

finally led to the demise of all embryos including those which had formed outgrowths.

Zinc appeared to enhance rather than inhibit the toxic effect of copper on cultured mouse blastocysts. Thus, it seems unlikely that competition for cellular transport sites or for binding sites on proteins, such as enzymes, contributes significantly to Zn-Cu antagonism in animals. Instead it appears likely that Zn-Cu antagonism occurs entirely at the level of intestinal absorption<sup>9,10</sup> and storage in the liver<sup>8</sup>. It remains possible that higher levels of  $Zn^{++}$  would protect blastocysts against  $Cu^{++}$  (e.g. 200  $\mu M$   $ZnCl_2$  with 50  $\mu M$   $CuCl_2$ ). However, this could not be tested in the present studies since addition of enough  $ZnCl_2$  to make a 200  $\mu M$  solution in MEM produced a precipitate of unidentified composition.

It was demonstrated here and previously<sup>13</sup> that BSA protects blastocysts from the potentially toxic effects of cupric ions. This protein also protects these embryos against the

toxic effects of copper wire in vitro<sup>11</sup>. The protein in 10% FCS was apparently adequate to protect blastocysts against 100  $\mu M$   $Zn^{++}$  (table vs fig. b). The high concentration of protein which prevails in the rat uterus at the time of implantation (42 mg/ml)<sup>16</sup> probably renders  $Zn^{++}$  (120  $\mu M$ )<sup>16</sup> and other potentially toxic substances which may also be constituents of uterine fluid harmless. In fact, Hurley and collaborators<sup>16,21</sup> have argued that a diminution in the zinc concentration in uterine and oviductal fluid to about 60  $\mu M$ <sup>16</sup> is detrimental to the development of preimplantation embryos. The full physiological significance of the relatively high concentrations of protein present in uterine fluid near the time of nidation remains to be determined. However, most previous studies of implantation-like events in vitro, where the protein concentration in the medium was an order of magnitude lower than the 42 mg/ml in uterine fluid<sup>12,19,22-24</sup>, must now be interpreted with added caution.

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## Acoustic differences between populations of western and eastern Bonelli's Warblers (*Phylloscopus bonelli*, Sylviidae)<sup>1</sup>

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**Summary.** Sonographic analyses of songs and calls of Bonelli's Warbler (*Phylloscopus bonelli*) show important structural differences between the geographically isolated western (*P.b. bonelli*) and eastern (*P.b. orientalis*) populations. Playback experiments in the field and some morphological differences suggest that these populations may be separating into 2 species.

The 2 populations of Bonelli's Warbler appear to exist as separate groups. The western subspecies (*Ph.b. bonelli* Vieillot 1819) inhabits Western Europe and the western Mediterranean basin including the Alps, Black Forest, Apennines, France, the Iberian Peninsula, and the Atlas region. The eastern subspecies (*Ph.b. orientalis* Brehm 1855) is a regular summer bird of Bulgaria and northeastern Greece while scattered populations are found in parts of southern Greece, Anatolia, Syria, and the Lebanon<sup>3</sup>.

There are no breeding records from the Adriatic coast region including Albania and Yugoslavia (except 1 record in Hercegovina<sup>3</sup>). The wintering areas of the 2 subspecies are situated in the Sahel region south of the Sahara and appear to be disjunct as well<sup>4,5</sup>.

There are small morphological differences with regard to the average wing length, relative length of primaries, and coloration<sup>6</sup>.

The present paper describes an experimental analysis of the difference in call structure and call recognition in these 2 subspecies.

The main call note of *Ph.b. bonelli* may be characterized as a soft prolonged 'doo-éoo' differing from other *Phylloscopus* calls (e.g. *collybita*, *trochilus*, *sibilatrix*) by its descending pitch at the end (fig. 1a). It is used in contact and many arousal situations.

The different call of *Ph.b. orientalis* was first described by Reiser<sup>7</sup> and Peus<sup>8</sup> and, more recently, by Géroudet<sup>9</sup> and Bergmann<sup>10</sup>. Calls of this subspecies were recorded mainly in a breeding population north of Alexandroupolis, Thracia, Greece. There are additional records from birds presumably on migration from Cyprus, Karpathos, and the south coast of Anatolia. These calls are much shorter and harsher than the calls of the western subspecies (fig. 1b). They were represented by Reiser<sup>7</sup> as a 'tüp' (in German),

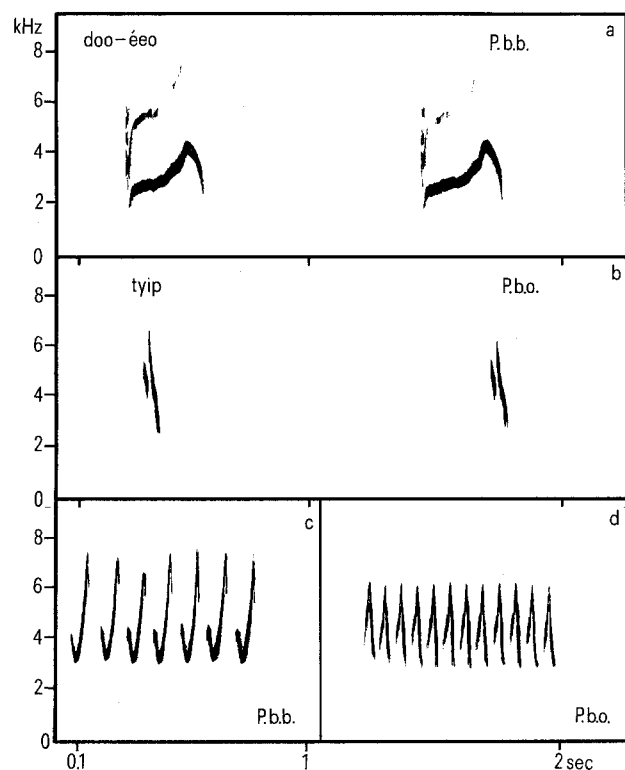


Figure 1. Sonograms of corresponding calls and songs to 2 subspecies of Bonelli's Warbler: *a* 'doo-éeo' (German düte) call of western subspecies *Phylloscopus b. bonelli* (P.b.b.) (recorded 6.6.1979, Garmisch-Partenkirchen, Southern Germany); *b* 'tyip' call of eastern subspecies *Phylloscopus b. orientalis* (P.b.o.) (recorded 16.5.1976, Thracia, Greece); *c* song strophe of western subspecies P.b.b. (recorded 6.6.1979, Garmisch-Partenkirchen, Southern Germany); *d* song strophe of eastern subspecies P.b.o. (recorded 16.5.1976, Thracia, Greece).

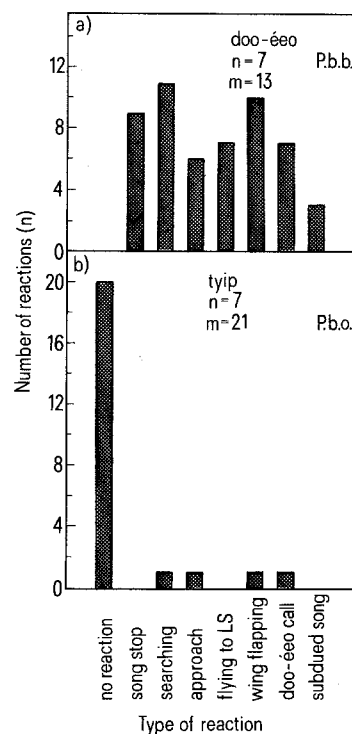


Figure 2. Reactions of western Bonelli's Warblers (*Phylloscopus b. bonelli*) to playback of *a* western 'doo-éeo' and *b* eastern 'tyip' call dialect. n, number of individuals tested; m, number of tests; LS, loud speaker.

by Peus<sup>8</sup> as 'tsjapp', and by Géroutet<sup>9</sup> as 'tsiehp' or 'tsioup'. A sonographic analysis shows that the call consists of a short nearly vertical stroke descending from about 6 to 3 kHz (kc/sec) preceded by a shorter and more vertical stroke between about 5 and 4 kHz. Intra-population and intra-individual variation appears to be small. It is heard in situations as unspecific as the 'doo-éeo' call of *Ph.b. bonelli*, e.g. from resting birds on migration<sup>10</sup> and from birds disturbed in their breeding territory<sup>11-13</sup>.

The releasing value of eastern and western calls was tested by playback experiments in June, 1980, in a breeding population of *Ph.b. bonelli* near Garmisch-Partenkirchen, Southern Germany (fig. 2). The birds reacted to the replay of western calls, i.e. of the same subspecies *b. bonelli*, by territorial reactions such as flying to the sound source (loud speaker), searching for the rival, prolonged and high intensity wing flapping, repeated calls at short intervals, and singing somewhat disturbed and low-voiced full song strophes (fig. 2a). In contrast, playing back of eastern call series, i.e. *b. orientalis*, did not elicit any such responses (fig. 2b). Individuals even continued to sing without any change in song pattern<sup>14,15</sup>.

Song patterns, though much more variable within both populations, appear to exhibit consistent differences as well<sup>16</sup> (fig. 1, c and d). Replay of a typical eastern song (fig. 1d) equally failed to elicit territorial reactions in the members of the western breeding population.

Several factors point to the possibility of regarding the 2 populations as semi-species if not 2 separate species: There are morphological differences, the differences of call and song structure, and the lack of reaction of western Bonelli's Warblers to eastern acoustic signals. As the lack of reaction to alien dialects need not be symmetrical, as has been shown in *Phylloscopus collybita* populations<sup>17,18</sup>, it would be of great interest to determine if eastern Bonelli's Warblers do react to songs and calls of the western subspecies. If these 2 separate populations come into close contact the differences already established might be strong enough to initiate or facilitate speciation<sup>19,20</sup>.

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