringed in May or June, so during the breeding season. Indeed, the ringing effort was important in Norway where Svein Dale has monitored a breeding population during many years. Seven of these ring recoveries have been obtained between 1993 and 2005.

Sweden provided 7 recoveries, of five individuals ringed during the breeding season, and two ringed at the end of August on islands known as migratory stopovers. These two birds probably came from further north.

Of the 9 recoveries from **Finland**, 3 have been recovered in the spring in Lot-et-Garonne and Gers. 8 of these recoveries were obtained before 1973, and only one later, in 1987. These Finnish recoveries indicate that some birds breeding in Finland do migrate by Les Landes i.e. the Atlantic route, though data from light loggers indicate that these are a minority of the total national population. It also was true when the Finnish breeding population was far larger then nowadays, as it declined by e.g. 90% during the last decades.

One chick ringed in **Estonia** was caught two and a half month later in Les Landes in 1991

Russia provided one ring recovery, of a bird ringed at Gumbartitsy, Leningrad region (60.68°N, 32.95°E), but late in August (26 August 1986) so that bird was already on migration, before being captured on 21 September in Les Landes.

One bird ringed in **Poland** in May was recovered in Les Landes in September four years later, and three birds ringed in **Germany** in May-July were also captured in Les Landes in September of the same year (2) or the year after ringing (1).

7 of the 8 recoveries from **Italy** are birds ringed during the spring migration in Italy (end of April early May), and recovered another year in Les Landes. Only one bird was ringed during the autumn migration in Italy (23 August 1993), and captured three weeks later in Les Landes (14 September), suggesting that a few individuals might sometimes switch from the Mediterranean to the Atlantic route.

The six recoveries from **Belgium** are of birds ringed there during the autumn migration, so of birds in migration along the Atlantic route.

Figure 16 maps the links between the ringing and the recovery locations of all ortolan bunting individuals currently in the database held by the CRBPO at MNHN.



VI – CONCLUSIONS

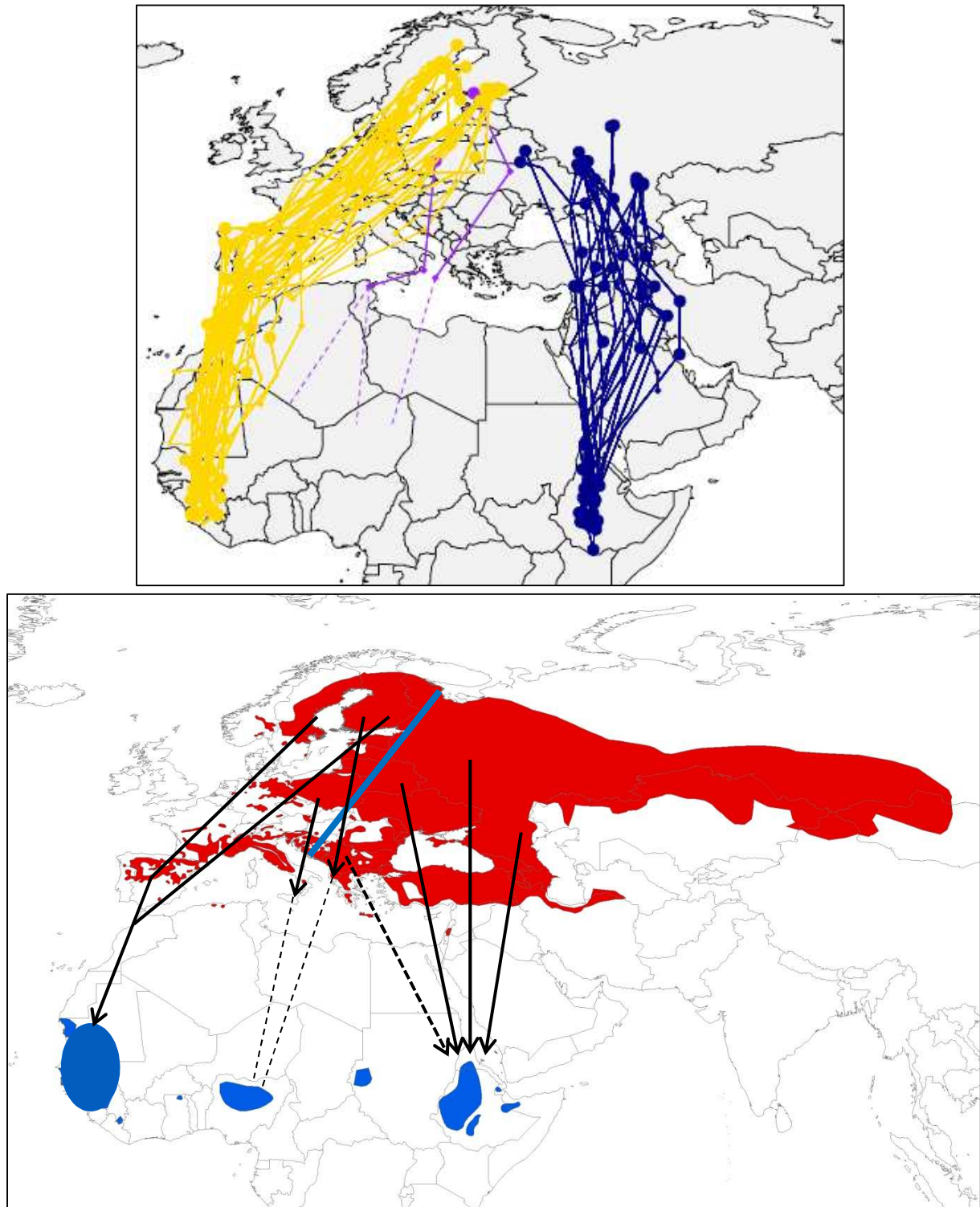
INTIGEO loggers provided 24 complete autumn tracks for 22 individuals breeding in Sweden, Finland, Lithuania, Belarus and Russia. These tracks identified two clearly separated flyways. Birds breeding in Belarus and at three sites in Russia all used an eastern flyway over the Caucasus and the Middle East, to winter in Ethiopia and Eritrea. Birds breeding in Lithuania, Finland and Sweden used a western flyway, following two different routes. Most tracks from Sweden, and all tracks from Germany ($n = 16$) obtained by Petra Bernardy go over south-west France, along an Atlantic route. A few tracks from Sweden and tracks from Finland and Lithuania followed a more continental route, over eastern Poland to northern Italy, following a Mediterranean route along the coasts of France and/or Spain – thus not visiting south-west France. Indeed, it seems that within the western flyway, populations breeding more to the west use the Atlantic route (Norway, Germany, most of Sweden, and probably part of western Poland), while populations breeding more to the east use the Mediterranean route (Baltic states, Finland, and probably eastern Poland).

The few short tracks (early autumn migration until the equinox) obtained from SOI loggers confirm the Mediterranean route for one individual from Poland and three individuals from Finland, and an eastern flyway for three individuals from Russia. As expected, two birds from Spain used the western flyway, as did one male from France in a spring migration. Furthermore, one individual from Norway did winter in Guinea, close to the wintering sites of all individuals using the western flyway.

Most interesting, one bird from western Poland and one bird from western Finland started to migrate southwards until the end of the logger recordings, i.e. 6 September and 30 August, respectively. While the real latitudes where these tracks stopped can be disputed (Libya versus south-east Europe), we can be confident in the estimated longitudes, revealing that these two individuals used a third flyway over central Europe, probably reaching Italy and Greece, respectively, by early September. The undoubtedly southward direction of these tracks suggests that these two individuals could head to the wintering range of the species in northern Nigeria.

Figure 17 gives a graphical summary of the results obtained with the light loggers.

Figure 17. Synthesis of the migration flyways used by ortolan buntings studied during our research program. Above, tracks obtained with light loggers, three colours for the three flyways. Below: simplified representation of the three flyways; breeding and wintering ranges from BirdLife International, modified in West Africa to fit our results from light loggers. The blue line shows the east-west migration divide. The black lines show the routes followed by tracked individuals (except for the dashed arrow which is inferred from isotopic results). The dashed lines are the suspected directions taken by the two individuals following the central flyway.



APPENDIX II - STABLE ISOTOPES ANALYSES

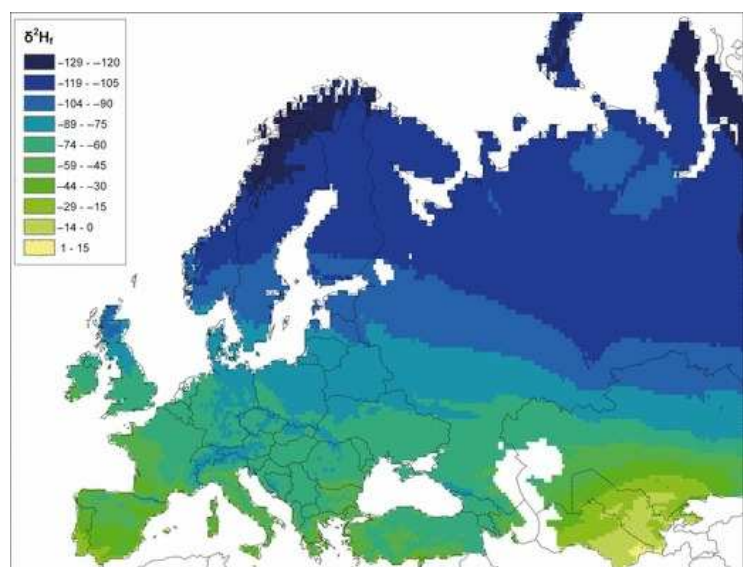
[Note: Methodological details and isotopic assignments realized by Kevin Kardynal, from Keith Hobson's lab]

Hydrogen isotopes (deuterium): from rainwaters to feathers

On continents, hydrogen isotopes concentrations are known to be spatially structured according to their concentrations in rainwaters. Bird feathers grown at a given place have hydrogen isotopic concentrations proportional to that of the environment where they have grown, i.e. to those concentrations in rainwaters. Hence, by measuring the deuterium concentration in a bird feather, it is possible to infer a probabilistic geographical space where this feather might have grown. Indeed, a spatially-explicit likelihood assignment method (Royle and Rubenstein 2004, Hobson *et al.* 2009) can be used to delineate probable areas of potential origins for Oortolan Bunting feather samples, converting an amount-weighted growing season precipitation surface (Bowen *et al.* 2005) to a feather isoscape using a calibration equation (Procházka *et al.* 2013). The latest equation is the transfer function of the deuterium from the environment to the feather. Assignments of origin are performed only within the known range of the species, so only pixels where we know that feathers can have grown/moulted are examined.

In Europe, deuterium (hydrogen isotope) concentrations have a clear latitudinal structure (Bowen *et al.* 2005), so that the feather deuterium isoscape too (Fig. 1). So this approach first provides a longitudinally-wide belt where the deuterium concentrations are similar to those of the environment where the feathers have grown; this does not mean at all that all birds which have grown their feathers in such a belt are concerned. It simply means that all birds that have grown their feathers in that belt have similar isotopic signatures in their feathers.

Fig.1. Feather $\delta^2\text{H}$ isoscape for Europe using the growing season amount-weighted precipitation $\delta^2\text{H}$ surface (Bowen *et al.* 2005) and a calibration relationship linking feather and precipitation $\delta^2\text{H}$ (Hobson 2011).



Wintering grounds of breeding birds

To help determining the wintering grounds of breeding birds from various populations, we also realized hydrogen isotopic measurements of keratin from feathers collected on breeding birds captured on their breeding territory. The Ortolan Bunting moults body coverts twice each year: before the autumn migration on the breeding grounds (August), and before the spring migration on the wintering grounds. Hence, breeding birds in May-June have body coverts moulted on their African wintering grounds.

We analyzed samples (scapulars or body coverts) obtained from territorial males captured on their breeding grounds in May-June 2013-2014-2015 in various countries (total then yearly sample sizes given in brackets): Belarus (n=43; 0-21-22), Finland (n=139; 53-33-53), France (n=34; 11-14-9), Lithuania (n=43; 0-21-22), Poland (n=31; 0-18-13), Serbia (n=7; 0-0-7), Spain (n=19; 0-10-9), and Russia (n=192; 0-60-132). We also analyzed body feathers of migrants captured in spring (April 2015) in Kuwait (n=45) and Israel (n=10) to similarly assign their feathers to potential wintering grounds.

Breeding origin of autumn migrants

To help determining the geographical origin of ortolans migrating across France and over Kuwait, we realized hydrogen isotopic measurements of keratin from feathers taken on live birds captured during migration and later released in the wild. In that species, autumn birds have either tail feathers grown in the nest (for juveniles) or moulted on the breeding grounds before the fall migration (for adults). We therefore sampled migrants in France in August-September (in four years: 2012, 2013, 2014, 2015; n=40) and in Kuwait in September 2014 (n=78).

Each year, the hunting police authorities seize ortolan buntings in south-west France. Such seized birds are then kept in a local wildlife care center, before being released in the wild (if in good health condition) when the judicial authorities order. These seized birds comprise both wild birds just captured and live dummies kept in captivity for one year or more. Such seized birds have been sampled (one tail feather; n = 274) when being ringed at the care center just before being released in the wild. We divided these seized birds in three groups: (1) first-calendar individuals (n = 34; hatched in the year, so wearing in September feathers grown for sure in the wild in the summer at their hatching site); (2) attested dummies (n=21; so with feathers grown in captivity for sure), including all individuals with coloration anomalies (one or more white secondary or primary, fuliginous body coverts) and one male kept in a care center since one year; (3) unknown status (n=179), which should be a mix of non-obvious dummies and wild adults. Whatever the status of all individuals, they all have hatched in the wild (there is no captive breeding of the species), but their feathers have grown either in the wild or in captivity.

Sampling to calibrate the transfer equation

In adults, tail feathers were supposed to be moulted once a year, before the autumn migration at the breeding grounds. Juveniles have tail feathers grown in the nest, i.e. at their hatching site. Therefore, we expected breeding birds in May-June to have approx. 10 month old tail feathers, grown the previous summer on their hatching/breeding sites. We then realized hydrogen isotopic measurements of tail feathers collected on breeding males across the continent (5 or 6 males per sampled site across 10 countries for a total of 100 samples). The idea was to determine a calibration equation for the Ortolan Bunting and to verify that it is similar to the calibration equation obtained for the Eurasian Reed Warbler (*Acrocephalus scirpaceus*; $d^2H_f = -10.29 + 1.28*d^2H_p$; Procházka *et al.* 2013), and usually used in assignments to origin for European birds. This formula expresses the transfer of deuterium from rain water to feather's keratin.

Hydrogen isotopic measurements

Feathers were cleaned in 2:1 chloroform:methanol solvent rinse and prepared for δ^2H analysis at the Stable Isotope Laboratory of Environment Canada, Saskatoon, Canada. The δ^2H of the non-exchangeable hydrogen of feathers was determined using the method described by Wassenaar & Hobson (2003) based on two calibrated keratin hydrogen-isotope reference materials (CBS, KHS). Hydrogen isotopic measurements were performed on H_2 gas derived from high-temperature (1350 °C) flash pyrolysis (Eurovector 3000; Milan, Italy) of 350 ± 10 ug feather subsamples and keratin standards loaded into silver capsules. Resultant separated H_2 was analysed on an interfaced Isoprime (Crewe, UK) continuous-flow isotope-ratio mass spectrometer. Measurement of the two keratin laboratory reference materials corrected for linear instrumental drift were both accurate and precise with typical within-run mean $\delta^2H \pm$ SD values of $-197 \pm 0.79\text{‰}$ ($n = 5$) for CBS and $-54.1 \pm 0.33\text{‰}$ ($n = 5$) for KHS. All results are reported for non-exchangeable H expressed in the typical delta notation, in units of per mil (‰), and normalized on the Vienna Standard Mean Ocean Water–Standard Light Antarctic Precipitation (VSMOW-SLAP) standard scale.

Assignment to Origin

To delineate probable areas of potential origins for Ortolan Bunting feather samples, we used a spatially-explicit likelihood assignment method (Royle and Rubenstein 2004, Hobson *et al.* 2009). To this end, we converted an amount-weighted growing season precipitation surface from Bowen *et al.* (2005) to a feather isoscape using the calibration equation for Eurasian Reed Warblers ($d^2H_f = -10.29 + 1.28*d^2H_p$) from Procházka *et al.* (2013) because a similar equation for Ortolan Buntings is not available. The residual standard deviation (SD = 10.36‰) from the linear regression model used to calibrate the precipitation surface for Eurasian Reed Warbler feathers was included in the assignments as an estimate of error. We obtained the digital distribution map of ortolan buntings from BirdLife International

and NatureServe (2011) and modified it to include known breeding areas in Kazakhstan (see map at <http://www.birds.kz/v2taxon.php?s=577&l=en>). For assignment to origin analyses for museum feather samples, we also modified the current range of the species to include the whole of Western Europe where the species might have bred previously before its strong decline. Ortolan buntings moult their feathers either on the breeding or wintering grounds, therefore assignments to origin analyses only included the species breeding or wintering range, respectively. In separate analyses, potential areas of origin for birds caught in the western and eastern flyways were restricted to their respective breeding areas (e.g. west or east of a latitudinal migration divide following political borders) based on results from geolocators data and genetic structure of breeding populations.

We used an odds ratio of 2:1 to assign feathers to potential hatching origin where cells in the isoscape in the upper 67% of probabilities were considered as likely (1) origins and all others were considered unlikely (0; Hobson *et al.* (2009)). We first realized assignments by country (grouping birds captured in a same country), then we grouped countries based on statistical analyses, grouping birds with similar values of deuterium concentrations (country effect in GLMs). Assignments conducted for feather samples resulted in a spatially explicit binary surface for individual birds, which were subsequently summed across assignments for all other individuals within a group to represent potential origins for that group. Manipulation of digital files and assignment to origin analyses were conducted using multiple packages including ‘raster’ v.2.3 (Hijmans 2015), maptools v0.8 (Bivand and Lewin-Koh 2015) and ‘permute’ (Simpson 2015) in the R statistical computing environment v3.3.1 (R Core Team 2016) and ArcMap 10.1 (ESRI 2012).

RESULTS

Calibration: where ortolans moult their tail feathers?

Tail feathers taken on breeding birds have the same deuterium concentrations than the scapulars of the same birds (see Fig. 2a; Pearson’s correlation coefficient $r=0.80$, $n=65$, for those individuals with deuterium measures in scapulars and tail feathers). This reveals that scapulars and tail feathers have been moulted in similar isotopic environments, hence that all tail feathers are moulted on the wintering grounds (i.e. all 12 and not 0 to 6 as reported in Demongin 2016).

For the ortolans captured in Kuwait during the spring migration, the same is found (see Fig. 2b; Pearson’s correlation coefficient $r=0.81$, $n=43$, excluding two outliers with a $\Delta d2H > 30$ between scapulars and the tail feather, attesting that these two feather types have moulted at different sites). This confirms that 43 out of these 45 individuals moulted their tail feathers on the wintering grounds.

To conclude, it was not feasible to calibrate a transfer equation with the analyzed samples. To do so, it would be necessary to collect samples on chicks in the nest or freshly fledged

juveniles. The main discovery of this analysis is that ortolan buntings do molt their tail feathers also in Africa, i.e. moult the tail twice each year. This is supported by the fact that ringers collected either a central or an outer tail feather, and that both types have similar isotopic signatures.

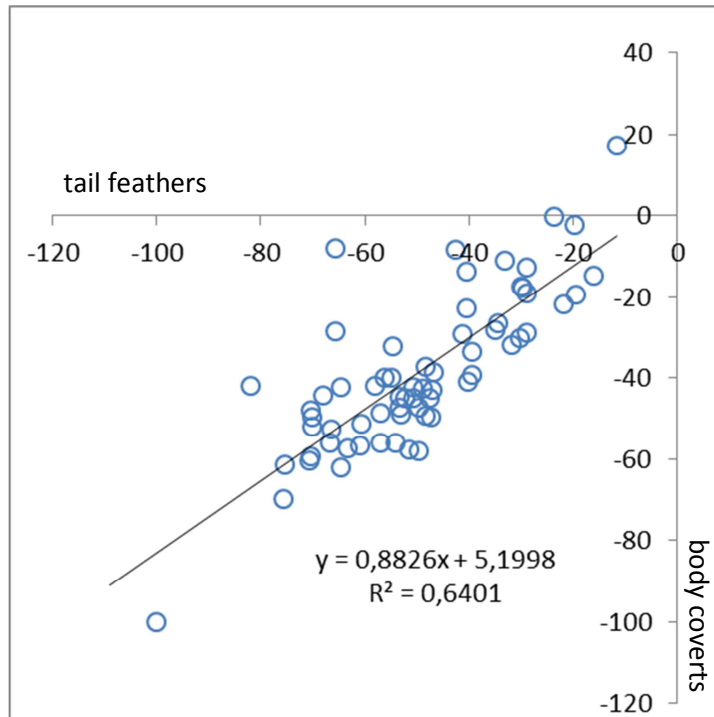


Fig. 2a. Deuterium concentration in feathers of 65 ortolan buntings captured in various countries in Europe (Belarus, Finland, France, Lithuania, Poland, Russia, Serbia, Spain) during the breeding season (May-June), in tail feather (horizontal axis) and body coverts (vertical axis).

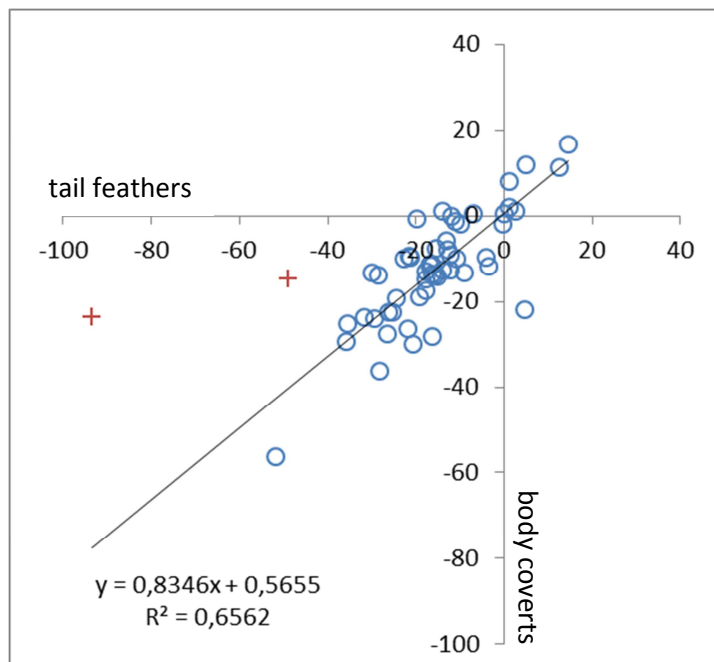


Fig. 2b. Deuterium concentration in feathers of 45 ortolan buntings captured in Kuwait in April 2015 during the spring migration, in tail feather (horizontal axis) and body coverts (vertical axis).

Wintering grounds

The deuterium concentrations in scapulars/ body coverts (moulted on African wintering grounds) of breeding /spring migrating birds revealed two distinct groups of countries: (1) Belarus, Russia and Serbia, together with spring migrants captured in Israel and Kuwait; (2) breeding birds of Finland, France, Lithuania, Poland and Spain. These two groups correspond to individuals having moulted in similar isotopic environments, so probably in the same geographical areas.

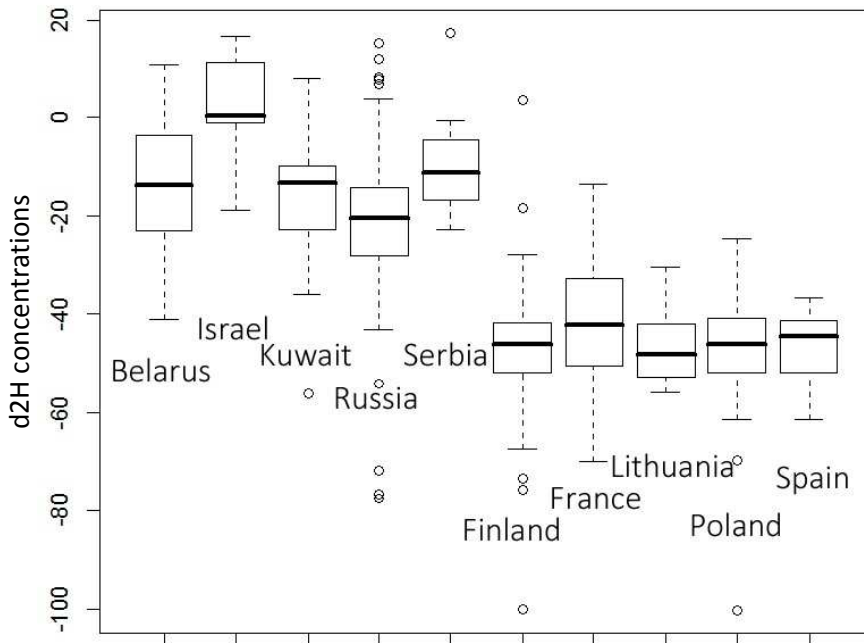


Fig. 3. Boxplot of average deuterium concentrations in body coverts / scapulars of ortolan buntings captured during the breeding season, by country where birds have been captured.

Probabilistic assignments to potential wintering grounds within known winter range

The following panel (Fig. 4) shows graphical outputs of the geographical assignments to the winter range of individuals captured in countries of the first group identified in the previous boxplots: (a) Belarus (n=43), (b) Serbia (n=7), (c) northern Russia (Vladimir, n=13), (d) southern Russia (Belgorod and Volgograd, n=179), (e) Israel and Kuwait (n=55), and (f) all individuals (n=297).

Then, Fig. 5 shows graphical outputs of the geographical assignments to the winter range of individuals captured in countries of the second group: (a) Finland (n=139), (b) France (n=35), (c) Lithuania (n=21), (d) Poland (n=24), (e) Spain (n=19), and (f) all individuals from these countries (n=238).

These outputs clearly show that the two groups correspond to a segregation between birds wintering in East Africa (group 1, eastern populations), and birds wintering in West Africa (group 2, including, Scandinavia, Finland, Baltic states and Western Europe).

Fig. 4. Geographical assignments of ortolan buntings (captured during the breeding season) to pixels of the winter range where they might have moulted their body coverts / scapulars. Individuals breeding in (a) Belarus, (b) Serbia, (c) Northern Russia, (d) Southern Russia, (e) Kuwait and Israel, and (f) all these individuals together.

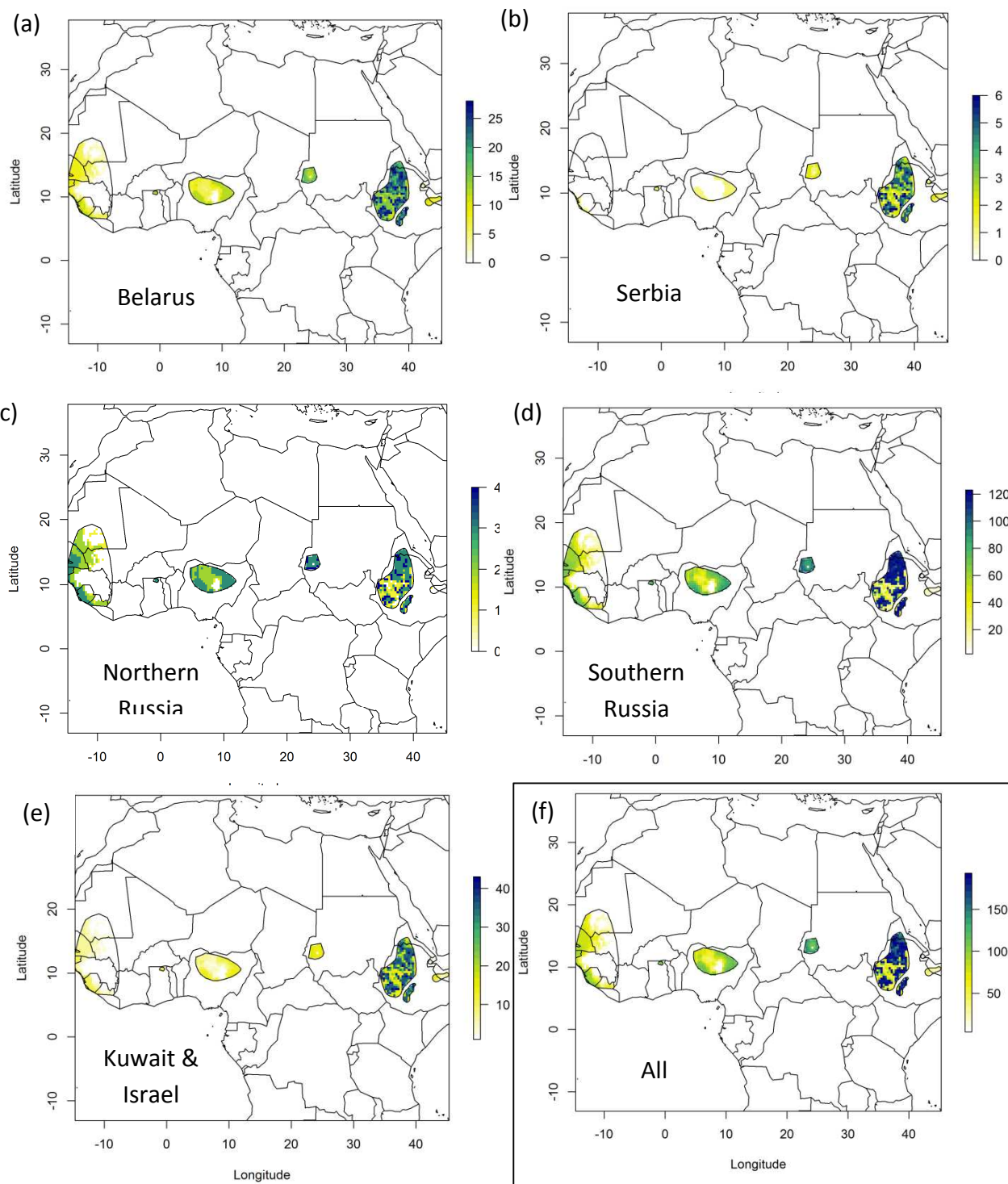
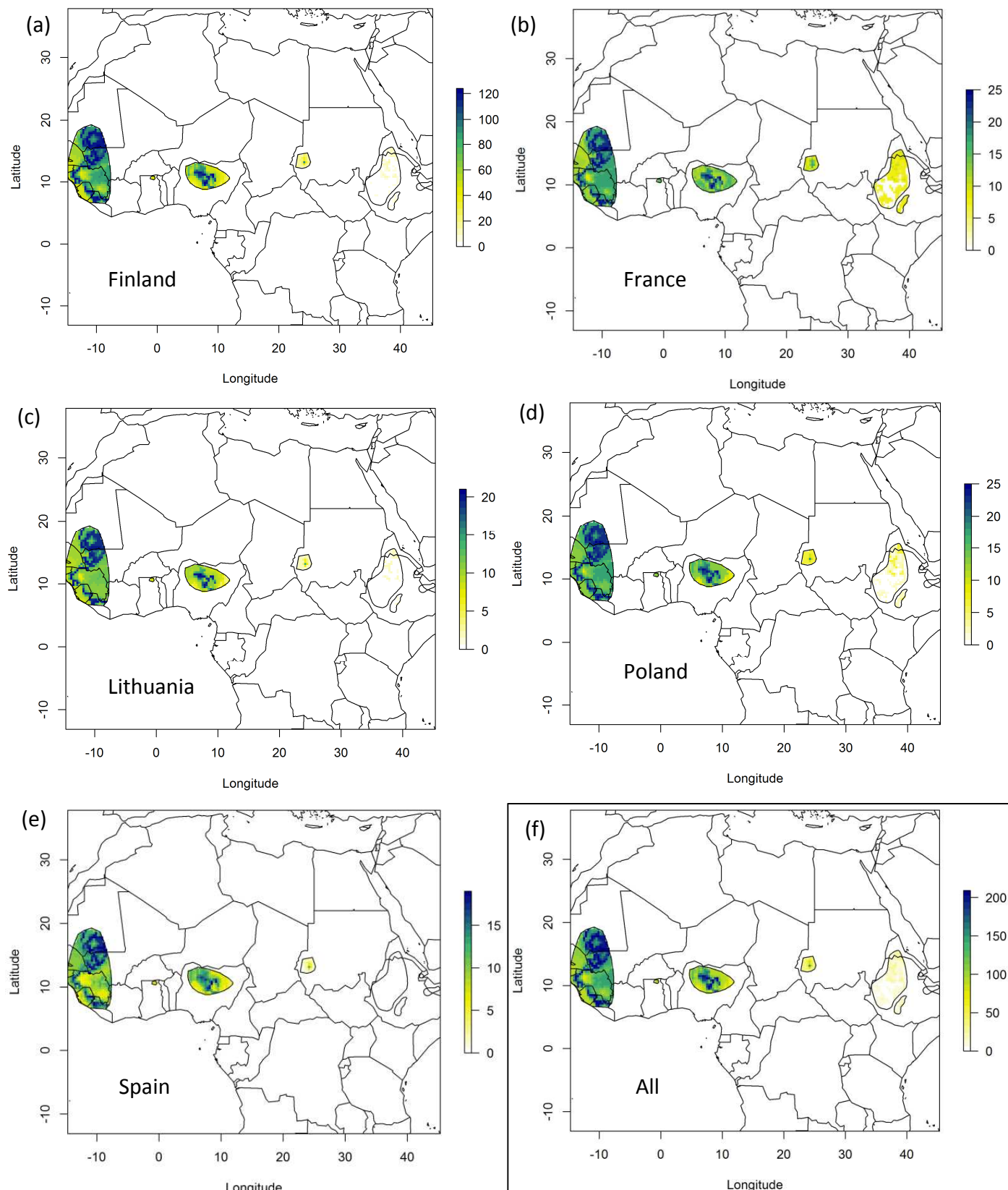


Fig. 5. Geographical assignments of ortolan buntings (captured during the breeding season) to pixels of the winter range where they might have moulted their body coverts / scapulars. Individuals breeding in (a) Finland, (b) France, (c) Lithuania, (d) Poland, (e) Spain, and (f) all these individuals together.



Probabilistic assignments of autumn migrants using the eastern flyway

To determine the latitudinal origin of migrants captured in September 2015 along the eastern flyway (in Kuwait), an assignment to origin analysis was performed on samples collected by CRBPO ringers on wild birds captured with mist-nets while migrating. A first map was obtained from the 78 individuals captured in Kuwait across the whole breeding range of the species (Fig. 6a). When restricting the assignment to countries with populations using the eastern flyway (i.e. wintering in East Africa; Fig. 6b), the possible geographical origin of these migrants appears as being mainly southern Russia, southern Ukraine and northern Romania, as well as Turkey. Given the respective population sizes breeding in these countries, we can consider that most migrants captured in Kuwait have a Russian origin, as almost all Russian ortolans (2 to 4.3 million pairs) breed within the pale green to dark blue areas depicted in Fig. 6b. From an isoscape analysis, Turkish (0.5-1 million pairs) and Romanian (225.000-550.000 pairs) populations probably also use this flyway. Ukraine holds only 58.000-67.000 pairs.

We can probably consider these probabilistic maps as also illustrating the breeding densities of the species across the eastern range. This would hold true if all birds from the depicted range do use this eastern flyway. In that case, it would suggest that densities in northern Russia, as well as in Kazakhstan and Mongolia, are very low, as birds breeding there represent a very small proportion of the migrants visiting the Middle East and the Arabian Peninsula. It would also confirm that Turkey holds a very large population, and that Romanian birds are mainly distributed in the north of the country. Birds from the Far East might be few, or might use a more eastern route (UAE, Oman) while migrating to Ethiopia.



Ringed site at Al Abraaq farm, Kuwait, April 2015 (photo F. Jiguet)

Fig. 6a. Assignment to origin map for 78 ortolan buntings captured in Kuwait in September 2014, representing a sample of migrants using the eastern flyway. Assignment performed here within the complete species breeding range, from Spain to Mongolia.

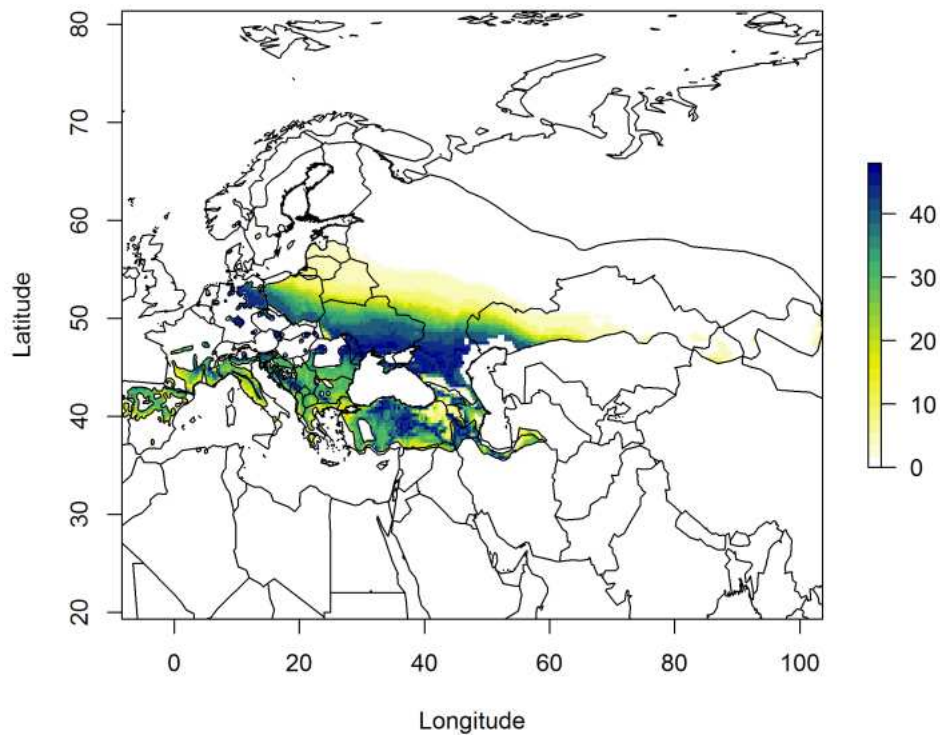
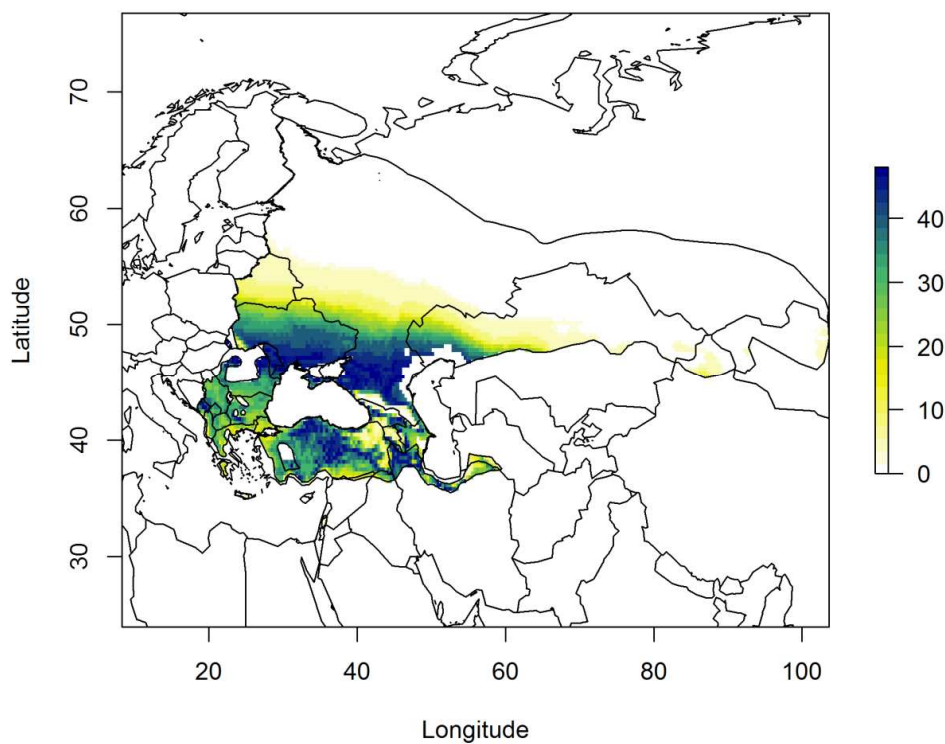


Fig. 6b. Same assignment to origin map when restricting the geographical area to countries with populations known to use the eastern flyway (results from genetic results in the present study).



Probabilistic assignments of autumn migrants using the western flyway

To determine the latitudinal origin of migrant captured in August-September 2012-2015 along the western flyway (in France, so a western Atlantic flyway), an assignment to origin analysis was performed on samples collected by CRBPO ringers on wild birds captured in France. A first map was obtained from the 40 individuals captured by ringers with mist-nets (Fig. 7a). A second map was obtained when adding to this sample the first-year birds seized by the police (n=34), which have been ringed in care center before being released in the wild (and wore first-generation tail feathers). So the second map (Fig. 7b) was obtained from 74 individuals. When restricting the assignment to countries with populations using the western flyway (i.e. wintering in West Africa; Fig.7c), the possible geographical origin of these migrants appears as being mainly Poland and Germany, and to a lesser extent France, the Baltic States and southern Sweden. Very few individuals seem to come from Norway (restricted breeding population), Finland (use a more continental route?), or northern Russia (use the eastern flyway, very low population density). Again, it seems that such a map – restricted to the countries with populations using the western flyway – might mirror local population densities, as the largest populations in the area of concern are located in Poland. The only exception here is Finland, which holds 7,000-19,000 pairs with a very low probabilistic contribution to migrants captured in France. Geolocators will reveal how and where these Finnish birds do migrate.

Fig. 7a. Assignment to origin map for 40 ortolan buntings captured in France in August-September 2012-2015 by CRBPO ringers with mist-nets, representing a sample of migrants using the western Atlantic flyway. Assignment performed here within the complete species breeding range, from Spain to Mongolia.

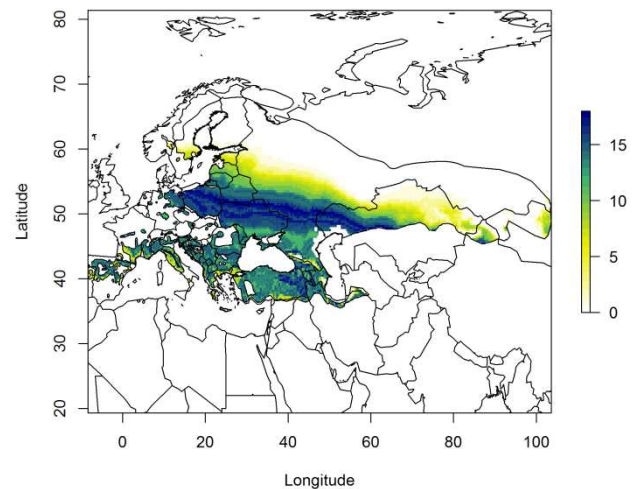
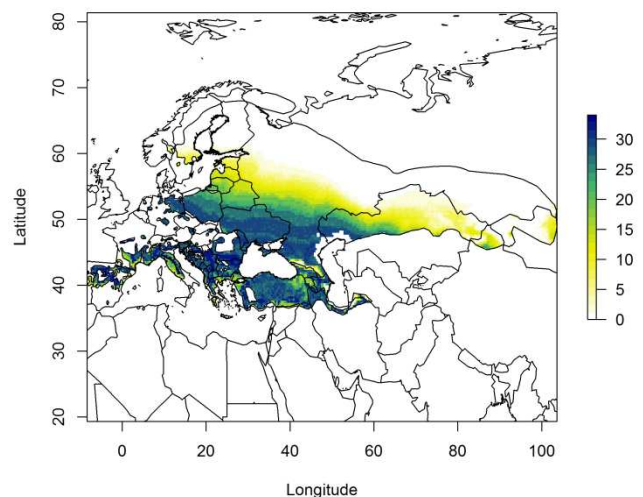
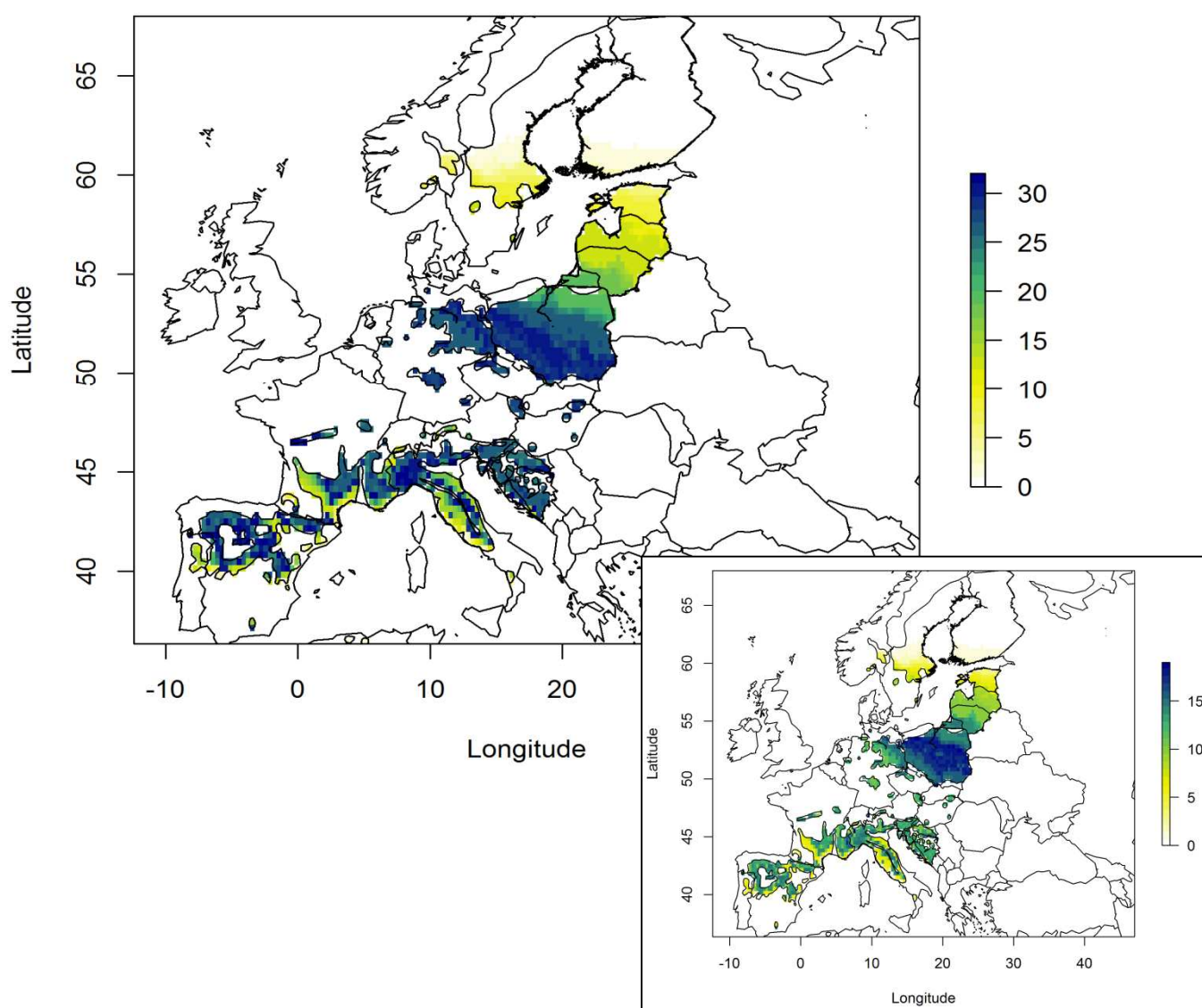


Fig. 7b. Assignment to origin map for 74 ortolan buntings ringed in France in August-September 2012-2015 by CRBPO ringers, including 40 individuals captured with mist-nets, and 34 first-calendar year birds seized by the hunting police and ringed before being released in September.



These maps illustrate potential areas where feathers of the considered ortolans might have grown, i.e. they identify all areas with similar deuterium conditions as those where the feathers have grown. Back-crossing these assignments with other information on potential areas of origin of migrants is necessary to get a more realistic picture. In this study, isotopic assignments to winter range, geolocators and genetics allowed to identify a migration divide, as birds breeding in Serbia, Belarus and northern Russia do winter in Ethiopia. Fig. 7c depicts the result of the same assignment of the 74 migrants to the part of the breeding range holding populations using the western flyway. Fig. 7d is the same but for the 40 mist-netted individuals only, and confirm the mainly Polish origin of migrants flying over France.

Fig. 7c (insert 7d). Assignments to origin of 74 wild-born ortolan buntings captured during autumn migration in France in 2012-2015. Assignments restricted to countries with populations using the western flyway.

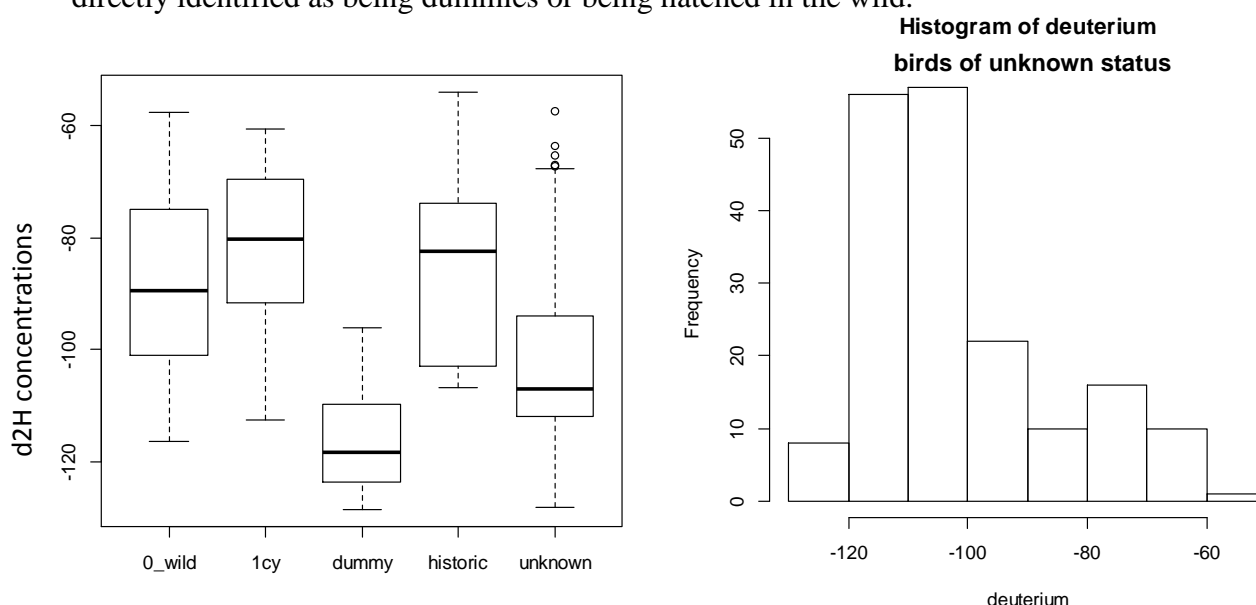


Explanations of differences with previous assignation maps

In September 2015, the hunting police seized numerous ortolans, with obvious dummies which were captive for a while, and thus having all feathers grown in captivity. This was a good opportunity to know the deuterium concentrations in captive-grown feathers of ortolan buntings, to further decide whether it is pertinent to include some seized birds within the samples used to identify the origin of migrants. As it appears that these dummies' feathers had very low deuterium concentrations, we further compared the deuterium concentrations of four groups of birds captured in France during the autumn migration: wild birds captured with mist-nets by ringers ($n=40$), 1st calendar year seized birds ($n=34$), obvious dummies ($n=21$; 20 seized birds with obvious pigmentation default, and one ringed bird kept in captivity for one year in a care center, with a d^2H value of -102.72) and all other seized birds of unknown status ($n=179$). For a further historic comparison, we also included here a fifth group, of feathers taken on museum specimens collected in August-September in south-west France ($n=15$), hold at MNHN and at the Museum of Natural History in Bayonne, to see if historic migrants had similar isotopic signatures to current migrants.

An ANOVA performed on these five groups revealed no difference between the reference group (wild birds) and first calendar year birds ($t=1.79$, $P=0.07$) and historic specimens ($t=0.69$, $P=0.49$), while dummies ($t=-6.77$, $P<10^{-10}$) and birds of unknown status ($t=-5.08$, $P=7.10^{-7}$) both had far lower values of d^2H (model captured 27% of total variance). Fig. 8a below is a boxplot of these groups. The 'unknown' group is clearly intermediate between wild and captive birds, while the histogram of d^2H in this group (Fig. 8b) revealed a majority of individuals with very low values of d^2H (dummies = captive birds) and a second lower peak of birds with higher d^2H values (seized birds with feathers grown in the wild, so wild birds captured recently).

Fig. 8. (a) Boxplot of d^2H values in the five groups of birds captured or seized in France in autumn. 1cy = first calendar year birds. **(b)** Histogram of d^2H values in ortolan buntings' feathers of the group reported as of 'unknown status', i.e. seized birds which could not be directly identified as being dummies or being hatched in the wild.



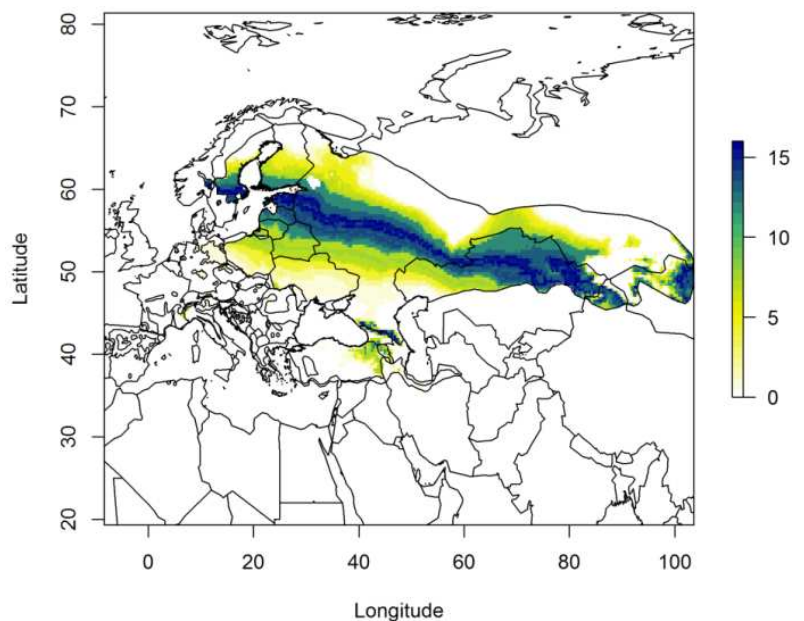
As a consequence, we decided not to include the majority of the seized birds in the isotopic assignments to origin analyses, except for those individuals identified as first calendar year birds, i.e. birds hatched a few months before for sure in the wild, which were captured during their first fall migration by hunters, further seized by the hunting police, brought to a wildlife care center, and ringed by a CRBPO ringer before being released in the wild.

Previous maps, released in intermediate summaries of this study, which depicted former temporary assignments to origin, are now considered as flawed by possible captive-grown feathered individuals, which according to the histogram in Fig. 8b might represent a large proportion (~75%) of that sample.

Fig. 9. Examples of individuals identified as decoys (plumage color aberrations, extended papilloma on toes...) (Photos by David Lambottin- ONCFS, at the Alca Torda wildlife care center, September 2015):



Fig. 10. Erroneous assignment to origin of feathers sampled on known decoys (n=21), as they drunk tap water, not rainwater, so that the deuterium concentration cannot be used to assign an origin to their feathers.



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APPENDIX 3 – GENETICS REPORT

The aim of the genetic part of the program was to map the genetic diversity of European breeding populations, and to further use this mapped pattern to assign autumn migrants captured on the western flyway (south-western France) and the eastern flyway (Kuwait) to breeding areas. Further back-crossing of the results with data obtained from stable isotopes and geolocators allowed to better assign migrants to genetically related breeding populations.

METHODS

Data preparation

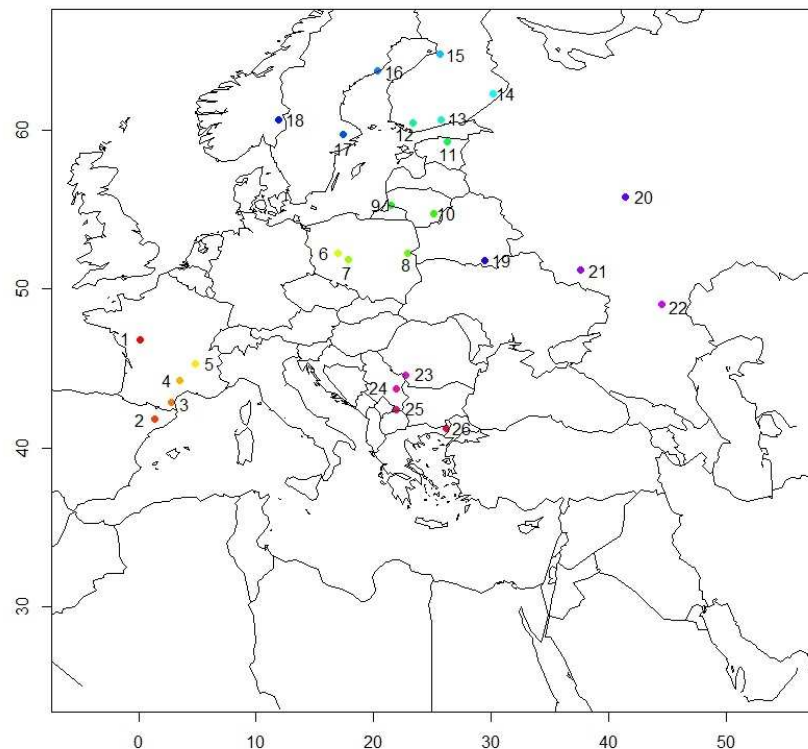
A total of 1127 samples, including duplicates, were genotyped for the 24 microsatellite loci. Genotypes were examined in Genemapper v5 ((Applied Biosystems, USA; see <https://tools.thermofisher.com/content/sfs/manuals/4476603A.pdf>) to determine peak alleles and raw allele sizes were exported to AutoBin (INRA BioGeCo, Bordeaux). This Excel macro examines the size difference between contiguous alleles and detects gaps to infer allele binning. Binning was carefully inspected and manually corrected when necessary. DNA amplification success was calculated for each sample and each locus. We re-amplified and genotyped 113 individuals to estimate genotyping error. We also used CERVUS (Marshall et al. 1998, Slate et al. 2000) to identify 30 further replicated genotypes that would correspond to recaptures in successive years. The 143 replicated genotypes thus represent a 12.69% sub-sample of the dataset.

Microsatellite characteristics and genetic diversity

Birds captured at a single breeding site were considered as sampling site. Where breeding densities were low, such as in Finland, individuals were sampled over larger geographical scales (up to 108 km) but grouped together as sampling sites. Sites with less than eight individuals were excluded from population-level analyses. The resulting breeding dataset consisted in 555 individuals sampled at 19 breeding sites for population level analyses and 575 individuals from 26 sites for individual level analyses (Figure 1; see Table 1).

GENEPOP 4.0 (Raymond & Rousset 1995) was used to test loci in departure from linkage equilibrium at each site (Markov chain parameters: 10,000 dememorisation, 100 batches, 5,000 iterations) and sequential Bonferroni correction for multiple tests was applied. We tested the correlation between the number of homozygotes and of missing data across samples and loci to determine whether any putative allelic dropout was due to low DNA quantity or poor DNA quality (correlation across samples) or to locus specific factors including null alleles. Tests were carried out in MICRODROP (Wang et al. 2012). The presence of scoring errors or null alleles was determined for each locus and each site using MICROCHECKER (van Oosterhout et al. 2004). Finally, we conducted 456 exact tests for deviation from Hardy-Weinberg equilibrium (HWE) for each locus (24) and each site (19) with 1000 Monte Carlo replicates using the PEGAS package in R (cran.r-project.org/web/packages/pegas/pegas.pdf). Bonferroni correction for multiple tests was applied to the nominal 5% p-value ($p=0.00011$).

Fig 1. Sampling locations of breeding ortolan buntings used in the genetic study.



Further analyses were carried out without three loci that displayed high frequency of null alleles and deviated from HWE. Genetic diversity indices were obtained for each sampling site in GENETIX 4.05.2 (Belkhir et al. 1996-2004) and FSTAT (Goudet 1995). Estimated F_{ST} averaged over loci (Weir & Cockerham 1984) and the 95% confidence intervals (CIs) after 1000 bootstrap over loci were calculated in GENETIX. A randomized G-test was performed with 1000 replicates to test for genetic differentiation among sites in R.

Population structure

Two methods were used to uncover genetic population structure. First, the Bayesian clustering program STRUCTURE (Pritchard et al. 2000) allowed the assignment of the 575 breeding individuals to K populations by minimizing deviations from HWE. Since the differentiation index F_{ST} was very low, indicating weak structure and likely high admixture, we used a correlated allele frequency and admixture model. We ran the program for one to six clusters using a burn-in of 5×10^5 iterations followed by 10^6 Markov Chain Monte Carlo iterations. Each K value was run five times with and without population information (sampling sites as prior). The optimal number of clusters K was obtained from ΔK , based on the rate of change in the log probability of data in successive K values (Evanno et al., 2005) as implemented on STRUCTURE HARVESTER (Earl and VonHoldt, 2012). Secondly, a multivariate method, discriminant analysis of principal components (DAPC) (Jombart et al. 2010) was applied to the breeding dataset. DAPC is free from population genetic assumptions and inferences are made on allelic similarity. It summarizes genetic variability of individuals within groups while optimizing group discrimination. Sampling sites were used as the grouping variable. The first 115 principal components (PC) were retained in the data transformation step, corresponding to 84.2% of genetic variance, and three discriminant functions were saved for further analyses. Analyses were carried out using the ADEGENET 2.0.1 package in R 3.3.1.

Consensus on population structure was reached based on results from these two methods. Individuals were assigned to one of the clusters defined (hereafter called populations) and tested in

an analysis of molecular variance (AMOVA) using the POPPR 2.2.0 package in R. Sampling sites with less than eight individuals were excluded from this analysis. Log-likelihood G tests for differentiation were performed between and within populations using 10,000 permutations.

We also tested a clustering based on these analyses and on prior knowledge of migratory corridors that we hypothesized to influence population structure. An AMOVA indicated the hierarchical repartition of genetic variance and cross-validation was performed. The full breeding dataset (575 individuals) was randomly split into a training and a validation dataset by assigning 70% of individuals from each sampling site to the training set (402 individuals) and the remaining 30% (173 individuals) to the validation set. The training set defined the genetic makeup of the clustering to be tested, and individuals from the validation set were assigned one of these populations by the program GENECLASS (Piry et al. 2004) using the Bayesian method described by Rannala and Mountain (1997). The process was repeated ten times.

Pairwise F_{ST} between the populations were calculated by the HIERFSTAT package in R to assess the level of connectivity.

Isolation by distance was tested between sampling sites with over eight individuals across the whole range, and within each defined population. Mantel tests were performed between matrices of linearized pairwise F_{ST} (ref) and log-transformed geographical distances using the HIERFSTAT package in R setting the number of repetition to 1,000.

Contemporary gene flow

The magnitude and direction of contemporary of gene flow occurring between the consensus populations were estimated using the program BAYESASS 3.0.1 (Wilson & Rannala 2003). The simulation was run with 2×10^7 iterations, with the first 10^6 iterations discarded as burn-in. Samples were collected every 1000 iterations. Allelic frequencies coefficient was set at 1, inbreeding coefficient at 0.9, and migration rate at 0.15. These mixing parameters ensured the acceptance rates were between 0.2 and 0.6. The trace file was examined in TRACER 1.5 to ensure mixing and convergence of the chains (Rambaut & Drummond 2009).

Genetic assignment of migratory individuals

The 396 individuals captured during migration in south west France, Kuwait and Israel were assigned to one of the clusters defined previously by the program GENECLASS using the Bayesian method (Rannala & Mountain 1997). Samples from France were obtained from wild migratory birds, from long-term captive birds used by traditional hunters to attract migratory individuals (decoy birds), putative wild migratory birds captured by hunters and seized or released by local authorities, and individuals of unknown status. Decoy birds might be selected by hunters for their success at attracting migratory individuals (e.g. selected for their dialect (Osiejuk et al. 2003)) and could thus originate from the largest population rather than reflect the diverse origins of migratory birds passing through SW France. Similarly, birds captured by hunters could be those attracted to the decoys and thus from the same breeding population. We therefore performed Chi-square tests to compare the distributions of origin population assignment for the different migratory categories, and pooled categories whenever the tests were not significant.

We also performed genetic assignment on individuals captured in south west France and grouped based on their status, as : (1) wild migrants (captured in the wild with mist-nets and ringed by CRBPO ringers), (2) 1st calendar year individuals seized by the hunting police (hatched and grown the same year in the wild); (3) dummies (adults obviously kept in captivity during their last moult because of aberrant colours, e.g. blackish plumage, or one or more totally white remiges); (4) status unknown (adult seized birds which could be dummies or wild migrants; according to their isotopic signature, 75% of them could be captive decoys). Chi-square tests were used to compare the distributions of origin population assignment, especially for comparing individuals coming the wild for sure (wild + 1st calendar year; n=73), coming from captivity for sure (dummies, n=21), and of unknown status (n=172). These are the same birds grouped in the isotopic analyses.

RESULTS

Data preparation

813 samples out of 1,127 amplified at all loci, giving a 72.14% success rate after only one PCR. Most samples that failed to amplify did so for only one locus (190 samples), and only 3.02% of samples failed to amplify at ten or more loci. Thirteen samples were removed from the dataset because of amplification failure for over eleven loci. No locus showed excessive amplification failure rate (mean \pm SD = $3.25 \pm 1.71\%$, range: 1.69-9.49%).

Ninety-one replicates had identical genotypes, corresponding to a 63.64% correct typing rate. Of the 53 samples that displayed typing errors, 80.95% were mistyped at only one locus, mostly as homozygote rather than heterozygote due to weak amplification of the second allele, and no individual was mistyped at more than four loci (occurred in only one sample). No locus showed excessive typing error (mean \pm SD = $2.04 \pm 1.65\%$, range: 0-6.38%).

The final dataset consisted of 971 unique individuals typed for a minimum of 14 loci, including 575 breeding birds and 396 birds captured during migration in south-west France (266 individuals) and in the putative eastern corridor (10 individuals in Israel, 120 in Kuwait).

Microsatellite characteristics and genetic diversity

Only ten tests for linkage disequilibrium out of 5,244 (24 loci, 19 sites) were significant. No significant correlation between the number of homozygotes and of missing data was found at the sample level ($r=-0.137$, $p=0.998$), indicating that any allelic dropout was not likely due to DNA quality or quantity. In contrast, significant correlation was detected at the locus level ($r=0.446$, $p=0.021$), indicating that allelic dropout could be due to null alleles. Four microsatellites displayed moderate to high null allele frequencies (>0.2) in over four sites, and in particular Embhort12 had high frequencies of null alleles in most sites. Fifty-three tests for deviation from HWE were significant after Bonferroni correction and due to deficiency in heterozygosity. Three loci were out of HWE in nearly 50% of populations, likely due to the presence of null alleles and were thus removed from further analyses (Embhort05, Embhort08 and Embhort12) (see Table 2).

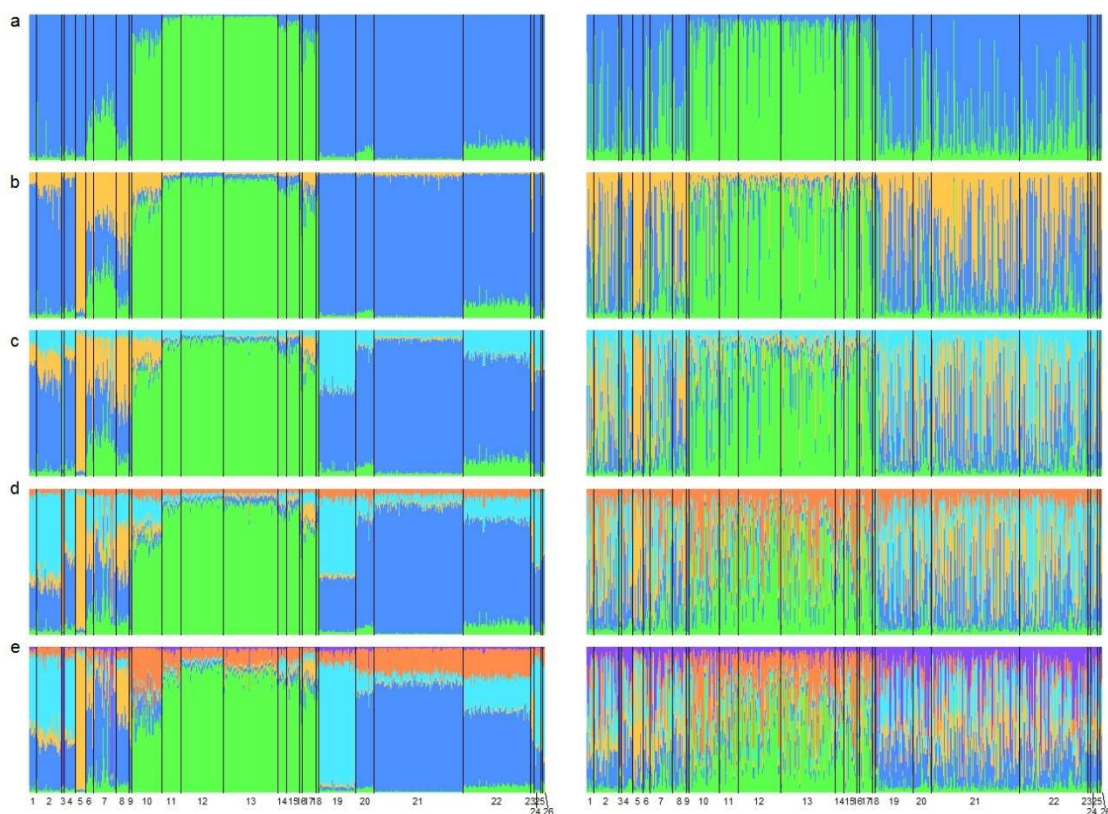
Mean allelic richness was high and similar for all sites (mean \pm SD = 6.28 ± 0.24). Observed heterozygosity was moderate and consistent across all sites (mean \pm SD = 0.730 ± 0.022), and was significantly lower than expected heterozygosity (mean \pm SD = 0.803 ± 0.025 , $p<0.01$). Inbreeding indices F_{IS} were low to moderate for all sites (mean \pm SD = 0.101 ± 0.048) and significantly different

from zero after 1,000 bootstraps for 14 out of 19 populations. Estimated F_{ST} over loci was low at 0.011 but significant (95% CI = 0.010 - 0.013) and the G-test was significant ($p < 0.001$), indicating that breeding sites were not part of a panmictic population.

Population structure

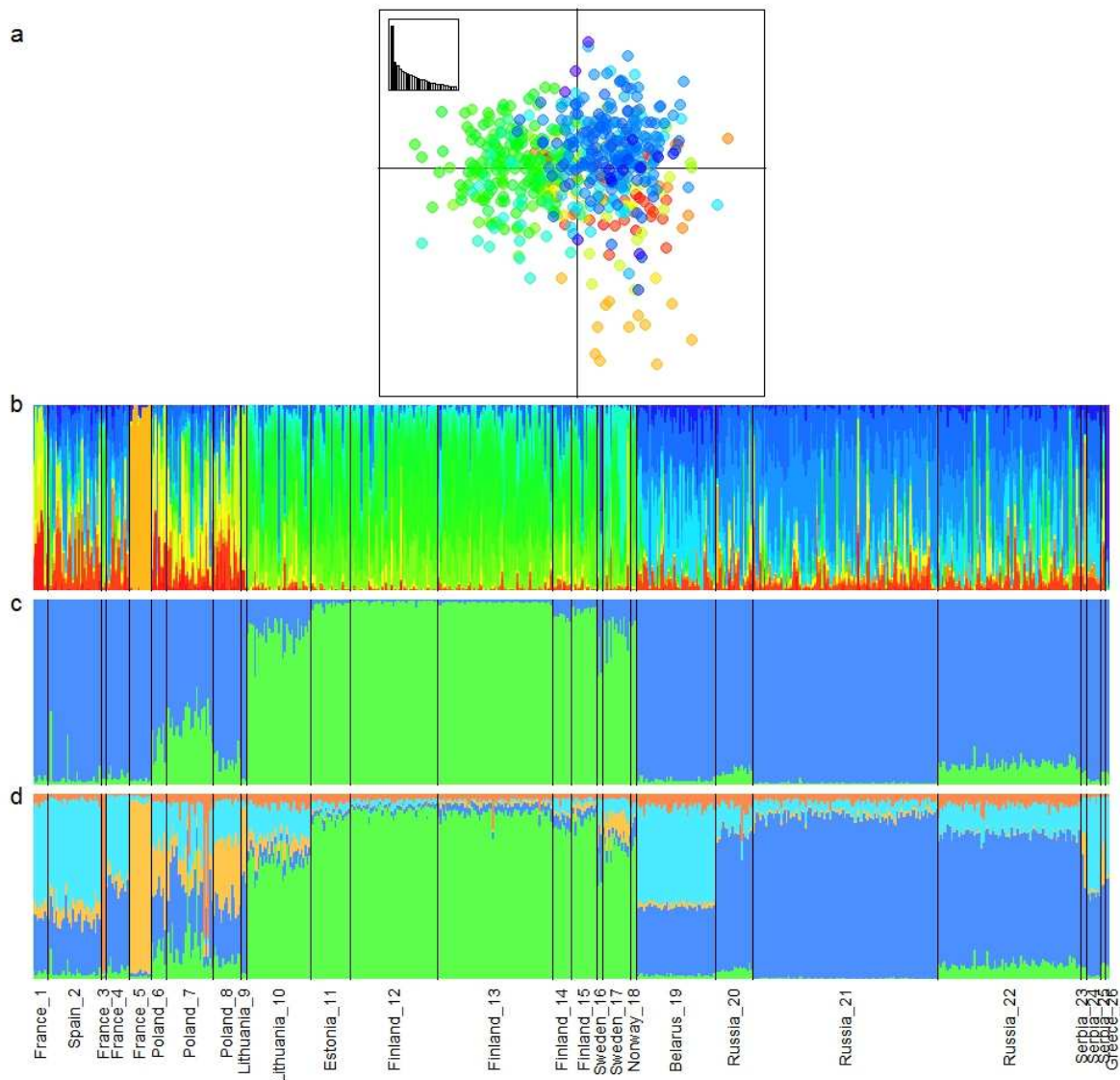
Bayesian modelling. Genetic structure was consistent with sampling geography. The prior and non-prior Bayesian models indicated similar patterns although the non-prior model displayed large levels of admixture that muddled the pattern (Figure 2). At $K = 2$ (two clusters), breeding sites from northern Europe (Scandinavia, Estonia and western Lithuania: sites 10 to 18) formed one cluster, while western, central, eastern and southern Europe formed a second one. At $K = 3$ (three clusters), the north-south clustering remains but the Drôme site from France (site 5) stood out as an independent cluster. At $K = 4$, the French samples from Corbières (site 3) distinguished themselves from the other clusters – but samples from this site are chicks from a single nest. At $K = 5$ and 6, the Belarus breeding site separated from the eastern cluster while the western and central sites displayed large admixtures. The standardised second-order rate of change ΔK indicated that the most likely number of clusters describing the data were two for both prior and non-prior models. As this method only distinguished higher structuring level, each cluster was then run separately with the same settings. No obvious geographical substructure was supported for northern Europe at the exception of larger admixture in Lithuania, possibly indicating a contact zone (the eastern Lithuania site was assigned to the southern cluster). In contrast, the optimal number of clusters for the southern group was four (prior model), consisting of the Drôme and Corbières sites, the Russian sites, and an admixed group gathering western, central and southern Europe as well as Belarus (Figures 2 and 3).

Fig.2 Bayesian posterior probability of membership to one of K populations obtained by STRUCTURE, using breeding sites as prior for the left column and no prior on the right column. Each vertical line represents an individual. Solid lines separate sites the individuals were sampled from. The sites are ordered according to their geographical location. a) $K = 2$, b) $K = 3$, c) $K = 4$, d) $K = 5$, e) $K = 6$.



Multivariate analysis. The discriminant analysis on sampling sites indicated a similar pattern of clustering. Most of the discrimination occurred on the first axis and distinguished northern Europe from western, central, eastern and southern Europe. The second axis isolated the Drôme samples from the southern cluster. Within these clusters, admixture seems high, and individuals cannot be reliably assigned to the breeding site they were captured from (41.57% of correct assignment after leave-one-out cross-validation) (Figure 3).

Fig. 3. Individual-based analyses on microsatellite data using multivariate (a and b) and Bayesian (c and d) assignment methods. (a) Scatterplot of individual principal components on the first two axes. Each site is colour coded as Figure 1. (b) Posterior probability of membership to one of the sites after discriminant analysis and leave-one out cross-validation. Each vertical line represents an individual. Solid lines separate sites the individuals were sampled from. The sites are ordered according to their geographical location and colour coded as in (a). (c) Bayesian posterior probability of membership to one of two populations obtained by STRUCTURE. Individuals and roots are displayed in the same order as in (b). (d) Bayesian posterior probability of membership to one of five populations obtained by STRUCTURE.



AMOVA and genetic differentiation among clusters. Based on these results, we considered three clusters (hereafter population): northern population (Scandinavia, Finland, west Lithuania and Estonia: sites 10 to 18), southern population (western, central, eastern and southern Europe: sites 1 to 4, 6 to 9 and 19 to 26) and the Drôme population (site 5). AMOVA indicated that most genetic variation occurred within sampling sites (97.57%, $\Phi_{ST} = 0.024$, $p < 0.001$), but still marginally supported the clustering, with larger genetic variation between populations than between sites within populations (1.39% $\Phi_{CT} = 0.014$, $p < 0.001$ vs 1.04% $\Phi_{SC} = 0.011$, $p < 0.001$) (Table 3).

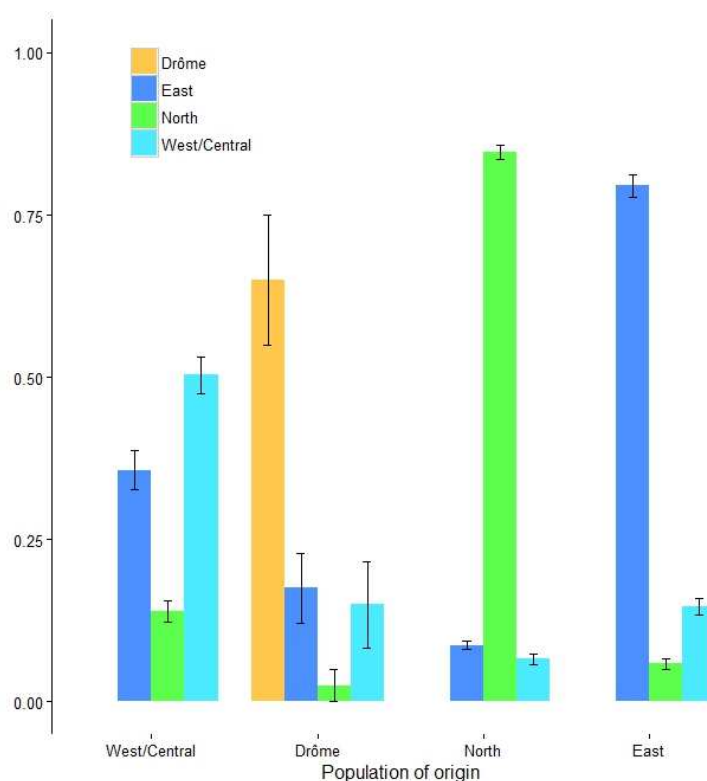
Our knowledge of western and eastern flyways led us to suspect some weak sub-structuring within the southern population, which was confirmed by the STRUCTURE and DAPC analyses. Hence we also considered four populations: the northern population, a western/central one (France, Spain, Poland and east Lithuania), the Drôme population, and an eastern one (Russia, Belarus, Serbia, and Greece). The AMOVA also marginally supported this clustering with larger genetic variation between populations than between sites within populations (1.23% $\Phi_{CT} = 0.012$, $p < 0.001$ vs 0.93% $\Phi_{SC} = 0.009$, $p < 0.001$) (Table 3).

In contrast, the putative flyways (western Atlantic: France, Spain, Norway, Sweden; western Mediterranean: Poland, Lithuania, Estonia, Finland; eastern: Russia, Belarus, Serbia, Greece) were not supported as contributing to genetic structuring (larger genetic variation between sites within flyways than between flyways (0.74% $\Phi_{CT} = 0.007$, $p < 0.001$ vs 1.28% $\Phi_{SC} = 0.013$, $p < 0.001$) (Table 3).

Pairwise F_{ST} demonstrated weak differentiation among populations with F_{ST} no higher than 0.011 and especially between the west/central population and the eastern one, while the Drôme and the northern populations seemed marginally more differentiated (Table 4 and 5).

Cross-validation. We performed a cross-validation on the four populations that supported the strength of the northern and the eastern clusters with an average of 84.68% and 79.48% of individuals correctly assigned (Figure 4). Correct assignment was also high for the Drôme population (65.00%) with mis-assigned samples mostly allocated to the eastern and the western/central populations. Moderate correct assignment to the west/central population (50.33%) with a large contribution of the eastern population (35.67%) suggests low differentiation between these populations.

Fig 4. Cross-validation of the four populations. 30% of individuals were assigned to the populations described by 70% of the dataset.



Isolation by distance. No isolation by distance was detected on the full dataset and within the southern population. When splitting the southern cluster into west/central and eastern populations, no correlation between genetic and geographic distances was found within the west/central cluster. In contrast, marginally significant isolation by distance was detected in the eastern and northern populations ($p = 0.046$ and $p = 0.045$ respectively).

Contemporary gene flow

Recent migration (in terms of immigration and emigration between breeding populations) rates were estimated as the mean proportion of individuals moving between the four defined populations per generation (Table 6). The disequilibrium-based program inferred a nearly complete lack of contemporary gene flow towards the eastern population and towards the northern population. Emigration rates from the northern population were very low (mean 1.97%), indicating that this population was isolated from the remaining Europe. Similarly, emigration rates from west/central and Drôme were close to zero. In contrast, emigration rates from the eastern population were high (mean 19.50%), especially towards west/central and Drôme populations. The eastern population would therefore act as a source population for Western Europe while northern Europe now appears genetically disconnected from the rest of Europe.

Genetic assignment of migratory individuals

Assignment of migrants (captured and sampled along the western flyway (France) and eastern flyway (Kuwait and Israel)) to their breeding population was performed on the four defined populations to reflect both the higher level, and thus stronger, genetic structure and our knowledge of migratory flyways. We found no significant difference in the distribution of assigned individuals to breeding populations among the different categories of French migratory birds ($X^2 = 16.601$, $p = 0.165$). Birds captured in France were thus all pooled to represent the western flyway. Significant differences in assignment were reported between individuals captured in the eastern and western flyway ($X^2 = 30.565$, $p < 0.001$). Hence, 67.69% of migratory birds using the eastern flyway were assigned to the eastern population, and 23.84% to the west/central one (Figure 5). In contrast, assignment of individuals from the western flyway was more equally distributed among populations, with 39.10% assigned to west/central population, 38.72% to the eastern one and 21.80% to the northern one. Only one individual captured in the western flyway seemed to breed in Drôme.

Stable isotopes and geolocators data indicated that individuals caught in south west France did not originate from the eastern population. We therefore re-ran the assignment for the western flyway and removed the eastern population as potential breeding origin. Individuals originated mostly from the west/central population (66.54%), and from the north (33.08%) (Figure 6).

Figure 5. Assignment of individuals sampled during migration in the eastern or the western flyway to a breeding population.

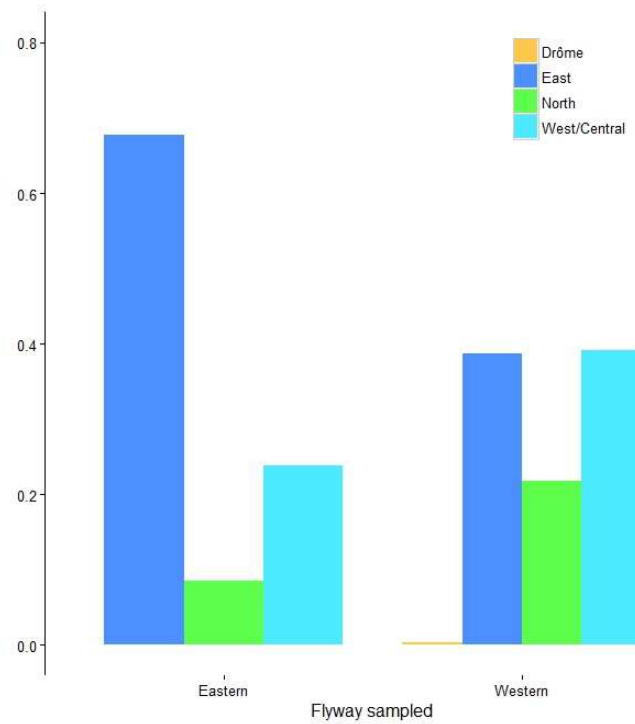
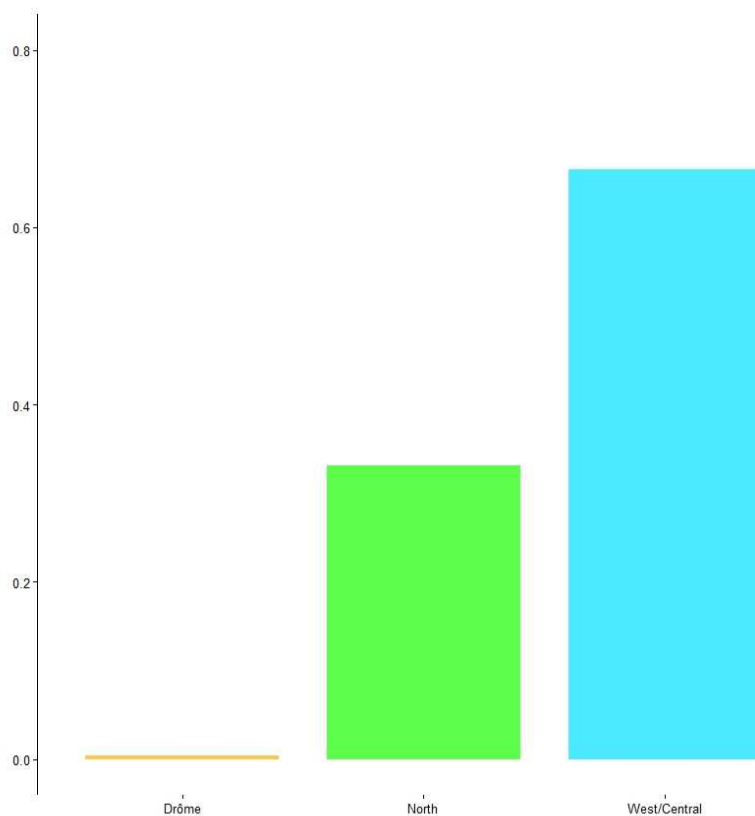


Fig 6. Assignment of individuals sampled during migration in the western flyway to a breeding population other than the eastern cluster. Stable isotopes and light-level geolocators excluded an eastern breeding origin for occidental migrants.



Genetic assignment of seized vs wild ortolans

When analyzing the assignment distributions of the four groups of autumn migrants of the western flyway (wild, 1st calendar year, dummies, unknown status) to the genetic populations using the western flyway, no significant differences were found in the assignment distribution of the different isotopic groups ($\chi^2 = 2.827$ $p = 0.830$; Figure 7), and especially between the 1A and the wild groups ($\chi^2 = 1.382$ $p = 0.240$; Figure 7) which were thus pooled. Individuals captured along the western flyway thus originated mostly from the west/central population (62-67%), and 33-38% coming from the northern population (Figure 8). Only one individual of unknown origin had a Drôme genotype.

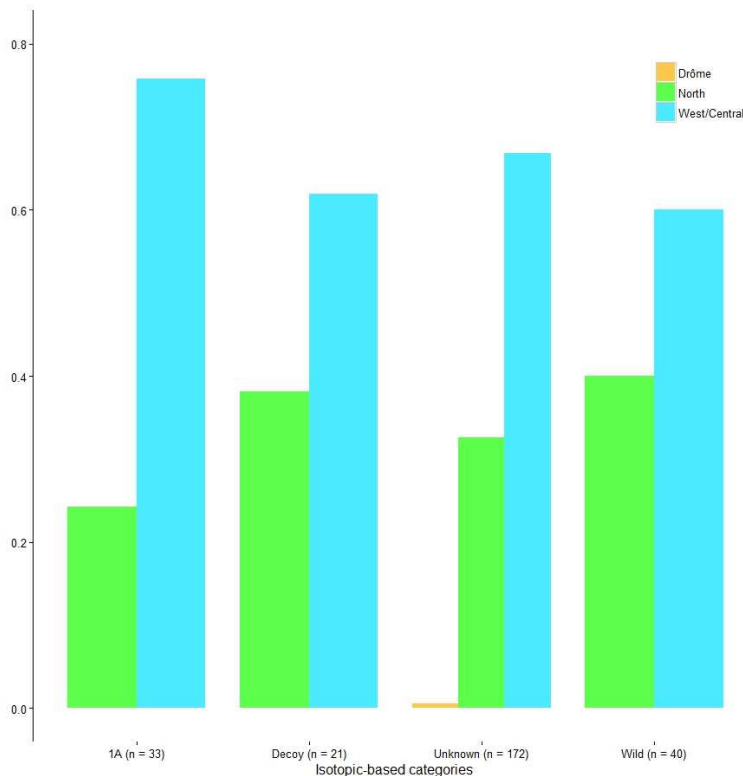


Fig 7. Assignment of individuals sampled during migration in the western flyway to a breeding population within those using the western flyway. 1cy = first calendar year.

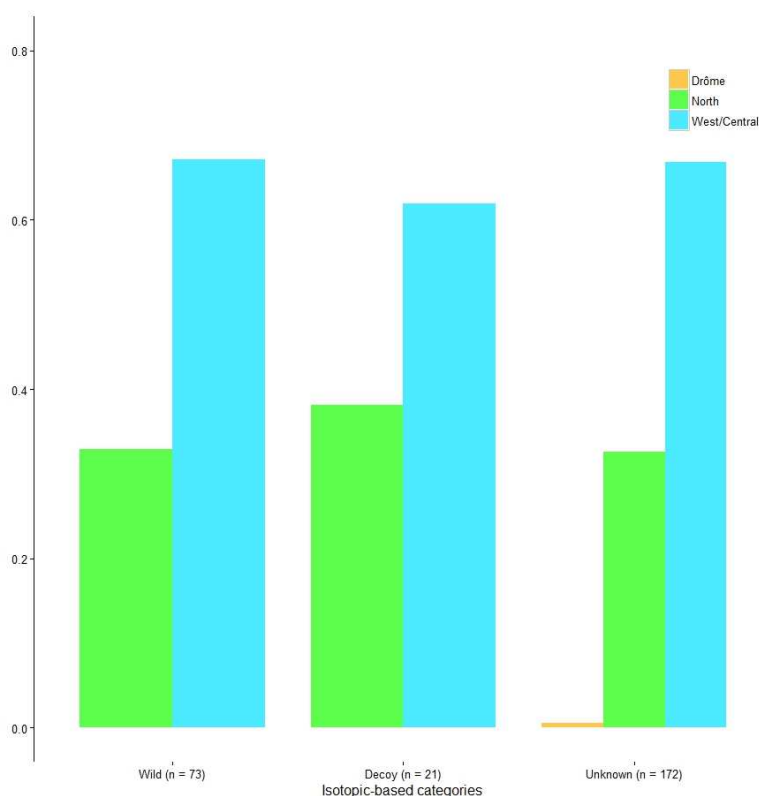


Fig 8. Assignment of individuals sampled during migration in the western flyway to a breeding population within those using the western flyway. Same as Figure 7 but pooling groups 'wild' and '1st calendar year'.

DISCUSSION

Main message: there is a genetic structure, including a possibly recently isolated population in the north and an east-west source-sink dynamics. Only few western migrants likely originate from Eastern Europe –if any.

- There is low but significant population structure across Europe consisting of four populations: north, east, west/central and a single French breeding site in Drôme.
- Little contemporary gene flow occurs between the North population and the rest of Europe. North Europe seems to be recently genetically disconnected.
- Contemporary gene flow was detected across the rest of Europe, with the eastern population acting as a genetic source.
- No idea why Drôme is standing out. Multiple data checks indicated this is not an artefact.
- No genetic differentiation among putative Atlantic and Mediterranean flyways, however eastern and western flyways contribute to a certain extent to genetic structuring.
- Migratory birds from eastern flyway largely assigned to Eastern breeding origin.
- Migratory birds from western flyway assigned to the different populations more equally. However, the large proportion of eastern genotypes in west/central breeding population indicates that eastern breeding origin is overestimated and west/central origin underestimated.
- Restricting the genetic assignment to breeding populations really using the western flyway indicate that 2/3 originate from west/central breeding populations, 1/3 from northern breeding populations. These proportions are coherent with the reported breeding population sizes in these two areas, especially if breeding success is higher in the west-central group, and if the Polish population size is lower than previously reported (see update by Jakub Kosicki, proposing that 150,000 breeding pairs in Poland, after extrapolations from a national sampling survey revealing a mean density of 0.49 pair/km²), or if Polish breeders do not all use the western flyway (see results from geolocators).

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Table 1. ^a Amplification failure rate calculated from 1127 genotypes. ^b Genotyping error rate calculated from 143 replicated genotypes. Percentage of populations significantly out of Hardy-Weinberg equilibrium for each locus. Adjusted nominal level (5%) is 0.00011 (19 populations, 24 loci). Percentage of populations with over 20% null alleles for each locus. In bold, loci removed from further analyses.

Locus	Amplification failure ^a	Genotyping error ^b	Deviation from Hardy-Weinberg	>20% null alleles
Embhort01	3.90%	6.38%	26%	0%
Embhort02	2.48%	0.71%	0%	0%
Embhort03	2.57%	0.71%	0%	5%
Embhort04	2.66%	2.13%	0%	0%
Embhort05	6.39%	4.26%	47%	21%
Embhort06	3.28%	4.26%	0%	0%
Embhort07	4.17%	0.71%	11%	5%
Embhort08	9.49%	0.71%	47%	47%
Embhort09	3.11%	1.42%	5%	0%
Embhort10	5.41%	1.42%	32%	16%
Embhort11	1.86%	4.96%	0%	0%
Embhort12	2.84%	3.55%	47%	68%
Embhort13	2.48%	2.13%	16%	21%
Embhort14	2.48%	2.84%	0%	0%
Embhort15	2.48%	1.42%	0%	0%
Embhort16	3.02%	0.71%	11%	0%
Embhort17	1.95%	0.71%	5%	0%
Embhort18	1.95%	0.71%	5%	0%
Embhort19	2.75%	2.13%	5%	0%
Embhort20	2.75%	0.00%	0%	0%
Embhort21	1.69%	1.42%	0%	0%
Embhort22	2.66%	0.71%	11%	5%
Embhort23	2.66%	1.42%	11%	11%
Embhort24	2.93%	3.55%	0%	0%

Table 2. Sample size (N), mean number of alleles (A), mean allelic richness (AR), observed (H_O) and expected (H_E) heterozygosity, and fixation indexes (F_{ST} and F_{IS}) (Weir and Cockerham 1984) over 21 microsatellite loci. * indicates significantly different from 0 at p<0.05 after 1000 bootstraps. Indices were not evaluated for breeding sites which sampling size was under eight individuals.

Breeding site	N	A	A _R	H _O	H _E	F _{IS}	F _{ST}
Drôme							
France 5	12	7.2	5.6	0.733	0.756	0.073	-
West/Central							
France 1	8	7.0	6.2	0.769	0.778	0.080	-
Spain 2	28	11.6	6.5	0.708	0.830	0.165*	-
France 3	3	-	-	-	-	-	-
France 4	12	9.0	6.6	0.740	0.812	0.133*	-
Poland 6	8	7.0	6.2	0.685	0.781	0.190	-
Poland 7	25	11.2	6.4	0.727	0.812	0.126*	-
Poland 8	15	9.4	6.4	0.713	0.807	0.151*	-
Lithuania 9	3	-	-	-	-	-	-
All West/Central sites	102	9.2	6.4	0.724	0.803	0.144*	0.006*
North							
Lithuania 10	34	11.2	6.2	0.731	0.816	0.120*	-
Estonia 11	21	10.0	6.1	0.700	0.790	0.138*	-
Finland 12	47	12.4	6.2	0.758	0.818	0.085	-
Finland 13	61	12.7	6.2	0.713	0.814	0.133*	-
Finland 14	10	7.4	6.0	0.760	0.764	0.061	-
Finland 15	14	8.9	6.2	0.722	0.793	0.128*	-
Sweden 16	2	-	-	-	-	-	-
Sweden 17	15	9.0	6.1	0.719	0.797	0.132*	-
Norway 18	3	-	-	-	-	-	-
All North sites	207	10.2	6.2	0.729	0.799	0.116*	0.007*
East							
Belarus 19	42	12.9	6.5	0.753	0.833	0.108*	-
Russia 20	20	10.5	6.3	0.726	0.809	0.127*	-
Russia 21	99	16.0	6.6	0.747	0.844	0.120*	-
Russia 22	76	15.3	6.5	0.742	0.834	0.118*	-
Serbia 23	3	-	-	-	-	-	-
Serbia 24	2	-	-	-	-	-	-
Serbia 25	8	7.4	6.4	0.732	0.771	0.117	-
Serbia 26	3	-	-	-	-	-	-
All East sites	253	12.4	6.5	0.740	0.818	0.118*	0.005*
All sites	575	10.3	6.3	0.730	0.803	0.121*	0.011*

Table 3. Analysis of Molecular Variance for three clustering: three populations (consensus from STRUCTURE and DAPC analyses), four populations (consensus from STRUCTURE and DAPC analysis and prior knowledge of migratory corridors), three populations (test for three putative migratory corridors).

Source of variation	Sum of squares	Variance components	Percentage variation
Three populations (Drôme, North, South)			
Between populations	102.903	0.225	1.387
Between sites within populations	328.752	0.169	1.042
Within sites	8490.125	15.840	97.571
Four populations (West/Central, Drôme, North, East)			
Between populations	132.334	0.198	1.225
Between sites within populations	299.321	0.150	0.929
Within sites	8490.125	15.840	97.847
Three flyways (Western Atlantic, Western Mediterranean, Eastern)			
Between flyways	88.387	0.120	0.744
Between sites within flyways	389.157	0.207	1.283
Within sites	8427.543	15.840	97.973

Table 4. Pairwise F_{ST} among the three populations

	South	Drôme
Drôme	0.004	-
North	0.005	0.008

Table 5. Pairwise F_{ST} among the four populations

	West/Central	Drôme	North
Drôme	0.011	-	-
North	0.006	0.007	-
East	0.003	0.005	0.006

Table 6. Contemporary gene flow among populations as percentage of genetic migrants per generation ($\pm 95\%$ confidence interval)

	From			
	West/Central	Drôme	North	East
<i>To</i>				
West/Central	66.98 (± 0.61)	0.31 (± 0.61)	2.69 (± 2.55)	30.02 (± 2.67)
Drôme	2.11 (± 3.84)	68.75 (± 3.86)	2.62 (± 4.63)	26.52 (± 6.61)
North	0.16 (± 0.31)	0.16 (± 0.31)	97.72 (± 2.06)	1.97 (± 2.02)
East	0.13 (± 0.25)	0.13 (± 0.25)	0.56 (± 0.86)	99.18 (± 0.92)

Appendix 4 – Official guidelines of the research program



Programme de recherches sur la migration du Bruant ortolan



**Cahier des charges pour une étude sur la stratégie de migration du Bruant ortolan en
France et en Europe**

V3. Version mise à jour le 21 février 2013

En partenariat avec l'Office National de la Chasse et de la Faune Sauvage



Rédigé par Frédéric Jiguet (MNHN), relu par Jean-Marie Boutin (ONCFS)

A la demande de la Ministre de l'Écologie, du Développement Durable et de l'Énergie, le Muséum National d'Histoire Naturelle (MNHN) a participé à une réunion en préfecture des Landes le 5 octobre 2012 pour un échange en vue de l'élaboration par le MNHN d'un cahier des charges portant sur une étude de la migration du Bruant ortolan. Cette espèce fait l'objet d'un programme de recherches lancé depuis plus d'un an qui implique des chercheurs de plusieurs pays européens (Comolet-Tirmand et al. 2012). Le présent document indique les développements prévus de ce programme pour couvrir de manière exhaustive les thématiques scientifiques importantes dans le contexte de la migration de l'espèce en France et en Europe. Il intègre les éléments proposés par la Fédération Départementale des Chasseurs des Landes, concernant notamment l'origine des migrateurs visitant les Landes, les voies de migration des populations russes, les sites majeurs de halte migratoire et la mise en évidence des principales zones d'hivernage. Ces questions seront abordées en utilisant des techniques modernes (électronique embarquée, analyses chimiques, génétique) en partenariat avec les meilleurs spécialistes de ces outils en France (génétique), en Europe (photomètres) ou au monde (isotopes). Ce réseau d'experts permettra au groupe de scientifiques internationaux participant (Norvège, Suède, Finlande, Russie, Pologne, Estonie, Espagne, Israël) de travailler dans les meilleures conditions pour lever les inconnues sur l'écologie de la migration de l'espèce.

Le programme de recherches développé dans ce document permettra d'étudier différents aspects de la migration du Bruant ortolan en France et en Europe :

- voies migratoires et zones d'hivernage des différentes populations nicheuses européennes ;
- origine et flux des ortolans migrant par le sud-ouest de la France ;
- sites majeurs et durée des haltes migratoires sur chaque voie migratoire, en automne comme au printemps ;
- importance des populations biélorusses et russes dans le flux passant par les Landes.

Ce programme s'articule autour de trois outils, les photomètres (GLS), les analyses d'isotopes stables et l'analyse de marqueurs génétiques, expliqués dans la suite du document.

I. Pose de GLS sur les zones de reproduction

Qu'est-ce qu'un GLS ? C'est une puce électronique dotée d'un capteur de luminosité, que l'on appelle photomètre géo-localisateur. Attaché sur le dos d'un oiseau, cet appareil enregistre en permanence l'heure et l'intensité de la lumière. Quand on recapture l'oiseau, et que l'on extrait les données stockées dans la puce, on peut connaître, pour chaque jour passé avec le GLS sur le dos, la durée du jour – et donc la latitude – et les heures de lever et de coucher du jour – et donc la longitude. La précision des coordonnées obtenues, de l'ordre de 100km, permet de localiser assez précisément l'oiseau. C'est en tout cas largement suffisant pour déterminer les voies de migration, les zones de halte migratoire et d'hivernage de chaque individu suivi.

La superposition des positions obtenues pour tous les oiseaux permet de déterminer les principales voies migratoires et la proportion des oiseaux qui y passent, les zones de concentration des oiseaux en halte migratoire, et les zones d'hivernage.

Récupérer les informations enregistrées dans le GLS nécessite de recapter l'oiseau qui l'a porté. Il faut donc que cet oiseau soit toujours vivant, qu'il soit retrouvé, et qu'il se laisse capturer. Pour maximiser la probabilité de récupération, nous équiperons uniquement des adultes (qui ont un meilleur taux de survie ; Dale & Steifetten 2010), mâles territoriaux (plus forte probabilité de revenir sur le même territoire l'année suivante), sur les sites de reproduction. Les oiseaux seront aussi équipés d'une bague colorée permettant de les repérer à distance pour mieux détecter leur retour.

Le principe de l'étude par GLS

Equiper une vingtaine d'oiseaux dans chacun des sites de capture retenu (en période de reproduction), le long d'un gradient Ouest-Est, pour savoir quelle proportion de chaque population utilise quelle voie migratoire, et déterminer une éventuelle ligne de partage de populations migrant vers l'Ouest ou vers l'Est. Capturer et baguer une dizaine à une vingtaine d'individus qui ne seront pas équipés, et qui serviront de lot témoin pour vérifier que le taux de retour des oiseaux équipés est le même que celui d'oiseaux non équipés.

A quelles questions les GLS permettront-ils de répondre ?

Quelles voies de migration sont empruntées par chaque population nicheuse ?

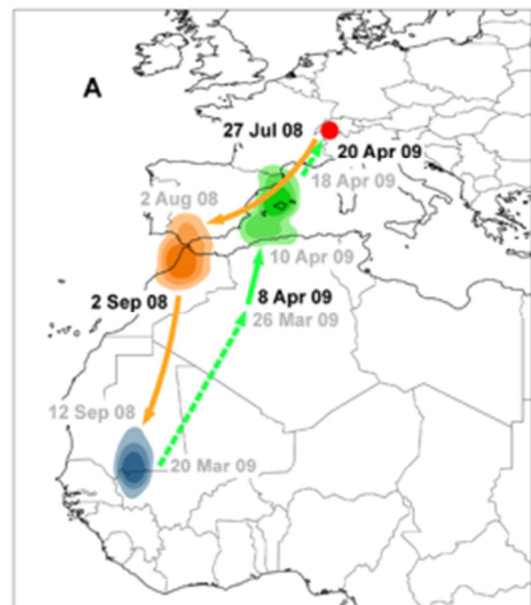
Quels sont les sites majeurs de halte migratoire le long de chaque voie ?

Quelle est la durée des haltes ?

Quels sont les sites d'hivernage ?

Sont-ils les mêmes pour les populations catalanes qui augmentent depuis plusieurs années (Brotons et al. 2008) ?

Le matériel que nous voulons utiliser a permis, dans d'autres études, de répondre à ce type de questions par exemple pour la Huppe fasciée. La Figure ci-contre extraite de (Bächler et al. 2010) montre les trajets empruntés et les zones de stationnement d'une Huppe fasciée (*Upupa epops*) baguée en Suisse le 27 juillet 2008, qui a stationné du 2 août au 2 septembre près de



Gibraltar, pour hiverner du 12 septembre 2008 au 20 mars 2009 dans le sud de la Mauritanie, avant de stationner du 26 mars au 8 avril en Algérie, puis vers les Baléares, pour un retour sur son site de nidification le 20 avril 2009. Nous aurons ce type de suivi pour de nombreux ortolans provenant de différentes zones de reproduction.

Une limite à cet outil : pendant deux à trois semaines autour des équinoxes (20-21 septembre, 20-21 mars), la durée du jour est équivalente à celle de la nuit et évolue très peu de jour en jour, et il devient alors difficile de déterminer la latitude. Par contre, la longitude sera toujours connue, donc la voie migratoire des oiseaux durant cette période (Sud-Ouest de la France ou plus à l'Est) restera connue.

Où ?

Nous souhaitons équiper une vingtaine de mâles sur chaque site de capture, et répartir les sites de capture entre différentes populations nicheuses européennes, le long d'un gradient est-ouest allant si possible jusqu'à l'Oural. Les sites déjà retenus sont :

- province d'Umea, Suède (resp. Gunnar Selstam)
- Norvège (resp. Svein Dale), site utilisé en 2012 mais qui ne le sera plus (population presque éteinte)
- Finlande (resp. Markus Piha)
- Pologne, 2 sites : Poznan et Siedlce (frontière biélorusse) (resp. Michal Skierczynski)
- Estonie : 1 site (Jaanus Elts)
- Biélorussie : site à définir avec l'OMPO qui y a des contacts
- Russie : 2 ou 3 sites à définir avec l'ONCFS et l'OMPO qui y ont des contacts
- France : causse Méjean, Lozère (resp. Jocelyn Fonderflick, CEFE-CNRS Montpellier)
- Espagne, Catalogne (resp. Lluís Brotons, Catalan Forest Technology Center)

Quand ?

La période de chant très actif des mâles est assez courte, entre mi-mai et mi-juin selon les populations. Nous interviendrons à cette période, l'année N pour poser les GLS, l'année N+1 pour les récupérer.

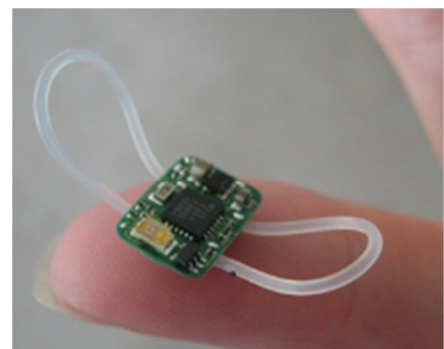
En 2012, 10 GLS ont été posés en Norvège, 19 en Suède.

En 2013, 20 GLS seront posés sur chaque site de Suède, Finlande, Pologne, Estonie, France et Espagne (pas assez d'oiseaux dans la population suivie de Norvège, qui n'accueillait plus que 20 mâles en 2012). 200 GLS seront donc nécessaires. Les contacts seront consolidés en Russie et Biélorussie pour organiser le terrain en 2014.

En 2014, il faudra retourner sur ces sites pour récupérer des GLS, et en reposer 20 par site. 200 GLS seront à nouveau posés.

En 2015, récupération des GLS posés en 2014 sur tous les sites.

Comment ?



Le matériel : mis au point par la station ornithologique de Sempach, en Suisse, les GLS que nous utilisons pèsent 0.6g ou 1g, harnais compris, ce qui représente de 2 à 5% du poids d'un Bruant ortolan. Ce matériel a déjà été utilisé sur d'autres petits oiseaux (Bächler et al. 2010, Salewski & Podula 2010). Le GLS se fixe grâce à deux boucles qui se placent autour des cuisses, le GLS étant positionné sur le dos de l'oiseau, comme un sac à dos (voir photos).

Année N : capture au filet sur le poste de chant du mâle, avec leurre acoustique. Sur chaque site 20 mâles seront bagués et équipés d'un GLS, 10 à 20 autres mâles seront seulement bagués et serviront de lot témoin pour vérifier que le taux de retour de migration en année N+1 est similaire pour les oiseaux équipés et non équipés. En plus de la bague métal Muséum, nous envisageons de poser une bague couleur portant un code simple, comme cela a été fait en Suède en 2012, pour permettre de repérer plus facilement les mâles à distance l'année N+1.

Année N+1 : recherche de mâles bagués sur les territoires occupés l'année N et au sein de la population locale. Capture des mâles équipés de GLS au filet sur leur poste de chant, avec leurre acoustique. Nous espérons un taux de récupération de 25% minimum, soit 5 GLS récupérés par an et par site, ce qui permettra sur les deux années de pose de récupérer 10 GLS par site suivi.

Photo. Un mâle de Bruant ortolan suédois portant une bague métal (muséum) et une bague colorée portant un code permettant d'identifier l'individu à distance (© Gunnar Selstam).

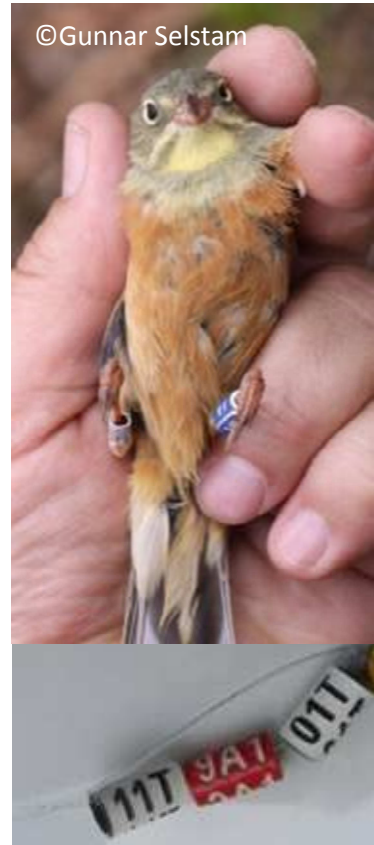


Photo. Un mâle de Bruant ortolan équipé d'un photomètre géo-localisateur, Norvège, juin 2012 (© Svein Dale)



II. Dosage d'isotopes stables dans les plumes

Les études réalisées à l'aide de photomètres seront complétées par des analyses de concentrations en isotopes stables dans les plumes des oiseaux. Hydrogène (deutérium) et oxygène, carbone et azote présentent différents isotopes dans la nature dans des concentrations variables selon le lieu, de grands gradients continentaux existent selon par exemple l'origine et la quantité des précipitations atmosphériques (pour le deutérium), le type de plantes qui assimile le dioxyde de carbone (carbone). Voir par exemple Prochazka et al. (2008) pour un exemple sur le carbone de plumes muées en Afrique utilisé pour définir une ligne de partage de voies migratoires des rousserolles effarvates européennes). Le dosage du deutérium présent dans une plume permet par exemple de déterminer une latitude à laquelle cette plume a poussé, mais reste peu informatif pour la longitude, étant donné la structuration spatiale de cet isotope dans l'environnement en Europe. Il faut donc multiplier les isotopes considérés pour obtenir des résultats intéressants notamment sur la zone russe. Des publications récentes expliquent l'intérêt d'utiliser plusieurs isotopes dans des analyses similaires réalisées en Amérique du Nord (Hobson et al. 2012) et en Europe (Popa-Lisseanu et al. 2012). Pour les zones de reproduction, nous essaierons un dosage des isotopes d'oxygène.

Figure. Concentration en deutérium dans l'environnement. Une structuration en bande latitudinale est évidente, mais cet isotope ne permet pas de séparer des plumes ayant poussé en Norvège, en Suède ou en Russie (d'après Hobson et al. 2004, figure extraite de Marquiss et al. 2012).

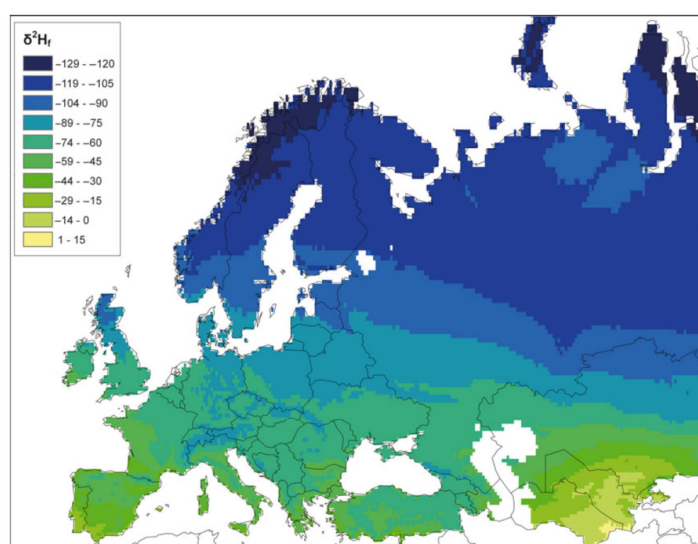
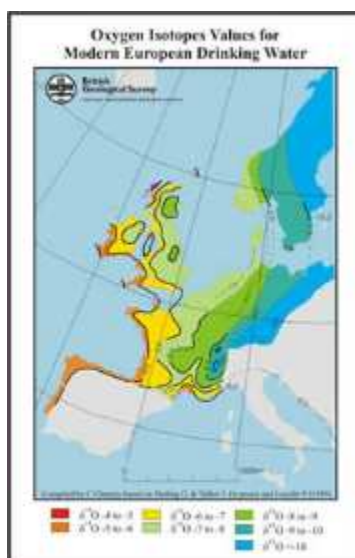


Figure. Les isotopes d'oxygène ont une structuration spatiale plutôt longitudinale, qui, croisée avec le deutérium, permet déjà de mieux localiser l'endroit où une plume a poussé.



La mue des bruants ortolan

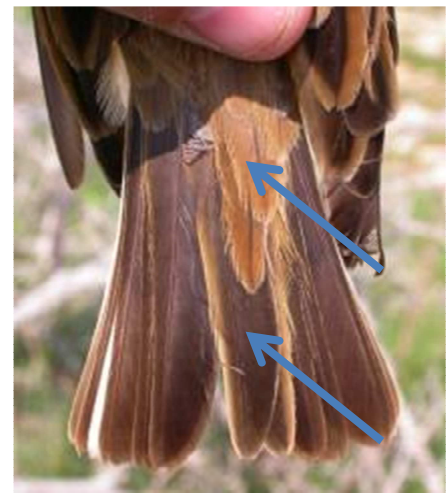
Les jeunes ortolans portent en automne des plumes de première génération, qui ont poussé sur le site où ils sont nés.

Les adultes font une mue complète (ils changent toutes leurs plumes) en fin d'été avant de partir en migration, donc sur leur zone de reproduction.

En hiver, les ortolans muent à nouveau les plumes de contour, du corps, mais pas les rémiges ni les rectrices. Parfois, quelques rémiges secondaires et quelques grandes couvertures peuvent être muées en hiver si elles ne l'ont pas été en fin d'été. Quand les ortolans reviennent en Europe au printemps, les plumes du corps, dont les scapulaires, sont neuves et ont poussé en Afrique, chez les jeunes nés l'année d'avant comme chez les oiseaux plus âgés.

Le principe :

- Prélever une rectrice centrale, 2 couvertures sus-caudales et 2 couvertures sous-caudales (voir photo ci-contre) sur des oiseaux capturés en France en automne (âge et sexe seront déterminés, la biométrie sera réalisée) pour déterminer une zone de pousse possible de la plume ; la plume prélevée repoussera rapidement pendant la halte migratoire pré-saharienne ; si on coupe la rectrice à sa moitié, elle ne sera pas changée avant la mue complète de l'été suivant, ce qui peut plus handicaper l'oiseau ; le même type de prélèvement sera effectué sur des ortolans migrateurs en Israël.
- Prélever une rectrice centrale, 2 couvertures sus-caudales et 2 couvertures sous-caudales, sur des oiseaux sur les zones de reproduction pour avoir des « témoins » des concentrations en isotopes des différentes zones de reproduction suivies (ces plumes ont poussé sur ces lieux de reproduction, l'année précédente).
- Prélever deux scapulaires de chaque côté sur les oiseaux capturés sur les zones de reproduction, car ces plumes ont été muées en Afrique, sur les lieux d'hivernage.



Les limites de cette méthode

En Afrique, les isotopes ne présentent pas de distribution spatiale aussi structurée qu'en Europe, il sera donc difficile de déterminer des zones d'hivernage précises avec cette méthode (voir Reichlin et al. 2010 & 2012 pour deux exemples sur la Huppe fasciée et le Torcol fourmilier), mais le fait que les oiseaux équipés de GLS feront l'objet d'une analyse isotopique permettra de préciser les résultats isotopiques.

Laboratoire(s) d'analyse

Nous prévoyons de faire analyser les échantillons dans un laboratoire canadien, celui de Keith Hobson (au Canada), avec lequel nous travaillons déjà depuis de nombreuses années (tout comme l'ONCFS).

Taille d'échantillon

20 oiseaux (équipés de GLS et témoins) en mai-juin pour chaque population nicheuse, pour rectrices et scapulaires, ce qui fait $20 \times 10 \times 2 = 400$ échantillons

150 oiseaux capturés en automne dans la moitié Ouest de la France.

150 oiseaux capturés en automne en Israël, voie orientale.

Soit 700 échantillons, qui seront analysés pour 2 isotopes, donc 1400 analyses

Photo. Un mâle de Bruant ortolan photographié le 14 mai 2004 au Danemark (Knud Pedersen). Les trois grandes couvertures alaires les plus internes sont neuves (signalées par la parenthèse rouge) et contrastent avec les autres usées. Elles ont été muées sur les zones d'hivernage et peuvent servir aux dosages de concentrations isotopiques.



III. Marqueurs génétiques (microsatellites)

Si des voies de migration séparées existent entre des populations nicheuses occidentales et orientales, elles pourraient entraîner une certaine différenciation génétique. En d'autres termes, des oiseaux utilisant une même voie de migration partageraient un patrimoine génétique plus important. Il s'agit ici de comparer plusieurs marqueurs microsatellites polymorphes d'oiseaux échantillonnés sur les lieux de reproduction (variés) et sur les voies migratoires (Sud-Ouest de la France et Israël). Les résultats espérés : déterminer génétiquement l'origine la plus probable des migrants. Si les marqueurs génétiques sont très variables, cette technique pourrait donner des résultats plus précis que les isotopes stables, dans la mesure où le nombre de populations nicheuses échantillonnées serait suffisant.

Cette technique a été utilisée sur d'autres espèces pour définir par exemple une ligne approximative de démarcation entre populations utilisant une voie de migration occidentale et une voie orientale, chez le Rousserolle effarvée *Acrocephalus scirpaceus* (Prochazka et al. 2011), ou chez le Pouillot fitis *Phylloscopus trochilus* (Bensch et al. 1999, 2009).

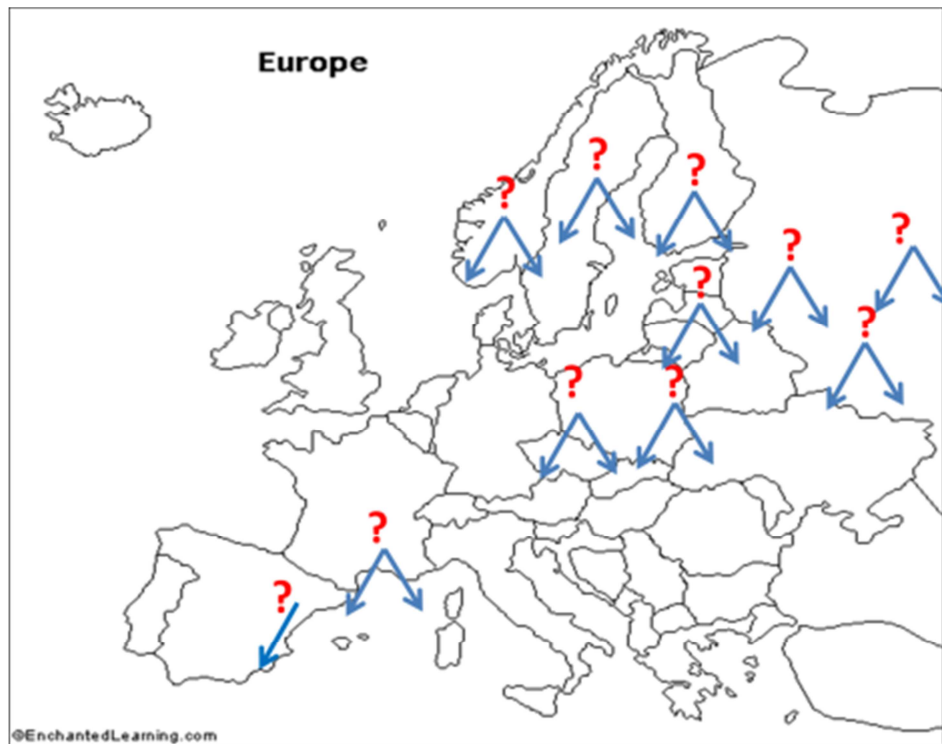
Les échantillons collectés pour analyse d'isotopes stables serviront aussi aux analyses génétiques, grâce aux fragments de peau présents à la base de la rectrice centrale. Nous espérons donc 700 individus échantillonnés, sur les sites de reproduction suivis (10 sites avec 40 oiseaux chacun soit 400), en halte migratoire sur la voie occidentale (150 dans le sud-ouest de la France), et sur la voie orientale (150 en Israël).

Si les populations reliques du centre-ouest de la France ne feront pas l'objet de pose de GLS, en raison de leur petite taille et de leur faible densité (risque de ne pas retrouver les oiseaux en année N+1), il sera envisagé de capturer quelques individus pour compléter l'échantillonnage de plumes pour les analyses isotopiques et génétiques.

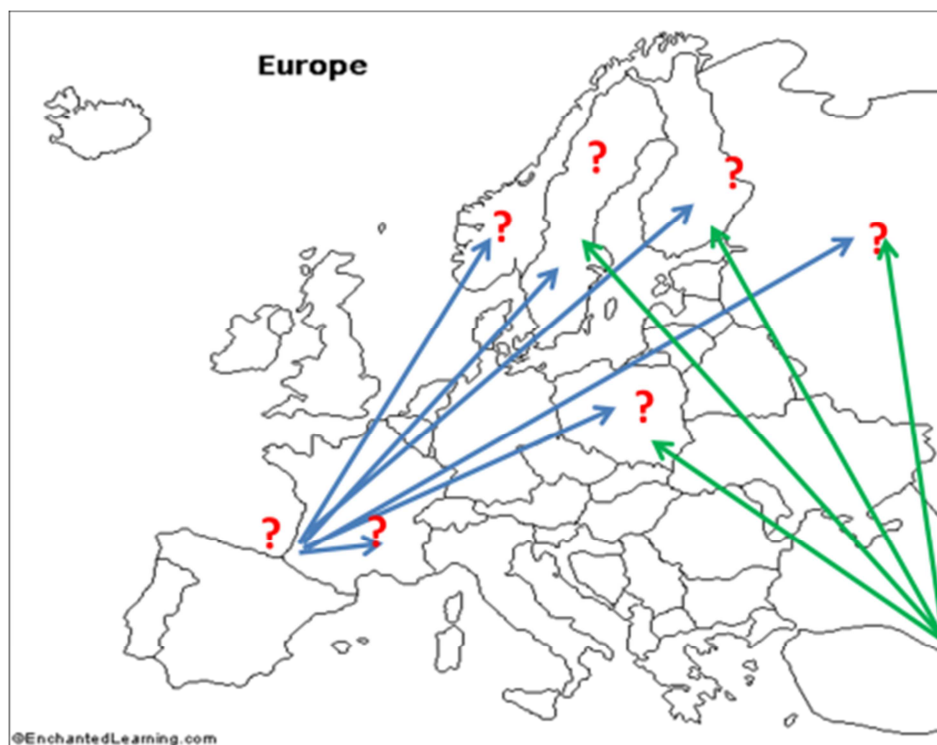
De plus, ponctuellement, des contacts seront pris pour compléter l'échantillonnage des populations nicheuses notamment en Asie Centrale, dans le Caucase, à la faveur de voyages ornithologiques de collègues bagueurs (Turquie, Géorgie, Azerbaïdjan, Kazakhstan). Ces échantillons de plumes permettront de mieux cerner la variabilité génétique des populations nicheuses.

Les travaux de génétique seront réalisés au Muséum National d'Histoire Naturelle, en partenariat avec le Service de Systématique Moléculaire, dirigé par Eric Pasquet. Ces travaux feront appel aux compétences scientifiques et techniques de la plate-forme, des techniciens et des chercheurs qui y sont associés.

Etudes par photomètres. Elles permettront de déterminer la proportion de chaque population nicheuse étudiée empruntant une voie de migration occidentale, orientale ou intermédiaire.



Etudes par analyses isotopiques et génétiques. Elles permettront de déterminer une origine probabiliste des individus capturés dans l'Ouest de la France et en Israël (comme exemple de voie migratoire orientale). Cela sera possible grâce aux références établies en suivant les populations nicheuses.



IV. Mise en œuvre de captures en France en automne

La capture du Bruant ortolan, espèce protégée inscrite à l'Annexe I de la Directive Oiseau, nécessite une autorisation en dérogation à la loi de protection de la nature, qui peut être délivrée par le Ministère de l'Ecologie après avis du CNPN, ou par le Muséum national d'Histoire naturelle de Paris (CRBPO), conformément à l'arrêté ministériel du 22 septembre 2008. Le prélèvement de tissu doit faire l'objet d'une demande d'autorisation particulière, au-delà de celle de capture et de baguage, que le CRBPO est habilité à traiter et à accorder, pour les bagueurs officiels de son réseau national (cf. arrêté ministériel du 22 septembre 2008). Depuis le 1^{er} janvier 2013, les captures et prélèvements de tissus doivent avoir reçu l'avis favorable d'un comité d'éthique, ce qui est le cas pour ce programme 'ortolans' (Comité d'éthique Cuvier, avis favorable valable pour 2013-2017).

Il est donc proposé que des sites de capture d'ortolan soient mis en place, dans l'Ouest de la France, entre le 15 août et le 15 septembre, à l'aide matoles agrainées et de leurres acoustiques, possiblement complétées avec des filets japonais (voir Girardot et al. 2009), chaque site étant sous la responsabilité unique d'un bagueur du CRBPO. L'utilisation de canaris domestiques ou de bruants d'espèces autres qu'ortolans comme appelants (mais pas d'ortolan) pourra être envisagée (voir Girardot et al. 2009).

Partenariats sur les stations de baguage

Il est souhaitable que les partenaires (FDC40, LPO) soient localement associés aux opérations de capture, dans les Landes ou ailleurs. Nous prévoyons de mobiliser des bagueurs pour mettre en place des sites de capture en région Aquitaine, Poitou-Charentes et Languedoc-Roussillon. Les bagueurs du CRBPO de ces régions seront consultés pour savoir qui est volontaire pour prendre la responsabilité d'un site de baguage. Certains de ces bagueurs sont agents de l'ONCFS et pourront être mobilisés dans le cadre de leur service.

Seuls les sites retenus et validés par le CRBPO pourront participer à l'opération, la validation étant valable pour une saison de capture (un automne), et la station étant sous l'entière responsabilité du bagueur CRBPO. Les emplacements de chacun de ces sites seront communiqués à l'ensemble des partenaires (préfecture, ONCFS, FDC40, LPO...) avant la mi-août.

Les sites de capture et l'installation des systèmes de capture seront élaborées avec les conseils de tendeurs, qui fourniront les matoles et les cages d'appelants, et qui pourront être aides-bagueurs sur les sites concernés.

Inutilité de poser des GLS sur les sites de migration

Etant donnée la probabilité quasi nulle de re-capturer une même année ou une année suivante un oiseau capturé en halte migratoire (0 individu sur 301 captures dans les Landes pour le programme de baguage de la FDC entre 2003 et 2008), il n'est pas envisageable de poser des GLS sur des oiseaux dans le Sud-Ouest, car aucun ne pourrait être récupéré.

Stations de baguage sur la voie orientale

En Israël, plusieurs stations de baguage standardisées capturent et baguent quotidiennement les oiseaux en période de migration : le Jerusalem Bird Observatory, Tzor'a Valley, l'International Bird Ringing Center à Eilat. Ces stations de baguage seront contactées pour estimer les tendances

d'évolution du nombre d'ortolans capturés et bagués en migration en Israël, ce qui donnera une indication sur l'évolution du flux utilisant une des voies orientales de migration. Ces oiseaux feront également l'objet de prélèvements de plumes pour compléter les analyses isotopiques et génétiques sur une voie de migration orientale.

Autres apports éventuels d'un baguage massif ?

Bilan sur les opérations de baguage réalisées en France et dans les Landes

Pour savoir s'il pourrait être intéressant de lancer en France un grand effort de capture et de baguage de bruants ortolans, il convient d'analyser les expériences mises en œuvre auparavant en France, notamment dans les Landes où l'effort de capture en migration est le plus fort. Dans ce département, des opérations de baguage ont eu lieu de 2003 à 2008 sous la responsabilité de Franck Ibanez, bagueur en possession d'un permis de baguage valide du CRBPO/Muséum Paris. Il y a conduit des opérations de baguage dans les Landes pour la FDC40 (voir Girardot et al. 2009). Comme tout bagueur, F. Ibanez envoie chaque année l'ensemble de ses données de baguage et de captures d'oiseaux déjà bagués (contrôles) au CRBPO. Ainsi, le CRBPO possède la base de données nationales des baguages, contrôles (oiseaux vivants) et reprises (oiseaux morts) d'oiseaux en France. Les chiffres avancés ci-dessous sont donc les chiffres officiels qui sont mis ici à disposition de la communauté scientifique.

D'après Girardot et al. (2009), le baguage était réalisé sur des sites avec matoles en utilisant des canaris comme appelants. Durant ces 6 années, 300 ortolans ont pu être bagués, mais aucun d'entre eux n'a été recapturé. En plus de ces 300 individus, un seul oiseau déjà bagué a été capturé (en 2003), porteur d'une bague norvégienne, et originaire de la population nicheuse suivie par Svein Dale. Ce taux de contrôle nul ne laisse aucun espoir quant à la possibilité de pouvoir utiliser l'outil du baguage pour étudier les durées de séjour et la prise de poids sur les sites de halte migratoire. En effet, dans les programmes de baguage classiques ayant ces objectifs (fauvettes paludicoles en roselières par exemple ; voir Julliard et al. 2006 pour un exemple sur le Phragmite aquatique), les taux de recapture sont de l'ordre de 5 à 10%. Ces oiseaux, bagués et pesés un jour puis recapturés et pesés à nouveau plus tard, permettent d'étudier la prise de poids, et par exemple de comparer la qualité de différents sites de halte migratoire pour la reconstitution des réserves énergétiques (graisse) nécessaires à la bonne poursuite de la migration. Dans le cas du Bruant ortolan, notre analyse des données déjà disponibles nous fait abandonner l'hypothèse d'organiser un baguage massif d'oiseaux en migration dans le sud-ouest pour étudier la qualité des sites de halte migratoire.

NB : si d'autres éléments n'ont pas été transmis au CRBPO (notamment des captures éventuelles d'autres oiseaux bagués durant la période 2003-2008, réalisées par le bagueur), il conviendrait de les faire parvenir s'ils sont susceptibles de modifier l'analyse présentée ici. Nous faisons ici allusion au document produit par la FDC40 en août 2012 intitulé « programme d'étude sur le bruant ortolan », signalant que (page 3) : « dans le cadre des opérations de baguage de la Fédération Départementale des Chasseurs des Landes, des Bruants ortolans venant de Russie, de Pologne et d'Allemagne ont été identifiés dans le département des Landes. »

Le taux de capture d'oiseaux bagués en dehors des Landes et l'absence de contrôle d'oiseaux bagués localement empêchent également d'utiliser l'outil du baguage pour estimer la taille du flux d'ortolan migrant par les Landes. Les modèles mathématiques de capture-recapture ne peuvent pas

être utilisés, et des méthodes plus simples de type 'règle de trois', utilisées pour estimer le flux d'alouettes baguées migrant par le sud-ouest, ou encore le nombre de *Phragmites aquatiques* s'arrêtant en France en automne (Jiguet et al. 2011), nécessite des contrôles d'oiseaux déjà bagués.

Il pourrait être intéressant d'augmenter la pression de baguage dans les pays au Nord de la France, sur les zones de reproduction, mais seulement si le nombre d'oiseaux bagués capturés dans le sud-ouest était important. La consultation de la base de données du CRBPO – mise à jour en octobre 2012 avec de nouvelles informations historiques provenant de centres de baguage étrangers - indique 26 captures en France d'oiseaux bagués sur les zones de reproduction depuis 1950. L'origine des 26 oiseaux bagués sur leurs sites de reproduction et retrouvés en France est la suivante : Norvège (9), Suède (6), Finlande (6), Russie (1), Estonie (1), Pologne (1), Allemagne (2). Ces chiffres dépendent bien sûr de la pression de baguage dans chacun de ces pays, mais plusieurs milliers d'ortolans ont été bagués en Finlande depuis 60 ans et le nombre de reprises en France ne laisse pas envisager qu'un baguage massif sur zones de reproduction pourrait aider à obtenir des reprises de bagues suffisantes en France.

En conclusion, il s'avère que la capture d'oiseaux sur les zones de halte migratoire ne permettra pas d'obtenir des recaptures locales (pour étudier le temps de halte migratoire) ou des contrôles étrangers (pour déterminer des zones d'origine), et n'a donc pas un intérêt scientifique majeur au-delà de la simple prise de données sur la biométrie, la masse des oiseaux, et bien sûr le prélèvement de tissus pour analyses isotopiques et génétiques.



Photo : baguage d'un mâle nicheur de Bruant ortolan sur le causse Méjean, en Lozère, France (©Frédéric Jiguet)

V. Gouvernance

Le projet sera réalisé sous la conduite du Muséum National d'Histoire Naturelle en partenariat avec l'ONCFS. Il nécessite de mettre en place 2 comités distincts pour assurer la gouvernance du projet :

- Un comité scientifique, composé de chercheurs, qui s'assurera de la pertinence et de l'excellence des recherches ; les porteurs du programme de recherches dans les principaux pays participeront à ce comité de pilotage : Raphaël Arlettaz (Suisse), Jean-Marie Boutin ou son représentant (ONCFS), Michel Alexandre Czajkowski (OMPO), Svein Dale (Norvège), Gunnar Selstam (Suède), Michal Skierczynski (Pologne), Markus Piha (Finlande), Jaanus Elts (Estonie), Jean-Philippe Siblet ou son représentant (Service du Patrimoine Naturel, MNHN). Ce comité sera présidé par Frédéric Jiguet (MNHN Paris) et se réunira une fois par an. Deux membres supplémentaires seront invités à participer à ce comité en tant qu'observateurs, l'un proposé par la FDC40, l'autre par la LPO Aquitaine ; ces deux membres devront comprendre et parler anglais, car les échanges au sein du comité scientifique se font dans cette langue ;
- Un comité de suivi, regroupant les différents acteurs du dossier 'ortolan' en France, partenaires et financeurs, qui pourrait être placé sous la responsabilité de M. le Préfet des Landes. Ce comité de suivi sera constitué de représentants du comité de pilotage (MNHN), de la Direction des Expertises du MNHN, de la Fédération Départementale des Chasseurs des Landes FDC40, du Conseil Général des Landes CG40, du Conseil Régional Aquitaine, de la DDTM40, de la LPO, de la SEPANSO, du MEDDE et d'autres financeurs ou partenaires éventuels, à la discrétion des services de l'état et de M. le Préfet. Le comité de suivi sera réuni deux fois par an et se verra présenter l'état d'avancement des recherches. Il est proposé de le réunir une fois fin juin et une fois en décembre.

Ces deux comités fonctionneront de manière indépendante tout en restant étroitement connectés. Le comité scientifique est responsable de la mise en œuvre du programme sur le terrain, avec l'aide du contractuel post-doc qui sera recruté. Il doit évaluer régulièrement les avancées scientifiques du programme, et peut décider de l'améliorer au besoin au regard de ses expertises d'étape, et éventuellement des remarques ou requêtes pouvant émaner du comité de suivi. Les deux observateurs du comité scientifique pourront saisir ce comité par écrit, et solliciter une réponse écrite, ces documents seront intégrés au rapport final. Le comité de suivi se réunira deux fois par an pour se voir présenter l'avancement des travaux de recherches, et pourra proposer des avis ou des conseils qui seront transmis au comité scientifique. Il ne pourra en aucun cas se substituer au comité scientifique, seul habilité à formuler des observations de nature à modifier l'orientation des travaux.

Atouts « politiques » de ce programme :

- Programme de recherches piloté par des chercheurs, dont les résultats seront publiés dans des journaux scientifiques internationaux ;
- Implique des chercheurs du reste de l'Europe, où l'ortolan est nicheur, qui seront membres du comité de pilotage ; cette dimension européenne, justifiée sur le plan scientifique, paraît en outre opportune dans le contexte tendu de mise en demeure ;
- Associe les acteurs locaux des Landes comme partenaires techniques et dans le comité de suivi ;
- Décontextualise l'étude, en ne se focalisant pas sur les Landes qui reste un lieu de conflit entre chasseurs traditionnels et associations de protection de la nature, ce qui devrait

permettre de faire des travaux de recherches de manière plus sereine, sans risque d'attiser les conflits locaux ;

- Fournit des résultats concrets en 3 ans, peut être prolongé au besoin ;
- Permet d'étudier l'éventuelle origine russe d'oiseaux en migration dans les Landes, et d'apporter quelques éléments sur l'évolution des populations russes.

Dans le cadre de ce projet, nous souhaitons aussi avoir la possibilité d'organiser des visites sur le terrain, notamment en Scandinavie et en Russie, de responsables des différents partenaires du projet, notamment des acteurs locaux du département des Landes (FDC, CG, CR, LPO, etc...). Cela permettra aux partenaires de voir comment le programme se met en place sur le terrain.

VI. Budgets

Les sommes seront versées au Muséum National d'Histoire Naturelle.

364.450€ TTC sur 3 ans

Ce budget est un ensemble cohérent dont le financement doit être assuré sur la durée des trois années, il ne peut être organisé sur la base d'une contractualisation annuelle renouvelable.

Coût du matériel GLS : 40.000€

400 GLS au total, 100€ HT par GLS, achetés auprès de la station ornithologique de Sempach, en Suisse.

Coût des bagues métal et couleur : 1.000€

environ 1000 bagues métal et 1000 bagues couleur

Coût des analyses isotopiques : 14.000€

10€ par analyse, 1400 analyses prévues

Coût des analyses génétiques : 7.000€

10€ par analyse d'échantillon, pour 700 échantillons

L'analyse scientifique de ces données génétiques sera réalisée dans le cadre du post-doctorat de recherches contractuel (identifier des marqueurs microsatellites, séquencer les échantillons, analyser les séquences obtenues et étudier leurs variabilités).

Coûts des missions sur le terrain (GLS) : 120.000€

40.000€ par an pendant 3 ans (2013, 2014 et 2015)

Ce sont les coûts de l'organisation et de la réalisation des missions sur le terrain en France et en Russie par les chercheurs français (MNHN et ONCFS), et une participation au financement des missions sur le terrain pour les sites situés en Espagne, Suède, Norvège, Finlande et Pologne, où les chercheurs associés au programme iront sur le terrain pour poser nos GLS.

Mise en place de stations de baguage en automne : 20.000€

Il s'agit de fournir du matériel aux bagueurs (filets, perches, nécessaire pour fabriquer des matoles), pour les captures à organiser dans l'Ouest de la France en août et septembre, durant deux années (2013 et 2014), pour espérer compléter l'échantillon nécessaire aux analyses isotopiques (150 oiseaux au total). Ces coûts n'incluent pas le temps du personnel technique déjà en poste qui prendra en charge ces stations de baguage. Il comprend par contre l'embauche de deux techniciens durant un mois chaque automne pendant trois ans en cas de besoin.

Valorisation de l'ensemble des données collectées : 50.000€

L'équipe de recherches en charge du projet envisage, pour analyser l'ensemble des données, de recruter, pour la dernière année du projet, un jeune chercheur en post-doctorat, dont le financement

se monte à 50.000€, un coût qui permettra de prendre en charge le salaire (48k€/an), l'achat d'un ordinateur et les coûts de publications scientifiques en accès libre.

Coordination et Gouvernance du projet : 25.000€

Réunion du comité de pilotage 1 fois par an (5.000€ par an pour réunir les chercheurs internationaux, pendant 3 ans)

Coordination des travaux au sein du MNHN et rédaction d'un rapport final par le Service du Patrimoine Naturel (10.000€)

[Réunion du comité de suivi des partenaires, 2 fois par an (juin et décembre). Estimation à 10.000€ sur 3 ans, proposition : à la charge de la Préfecture des Landes qui organisera les réunions]

Frais de gestion (10%) : 27.700€

TOTAL HT : 304.700€

TVA (19.6%) : $304.700 * 0.196 = 59.750€$

TOTAL : 364.450€

Ventilation du budget par an :

Poste	2013	2014	2015
Achat GLS	17.000	23.000	0
Bagues	1.000	0	0
Analyses isotopes	5.000	5.000	4.000
Analyses génétiques	0	3.500	3.500
Missions printemps	30.000	45.000	45.000
Missions automne	10.000	10.000	0
Contrat post-doctoral			50.000
Gouvernance	7.000	7.000	11.000
Frais de gestion 10%	7.000	9.350	11.350
TOTAL HT	77.000	102.850	124.850
TVA 19.6%	15.100	20.150	24.500
TOTAL TTC	92.100	123.000	149.350

Comme le financement du programme ne pourra démarrer avant le deuxième trimestre 2013, nous proposons une répartition des crédits comme suit, avec une répartition des dépenses qui seront à engager, par trimestre.

Ventilation trimestrielle des dépenses :

Poste	T2 2013	T3 2013	T4 2013	T1 2014	T2 2014	T3 2014	T4 2014
GLS	17.000			23.000			
Bagues	1.000						
Analyses isotopes			5.000				5.000
Analyses génétiques				3.500			
Missions printemps	30.000				45.000		
Mission automne		10.000				10.000	
Post-doc coordination							
Gouvernance	2.000	5.000		2.000		5.000	
Frais gestion	5.000	1.500	500	2.850	4.500	1.500	500
Total HT	55.000	16.500	5.500	31.350	49.500	16.500	5.500
TVA	10.780	3.240	1.080	6.140	9.700	3.230	1.080
TOTAL TTC	65.780	19.740	6.580	37.490	59.200	19.730	6.580

Poste	T1 - 2015	T2 2015	T3 2015	T4 2015
GLS				
Bagues				
Analyses isotopes			4.000	
Analyses génétiques			3.500	
Missions printemps		45.000		
Mission automne				
Post-doc coordination	12.500	12.500	12.500	12.500
Gouvernance			5.000	6.000
Frais gestion	1.250	5.750	2.500	1.850
Total HT	13.750	63.250	27.500	20.350
TVA	2.700	12.400	5.400	4.000
TOTAL TTC	16.450	75.650	32.900	24.350

2013 : 92.100€

2014 : 123.000€

2015 : 149.350€

TOTAL : 364.450€

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Appendix 5

Jiguet *et al.* (2016) An update of European population sizes and trends of the Ortolan Bunting (*Emberiza hortulana*). *Ornis Fennica* 93: 186-196

Review

An update of the European breeding population sizes and trends of the Ortolan Bunting (*Emberiza hortulana*)

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Following recent updates proposed by BirdLife International and further updates across Europe gathered in the context of a continent-wide study of the migration strategy of the species, we propose here an update of national population sizes and associated recent trends of the Ortolan Bunting (*Emberiza hortulana*). Previous estimates for the period 1999–2002 reported 5,200,000 to 16,000,000 breeding pairs, for an area extending east to European Russia, and south to the Caucasus and Turkey. The countries holding the largest populations were Turkey (3–10 million pairs) and Russia (1.5–5.0 million pairs). The updated results give approximately 3,319,000 to 7,057,000 pairs in Europe (for the period 2012–2014), representing a c. 50% decrease in numbers over the last decade. This decrease is partly due to overestimates proposed in previous reports for the key country, Turkey, which is now considered to support only 500,000 to 1,000,000 pairs. Russia still holds 2.0–4.3 million pairs, although with an estimated decline of c. 15–30% since 2000. Overall, within the 39 European countries assessed here, recent decadal trends (on average 2000–2012) in population size are reported as unknown in 15 countries, increasing in 2 countries (Germany and Serbia), stable or fluctuating in 6 countries, and decreasing in 16 countries including recent extinctions in Belgium, Hungary, Slovakia and the Netherlands. Overall, declining populations are mostly located in northern Europe, and fourteen of the 15 northern European countries with a known national trend have declining breeding populations, suggesting that northern breeders are of particular conservation concern.

1. Introduction

The intensification of agricultural practices across Europe is considered to be the major recent driver of bird population declines in farmland habitats (Donald *et al.* 2001). Loss of habitat heterogeneity and field margins, conversion of grassland to crops and widespread use of fertilizers, herbicides and pesticides have caused massive declines in food abundance and diversity, including seeds and invertebrates, for breeding birds (Vickery *et al.* 2001). In this context, the Ortolan Bunting (*Emberiza hortulana*) has suffered a major population decline across Europe in recent decades

(Goławski & Dombrowski 2002, Revaz *et al.* 2005, Vepsäläinen *et al.* 2005, Dale 2009, Menz & Arlettaz 2012). As it is a long-distance passerine migrant, population trends might also be influenced by potential pressures or changes in migration stopover and wintering areas (see Selstam *et al.* 2015).

A first step before trying to understand the potential causes of population declines is to quantify the losses. Here we propose an update of estimated national breeding population sizes, based on recent literature and extensive field work conducted in the context of a continent-wide study of the species' migration strategy.

2. Methods

2.1. Previous reference estimates

Baselines for this update are the numbers published by BirdLife International (2004), for the 39 countries listed in Table 1. According to this reference, the European breeding population was estimated to number 5,200,000–16,000,000 breeding pairs, for an area extending east to European Russia, and south to the Caucasus and Turkey. These numbers generally refer to population sizes estimated during the period 1999–2002. Countries holding the largest populations were reported to be Turkey (3–10 million pairs) and Russia (1.5–5.0 million pairs).

2.2. Updated estimates

To propose updated national population sizes for the 39 countries, we used three sources of information. The first one is the official reporting made by EU Member States under Article 12 of the Birds Directive (hereafter Art. 12), available on the website of the European Topic Centre on Biological Diversity (ETC-BD, 2015). The second source comes from ornithological experts participating in a continent-wide research program studying the migration strategy of the species, and their knowledge of national Ortolan Bunting populations, all of whom are co-authors of this paper.

The third one concerned non-EU countries, with recent estimates gathered by BirdLife International (2015) to prepare the recent update of the European Red List of Birds, a project funded by the European Commission. Population changes between the two periods reported in Table 1 were reprinted from BirdLife International 2015, except for Belarus as the updated information here reveals a different trend.

3. Results and discussion

3.1. National population sizes and trends

Table 1 presents an overview of the national population sizes as published by BirdLife International in 2004 (Birds in Europe) and the update by the ex-

perts co-authoring the present paper. We further discuss below the numbers and trends reported in Table 1 for countries for which we propose new data explaining and/or updating the figures published in BirdLife International (2015). Some of these countries have published national Red List status for the species (see IUCN 2012), which is reported here when available.

Belarus

Previous strongholds of the species in the south-east were visited in spring 2014 to locate singing males, and remnant populations were only found in the radio-contaminated exclusion area near Chernobyl. The total population is estimated to number at most between 1,000 and 3,000 males, which represents a decline of c. 34% in 12 years. BirdLife International (2015) reported 2,500 to 4,000 pairs in 2012.

Estonia

Only 200–400 pairs reported in 2014, a 90% decrease since BirdLife International (2004). A prime example of the magnitude of the decline comes from Lahemaa National Park, believed to be one of the strongholds of the species in Estonia, where c. 130 pairs bred at the end of the 1990s, but only 4 singing males were recorded during a thorough inventory in 2014 (data from the Estonian Ornithological Society). Art. 12 reporting mentioned 300–500 pairs in 2008–2012 (Eltis *et al.* 2013, BirdLife International 2015).

Finland

There were 9,400 to 25,000 pairs in 2006–2012, a period which does not overlap with the estimates obtained from 1998–2002, thereby representing a decline of c. 60% in c. 10 years. The population estimate in 2014 is, however, updated here to 7,000–19,000 pairs based on the steep and steadily continuing decline of c. 13% per year (Väisänen & Lehtikoinen 2013). The rate of decrease is derived from the national line transect and point count data. These monitoring data suggest a national decline of 78% from 2004 to 2014, which is quite similar to the decline observed in adjacent Estonia, but involves here a larger population. Since the start of the national breeding survey, the species has lost 98% of its breeding numbers in 31 years (Väisänen & Lehtikoinen 2013). The species is

Table 1. National breeding population sizes of the Ortolan Bunting across Europe. The trend is the recent trend taken from BirdLife International (2015). The countries are categorized into northern and southern Europe.

Country	Birds in Europe (2004)	Year	Update 2014	Year	Trend	Northern/ Southern
Albania	1,000–2,000	2002	1,000–2,000	2012	→	S
Andorra	4–10	2001	No new data	–	?	–
Armenia	15,000–30,000	2002	15,000–30,000	2012	?	–
Austria	15–25	2002	4–10	2012		N
Azerbaijan	20,000–100,000	2000	No new data	–	?	–
Belarus	2,500–4,000	2002	1,000–3,000	2014		N
Belgium	0	2000	0	2012	–	–
Bosnia & HG	?	–	1,500–2,000	2012	?	–
Bulgaria	25,000–50,000	2002	34,000–150,000	2012	F	S
Croatia	1,000–5,000	2002	3,500–6,000	2010	?	–
Czech Republic	100–200	2000	80–160	2012		N
Estonia	2,000–4,000	1998	200–400	2014		N
Finland	30,000–50,000	2002	7,000–19,000	2014		N
France	10,000–40,000	2002	5,000–8,000	2012		N
Georgia	present	–	–	–	?	–
Germany	5,600–7,000	1999	10,500–16,000	2009	↑	N
Greece	20,000–50,000	2000	20,000–50,000	2012	→	S
Hungary	10–15	2002	0	2012		S
Italy	4,000–16,000	2003	4,000–16,000	2012	?	–
Kosovo	500–1,000	2003	350–700	2014	?	–
Latvia	500–2,000	2000	144–7744	2004	?	–
Lithuania	200–800	2001	60–100	2012		N
Macedonia	3,000–10,000	2000	3,000–10,000	2012	?	–
Moldova	4,500–5,000	2000	2,000–3,000	2010	F	S
Montenegro	400–800	2003	400–800	2012	?	–
Netherlands	0–5	2000	0	2011		N
Norway	150–155	2002	20	2014		N
Poland	150,000–300,000	2002	197,000–298,000	2012		N
Portugal	500–2,500	2002	1,000–5,000	2012	?	–
Romania	125,000–255,000	2002	225,000–550,000	2013	?	–
Russia	1,500,000–5,000,000	2000	2,000,000–4,300,000	2013		N
Serbia	3,500–4,500	2003	26,000–42,000	2014	↑	S
Slovakia	0–5	1999	0	2012	?	–
Slovenia	200–300	2000	20–34	2012		S
Spain	200,000–225,000	1992	180,500–365,000	2012		S
Sweden	2,000–7,000	2000	2 600–5,000	2012		N
Switzerland	100–150	2002	0–1	2014		N
Turkey	3,000,000–10,000,000	2001	500,000–1,000,000	2014	→	S
Ukraine	58,000–67,000	2000	No new data	–		N
EUROPE	5,184,779–16,219,465	2004	3,318,882–7,056,959	2014	–	–

listed as Endangered on the Finnish national Red List (Rassi *et al.* 2010, Tiainen *et al.* 2016).

France

Recent analyses of the national breeding bird atlas (2009–2012) data provided an estimate of 5,000 to 8,000 pairs, distributed mainly in the south, a c.

68% decline compared to the estimate published in 2002 (10,000–40,000 pairs). Concomitantly, the national breeding bird survey reported a 50% decline for the period 2001–2014 (Jiguet *et al.* 2016). The numbers provided for Art. 12 reporting were overestimated because they were based on an extrapolation of only part of the final semi-quantita-

tive data collected during atlas work (2009–2012), whereas the final estimates of national population sizes were computed in 2014 (Issa & Muller 2015). So, contrary to BirdLife International (2015), the attested recent decrease can be considered as large (> 50%) and not moderate (20–49%), with the result that the species is now listed as Endangered on the French national Red List (IUCN France and MNHN in prep.).

Germany

Recent estimates imply a population increase, but it has been suggested that the previous reported estimates were much too low (Dürr & Ryslavý 2009). The current estimates are based on a much larger sample size and improved knowledge gained during field surveys for the recent breeding bird atlas (Gedeon *et al.* 2014). As to trends, there are also contradictory elements, as the species is still increasing in its current German strongholds (for example in eastern Lower Saxony), also due to conservation efforts, whereas in other areas numbers are decreasing. This might explain the stable trend found by the national breeding bird survey, and the stable long-term trend reported for Germany by BirdLife International (2015). In 2007, the set-aside scheme of the EU was abandoned in Germany; until then the Ortolan Bunting had benefited from improved habitat structure in suitable sandy habitats, but also from the tilling of grasslands in mosaic landscapes and from other conservation actions. In most parts of Germany, the species shows clear declines and even area losses. At the distribution edges, the set-aside scheme has not had the same positive effects on the species, and numbers have been continually declining. Finally, even if a positive population trend in the species' German stronghold is evident, there is a sex bias towards males indicating that reproduction is reduced (Gedeon *et al.* 2014), which is a clear sign of a species with fragmented, "unhealthy" populations (Dale 2001).

Norway

Norwegian counts represent males. The number of males was 152 in 2002 and 20 in 2014, representing a 87% decline. For pairs (so considering females), the trend is a decline of 88% (82 in 2002 to probably 10 females in 2014). BirdLife (2004) reported 150–155 pairs in 2002 but these figures are

in fact related to the number of males. The species is listed as Critically Endangered on the Norwegian national Red List (Kålås *et al.* 2015).

Poland

Despite the previous upper and lower values of the population size estimate being not dissimilar to the current one (see Table 1), the population is known to have declined by 20% during the first decade of the 2000s (Kosicki & Chylarecki 2012), hence the negative trend reported for that country.

Russia

BirdLife International (2004) reported 1.5–5.0 million pairs in European Russia as a whole in 2000. In the Rostov region in the late 1990s, the Ortolan Bunting was recorded in 48 of 54 surveyed 50×50 km squares. It was nesting in 25 squares, and probably nesting in another 22 squares. The number of pairs was estimated at 10,000–100,000 for 32 squares, at 1,000–10,000 pairs for 12 squares (Belik 2000). Subsequently, in southern Russia, numbers have decreased in the Stavropol (*vide* Lyubov Malovichko), Rostov and Voronezh regions. Numbers have remained stable in Belogorie reserve in the Belgorod region, but this population is confined to steppe with shrubs and trees that represents less than 1% of the region. In northern Russia, the species colonized the Karelian Isthmus and areas to the southeast (Leningrad, Vologda, Kostroma, Nizhny Novgorod to Perm) during 1930–1950, but started to decline in the early 1980s. It has since almost disappeared as a breeder and migrant in the Karelia and Leningrad regions (*vide* N.V. Lapshin, G.A. Noskov & T.A. Rymkevich). It has become rare or threatened elsewhere, perhaps even in the greater Moscow region where small numbers are sporadic. The Ortolan Bunting is classified in categories 2–4 in the Red List of threatened birds in Eastern Fennoscandia (1998) and in most parts of the northwestern and central Russia: Ryazan (2001), Leningrad, Tula and Tver (2002), Yaroslavl (2004), Vologda and Kaluga (2006), Moscow (2008), Kostroma (2009), Kaliningrad (2010), Vladimir, etc. (*vide* V. V. Romanov).

Overall in southern Russia, from Rostov and Volgograd southwards to Dagestan, the current breeding population is estimated at 1–3 million pairs, representing a decline of 10–20% in the last

ten years. The population in the rest of European Russia was estimated at 1.0–1.3 million pairs in 2012, localized in a southern belt of steppe habitats ranging from Belgorod to Orenburg. In this area, the species is considered in decline too. Therefore, a total of 2.0–4.3 million pairs are estimated to breed in Russia, but largely located in southern districts. Alexander Mischenko, Viktor Belik and other Russian colleagues (BirdLife International 2015) estimate that the number of Ortolan Buntings breeding in European Russia has decreased by 15–30% since 2000.

Serbia, Montenegro and Kosovo

BirdLife International (2004) reported 4,500–6,500 pairs for the former ‘Serbia’, which now comprises three countries, for which separate estimates for 2004 have been calculated (Puzović *et al.* 2003). Using data for the period 1998–2003, the population size could be divided into 500–1,000 pairs in Kosovo, 400–800 pairs in Montenegro and 3,500–4,500 pairs in Serbia. Recent estimates for 2014 amount to 350–700 pairs in Kosovo, 400–800 pairs in Montenegro, and 26,000–42,000 pairs in Serbia. In this context, it is difficult to infer population trends, as former estimates were certainly underestimates, although the Bird Protection and Study Society of Serbia (in litt. to BirdLife International) reported a recent increase in breeding numbers (by c. 10%), probably due to a reduction in agricultural land use and associated pressures.

Spain

The population is now considered to comprise 180,500–365,000 pairs and the species is reported to have colonized many post-fire Mediterranean areas, although overall a slight decrease was reported (–13% for 1998–2012; BirdLife International 2015), even if the population indices reveals strong fluctuations (see http://www.magrama.gob.es/es/biodiversidad/temas/inventarios-nacionales/escribano_hortelano_tcm7-219856.pdf). Trends can vary regionally, and in Catalonia, the SOCC (Seguiment d’Ocells Comuns a Catalunya) reports an overall decrease of –54% from 2002 to 2014 (<http://www.sioc.cat/fitxa.php?sci=0&sp=EMBHOR>).

Sweden

Recent estimates give 2,600–5,000 (probably 4,000) pairs in 2012, i.e. within the range of the previous estimate of 2,000–7,000 pairs (in 2000), although the species is considered to have declined nationally by c. 38% (for results of the Swedish national breeding bird survey see <http://www.fageltaxering.lu.se/node/35785>). The occupied range in Sweden has decreased every year since 2000. The species is listed as Vulnerable on the national Red List (ArtDatabanken 2015).

Switzerland

A lone singing male was detected in spring 2014, and again intermittently in 2015. As the national population was estimated at 100 to 150 pairs in 2002, the species is listed as Critically Endangered on the Swiss national Red List (Keller *et al.* 2010).

Turkey

BirdLife International (2004) reported 3–10 million pairs in Turkey in 2001, which represented c. 60% of the total European population. In an earlier review, Tucker & Heath (1994) reported even wider limits of 1–10 million pairs, based on discussion with observers active in the country in the late 1980s/early 1990s. Kirwan *et al.* (2008) reported that the species is a widespread and common summer visitor, with apparently stable populations, mainly breeding in uplands between 750 m and 2,600 m (exceptionally 3,300 m in the extreme east), more locally at lower altitudes in western and northern coastal regions. The species is completely absent as a breeder from large parts of central and western Turkey, and is local in Thrace (European Turkey). In some suitable areas, its altitudinal range can be much more restricted. For example on Uludağ, in western Turkey, the species is a common breeder between 300 and 1,200 m, but does not occur at higher elevations (Jetz 1995). Unfortunately, both qualitative and quantitative data on the species’ density in Turkey are still extremely scarce, especially at sites within the core range. The largest and most robust dataset (still lacking in density information) that we possess is for south-east Anatolia, an area of 75,358 km², or almost 10% of the Turkish landmass. Here, bird surveys were conducted in 657 squares, or 81.5% of the region, in 2001–2003 (Welch 2004). The survey recorded Ortolan Bunting in 52 of the

657 squares, widely scattered across the region, or 7.9% of all surveyed localities. However, breeding was not confirmed in any squares and was considered to be probable in just 17 squares, or c. 2.5% of all surveyed areas. Records in many other areas often involved migrants. Nevertheless, the species was observed in c. 50% of the 30 key areas for biodiversity (not only birds) identified during the project. In far eastern Turkey, the range of Ortolan Bunting overlaps with that of Grey-necked Bunting (*Emberiza buchanani*), whose population in Turkey is estimated by BirdLife International (2004) at 6,000–18,000 pairs. In this region, Ortolan is either replaced by Grey-necked, or occurs at lower densities (Kirwan *et al.* 2008). In 1993, GMK surveyed (using 1 km-long line transects) four low-elevation localities with suitable habitat in this region of overlap, recording Grey-necked at all four, but Ortolan at just one, where its numbers were c. 25% lower than Grey-necked. The Ortolan Bunting is generally mapped (in field guides and handbooks) as present at all of these areas in eastern and south-eastern Anatolia, despite being absent locally from many apparently suitable areas. As c. 15–20% of the country constitutes wholly unsuitable habitat for Ortolan Bunting (based on the map in Kirwan *et al.* 2008, all in western, south-western, northern and central Turkey) and Turkey's total land area is 779,452 km², this means that a minimum of 117,000 km² of the country might completely lack the species. In other words, if 10,000,000 pairs breed in Turkey, their density is > 15 pairs per km², or more than 4.5 pairs per km², if the lower limit of the BirdLife estimate was to be applied. Given that surveys (cited above) from parts of Turkey where the species is thought to be generally common demonstrate widespread absence at the local scale, these figures are, at least on the basis of the available evidence, far too optimistic. As the range size of Grey-necked Bunting in Turkey is perhaps just 5–10% of that of Ortolan Bunting and the two species probably occur at generally similar densities (albeit perhaps locally higher in Grey-necked), if the upper limit of the BirdLife estimate for the Grey-necked was correct and was extrapolated to the range of Ortolan Bunting, then the population of Ortolan might be no more than 360,000 pairs. Further anecdotal evidence of the species' true abundance in Turkey comes from Cyprus, where the species does not

breed, but recent estimates (based on data collected between 2003 and 2013) suggest that Ortolan Bunting is only the 45th most abundant passage migrant, with a total of 152 records involving 550 birds (Richardson 2014).

Without robust data, including specific density estimates, from more areas across the species' range in Turkey, however, it is impossible to say more than the BirdLife International (2004) upper limit of population appears likely to be an overestimate by a factor of ten. For now, we suggest that a population of 500,000 to 1,000,000 pairs is a much more reasonable estimate, and even this could be too high. This represents a reduction of 87%, which should be considered as a re-estimation of the population size, not an attested population decrease.

3.2. European population sizes and trends

According to these updates, we can propose a new estimated European population size for the Ortolan Bunting, by summing the most recent national population sizes summarized in Table 1 (taking values published by BirdLife International in 2004 where no updates are available). The result of this exercise is that approximately 3,319,000 to 7,057,000 pairs of Ortolan Bunting are estimated to breed in Europe in the period 2012–2014, compared to 5,185,000 to 16,240,000 reported in 2002 (BirdLife International 2004). As this difference is partly due to earlier overestimates, it is safer to exclude Turkey (unrealistic, inflated estimates in the past) to obtain a more realistic trend estimate between these two dates, and to consider cautiously countries for which we have no estimate for either the early 2000s or the early 2010s (i.e. Andorra, Azerbaijan, Bosnia-Herzegovina, Georgia, Ukraine). For all other countries with recent reliable information, a comparison of the geometric means of the upper and lower values of national population range sizes does not reveal the decline reported by experts, probably because earlier estimates were less precise. The case of Russia illustrates this well: the population size changed from 1.5–5 million to 2–4.3 million pairs, with a trend of geometric means of +7%, while all Russian experts agree upon an overall decline of 15–30% in

numbers, with no region where the species might have increased in numbers in the 2000s, but also regions where the species has almost disappeared.

Overall, within the 39 countries listed in Table 1, the breeding populations of Ortolan Bunting have unknown trends in 15 countries, are increasing in 2 countries (Germany and Serbia), stable or fluctuating numbers in 6 countries, and decreasing in 16 countries, including probable recent extinctions in Hungary, Slovakia and the Netherlands, plus Belgium, where the extinction is confirmed, as well as likely extinction in Switzerland. Nearly 40% of the countries have no monitoring data to permit estimation of a population trend for the species, while most long-lasting or recent declines were detected due to general breeding bird surveys (France, Finland, Sweden) or dedicated monitoring of the species in some strongholds (Russia). This highlights how important good national monitoring data are to monitor the changes of such species across Europe.

The trend in breeding numbers is however spatially variable. Some southern countries have stable, fluctuating or increasing populations (Serbia, Greece, Bulgaria and possibly Turkey), almost all western and northern European populations are declining dramatically (Fennoscandia, Poland, Baltic states, France), while eastern populations, including Belarus, Ukraine and the Russian strongholds of the species, have faced important declines during the last decade. If we divide the 39 European countries into two groups according to their latitude (with a split at around 45–46°N; see last column of Table 1 for the classification of each country with a known trend within a group), it appears that the trends in breeding numbers differ between northern and southern countries, with more declines in the north (14 countries with declining populations compared to 1 increasing/stable/fluctuating in the northern group; 3 declining compared to 6 stable/fluctuating/increasing in the southern group; Fisher's exact test, $P = 0.0037$). There are more southern countries with unknown trends, because of missing or incomplete data. However, the quality of the data used to estimate the reported trends (as poor, medium or good) is the same irrespective of the direction of the trend (negative, or not negative e.g. stable, positive or fluctuating; Fisher's exact test, $P = 0.15$).

The reasons for such a strong decline in some northern countries are not clear, but probably involve multiple factors. As reasons for declines in the species, Menz & Arlettaz (2012) mentioned habitat loss, climate change on the breeding grounds, altered population structure and dynamics, hunting on migration, and environmental changes in the wintering areas. Published studies show that factors on the breeding grounds may have had a negative effect on populations, especially the loss of small-scale environmental heterogeneity in farmland landscapes (Vepsäläinen *et al.* 2005, 2007). However, the Ortolan Bunting has also decreased in areas where major changes have not occurred, e.g. in Finland and Sweden. This fact and occasional population crashes (e.g. –20% in 2006–2007 in Finland) makes it plausible that the major drivers of the decline are occurring along the migration flyway(s) or on the wintering grounds, including anthropogenic and climate change-driven habitat deterioration. Northern and southern breeding populations certainly face different changes in their habitats and environmental conditions, as ongoing land use and climatic changes could have different impacts on birds in southern and northern Europe (Barbet-Massin *et al.* 2012, Thuiller *et al.* 2014). Northern and southern populations might also have different migration routes and wintering grounds, and thus face different pressures along their migration flyways, as might do populations using the eastern or the western flyways. Future research that attempts to identify which populations use which flyways, and their respective wintering areas, could shed new light on the varying fates of European breeding populations. Finally, many breeding populations have become small and isolated, often with a biased sex ratio because of a lack of females, so that there are few management options that may help recovery. In Norway, a long-term individual-based monitoring of breeding populations revealed that the decline was caused by a normal behaviour of female dispersal, and not by poor breeding success or low survival (Dale 2009). Natal dispersal is higher in females (Dale 2001), while such a breeding dispersal might be maladaptive in the context of habitat fragmentation and population isolation (no close sites with displaying males), and could amplify the ongoing declines.

3.3. Conclusion

Currently, the Ortolan Bunting is evaluated as Least Concern (with extinction) on the IUCN Red List (BirdLife International 2015), because the species has a large range and a large population size. While a population decline is recognized, this is not considered sufficiently rapid to reach the thresholds for Vulnerable status. The recent information gathered for Article 12 reporting and for this paper suggests strong ongoing declines, at least in northern and eastern countries. Therefore the regional status of the species could be revisited, based on direct observations and an index of abundance appropriate to the taxon (IUCN 2012). In order to complete this exercise and permit the species' global status to be re-evaluated, equivalent population data are also required for the rest of the breeding range in Central Asia and the Middle East. The same is needed also in European countries where the trend is currently reported as unknown, highlighting the importance of operating robust national monitoring schemes to efficiently monitor changes in bird numbers across a species' range. All this calls for urgent research that provides the necessary evidence-base about species decline across Europe so as to prompt proper conservation action.

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Peltosirkun kannankoot ja -muutokset Euroopassa

Peltosirkku on harvinaistunut monin paikoin Euroopassa, minkä vuoksi on tärkeää saada ajan-

tasaista tietoa lajin kannankootista ja -muutoksista mahdollisimman laajasti lajin esiintymisalueelta. Koostimme tähän artikkeliin päivitettyt arviot peltosirkun kansallisista kannankootista ja -muutoksista. Nämä perustuvat peltosirkun mantereenlaajuisen muuttostrategiatutkimuksen yhteydessä kerättyyn tietoon sekä BirdLife Internationalin julkaisemiin katsauksiin.

Aiemman, vuosien 1999–2002, arvion mukaan peltosirkun kannan esitettiin olleen 5,2–16 miljoonaa paria Euroopan, Euroopan puoleisen Venäjän, Kaukasian ja Turkin kattamalla alueella. Suurimpien populaatioiden esitettiin tuolloin olevan Turkissa (3–10 miljoonaa paria) ja Venäjällä (1,5–5,0 miljoonaa paria). Nyt päivitettyjen tulosten mukaan alueen kokonaisparimäärä arvioitiin vuosina 2012–2014 vuosikymmenen takaiseen parimääräarvioon verrattuna n. 50 % pienemmäksi (3,3–7,1 miljoonaa paria). Vähennys johtuu osittain aiemmasta Turkin populaatiokoon yliarviosta, sillä nykyinen arvio Turkin populaatiokoolle on vain 0,5–1,0 miljoonaa paria. Venäjän Euroopan puoleisen osan kannan arvioidaan olevan yhä suuri käsittäen 2,0–4,3 miljoonaa paria, vaikka kanta on siellä vähentynyt 15–30 % vuodesta 2000.

Viimeisen n. kymmenen vuoden (keskimäärin vuodesta 2000 vuoteen 2012) kannanmuutos 39 maassa osoitti, että peltosirkun kanta väheni 16 maassa sisältäen neljä maata, joista laji on äskettäin kuollut sukupuuttoon (Belgia, Unkari, Slovakia ja Alankomaat). Laji runsastui kahdessa maassa (Saksa ja Serbia), kuudessa maassa kannankehitys oli vakaa tai vaihteleva ilman selvää pitkäaikaismuutosta, ja 15 maan osalta kannanmuutosta ei oltu arvioitu. Peltosirkun suojelutilanne on erityisen huolestuttava Pohjois-Euroopassa, missä 15 arvioidusta maasta 14:ssä pesimäkannat ovat vähentyneet.

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