Taxonomic research update

Greenish Warbler, 'Two-barred Greenish Warbler', and the speciation process

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ABSTRACT This Taxonomic Research Update draws birdwatchers' attention to the work of Irwin et al. (2001) on Greenish Warblers Phylloscopus trochiloides. Their research shows that two subspecies, P. t. viridanus and P. t. plumbeitarsus, both of which occur as vagrants in western Europe, do not interbreed in the region where their ranges overlap because they do not recognise each other's songs. The subspecies of Greenish Warbler are perhaps the best example of an avian 'ring species', and in this case sexual selection for increased complexity of song is driving the speciation process.

Introduction

The Greenish Warbler Phylloscopus trochiloides is a polytypic species that breeds across much of temperate Asia, and as far west as eastern Europe. Six subspecies are recognised (Ticehurst 1938), their breeding distributions being shown in fig. 1. Greenish Warblers of the subspecies viridanus are regular vagrants to western Europe, although P.t.plumbeitarsus ('Two-barred Greenish Warbler') and P. t. nitidus ('Green Warbler') have also occurred there (Dean 1985; see also pages 284-288).

The Greenish Warbler complex is one of the few putative avian examples of a 'ring species'. Ring species, most influentially described by Mayr (1963), are deemed to be a possible result of one species expanding its range in a ring-like manner around an area of unsuitable habitat (see fig. 2).
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Fig. 2. Hypothetical formation of a ring species. Panel A represents the ancestral distribution of a species, shown in green. In B, the species’ range starts to expand, perhaps as a result of climatic amelioration, around the sides of an area of unsuitable habitat (in this case, a mountain range): as expansion proceeds, populations on each side of the mountain range evolve genetic and morphological differences (represented by blue and yellow), either by chance or because the selection pressures on each one are different; nevertheless, there is limited gene flow and intergradation between adjacent populations (represented by double-headed arrows). In C, the opposite ends of the ring meet on the far side of the area of unsuitable habitat: the races of the ring species at these terminal points of the chain have diverged to such an extent that they are reproductively isolated, i.e. they do not interbreed, and thus behave as separate species; gene flow is maintained between all other races in the ring.

Under certain conditions, a chain of racial variants may arise, each race able to breed and intergrade with its neighbours, except for those co-existing at the two overlapping ‘ends’ of the ring, which have diverged so much that they behave as different species.

The conditions that lead to the formation of a ring species are not fully understood, and the validity of some of the classic examples, such as the Herring Gull *Larus argentatus* / Lesser Black-backed Gull *L. fuscus* circumpolar ring, and the Eurasian ring of Great Tits *Parus major* / Grey Tits *P. afer*, has been seriously challenged by new taxonomic data. Similarly, although Greenish Warblers of the subspecies *viridanus* and *plumbeitarsus* have been reported not to interbreed (Cramp 1992), there has been considerable ambiguity concerning the relationship between the two, no thorough analysis of which had been undertaken until recently, with the results published in the journal *Nature* (Irwin et al. 2001).

**Recent research**

Many of the fundamental gaps in our knowledge about the subspecies of Greenish Warbler have been addressed in the *Nature* paper mentioned above. Irwin et al. used behavioural, morphological and genetic data to show that *viridanus* and *plumbeitarsus* apparently do not recognise each other as conspecifics, and that there is no detectable gene flow between the two. They are linked, however, around the southern edge of the Tibetan plateau by a ring of intergrading, intermediate subspecies. Greenish Warblers hence fulfil many of the critical requirements of a ‘typical’ ring species.

In the study, Greenish Warblers of all subspecies were observed at 29 sites throughout the entire range. Various biometric and plumage characters were recorded, sonograms were constructed and analysed, the responses to songs of the same and of different subspecies were measured, and blood samples were taken for genetic analysis.

Northern races of Greenish Warbler, *viridanus* and *plumbeitarsus*, have longer and more complex songs than the nominate southern subspecies *trochiloides*. The authors demonstrated parallel gradients of increasing song length and complexity on both the eastern and the western sides of the Tibetan plateau. By breaking down the songs into their principal components, they were able to show that there are smooth clines in song structure from *trochiloides* to *viridanus* on the west side of the ring, and from *trochiloides* to *plumbeitarsus* on the east side. It is presumed that, during the
species' northward range expansion following glaciation, sexual selection encouraged this increasing complexity, favouring males with more elaborate songs. Although there have been parallel pressures to evolve longer, more complex songs, however, the songs have developed differently on each side of the Tibetan plateau: *viridanus* have songs of high frequency range, composed of three or four long song units, while *plumbeitarsus* have songs with a lower frequency range, composed of seven or eight shorter units. This divergence, although smooth and gradual, has, therefore, led to the manifestation of noticeably different songs in *viridanus* and *plumbeitarsus*.

What is perhaps more interesting is that the differences in song structure that we hear are of biological significance to the birds themselves. Typically, male passerine birds exhibit a strong territorial response to playback of songs which, although not identical to their own, they recognise as coming from a competing male of the same species. If they do not recognise the song as being that of a competitor, for a territory or a mate, they do not react.

Irwin *et al.* subjected male Greenish Warblers of each subspecies to playback of the songs of all other subspecies, and measured the strength of their response. They found that, in general, Greenish Warblers reacted strongly to the songs of their own or neighbouring populations or subspecies, but less strongly to those of more distant populations. For example, *trochiloides* recognised *ludlowi* and *obscuratus* as being from the same species, but showed a weaker response to *viridanus* songs; *ludlowi* showed a strong response to many *viridanus* and *trochiloides*, but a weaker response to *obscuratus*; and so on. In general, individuals reacted aggressively to the songs of subspecies which occurred within approximately 1,500 km of their own territory, but less strongly or not at all to songs of those beyond that range, presumably because the latter's songs were too different from their own. One notable exception was the reaction of *viridanus* to *plumbeitarsus*. Individuals of these two subspecies within their zone of overlap showed no response to each other's songs, and, indeed, sometimes shared territories. The clear, measurable differences in their songs, as a result of their being at the opposite ends of the ring of subspecies varia-
tion, are therefore also registered by the birds themselves. They behave as different species.

To determine whether this incipient speciation of the Greenish Warblers was reflected in their genetic history, a phylogenetic tree was drawn up. This is based on an analysis of 1,200 base pairs of mitochondrial DNA (mtDNA) from 149 individuals of all races, taken from sites throughout the entire range of the species. These data are summarised in a simplified form in fig. 3. This gene tree demonstrates a deep genetic split between western and eastern birds. In the zone of overlap of viridanus and plumbeitarsus in Siberia, genetic differences were fully in accordance with differences in physical appearance: i.e. all viridanus had the 'western-clade' DNA, and all plumbeitarsus had the 'eastern-clade' DNA. There is therefore no evidence of gene flow between the populations of viridanus and plumbeitarsus in Siberia, a finding which supports the behavioural evidence derived from the song-playback experiments.

Interestingly, the boundary between the eastern and western genetic lineages falls within the range of one of the subspecies, ludlowi. Around this boundary, there are no morphological or song differences between warblers carrying the eastern-clade DNA and those with the western-clade DNA structure, and the playback experiments confirm that the birds do not distinguish between individuals carrying the different mtDNA lineages. While direct gene flow between viridanus and plumbeitarsus in Siberia does not occur, it would seemingly be possible for gene exchange to take place 'via the back door' (i.e. through the chain of intermediate subspecies encircling the southern edge of the Tibetan plateau). Nevertheless, gene flow around this ring is apparently not great enough to disrupt the genetic differentiation of the Greenish Warbler populations; this is presumably a classic case of 'isolation by distance'.

The trends in other genetic (microsatellite) and morphological (width of the greater-covert wing-bar) characters were followed for individuals in the ring of subspecies from viridanus, through ludlowi, trochiloides and obscuratus, to plumbeitarsus. Again, an apparent lack of gene flow in the Siberian overlap zone was demonstrated, but there was significant continuing or recent gene flow between adjacent subspecies along the southern side of the ring.

**Discussion**

The classic explanation of the Greenish Warbler as constituting a ring species would postulate that the ancestral populations inhabited the forested sub-Himalayan region. During the Ice Ages, much of Siberia would

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**Fig. 3.** A gene tree, derived from analysis of mitochondrial DNA, showing the deep genetic split between eastern and western subspecies of Greenish Warbler *Phylloscopus trochiloides*. For clarity, the shaded boxes represent clusters of genetically similar individuals. A western-clade DNA (§) contains all the individuals of *viridanus* and *nitidus* and a proportion of the *ludlowi* populations, whereas an eastern-clade DNA ($) is representative of other *ludlowi* individuals, as well as all *trochiloides*, *obscuratus* and *plumbeitarsus*. Individuals labelled *ludlowi/trochiloides* were taken from the *ludlowi* end of the intergrade zone between those two races. See text for discussion.
Tibetan plateau (where the birds recognised each other as potential mates and interbred), and one to the north (where they did not recognise each other).

There are some controversial differences between the two models outlined here, but birdwatchers need not be too bothered about them. The conditions that lead to the formation of a ring species may well, in practice, involve periods of range-splitting and subsequent divergence in allopatry. If we think of the evolution of the subspecies of Greenish Warbler in terms of the classic ring species, based on expansion of an ancestral Himalayan population, but then superimpose the possibility that genetic divergence has been maintained, or at least helped along, by periods of range contraction and splitting during glacial periods, followed by secondary contacts between the races during range expansion, we are probably not too far away from a conceptual basis for understanding what has happened.

Males of plumbeitarsus and viridanus do not recognise each other as conspecifics; there are no intermediate individuals in the Siberian overlap zone; and, while direct gene flow between the two would be expected to occur if females of one subspecies were mating with males of the other, none has been detected. Furthermore, although it has been possible until recently for gene flow to occur indirectly, via the ring of intermediate subspecies, this ring has now been broken by deforestation in China (fig. 1). There seems little chance that the separate evolutionary lines taken by viridanus and plumbeitarsus will ever again merge into one. Perhaps those birders who have seen plumbeitarsus, or 'Two-barred Greenish Warbler', are justified in following the line taken by BWP, giving this taxon separate specific status. Were such a split to be adopted, however, some or all of the southern subspecies might conceivably be
left in limbo, since it would be impossible to assign them to either Greenish Warbler or Two-barred Greenish Warbler.

It is often assumed that a complete understanding of the stages by which speciation occurs is not possible, because no single study has demonstrated the evolution of a new species through all the 'grey-zone' intermediates (as shown diagrammatically in Collinson 2001). The process by which one species may, over time, split into two increasingly divergent populations which eventually become species, fully isolated reproductively, is illustrated neatly, however, by ring species. All the intermediate stages are extant and represented in geographical space. Examples of ring species such as the Greenish Warbler are perhaps, therefore, the most graphic argument to counter the views of those people who continue to believe that Darwinian evolutionary theory cannot explain how one species becomes two.

References


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