Assortative mating also indicates that common crossbill *Loxia curvirostra* vocal types are species

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Compared to most other birds, the taxonomy of crossbills (*Loxia*) is still highly unsettled. However, much progress seems to be achievable when data on vocalisations is included. In a recent paper, Summers et al. (2007) argued that strong assortative mating indicated that parrot crossbill *Loxia pytyopsittacus*, Scottish crossbill *Loxia scotica* and common crossbill *Loxia curvirostra* behave as good species when breeding in sympatry. Here I argue that their data, when placed in the context of other studies, also indicate that three vocally differentiated European populations within the common crossbill are species (following the biological species concept of Mayr (1963): species are groups of interbreeding natural populations that are reproductively isolated from other such groups). If this tentative conclusion remains to be upheld, it might have large repercussions for our understanding of the speciation process as well as for a number of more applied issues such as the discovery and description of biodiversity and the conversation of mobile, cryptic species.

The common (red) crossbill *Loxia curvirostra* has long challenged taxonomic stability and customs, and seems to continue to do so. In North America various treatments have tried to mould morphological variability into geographically defined subspecific units. However, a frequent finding was that birds collected at the same locality often varied much more in morphology than did birds collected at opposite ends of the continent, or that birds collected one year differed dramatically from birds collected another year at the same locality (reviewed in Groth 1993a). The inclusion of vocal characters has proven to be a major step forward in unravelling these unusual patterns, as is often the case for morphologically similar birds with overlapping distributions (Remsen 2005). Work by Groth and Benkman has shown that at least 9 vocal types are involved. These types differ in vocalisations, morphology, ecology and genetic markers (Groth 1988, 1993a, Benkman 1993a, 1999, 2003, Parchman et al. 2006, Smith and Benkman 2007). Generalising and extrapolating across these and other, more anecdotal studies (refs. in Adkisson 1996, Groth 1993a, Knox 1990, 1992), most of these vocal types roam over vast areas (sometimes spanning the width of the continent) in search of crops of conifer cones that fit their bill morphologies. Doing so, they regularly occur in sympatry (refs. in Knox 1990, 1992, Groth 1993a, Adkisson 1996). For at least some of these types it has been established that interbreeding during sympatry is rare (reviewed in: Knox 1990, 1992, Groth 1993a, b, Smith and Benkman 2007), and that females prefer males of the same

call type even when they are identical in plumage and bill size (Snowberg and Benkman 2007). Hence, even though many details are absent and much data still needs to be collected, at this moment the most parsimonious interpretation of all findings is that these vocal types represent evolutionarily separated lineages. Note that vocal types are not vocal dialects as observed in many other songbirds, since birds of different dialects have allopatric breeding distributions, do not differ in morphology, ecology and genetic markers, and do not show assortative mating.

The strongest example of evolutionary separation is provided by vocal type '9', the crossbill endemic to the South Hills and Albion Mountains of southern Idaho. Smith and Benkman (2007) recently showed that the total degree of reproductive isolation between this type and the most commonly co-occurring and genetically closest type 2 and type 5 (Parchman et al. 2006) amounts to 0.9975 and 0.9998, respectively, on a scale from 0.0 = no isolation to 1.0 = complete isolation. With such very high levels of reproductive isolation, it seems fully justified to formally describe this highly localised vocal type as a separate species. As far as I know, it would then constitute the first bird species in the world that is not diagnosable by plumage, morphology, genetics or location of collection (due to overlap with other types); i.e. museum specimens without deposited recordings of the same live bird are not identifiable (!).

But before hastily deciding that vocal differentiation between common crossbill populations is always enough to

assign them specific status, we should realise that even the specific status of vocally differentiated parrot *L. pytyopsittacus*, Scottish *L. scotica* and common crossbills (Robb 2000) in northern Europe is less established than some may think. Some authors do not recognise the distinctively larger parrot crossbill as a separate species (e.g. Eck 1981). Indeed, despite large geographical overlap in breeding distribution, there are hardly any quantitative studies to evaluate any lack of hybridisation (but see Summers et al. 2007). Moreover, Piertney et al. (2001) could not find any genetic differentiation between these three taxa in presumably neutral nuclear microsatellites and mitochondrial DNA sequences, which is highly unusual for birds holding species rank (but can happen if differentiation occurred very recent). Summers et al. (2007) took a step back from the current taxonomy of this trio, and tested alternative explanations ranging from different species to genetic polymorphism to phenotypic plasticity. In support of the current taxonomy, their data indicated that bill size was heritable, and that birds with different bill sizes mated strongly assortative. Birds giving distinct calls were previously shown to have distinct bill morphologies (Summers et al. 2002), and additional birds that had not been caught and measured mated strongly assortative by calls and thus by inference also by morphology, providing further support for species rank for each of the three groups discussed.

However, their results also support that differentiation in vocalisations is correlated to reproductive isolation. And as shown for some North American vocal types (Groth 1993b, Smith and Benkman 2007, Snowberg and Benkman 2007). Table 2 of Summers et al. (2007) also shows strong assortative mating between common crossbills belonging to different recently discovered European vocal types (Robb 2000, Summers et al. 2002, Constantine and the sound approach 2006). Based on the diagnostic excitement calls Summers et al. (2007) noted five mixed pairs out of 99 pairs involving parrot, Scottish or common crossbills (5.1% hybridisation), but did not detect a single mixed pair among 38 pairs of common crossbill vocal types (0.0% hybridisation; see also Robb 2000 and Edelaar et al. 2003, 2004 for additional reports on lack of mixed pairing). These percentages may change somewhat if larger datasets such as that of Smith and Benkman (2007) are collected, but while I agree with the interpretation of Summers et al. (2007) that the three taxa they discussed are distinct species, I see no obvious reason why their interpretation should not be extended to apply to the common crossbill vocal types as well. In fact, one might argue that the parrot crossbill is 'just' another vocal type belonging to the complex of European common crossbill vocal types: it does not differ in DNA (Piertney et al. 2001), nor in plumage (Cramp and Perrins 1994), but does show differentiation in vocalisations (Robb 2000, Summers et al. 2007), has geographic overlap with other vocal types (Robb 2000), and it shows strong assortative mating with respect to calls in sympatry (Summers et al. 2007). It current status as a separate species is then only because it happens to be so differentiated in bill size that the absence of morphological intermediates allowed for species recognition prior to the modern inclusion of vocal characters.

The main argument against equating strong assortative mating by calls with strong reproductive isolation among vocal types is that many Cardueline finches can match their calls between mates by imitative learning (Mundinger 1970, 1979). At the extreme, Mundinger (1970) reported how a male pine siskin *Carduelis pinus* had adopted the flight call of his heterospecific European siskin *C. spinus* female partner. Matching of detailed call structure between mates of the same vocal type has also been documented in crossbills (Groth 1993b, Smith and Benkman 2007). So it could be possible that two birds initially belonging to different vocal types subsequently match their calls when forming a mixed pair and thus falsely appear to us as a pure pair. In the absence of large morphological differences between the vocal types that could be used as a parallel check for mixed pairing (as done by Summers et al. 2007), we would never be able to find mixed pairs (except perhaps during the initial formation of pair bonds prior to or during the process of call matching). However, there are a number of cases that argue against the possibility that call matching explains the observed strong assortative mating among vocal types. Summers et al. (2007) describe a case of a successfully breeding mixed pair of birds each giving either Scottish or parrot crossbill calls for two consecutive breeding seasons. Relative to the calls of the different vocal types of common crossbills, calls of parrot and Scottish crossbills are very similar (see Fig. 1 in Summers et al. 2007), yet call matching between mates apparently did not occur. Similarly, Smith and Benkman (2007) found very strong assortative mating between sympatrically breeding vocal types in North America, but also found six mixed pairs (out of 428 pairs, 0.9%), again suggesting that call matching does not always and perhaps never results in apparent pure pairs that are actually mixed pairs matching their calls. The same is true for additional mixed pairs documented by Summers et al. (2007). Additionally, Groth (1993b) reported that in captivity two experimentally forced mixed pairs of North American vocal types that successfully raised young had not changed their initial calls in order to match the call of their mate. Finally, if call matching between mates of different vocal types was common, then the dispersive nature of northern crossbills (Payne 1987, Cramp and Perrins 1994, Newton 2006, Marquiss et al. 2008), coupled with regular breeding in sympatry (refs. in Knox 1990, Groth 1993a, b, Adkisson 1996, Questiau et al. 1999, Robb 2000, Edelaar et al. 2003, Smith and Benkman 2007, Summers et al. 2007) would quickly erase any vocal differentiation between populations and lead to a more homogenous population giving a single kind of call. This would be true whether calls are learned or genetically inherited from parents. This same argument of observed distinctiveness under expected homogenisation is often used to decide over the taxonomy of birds, and was used to formally recognise the Scottish crossbill as a distinct species (Knox 1990). Hence, to the extent that call matching can be reliably excluded as an alternative explanation for the observed strong assortative mating by calls, the data of Summers et al. (2007) indicate that the three European common crossbill vocal types included in their study also are different, reproductively isolated biological species.

What could be done to strengthen this still somewhat weak but exciting conclusion? Below I give a number of more feasible or promising suggestions, with a focus on calls, assortative mating, and call matching.

First of all, more field data on assortative mating between these and other vocal types are needed. For some vocal types there is no data at all on assortative mating, so the conclusion that vocal differentiation always translates to strong assortative mating for all vocal types cannot yet be drawn. For example, it is possible that mixed mating is commoner between some vocal types than between others, thus creating the opportunity for gene flow across the complex that might explain the surprising lack of genetic differentiation encountered so far. An example of testing for (and finding) assortative mating in captivity is given in Snowberg and Benkman (2007).

Second, we should test for the presence of call matching in the wild. This is best done by catching, recording, vocally classifying and marking individuals prior to breeding, and then later look for and record breeding pairs as indicated by behaviour or nests/chicks to see if any calls have changed (see e.g. Smith and Benkman 2007). The simplest scheme (not requiring reading a full individual marking which can be difficult, time-consuming, or prone to error) would be where each vocal type receives its own mark (say, a ring on the left or right leg in the case of only two types, or a ring of a particular colour if more types are present). If all pairs are found to consist of birds bearing the same mark (i.e. to belong to the same vocal type), strong assortative mating by calls has been confirmed, even if no recordings are obtained. In addition, if a pair bearing different marks is encountered, we can record their calls again to test if one of the mates has matched its call to the call of its partner. Since Summers et al. (2007) found that in one case birds from a mixed pair had similar excitement calls but different flight calls, it is worth to analyse both kinds of vocalisations.

However, if it is confirmed that mixed pairs are relatively rare in the wild, we need more examples of forced mixed pairs in captivity along the lines of Groth (1993b) to experimentally determine the percentage of mixed pairs that do match their calls (if any). If several vocal types are combined, we cannot only test whether these percentages perhaps differ between certain combinations of vocal types, but also if the likelihood of matching is related to similarity of calls, ecology, geographical distribution or genetics. This would be a large research effort, but since such experimental mixed matings so far have been done with only two pairs belonging to North American vocal types, even small samples (especially of European vocal types) would yield immediate means to interpret the data on assortative mating obtained in the wild.

Next, we need to figure out when and from whom calls are learned before we can be sure that the calls are always learned from their parents and thus could be viewed as reliably (vertically) inherited. For example, if calls of independent young can still switch prior to breeding, then even complete assortative mating by calls does not mean that vocal types are genetically isolated and evolutionarily independent. In the field this could be studied by recording parents and their (marked) offspring later in life (easier in resident populations), in order to calculate 'heritabilities' for calls (at different ages if possible). In the laboratory specific experiments could be designed in order to follow the ontogeny of calls. For example, cross-fostering of fledglings has shown that flight calls are not genetically inherited but learned from foster parents (Groth 1993b). But what if chicks are placed within flocks of other vocal types later in life, do they keep their own calls or do they switch? Does this depend on the age of the chicks, and on the relative abundance of own versus other vocal types?

If vocal types do not interbreed and consist of evolutionary distinct lineages, we would expect additional differences to evolve between them, as has already been established in great detail for North American types (summarised above). Small but consistent differences in morphology have now also been documented for two sympatric European vocal types (Edelaar et al. 2008). In the absence of much interbreeding between vocal types, we would also expect non-selected genetic differences to build up over time, and thus continued efforts to test for differentiation of DNA remain relevant, perhaps using more sensitive markers such as AFLP's which showed genetic differentiation among North American vocal types (Parchman et al. 2006). Even better, the construction of molecular phylogenies would allow us to infer the historical relationships between the taxa and how traits such as biometry, calls and reproductive isolation might have evolved. Additional suggestions for studies on morphology and ecology can be found in Edelaar et al. (2003).

Finally, but in fact primary to all of the above, why would we care if crossbill vocal types are evolutionarily independent, good biological species, and whether they receive taxonomic status? For someone mainly interested in pattern and process of evolutionary relationships, the taxonomic status of populations are rather unimportant since evolutionary distinct populations maintain their relevance whether they are named species or subspecies, or even when unnamed (as long as they are recognised as distinct). Above I tried to highlight that the evidence at hand suggests that crossbill vocal types are such distinct populations, and if this possibility is tentatively accepted then it yields a number of interesting questions. Which evolutionary drivers could have contributed to their divergence (neutral forces, divergent natural/sexual/social selection?), and in which ecological and historical settings could these have acted? How do we explain their apparent sympatric occurrence despite great similarity: might there be more morphological and ecological difference then at first sight? Since crossbills are so dispersive, could this sympatry be a recent phenomenon, or did the vocal types evolve despite initial gene flow? Why did we not discover this cryptic diversity earlier, and what does that imply for our current understanding and methods of detection of (avian) biodiversity? If assortative mating and reproductive isolation among crossbill vocal types turns out to be only incomplete, should we perhaps recognise the vocal types as *sympatric* subspecies? However interesting and exciting these questions may be, their relevance depends on how we view the status of the crossbill vocal types.

In addition, if crossbill vocal types are recognised as evolutionarily independent populations, this might have positive implications for their conservation. For example, vocal type 9 from North America is strongly reproductively isolated from the genetically, ecologically and morphologically closest vocal types 2 and 5 (Smith and Benkman 2007), and is endemic to two small mountain tops in southern Idaho. Global warming leading to habitat change, forest

fires, and habitat mismanagement could compromise its continued survival. The species is now known to have evolved due to the absence of red squirrels *Tamiasciurus hudsonicus,* a food competitor (Benkman 1999, 2003). Introductions of squirrels are already responsible for the extinction of two similarly ecologically distinct crossbill populations/species (Benkman 1993b, 1999), and deliberate or accidental introduction of squirrels to the habitat of type 9 could mark its doom. Since much protective legislation depends on having species status or at least a scientific name, description as a separate species could activate such legislation. Similarly, some of the European vocal types have been recorded orders of magnitude less often than others: e.g. type D and F of Robb (2000) are exceedingly scarce and have not been recorded outside of The Netherlands and Belgium, while type E is relatively abundant in Great Britain yet scarce on the European continent. In the virtual absence of data from eastern Europe and beyond it is possible that on a Eurasian scale these types are not rare, but until further research shows this indeed to be the case it seems safer not to dismiss the possibility that these scarce vocal types, as type 9 from North America, might be specialised on a resource that is sparse or localised, and thus that they may be at a conservation risk. With every new indication supporting that the crossbill vocal types are separate species, these research questions and conservation issues become more pressing.

References

Adkisson, C. S. 1996. Red crossbill *Loxia curvirostra.* – In: Poole, A. and Gill. F. (eds). The Birds of North America, no. 256.

Benkman, C. W. 1993a. Adaptation to single resources and the evolution of crossbill (*Loxia*) diversity. – Ecol. Monogr. 63: 305–325.

Benkman, C. W. 1993b. The evolution, ecology, and decline of the red crossbill of Newfoundland. – Am. Birds 47: 225–229.

Benkman, C. W. 1999. The selection mosaic and diversifying coevolution between crossbills and lodgepole pine. – Am. Nat. 153: S75–S91.

Benkman, C. W. 2003. Divergent selection drives the adaptive radiation of crossbills. – Evolution 57: 1176–1181.

Constantine, M. and The Sound Approach 2006. The sound approach to birding – a guide to understanding bird sound. – Publ. by author.

Cramp, S. and Perrins, S. C. 1994. The birds of the western Palearctic. – Oxford University Press, Oxford.

Eck, S. 1981. Reflexionen über die Taxonomie westpalaearktischer *Loxia*-arten (Aves, Passeriformes, Fringillidae). – Zool. Abh. Staatl. Mus. Tierk. Dresden 37: 183–207.

Edelaar, P., Summers, R. and Iovchenko, N. 2003. The ecology and evolution of crossbills *Loxia* spp.: the need for a fresh look and an international research program. – Avian Sci. 3: 85–93.

Edelaar, P., Robb, M., Van Eerde, K., Terpstra, K., Bijlsma, R. and Maassen, E. 2004. Zijn er meerdere soorten 'gewone' kruisbek in Nederland? – Limosa 77: 31–38.

Edelaar, P. van Eerde, K. and Terpstra, K. 2008. Is the nominate subspecies of the common crossbill (*Loxia c. curvirostra*) polytypic? II. Differentiation among vocal types in functional traits. – J. Avian Biol. 39: 108–115.

Groth, J. G. 1988. Resolution of cryptic species in Appalachian red crossbills. – Condor 90: 745–760.

Groth, J. G. 1993a. Evolutionary differentiation in morphology, vocalizations, and allozymes among nomadic sibling species in the North American red crossbill (*Loxia curvirostra*) complex. – University of California, Berkeley and Los Angeles, California.

Groth, J. G. 1993b. Call matching and positive assortative mating in red crossbills. – Auk 110: 398–401.

Knox, A. G. 1990. The sympatric breeding of common and Scottish crossbills *Loxia curvirostra* and *L. scotica* and the evolution of crossbills. – Ibis 132: 454–466.

Knox, A. G. 1992. Species and pseudospecies: the structure of crossbill populations. – Biol. J. Linn. Soc. 47: 325–335.

Marquiss, M., Hobson, K. A. and Newton, I. 2008. Stable isotope evidence for different regional source areas of common crossbill *Loxia curvirostra* irruptions into Britain. – J. Avian Biol. 39: 30–34.

Mayr, E. 1963. Animal species and evolution. – Belknap Press, Cambridge, MA.

Mundinger, P. C. 1970. Vocal imitation and individual recognition of finch calls. – Science 168: 480–482.

Mundinger, P. C. 1979. Call learning in the carduelinae: ethological and systematic considerations. – Syst. Zool. 28: 270–283.

Newton, I. 2006. Movement patterns of common crossbills *Loxia curvirostra* in Europe. – Ibis 148: 782–788.

Parchman, T. L., Benkman, C. W. and Britch, S. C. 2006. Patterns of genetic variation in the adaptive radiation of New World crossbills (Aves: *Loxia*). – Mol. Ecol. 15: 1873–1887.

Payne, R. B. 1987. Populations and type specimens of a nomadic bird: comments on the North American crossbills *Loxia pusilla* Gloger 1834 and *Crucirostra minor* Brehm 1845. – Occ. Pap. Mus. Zool. Univ. Mich. 714: 1–37.

Piertney, S. B., Summers, R. W. and Marquiss, M 2001. Microsatellite and mitochondrial DNA homogeneity among phenotypically diverse crossbill taxa in the UK. – Proc. R. Soc. B 268: 1511–1517.

Questiau, S., Gielly, L., Clouet, M. and Taberlet, P. 1999. Phylogeographical evidence of gene flow among common crossbill (*Loxia curvirostra*, Aves, Fringillidae) populations at the continental level. – Heredity 83: 196–205.

Remsen, J. V. 2005. Pattern, process, and rigor meet classification. – Auk 122: 403–413.

Robb, M. 2000. Introduction to vocalizations of crossbills in north-western Europe. – Dutch Birding 22: 61–107.

Smith, J. W. and Benkman, C. W. 2007. A coevolutionary arms race causes ecological speciation in crossbills. – Am. Nat. 169: 455–465.

Snowberg, L. K. and Benkman, C. W. 2007. The role of marker traits in the assortative mating within red crossbills, *Loxia curvirostra* complex. – J. Evol. Biol. 20: 1924–1932.

Summers, R. W., Dawson, R. J. and Phillips, R. E. 2007. Assortative mating and patterns of inheritance indicate that the three crossbill taxa in Scotland are species. – J. Avian Biol. 38: 153–162.

Summers, R. W., Jardine, D. C., Marquiss, M. and Rae, R. 2002. The distribution and habitats of crossbills *Loxia* spp. in Britain, with special reference to the Scottish crossbill *Loxia scotica*. – Ibis 144: 393–410.