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Population structure and migratory directions of Scandinavian bluethroats *Luscinia svecica* – a molecular, morphological and stable isotope analysis

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Many species of birds show evidence of secondary contact zones and subspeciation in their Scandinavian distribution range, presumably resulting from different post-glacial recolonization routes. We investigated whether this is the case also in the Scandinavian bluethroat *Luscinia svecica*, a species that has been suggested to consist of two separate populations: one SW-migrating and long-winged (*L. s. gaetkei)* breeding in southern Norway, and one shorter-winged ESE-migrating (*L. s. svecica*) in northern Scandinavia. We sampled males at eleven breeding sites from southern Norway to northernmost Sweden. There were no morphological differences or latitudinal trends within the sample, neither were there any genetic differences or latitudinal trends as measured by variation in AFLP and microsatellite markers. Stable isotope ratios of throat feathers moulted on the wintering grounds showed no, or possibly marginal differences between birds from southern Norway and northern Sweden. We also re-measured old museum skins that in previous studies were classified as *L. s. gaetkei*, and found marginally longer wings in birds from the southern part of the Scandinavian breeding range. The difference, however, was much smaller than proposed in earlier studies. We conclude that there is no evidence of a genetic population structure among Scandinavian bluethroats that would suggest the presence of a zone of secondary contact. Finally we discuss whether the presumed subspecies *gaetkei* ever existed.

The Pleistocene glacial periods have resulted in repeated withdrawal of fauna from areas in the north to different refuges in the south for extensive periods of time (Hewitt 2002). The location of these refuges, how isolated they have been from each other as well as subsequent post-glacial recolonisation patterns, have had strong impact on the level of population differentiation seen today (Merilä et al. 1997, Taberlet et al. 1998). During periods of allopatry among populations in glacial refuges, genetic differences might have evolved that now prevent or restrict mixing of the gene pools when the populations are coming into the secondary contact as a result of post-glacial range expansions (Hewitt 1996, Taberlet et al. 1998).

In Scandinavia there are several species of land birds that are represented by two subspecies that appear to have colonized from two directions; from south over the Danish islands or from northeast through Finland around the Gulf of Botnia. The willow warbler *Phylloscopus trochilus* (Bensch et al. 1999), the chiffchaff *Phylloscopus collybita* (Hansson et al. 2000, Lindström et al. 2007) and the redpoll *Carduelis flammea* (Ottvall et al. 2002) are well-established examples of such bidirectional colonization routes. However, based on patterns of morphological clines or gap distributions in central Scandinavia, many more species probably have colonized Scandinavia from two directions, e.g. yellow wagtail *Motacilla flava* and sedge warbler *Acrocephalus schoenobaenus* (SOF 2002).

Another bird species that has been suggested to be represented in Scandinavia by two subspecies is the bluethroat *Luscinia svecica* (one subspecies in southern Norway and one in northern Scandinavia). The overall picture of bluethroat presence in Scandinavia is undisputed. Only red-spotted bluethroats breed regularly in Norway, Sweden and Finland (in contrast to the white-spotted birds of continental Europe). The red-spotted birds occur commonly in sub-alpine birch forests, from southernmost Norway through the Swedish mountains up to northernmost Norway (Thingstad 1994, SOF 2002, Fig. 1), and then further east through Finland and northern Russia to the Bering Strait (Cramp 1988). The pre-dominant migration direction in autumn of the bulk of Scandinavian bluethroats *L. s. svecica* is no doubt ESE, towards yet unknown wintering grounds in southern Asia, as shown by numerous ringing recoveries (Ellegren and Staav 1990).

The presence of two subspecies of bluethroats in Scandinavia, with potentially different colonization routes, has been controversial for almost a century. Actually, there are two, possibly related controversies to be solved: 1) are

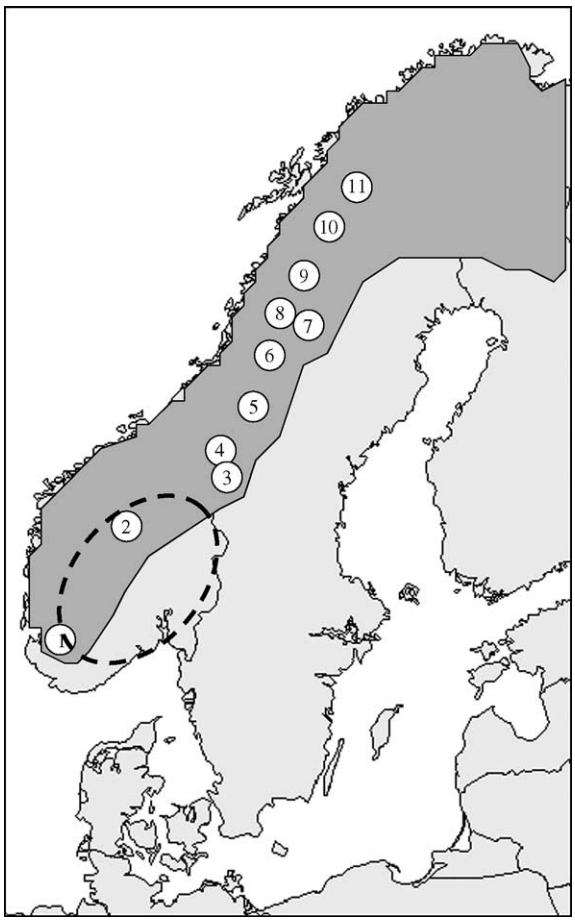

Fig. 1. Sampling site positions in Norway (1–2) and Sweden (3–11, see also Table 1). The shaded area is the present breeding range of red-spotted bluethroats (after Thingstad 1994, SOF 2002), the broken line indicate suggested distribution area of *gaetkei* by Lundevall (1950).

the bluethroats in southern Norway morphological or genetically different from other Scandinavian bluethroats thus reflecting the presence of a secondary contact zone in central Scandinavia? 2) Do south Norway birds migrate in another direction (SSW) than that of the rest of the Scandinavian birds (ESE)? For a detailed background to these questions, see Methods.

We decided to address the two questions in a more systematic way. First, morphological variation of bluethroats across Scandinavia was measured in the field, but also on museum skins. Second, if birds winter at widely separated wintering grounds (for example, tropical Africa and southern Asia), there is a chance that the ratio of stable isotopes in feathers moulted at their wintering ground will differ between the two subpopulations (Chamberlain et al. 2000). Third, and most importantly, historical isolation or reduced gene flow between populations might have resulted in genetic differentiations at neutral loci. We therefore examined genetic differences, using AFLP (amplified fragment length polymorphism) and microsatellite analyses. We chose these markers rather than mtDNA because north European bluethroats lack phylogeographic structure based on mtDNA (Questiau et al. 1998, Zink et al. 2003) but seem to have significant, though subtle, structure in microsatellites (Johnsen et al. 2006). If south Norwegian bluethroats belong to a population isolated from other Scandinavian populations, we expected to find differences in one or more of the investigated variables.

Methods

Background

Bluethroats in Scandinavia and the enigmatic gaetkei subspecies

The population differentiation of Scandinavian bluethroats was first addressed by Nilsson (1819). In a paper containing the scientific description of the chiffchaff, Nilsson suggested, in passing, that the northernmost breeding bluethroats in Scandinavia migrate east over Finland and Russia, and that the west Norway birds migrate over Germany, Holstein and Jutland. However, Nilsson (1819) presents no evidence or citation for this suggestion.

The formal description of a separate south Norway subspecies comes from Germany. Kleinschmidt (1904) named it *Erithacus* (*astrologus*) *gaetkei* and claimed that *gaetkei* breeds ''Fille-fjeld'', south of Jutenheimen in southern Norway, and migrates towards SW in autumn. The *gaetkei* birds were described as long-winged, ''easily 8.0 cm'', but the only direct comparison Kleinschmidt made with ssp. *svecica* is that *svecica* is said to have a paler blue throat than *gaetkei*.

There are several remarkable facts about Kleinschmidt's description. First, the individuals forming the basis of the formal description of *gaetkei* were trapped during migration on Helgoland, an island on the German North Sea coast. Second, Kleinschmidt cites no sources from the presumed Scandinavian breeding grounds. Third, the main reason for his subspecies description seems to be his admiration for the ornithologist Heinrich Gätke, who on Helgoland did pioneering work on bird migration in the second half of the 19th century. Gätke (1895) himself described the occurrence of bluethroats on the island, but does not mention a separate subspecies going to southern Norway. Nevertheless, Kleinschmidt names a bluethroat subspecies after him. It seems clear that Kleinschmidt's (1904) description of a new subspecies was substandard, but it has recurrently bothered scientists for more than a century.

Further descriptions of, and support for, *gaetkei* were presented by Weigold (1926) and Drost (1927), again based on birds from Helgoland. Red-spotted male bluethroats at Helgoland, all presumed to be *gaetkei*, were said to have wing lengths of 75–80 mm, in stark contrast to the 69–74 mm for *svecica* males. The origin of these *svecica* birds is never presented, but measurements seem to come from the same source; ''H. Br. B.'' (Drost 1927).

No clear reference had so far been given to birds from the presumed Scandinavian breeding grounds. However, Ekman (1922), refuted the idea of a separate subspecies in southern Norway. Steinbacher (1935) and Salomonsen (1949) compared museum skins from various places along the Scandinavian breeding grounds. They found males in south and central Norway to be only marginally longer-winged (72–80 mm) than birds from further north (70–78 mm; no means or statistics presented) and therefore refuted

the idea of a separate long-winged subspecies in southern Norway.

Nevertheless, Lundevall (1950) gave new life to *gaetkei* when measuring skins from various parts of the Scandinavian breeding range (many of the skins must have been the same that Steinbacher (1935) measured). He found that both males and females from southern Norway had on average four mm longer wings than northern birds, with hardly any overlap in absolute measures. Lundevall also mapped the southern Norway origins of the presumed *gaetkei* birds, which to our knowledge is the only kind of "evidence-based" reference to the presumed breeding haunts of *gaetkei*. Rendahl (1967) reviewed the situation in Scandinavia and supported Lundevall's idea of *gaetkei* in southern Norway, not least by pointing at an alleged bicentric breeding distribution. He suggested that Swedish birds from Härjedalen and south Jämtland probably also belong to the southern Norway population. Since Rendahl's work, *gaetkei* seems to have gone out of fashion. In the latest Norwegian breeding bird atlas *gaetkei* is not even mentioned. In addition, red-spotted bluethroats are shown to have a continuous breeding range in Norway (Thingstad 1994).

Migratory direction of Scandinavian bluethroats

Since the migratory direction has been coupled to subspecies or population status (Nilsson 1819, Kleinschmidt 1904), and a migratory divide in Scandinavian bluethroats would parallel the situation in other species, for example, the willow warbler (Hedenström and Pettersson 1987), the question of migratory direction also has bearing on the bluethroat subspecies discussion.

Staav (1975) and Ellegren and Staav (1990) reviewed available Nordic recoveries and concluded that only very rarely, if ever, do Nordic bluethroats migrate SW in autumn. This view was criticised by Anderssen and Gylseth (1992) who claimed, based on four Norwegian recoveries, that some Norwegian bluethroats indeed migrate towards southwest. From the recent Norwegian bird ringing atlas (Bakken et al. 2006) it seems clear that easterly directions nevertheless dominate. A total of 16 500 birds ringed resulted in 24 foreign recoveries, distributed as follows: Sweden 17, Finland 3, Russia 1, Denmark 1, Belgium 1 and Algeria 1. Almost all of these birds were ringed in southern Norway, that is, within the area suggested to be inhabited by *gaetkei* (Kleinschmidt 1904, Lundevall 1950). Obviously, southwesterly directions are very rare (at least after 1955, the year after which all recovered birds were ringed), especially when considering that the likelihood of getting a recovery from western Europe must be substantially larger than to get one from eastern Europe and Asia. In addition, both Ellegren and Staav (1990) and Bakken et al. (2006) reported a few birds ringed in northern Scandinavia that had flown in southerly and southwesterly directions. Thus, seemingly aberrant migratory directions may occur in both southern Norway and northern Scandinavian populations.

Skin studies

To evaluate the results of the old skin studies that suggested the occurrence of a morphological different subspecies in southern Norway we decided to re-measure all the skins used in these former studies (see above). Steinbacher (1935) and Lundevall (1950) measured bluethroat skins at the Zoological Museum in Oslo, Norway and at the Swedish Museum of Natural History in Stockholm, Sweden. OH visited these museums in 2002 and re-measured all skins dated 1884–1948 (most likely being the same skins that Steinbacher and Lundevall measured). The skins were also sexed and aged (1 yr – birds in their first calendar year, 2 yr – birds in their second calendar year, and 3 yr+ – birds in their third calendar year or older). Skins were also scored for primary moult status, since 2 yr and 3 yr+ birds moult their flight feathers on their breeding grounds in July–August, immediately after breeding (Svensson 1992).

Data collection in the field

Between 23 May and 25 June 2001, 11 different sites from Övre Sirdal (Norway) in the south to Abisko (Sweden) in the north (Fig. 1, Table 1) were visited by one of us (OH). The sites, numbered 1–11 from south to north, were evenly (when possible) distributed along a south–north transect within the Scandinavian breeding range. All but one site were located within 100 m of altitude from the tree line (the exception was site 8, Umfors, situated in pine forest at lower altitude). Five to ten singing males were caught and ringed at each site, using mistnets and playback. Throughout, the same song recording was used (recorded in Jokkasjaure, north Sweden (CD, Sten Wahlström, "Fågelsång. 197 nordiska arters läten", 2nd ed. 2000). Males seemed to

Table 1. Capture sites for bluethroats in 2001 (see also Fig. 1). Birds from sites with* where used for DNA analysis. Birds from all sites where used for morphology analysis.

Site	Site name	Latitude	Longitude	Capture date	n
1	Övre Sirdal*	59°00′N	06°45′E	23–25 May	10
2	Övre Heimdal*	61°30′N	08°50′E	22–25 June	10
3	Flatruet*	62°40′N	12°45′E	26–27 May	8
4	Storulvån*	63°10′N	12°25′E	28 May	10
5	Hotagen	64°00′N	13°45′E	16–17 June	5
6	Stekenjokk*	65°05′N	14°35′E	30–31May	10
7	Kraipe	65°40′N	16°20′E	13–14 June	6
8	Umfors*	65°50′N	15°00′E	31 may–1 June	10
9	Mierkenis*	66°40′N	16°10′E	2 June	10
10	Stora Lulevattnet*	67°40′N	17°25′E	3–4 June	8
11	Abisko*	68°25′N	19°00′E	5–6 June	10

have the same strong response at all sites. In Övre Heimdal (site 2) the mating season was over when the area was sampled and the males no longer responded to song. Instead mistnets were put in areas where bluethroats where known to hold territories. Most likely all captured birds were territory holders, and hence, it is unlikely that the trapping method (song playback vs more passive trapping) biased the sample.

All birds except two were aged as either second-year (born in 2000) or adult (born in 1999 or earlier), following the criteria given in Svensson (1992). The head-bill length was measured to the nearest 0.1 mm from the back of the head to the tip of the bill, using callipers. The length of the left wing was measured to the nearest full mm (method 3, Svensson 1992). The length of the right tarsus was measured to the nearest 0.1 mm using callipers, by bending the foot at the toes and the leg at the intertarsial joint, reading the distance between the extreme bending points (Alatalo et al. 1984). Tail length was measured by inserting a ruler between the tail feathers and the under-tail coverts and measured to the nearest 0.5 mm (Svensson 1992). All measurements were taken by OH.

Throat feathers (grown on the wintering grounds) from five individuals per site from two sites in Norway, two sites in the middle of the sampling range and the two northernmost sites in Sweden were used for stable isotope analysis (Table 1). From each bird 5–20 µl blood was collected, from the wing or the tarsus vein. The samples were stored in 500 µl SET-buffer (0.015 M NaCl, 0.05 M Tris, 0.001 M EDTA, pH 8.0) at ambient temperature for up to 3 weeks before permanently stored at -20°C.

Stable isotope analysis

The ratios (measured in δ-notation) of stable isotopes of certain elements in a feather reflect the biotic and abiotic characteristics of the area where the feather was grown (Hobson 1999a). Substantial differences in stable isotope values of feathers have been found for passerines wintering in different parts of Africa (Chamberlain et al. 2000, Evans et al. 2003, Pain et al. 2004, Yohannes et al. 2007). Because of the potentially large difference in ecological conditions between winter grounds in Africa and southern Asia (the suggested wintering areas for bluethroats) it is reasonable to assume that feathers grown in these two regions would contain different isotope ratios. Bluethroats moult their throat feathers in late winter, presumably on the wintering grounds (Svensson 1992). The analysis of $\delta^{15}N$ and $\delta^{13}C$ values followed the method described in Hobson (1999b).

Genetic analysis

DNA was extracted from blood using a standard chloroform/isoamylalcohol method (Sambrook et al. 2002). For the molecular typing we analysed birds from 9 of the 11 sites (i.e. sites with 8 or more sampled individuals) including a total of 86 samples. For the microsatellite locus (Pocc 5), which has been isolated in *Phylloscopus occipitalis* (Bensch et al. 1997) we used the protocol in Johnsen et al. (1998). The primer was fluorescein labelled and the amplified fragments were separated in a 6% denaturing polyacrylamide gel for 1–1.5 h at 30W. The position of the DNA fragments was visualised in a Vistra FluorImager.

The AFLP analysis was carried out following the protocol of Vos et al. (1995) with minor modifications as in Bensch et al. (2002a). In the pre-amplification, the following combinations of primers were used: E_T (5′ -GACTGCGTACCAATTCT-3′) and M_C (5′ -GATGAGTCCTGAGTAAC-3′). All the 86 samples were typed with two sets of selective primer combinations, E_{TGA}/M_{CGT} and E_{TCT}/M_{CGA}. The E-primers were labelled at the 5' end with fluorecein (Life Technologies) and the same gel procedures as for microsatellites were used.

Statistical analyses

We used MANOVA to simultaneously test for differences in multiple traits between groups, thus allowing corrections for age and sex. The analyses were performed on the untransformed trait values using SYSTAT 10.0 (Wilkinson 1998).

To test for presence of genetic population structure we used the program STRUCTURE 2.0 (Pritchard et al. 2000, Pritchard and Wen 2003), in which we combined the information from the co-dominant microsatellite locus with the 68 dominant AFLP loci. We searched for the number of populations (K) that produced the highest likelihood of the data (Ln P(D)), testing each K (1–6) ten times. We used the program Arlequin 3.01 (Excoffier et al. 1992, Schneider et al. 1997) to calculate overall (including all loci) and single locus pair-wise genetic differentiation (F_{st}) between sampling sites separately for the microsatellite and the AFLP data. F_{st} values were calculated between the two southernmost populations and the rest of the sampled populations.

Results

Morphology

Skins studies

In our efforts to repeat the study by Lundevall (1950) we measured the tail and wing length of 84 birds (28 females and 56 males) from northern Scandinavia (“northern”) and 44 birds (12 females and 32 males) from southern Norway (“southern”), of which we were certain of age, sex and sampling location. Among the 2 yr and 3 yr+ birds, none had newly moulted outer primaries, that is, they all carried last years' feathers in a more or less abraded state. For a few individuals, only one of the two measurements (wing and tail) could be obtained. The range in wing length for males and females (all ages combined) of southern and northern birds were: southern males 74–80 mm, southern females 71–77 mm, northern males 72–78 mm and northern females 69–75 mm (Fig. 2).

The overall model was significant (MANOVA, $F_{2,118} = 6.48$; $p = 0.002$) when controlling for sex and age. The main effect was clearly due to wing length ($F_{1,119} = 9.79$, $p = 0.002$), which was longer in southern than in northern birds (Fig. 3), and not due to tail length ($F_{1,119} = 0.22$, $p = 0.64$). The difference in average wing length was ca 1 mm between each of the age groups, 1 yr, 2 yr and 3 yr+ males, whereas the same comparison was not possible for females.

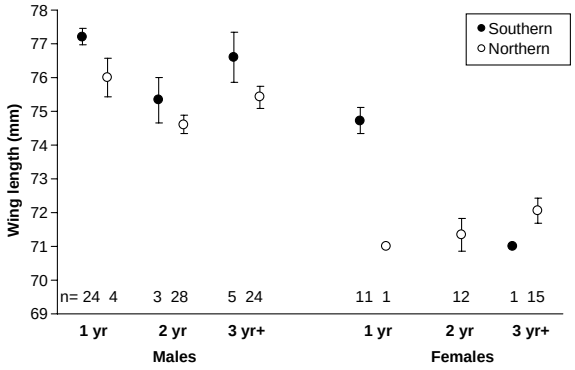

Fig. 2. Mean wing length (bars represent standard error) of bluethroat skins collected 1884–1948 (museum collections in Stockholm and Oslo), grouped according to sex, age and collection locality (sample sizes are given above the x-axis).

Unfortunately, for almost each age and sex group compared, the presumed subspecies samples were heavily unbalanced in number of skins making comparisons between average values imprecise (e.g. 3 southern birds and 28 northern birds among 2 yr males, and 1 vs 15 birds among 3 yr+ females, Fig. 2).

Birds in the field

There were no apparent differences or latitudinal trends in the five morphological traits across the sampling sites (Fig. 3). We used MANOVA to test whether the birds at sites 1 and 2 (from potential *gaetkei* breeding grounds) were morphologically different from the birds at sites 3–11. Overall, there was no statistical difference between the groups ($F_{5,89} = 1.87$, $p = 0.11$) while simultaneously controlling for age. Including also sites 3 and 4 as part of the presumed *gaetkei* breeding grounds (cf. Rendahl 1967) did not change the result ($F_{5,89} = 0.83$, $p = 0.55$).

Stable isotopes

When comparing $\delta^{13}C$ and $\delta^{15}N$ values of the throat feathers of "southern" (sites 1 and 2) and "northern" (sites 4, 5, 6 and 9) males, there was a tendency for a difference in the overall model (MANOVA, $F_{2,26} = 3.18$; $p = 0.058$,

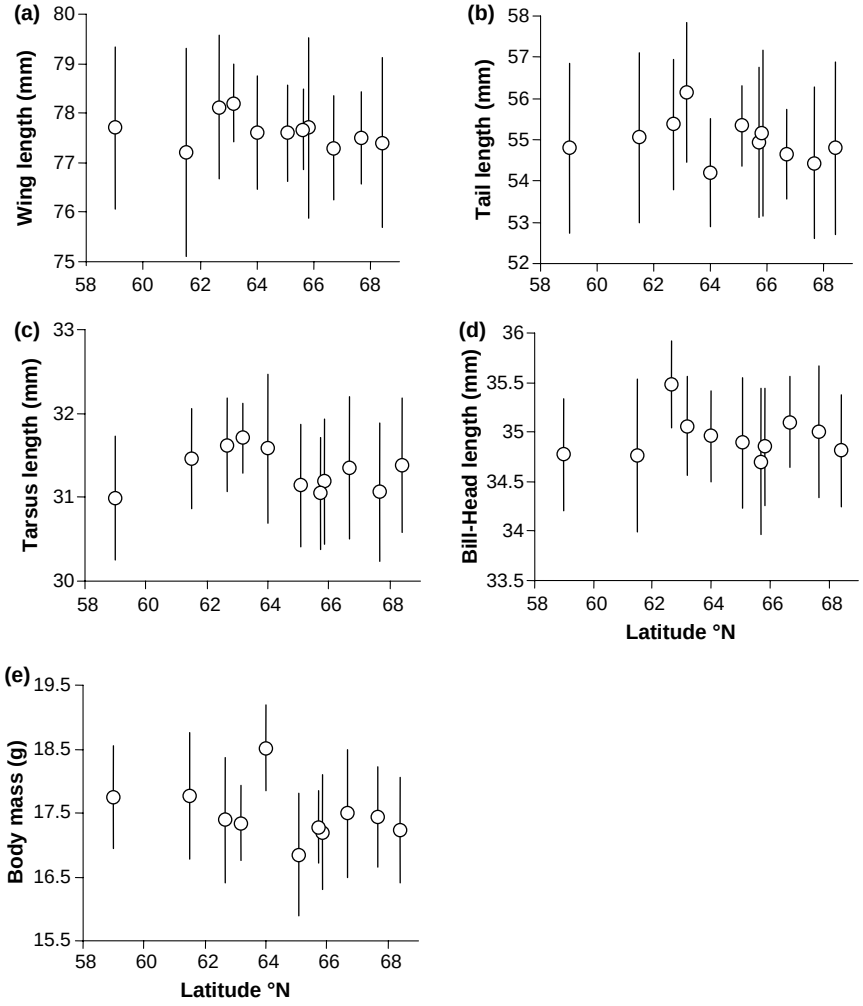

Fig. 3. Measurements (mean with standard error represented by bars) of bluethroat males from 11 sampling sites at different latitudes. (a) Wing length, (b) tail length, (c) tarsus length, (d) bill-head, (e) body mass. Individual wing and tail lengths were compensated for age, due to significant age differences.

Table 2. Mean values (±standard deviation) of $\delta^{13}C$ and $\delta^{15}N$ from throat feathers of bluethroats (*Luscinia svecica*). From each site feathers from 5 individuals was analysed. For site data, see Table 1.

Site no.	$\delta^{13}C$	$\delta^{15}N$
1	−20.96±3.73	6.71±2.42
2	−22.65±0.49	6.80±1.37
3	−22.41±1.57	7.72±2.11
4	−21.79±2.04	8.13±1.87
8	−22.92±1.41	7.23±1.60
11	−23.47±1.32	7.08±1.25

Table 2). For each element separately there were no differences between groups ($\delta^{13}C$: $F_{1,27}=1.18$, $p=0.29$; $\delta^{15}N$: $F_{1,27}=1.35$, $p=0.26$). When assigning also sites 3 and 4 to the "southern" birds (cf. the suggestion by Rendahl 1967), there was no difference between groups (MANOVA, $F_{2,26}=1.63$; $p=0.21$). Individual $\delta^{15}N$ and $\delta^{13}C$ values were significantly positively correlated to each other (Spearman rank correlation; $r_s=0.495$, $p=0.005$; Fig. 4).

Genetic analyses

The microsatellite locus Pocc5 showed 7 different alleles. All pairwise F_{st} values were low and the overall F_{st} was 0.0028 and not significantly different from zero. Of the 68 analysed AFLP fragments 59 were polymorphic. The vast majority of the pairwise F_{st} values were close to zero (mean $F_{st}=0.002$) and only one marker showed a moderately high F_{st} of 0.29 (Fig. 5). Although this moderately high F_{st} value is significant when analysed separately ($p<0.001$), this specific marker found in 3 out of 17 individual in the southern population and in non of the 59 individuals in the northern population and hence does not candidate to be an informative AFLP marker (cf. Bensch et al. 2002b). In the combined analyses using the program STRUCTURE we found consistently higher likelihood values for $K=1$ (Ln P (D) $=-1882$) than for $K\geq 2$ (Ln P (D) <-1917). Therefore, the most likely hypothesis includes all sites into one panmictic population.

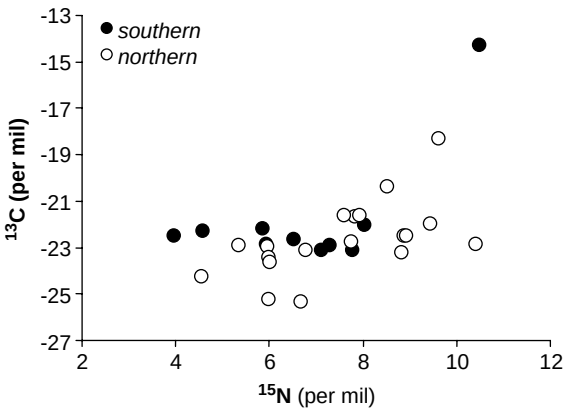

Fig. 4. The relationship between the isotope compositions of C and N in throat feathers of bluethroats *Luscinia svecica* caught in southern and northern Scandinavia. For location of the different site, see Fig. 1 and Table 1.

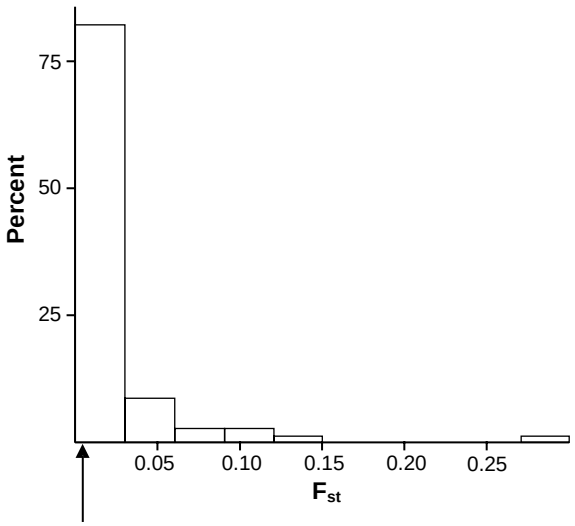

Fig. 5. Distribution of F_{st} values for individual AFLP fragments. The overall F_{st} value including all loci was 0.002. The arrow indicates the F_{st} value of the microsatellite locus Pocc5.

Discussion

Morphology

In the contemporary data set collected in 2001, none of the body size measurements showed any indication of differences related to latitude across Scandinavia (Fig. 2 and 3). Nor was there any evidence of the birds from southern Norway (sites 1–2) or southern Scandinavia (sites 1–4) differing from the more northern sampled populations. The lack of evidence for a morphological contact zone (Barton and Hewitt 1989, Bensch et al. 1999) favours the view that the Scandinavian bluethroats should be regarded as one homogenous population. However, when re-measuring the skins that Lundevall (1950) used to define *gaetkei*, we found a significant difference in wing length of ca 1 mm in adult males (2 yr and 3 yr+), which is similar to what Steinbacher (1935) and Salomonsen (1949) found. The difference, however, is much smaller than the 4–6 mm difference suggested to be diagnostic for *gaetkei* and *svecica* (Weigold 1926, Drost 1927) and found on skins by Lundevall (1950). It seems clear that Lundevall (1950) did not consider the age of the birds he measured. Most southern Norway birds happened to be juveniles in very fresh plumage, whereas most northern birds were adults in worn breeding plumage, when wing tips are abraded and therefore shorter (Svensson 1992, Fig. 2). This, however, still cannot fully explain the discrepancy between our and Lundevall's measurements. Whereas we found a considerable overlap between southern and northern birds, Lundevall (1950) found hardly any overlap at all, and accordingly, a much clearer difference in wing length between the study groups.

The lack of consistency between the analyses of birds from 2001 and the skins from 1884–1948, might of course be a result of sample stochasticity (type I error), not least due to the unbalanced sample sizes among the museum skins. However, we can not exclude that the old skins from southern Norway at least partly represent an

extinct, longer-winged, population (*gaetkei*?). Speaking against this is that the difference we found in wing length of ca 1 mm does not even come close to the difference of ca 4–6 mm suggested (on unclear grounds) by Weigold (1926) and Drost (1927).

In the older sources, *svecica* males were supposed to have wing lengths of 69–74 mm (Drost 1927) or 70–77 mm (Lundevall (1950). In Ammarnäs in Swedish Lapland (near site 7, well away from potential *gaetkei* territory), we have in 1983–2006 trapped ca 60 adult males (2 yr and 3 yr+) in worn breeding plumage, having wing lengths of 74–81 mm (Lindström unpubl.). No measurement technique was given in the older sources but we can assume that in former days they mainly measured shorter-winged (shrunk) skins and in addition, with a non-maximum technique. This could explain the ca 4–5 mm difference in absolute values for *svecica* males from then to now. However, the average wing length of *gaetkei* in the old days was suggested to be 5–6 mm longer than that of *svecica*, which with today's technique would correspond to 79–86 mm wings in males! At least the upper half of this range corresponds to wing lengths never encountered in wild-caught bluethroats. Therefore, the very long-winged individuals reported from Helgoland (Kleinschmidt 1904, Weigold 1926, Drost 1927) simply do not match any birds from southern Norway, neither presently (wild birds) nor a century ago (skins). Another explanation is needed for their presence than belonging to a *gaetkei* subspecies breeding in southern Norway.

Another possibility would be that we in our sampling of wild birds overlooked "*gaetkei*", but this seems unlikely since both of the locations visited in the southern Norway, as well as two southernmost Swedish locations, are inside the area suggested by Lundevall (1950) and Rendahl (1967) as the breeding range of *gaetkei*.

Overall we must conclude that two populations with dramatically different wing lengths do not occur in Scandinavia today and it is unlikely that they did earlier either.

Stable isotopes

The analysed throat feathers were most likely grown when the birds were still on the winter grounds. We found marginally or non-significant differences in $\delta^{13}C$ and $\delta^{15}N$ values between sample sites, the outcome depending on which sites to include as different populations. This gives no or only weak support for that birds from the different breeding sites used different wintering ground. However, whereas large differences would have been suggestive, we cannot exclude that wintering grounds as far apart as Africa and south central Asia would result in similar isotope ratios due to similarity in climate and habitat.

The significant positive correlation between $\delta^{13}C$ and $\delta^{15}N$ -values (Fig. 4) show that the two elements carry associated information about conditions at the wintering grounds (Møller et al. 2006), with the lower values reflecting more mesic, and the higher values more xeric habitats (Kelly 2000). To find out whether carbon and nitrogen isotope ratios can separate African and Asian wintering grounds for bluethroats, analyses of feathers collected from birds from known wintering grounds are needed.

Genetic structure and implications for migratory directions

The very low F_{st} values indicated that gene flow is high between all the visited locations. None of the sites differed from the others, neither when looking at the microsatellites nor at the AFLP fragments. This suggests that the Scandinavian population should be regarded as one single population.

Migratory directions in long-distance migrating passerines seem to be under tight genetic control (Helbig 1996). We have no data with direct bearing on migratory directions of the bluethroats we sampled, but with a high level of gene flow across Scandinavian populations, drastically different migratory routes (such as SW vs ESE) would probably be hard to maintain. Frequent hybridisation would presumably produce many offspring with intermediate migratory routes. Those hybrids would be likely to have substantial disadvantages as they are bound to follow a migratory route that may lead them to a suboptimal wintering ground (Helbig 1996). Accordingly, hybrids might fail to complete the migration and therefore not be able to reproduce themselves, although completely viable in all other respects. Such a scenario would result in restricted gene flow between the two subpopulations. Nevertheless, the willow warbler shows a sharp migratory divide in central Sweden, but despite this exhibits no difference in mtDNA and microsatellites (Bensch et al. 1999) a pattern also found in the north American species, black-throated blue warbler *Dendroica caerulescens* (Davis et al. 2006). This pattern has been suggested to result from shared ancestral genetic polymorphism in neutral DNA followed by recent divergence in genes encoding for migratory direction (Bensch et al. 2002b). Hence, lack of neutral genetic differences between south and north Scandinavian bluethroats is not sufficient for excluding that the two populations can have evolved different migratory directions.

Conclusion

The results from the three different techniques used in this study, suggest that the contemporary Scandinavian population of bluethroats should be regarded as one homogenous population. Hence, the results did not support the existence of a secondary contact zone nor did it support the existence of a separate subspecies of bluethroat in southern Norway ("*gaetkei*").

As far as the presence of a SW migrating population of bluethroats is concerned, we add no new information on migratory directions as such. Ringing recoveries have shown that at least in the last 50 yr the vast majority of bluethroats, also from southern Norway, are migrating in an easterly direction in autumn (Ellegren and Staav 1990, Bakken et al. 2006). We argue that the low F_{st} values, indicating high gene flow throughout the breeding range of red-spotted bluethroats, make it unlikely that a population of birds could maintain a genetically determined direction of S/SW.

Even if there is no population differentiation found today, what about the possibility that a ''*gaetkei*'' type of bluethroat once existed in southern Norway? The only data from Norway hinting in that direction are the slightly longer-winged skins measured by Lundevall (1950) and ourselves, and a few ringing recoveries in southwesterly direction (Anderssen and Gylseth 1992, Bakken et al. 2006). These possibilities are discussed above and none seem to weigh heavily in favour of ''*gaetkei*''.

What about the situation at Helgoland then, where *gaetkei* was first described (Kleinschmidt 1904)? Dierschke (2005) reviewed the occurrence of red-spotted bluethroats at Helgoland over the last 150 yr. Substantially more bluethroats were recorded on migration there in Gätke's time (second half of 1800s) and in the first half of the 1900s, than in the last 50 yr. The decline in numbers is obvious in both spring and autumn. Dierschke (2005) speculated that the autumn decline could possibly be explained by the disappearance of S/SW migrants from Scandinavia. Helgoland would have been a likely place for such birds to appear. The spring decline, however, does not fit well in such a scenario, because high numbers in spring were already in Gätke's days tightly connected to the occurrence of light south-easterly winds (Gätke 1895, Dierschke 2005). This is pointing more towards arrival from the southeast, as expected for the *svecica* subspecies.

Luscinia svecica gaetkei first ''appeared'' at Helgoland under very special circumstances (Kleinschmidt 1904). As far as we are concerned, its breeding grounds are not in southern Norway, and if they exist are yet to be found. We conclude that, today, there is no evidence of a secondary contact zone in the Scandinavian bluethroat population and that only one subspecies of red-spotted bluethroats breed in Scandinavia, *Luscinia svecica svecica.*

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