also reports of dead or injured birds as results of fights with other Bitterns (Percy 1932). L. Broberg has studied Bitterns during 50 years at Söderfjärden and at this site aggressive behaviour has been very rare. According to Broberg second year males are tolerated within the territory of a breeding male.

My first observation was most likely a fight between two males since both birds were of the same size and the attacks very aggressive. The second observation may have involved a male and a female since the birds were much different in size. But if so, this observation is not in line with Percy's statement that only territorial males fight each other. Since there is considerable size variation among males, it is hence likely that the second chase also involved two males.

The fact that I saw two fights in 2000 but none in any previous year is probably explained by the higher number of individuals at the locality in this year. The locality is probably not very good for the Bittern since only one male has been recorded in any year before, and hence the competition for a good territory may have been particularly strong. The two observations of Bitterns passing over also indicate that the number of birds searching a territory in this year may have been unusually large.

Svante Söderholm, Narvavägen 4, 115 23 Stockholm
e-mail: svante.soderholm@hem-pc.bip.net

https://doi.org/10.34080/os.v11.22869

A Chiffchaff *Phylloscopus c. collybita* with mixed Chiffchaff and Willow Warbler *Ph. trochilus* song – genetic evidence

STAFFAN BENSCH, LARS G. R. NILSSON, PER NOTHAGEN, PETER OLSSON & MIKAEL ÅKESSON

In many species, some individuals may sing the song of other, often closely related, species in addition to the species typical song. This phenomenon is known as mixed singing (Helb et al. 1985) and has been observed in primarily oscine passerines, the group of birds in which learning constitutes an important part of song development (Kroodsma 1982). When the two songs that are mixed come from morphologically similar species, such as the treecreepers (*Certhia familiaris* and *C. brachydactyla*) or *Phylloscopus* warblers (e.g. *P. collybita* and *P. trochilus*), it is often not clear whether the mixed singer is a pure genotype of one of the taxa that also expresses the song of the other species, or whether the mixed singer is a genetic hybrid (Helb et al. 1985). Here we report a *Phylloscopus* warbler singing the typical song of both Chiffchaffs and Willow Warblers, and demonstrate that the specimen was a pure Chiffchaff based on both mitochondrial DNA (mtDNA) and nuclear gene sequence data. This is the first time the species status of a mixed singer has been genetically determined.

The specimen was observed singing on 8 and 9 July 2000 at Lunds sewage farm at Värpinge (13´10° E, 55´40° N). During a one hour visit in the evening of 8 July, it was singing intensively from a dense shrubbery (privet *Ligustrum* sp., hawthorn *Crataegus* sp.) with a few larger trees (elm *Ulmus glabra*, chestnut *Aesculus hippocastanum*). Most song bouts consisted of varying number of chiffchaff-type units followed by a willow warbler song followed by another series of chiffchaff-type units. Sometimes, it sang only willow warbler songs and when doing this, the song strophes appeared to be interrupted prematurely and were then immediately followed by new song strophes without notable pauses. For certain periods it kept to Chiffchaff songs, and sometimes gave long series of the "tett tett" syllables with which Chiffchaffs normally initiate their song bouts. During the evening of 9 July, the bird sang a higher proportion Chiffchaff song than the pervious evening, although it had periods when it frequently mixed between the song of the two species. The song was recorded using a parabolic microphone and a minidisc recorder. A selected sonogram of a song which started with Chiffchaff types "tett-tett chiff-chaff", followed by a willow warbler song and then eight more "chiff-chaff" syllables, is given in Figure 1. Four recordings (13–84 seconds long) from a total recording of nine minutes can be found at http://user.tninet.se/~mwe231t/phcol/.

After recording the song of the bird, it was lured into a mist-net using song play-back of its own song. The bird appeared to be a typical Chiffchaff in heavily worn plumage, with the following measurements (following Svensson 1992): wing length 61 mm, tip of 2nd P equal to 5th P, 4th P longest with 3rd and 5th just slightly shorter, outer web of 3rd, 4th, 5th and 6th P emarginated, 1st P 5 mm longer than longest PC. The short and rounded wing, and emarginated 6th primary are characters that are typical of Chiffchaffs and fall outside the observed variation in willow warblers (Svensson 1992). The breast and belly had a clear yellowish tinge indicating subspecies

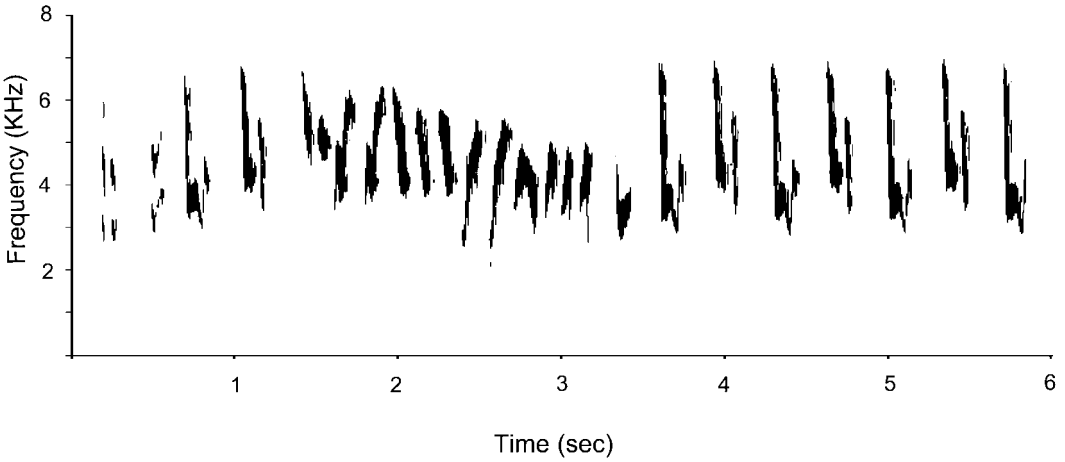

Figure 1. Sonogram of six seconds of continuous song of the mixed singing Chiffchaff. The image was produced with a resolution of 195 Hz and 5.1 ms, filtering off sounds below 2.2 KHz.
Sonogram visande sex sekunders sång av den blandsjungande gransångaren.

collybita (Svensson 1992). The bird was apparently one year old (2K) based on fresh central tail feathers contrasting with worn outer ones (Svensson 1992). The outermost tail feathers were collected for DNA analysis.

To extract DNA, about 5 mm of the base of one tail feather was placed in 100 µl Lysis buffer and 3 µl proteinase K at 55°C for three hours. The supernatant was then precipitated in ethanol (Laird et al. 1991). The final pellet was dissolved in 50 µl ddH_2O. We amplified a portion of the mitochondrial cytochrome b gene using universal primers (L14841 and H15149 Kocher et al. 1989) and an intron of the CHD-gene on the Z-chromosome with primers P2 and P8 (Griffiths et al. 1998). We employed direct sequencing (big dye sequencing kit) loaded on an ABI310 capillary sequencer.

The obtained fragment of the mitochondrial cytochrome b gene (306 bp excluding the primers) was tested against available DNA sequences in the GenBank data base using the BLAST search routine. The fragment matched perfectly with that of *Phylloscopus c. collybita* and showed four substitutions compared to *P. c. abietinus* (Helbig et al. 1996). Compared to willow warbler sequences, there were 19 substitutions. Hence, we conclude that the mother of the mixed singer was of the nominate subspecies of the Chiffchaff, the subspecies of Chiffchaff which now breeds frequently in southern Sweden (Hansson et al. 2000).

The intron of the CHD-gene on the Z-chromosome (219 bp sequenced) had not previously been studied in any *Phylloscopus* warbler. We therefore examined two male willow warblers (GenBank AF355150) and two male Chiffchaffs (GenBank AF355151). The mixed singer differed from the willow warbler by showing a G instead of a T at position number 53, and was at all positions identical to the analysed Chiffchaffs. Male birds have two Z-chromosomes, one inherited from its mother and one from its father. If the mixed singer was a hybrid, it would have received one Z-chromosome from a willow warbler father and one Z-chromosome from its Chiffchaff mother (the mtDNA analysis demonstrated that the mother was a Chiffchaff). This would have resulted in a double peak in the electropherogram at position 53. There was no sign of even a slight T -peak in the mixed singer, strongly suggesting that it only carried Chiffchaff Z-chromosomes. We therefore reject the hypothesis that this mixed singer was a F1 hybrid although we cannot exclude that it was a backcrossed individual (the result of a mating between a hybrid and a Chiffchaff).

Birds mixing the song of willow warblers and Chiffchaffs are widely reported in the literature (Gwinner & Dorka 1965; Schubert 1969; Haensel & Lippert 1976; Helb et al. 1985; Frost 1986; Wilson 1986) and where identity has been established, mixed singers seem almost always to be willow warblers (Cramp 1992). The most convincing case of a mixed singer being a hybrid is a bird from Scotland ringed in a nest fed by a male Chiffchaff and a female

willow warbler (Prato & Prato 1986). Given that the songs of the Chiffchaff and the willow warbler are clearly different and that the species appear to have been separated for several million of years as based on their mtDNA divergence (Richman & Price 1992), it is remarkable that some individuals have the capacity to express the song of the other species to such a perfection. Mixed singing, as a result of misdirected learning, is likely to promote hybridisation (Baker & Boylan 1999). However it remains to be established to what extent hybridisation between Chiffchaffs and willow warblers results in gene flow, as this requires that hybrids themselves reproduce successfully with pure genotypes of either of the parental species.

Acknowledgements

We thank Anders Hedenström and Darren Irwin for constructive comments on an earlier draft of the manuscript. Financial support for the genetic work was from the Swedish Natural Science Research Council to SB.

References

Baker, M. C. & Boylan, J. T. 1999. Singing behaviour, mating associations and reproductive success in a population of hybridizing lazuli and indigo buntings. Condor 101: 493–504.

Cramp, S. 1992. (ed.) Handbook of the Birds of Europe, the Middle East and North Africa. Oxford University Press, Oxford.

Frost, R. A. 1986. Phylloscopus warbler with songs of chiffchaff and willow warbler. British Birds 79: 340–341.

Griffiths, R., Double, M. C., Orr, K. & Dawson, R. J. G. 1998. A DNA test to sex most birds. Molecular Ecology 7: 1071–1075.

Gwinner, E. & Dorka, V. 1965. Beobachtungen an Zilpzalp-Fitis-Mischsängern. Vogelwelt 86: 146–151.

Haensel, J. & Lippert, W. 1976. Weidenlaubsänger, *Phylloscopus collybita* (Vieill.), mit Gesangsanteilen des Fitis, *Phylloscopus trochilus* (L.). Beitr. Vogelkd. 22: 26–37.

Hansson, M., Bensch, S. & Brännström, O. 2000. Range expansion and the possibility of an emerging contact zone between two subspecies of *Chiffchaff Phylloscopus collybita* ssp. *Journal of Avian Biology* 31: 548–558.

Helb, H.-W., Dowsett-Lemaire, F., Bergmann, H.-H. & Conrads, K. 1985. Mixid singing in European songbirds – a review. *Zeischrift für Tierpsychologie* 69: 27–41.

Helbig, A. J., Martens, J., Seibold, I., Henning, F., Schottler, B. & Wink, M. 1996. Phylogeny and species limits in the Palaearctic chiffchaff *Phylloscopus collybita* complex: mitochondrial genetic differentiation and bioacoustic evidence. *Ibis* 138: 650–666.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., Wilson, A. C. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. P*roc. Nat. Acad. Sci. USA* 86: 6196–6200.

Kroodsma, D. E. 1982. Learning and the ontogeny of sound signals in birds. In: *Acoustic Communication in Birds, Volume 2, Song learning and its consequences* (eds. Kroodsma DE, Miller EH) p. 1–23. Academic Press, New York.

Laird, P. W., Zijderveld, A., Linders, K., Rudnicki, M. A., Jaenisch, R. & Berns, A. 1991. Simplified mammalian DNA isolation procedure. *Nucl. Acids Res.* 19: 4293–4293.

Richman, A. D. & Price, T. 1992. Evolution of ecological differences in the old world leaf warblers. Nature 355: 817–821.

Da Prato, S. R. D. & Da Prato, E. S. 1986. Appearance and song of possible chiffchaff x willow warbler hybrid. British Birds 79: 341–342.

Schubert, M. 1969. Untersuchung über die akustischen parameter von Zilpzalp-Fitis-Mischgesängen. Beitr. Vogelkd. 14: 354–368.

Wilson, C. 1986. Chiffchaff with songs of chiffchaff and willow warbler. British Birds 79: 342.

Sammanfattning

En gransångare med blandad gran- och lövsångarsång

Hos många fågelarter kan man stundom påträffa enstaka individer som växlar mellan den egna artens sång och en närbesläktad arts sång. Hos artpar som är lika till utseende, t ex trädkrypare/trädgårdsträdkrypare och gransångare/lövsångare är det ofta omöjligt att avgöra om en ”blandsångare” är rena exemplar av den ena eller andra arten som lärt sig också den andra artens sång, eller om det rör sig om en hybrid mellan arterna. Vi rapporterar om en gransångare som sjöng både som gran- och lövsångare. Resultaten från genetiska analyser av mitokondrie- och kärn-DNA utförda på en stjärtpenna visar tillsammans med biometriska mått, att det rörde sig om en ren gransångare.

Fågeln observerades vid Lunds reningsverk 8–9 juli 2000. Sången inleddes oftast med en serie typisk gransångar ”silt-salt” varefter den inflikade mer eller mindre perfekta lövsångarstrofer, ofta omedelbart åtföljda av en nye serie ”silt-salt”. I Figur 1 visas ett sonogram på en utvald sångsekvens. Efter inspelning infångades fågeln med hjälp av slöjnät och play-back. Allmänt utseende och biometri pekade entydigt på gransångare av rasen *collybita*, bl a kort vinge (61 mm) samt ytterfansinskärning på sjätte handpennan (räknat utifrån). I samband med fångsten insamlades de yttre stjärtpennorna för DNA analyser.

Från basen av en stjärtfjäder extraherades DNA.

Ett avsnitt av mitokondriens cytochrom-b- gen amplifierades med hjälp av PCR. Det framtagna DNA:t sekvenserades och jämfördes med tillgängliga DNA-sekvenser i den internationella gendatabanken "GenBank". Sekvensen överensstämde perfekt med en befintlig sekvens från *P. c. collybita,* medan en sekvens från *P. c. abietinus* skiljde sig på fyra positioner. Eftersom mitokondrien nedärvs via mödernet kan vi alltså sluta oss till att modern till blandsångaren var en gransångare av den sydliga rasen collybita, vilken numer häckar allmänt i regionen.

Vi ville också försöka fastställa arttillhörigheten hos blandsångarens far. DNA sekvensen på en del av en gen som finns på Z-kromosomen (CHD-Z) undersöktes därför. Fåglars könskromosomer benämns Z och W. Hanar bär två uppsättningar av Z-kromosomen och betecknas därför ZZ, medan honor bär en Z och en W-kromosom och därför betecknas ZW (jämför XY-systemet hos däggdjur). Det undersökta avsnittet på CHD-Z genen har tidigare inte studerats hos Phylloscopus-sångare och därför undersöktes också två gransångare och två lövsångare. En unik skillnad på position 53 hittades mellan arterna och vår blandsångare överensstämde helt med gransångarsekvensen. Om blandsångaren hade varit en hybrid, skulle den ha fått en CHD-Z kopia från en lövsångarfar och en CHD-Z kopia från sin gransångarmor (mitokondrie-DNA analysen visade att modern var gransångare). Detta skulle ha uppenbarat sig som en sammansatt signal på den position i sekvensen för vilken gran- och lövsångare skiljer sig åt. Men blandsångaren visade en entydig gransångarsignal för nämnda position. Vi kan därför förkasta hypotesen om att blandsångaren var en hybrid av första generationen.

Staffan Bensch, Dept. of Animal Ecology, Ecology Building, S-223 62 Lund, Sweden
Lars G R Nilsson, Kämpagränden 21 B, 224 76 Lund, Sweden
Per Nothagen, Toftängsgatan 3, 212 38 Malmö, Sweden
Peter Olsson, Pure & Applied Biochemistry, Center for Chemistry and Chemical Engineering, Lund University, POB 124, 221 00 Lund, Sweden
Mikael Åkesson, Kämnärsvägen 2:169, 226 42 Lund, Sweden