Poznań, Sept 1st 2011

Adam Mickiewicz University

Tomasz S. Osiejuk

Prof. dr hab. Tomasz S. Osiejuk, Department of Behavioural Ecology, Adam Mickiewicz University,
Umultowska 89, 61-614 Poznań, Poland; Email: t.s.osiejuk@life.pl

Review of the master thesis

Vocalization of two nightingale species in their hybrid zone

by Jana Vokurková

The MSc thesis concerns song variation in two closely related nightingale species with special reference to the phenomenon of mixed singing. The analysed songs originate from the areas of sympatric and allopatric occurrence of *Luscinia luscinia* and *Luscinia megarhynchosi* In Czech Republic, Germany and Poland. Bioacoustics analyses are supported by information about possible genetic hybridization of recorded individuals. In general, the study is about one of the most important topics in evolutionary biology, namely the role of sexually selected signals in reproductive isolation between species.

Structure of the thesis. The MSc thesis starts with presentation of the contents and abstracts in both Czech and English language versions. Then we have chapter GENERAL INTRODUCTION in which MSc candidate presented the current state of knowledge about hybridization in birds and relationships between song as a sexually selected trait and hybridization. In the second part of this introduction both studied nightingale species are presented. Finally, aims of the thesis are given. The next part of the MSc thesis was called MANUSCRIPT DRAFT and is strictly dedicated to the description of methods and results of the study. MSc thesis ends with general CONCLUSIONS OF THE THESIS and four APPENDIXES. I devoted more space to describe the structure of the MSc thesis as it seems to be a bit

awkward and some content is unnecessarily repeated in both main parts (especially in introductions). However, this critique remark has secondary importance.

Methods. The MSc thesis is based on two different source of information: (1) analyses of song from recordings of two nightingales species or their hybrids and (2) genetic analyses of DNA sequences from sex chromosome Z of recorded males. The sample size understood as number of males whose songs and DNA were analysed is not big (34 males) but with good distribution among studied populations and sufficient to draw conclusions. Recordings analysed contain hundreds of song phrases, which allow for detailed characterisation of each individual repertoire and assigning males to particular group of singers (typical singer, mixed singer etc.) with high level of certainty. One of the most important challenges of this study was correct classification of song phrases as species-typical or mixed songs. Different species within *Luscinia* genus have moderate to large repertoire size, thus an objective comparison of hundreds of different phrases and classification to particular categories is a hard task. This was especially important for this study as MSc candidate planned to classify songs of two species and – likely – their genetic hybrids and/or mixed singer simultaneously. In general, I appreciate description of the methods of song analysis. It is precise and enables repeating similar analysis if somebody wants to focus on different populations of nightingales in the future. I have one little technical remark about the use of Excel macro for visual comparison of sonograms. As the macro is not added to the thesis, the idea of how it exactly works should be presented. It is difficult to assess if this macro was critical for assigning songs to particular classes or not (I suppose that it was not but this is only my guess).

Results. The most important results obtained are as follows: (1) both nightingales from allopatric areas sung songs typical for own species, (2) all trush nightingales from sympatric areas were mixed singer, (3) common nightingales practically do not sing phrases typical for trush hightingale (including males from sympatry area), (4) all interspecies hybrids are also mixed singers with significantly higher level of common nightingale phrases than non-hybrids mixed singers. The most important new findings of the thesis are (1) quantitative evaluation of the proportion of the trush nightingale mixed singers in sympatry areas, and (2) demonstrating that mixed singers not necessarily are interspecies hybrids (but simultaneously such hybrids had higher level of phrases from sibling species).

Discussion. The discussion section of the MSc thesis is generally well written and covers mainly consideration about origin and possible functionality of mixed singing in thrush nightingale in sympatry areas. MSc candidate discusses the results obtained from both possible song receivers' perspective, i.e. males (rivals) and females (mates). Considered are hypotheses about functional song convergence and erroneous learning with pointing out pros and cons of both explanations. I will not repeat different evolutionary scenario presented and focus only on weaker or omitted aspects.

A weaker point of the discussion is that candidate refer practically only to single other example of hybridizing and mixed singing species complex (namely, *Ficedula hypoleuca/Ficedula albicollis)* while there is obviously much more other examples available (e.g. in genus *Hippolais* Behav Proc 46, 151-158, *Certhia* –many papers, e.g. J Orn 113, 287-296, *Phylloscopus* – again many papers, e.g. J Orn 130, 455-473 and much more more if we leave Eurasia).

The second thing, which seems to be very interesting and was not considered enough thoroughly, is the difference in repertoire size between study species. It seems particularly interesting that the species, which has much more larger repertoires do not mimic the sibling species, while the sibling species is doing so, despite having an order of magnitude smaller repertoire. It seems that the ability to learn non-specific songs (or just new sounds) is something different than ability to remembering larger repertoires. The crucial question to answer is what kind of selection forces kept trush nightingale repertoire at moderate level (despite its ability to mimic) while the sibling common nightingale increased their repertoires so much. I understood that author had to focus on some selected aspects of results obtained but I suppose that for full understanding of what is going on in *Luscinia* sp. we have to know why and under what factors they repertoires have evolved.

I also lack information about repertoire size of particular individuals from different populations. I wonder if there were some within-species differences between sympatry and allopatry areas?

In summary, the work presented in the thesis significantly increase our knowledge about mechanisms and functions of mixed singing in *Luscinia* sp. and fully meet requirements for the degree of MSc.

prof. dr hab. Tomasz S. Osiejuk

Minor remarks (not for public reading)

p. 24 – Are there differences in singing styles of nightingales between night and day? As some recordings were taken at different time of the day there should be information with reference that it should not affect the results obtained.
p. 31 – "Ranozsek 2001" should be "Ranoszek 2001)
p. 33 – Despite the studied nightingale species are morphologically very similar, there is no prove that females are not able to distinguish between them visually (human vision does not reflect avian ones).
APPENDIX II – Time scale of the first sonogram (template song from catalogue) is different than all the others, hence it is hard to compare song to the "catalogue template"