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PERSPECTIVE

From song dialects to speciation in white-crowned sparrows

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The behavioural signals used in mate selection are a key component in the evolution of premating isolating barriers and, subsequently, the formation of new species. The importance of mating signals has a long tradition of study in songbirds, where many species differ in their song characteristics. In oscine songbirds, individual birds usually learn their songs from a tutor. Mistakes during learning can help generate geographic dialects, akin to those within human language groups. In songbirds, dialect differences can often be substantial and there is an intuitive connection between the evolution of song amongst populations at a small scale, and the more substantive song differences between bird species and presumably used in species recognition. However, studies investigating the concordance between putative genetic and behavioural boundaries have generated mixed results. In many cases, this is possibly a function of the poor resolving power of the genetic markers employed. In this issue of *Molecular Ecology*, Lipshutz *et al.* (2017) combine genomic markers with a robust behavioural assay to address the importance of song variation amongst white-crowned sparrow (*Zonotrichia leucophrys*) subspecies.

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At first glance, the white-crowned sparrow – a common North American passerine – appears a rather unremarkable songbird (Fig. 1A). It may seem surprising to learn, then, that this humble sparrow was at the centre of one of the 'raging controversies of the 1970s and 1980s' (Baker *et al.* 1982; Zink & Barrowclough 1984; Kroodsma 2005). The heart of this debate centred on the observation that, like human language, many songbirds exhibit regional dialects in their vocalizations. Dialects are observed in many bird species, although they are conspicuous and easily delineated in white-crowned sparrows, which is one reason they have been a popular study subject for the field.

The central questions at the time were as follows: what are the drivers of song dialects and what are their evolutionary consequences? The first part of this question was readily addressed: dialects are the result of limited dispersal and cultural evolution. Cultural evolution is, in many ways, analogous to genetic evolution, but in this case, inheritance can be vertical – from parent to offspring – as well as horizontal, between neighbours within earshot. Importantly, young songbirds, when learning from their tutors, will inevitably make mistakes, and these mistakes (termed 'cultural mutations') can spread throughout a population akin to genetic alleles. Thus, dialects are the cultural equivalent of population genetic structure, resulting (mostly) from limited dispersal and cultural drift.

The more difficult (and debated) question came from trying to understand the evolutionary *consequences* of dialects. In addressing this, researchers identified a paradox. Many species of birds differ strongly in their vocalizations and, between closely related species, song differences have been demonstrated to be used in recognition. For example, song responses have been quantified between species of *Sporophila* seedeaters (Benites *et al.* 2014) and *Setophaga* warblers (Kenyon *et al.* 2017). Moreover, song evolution has been linked the evolution of premating isolating barriers in birds (Slabbekoorn & Smith 2002; Price 2008). It is intuitive, then, that populations or subspecies that differ in song – possibly at the early stage of the speciation process – might also show a measurable preference for homo- versus heterotypic songs. This could result in reduced fitness following dispersal (i.e. males with a nonlocal song type might have a more difficult time finding a mate) and, subsequently, genetic differentiation amongst different singing groups could evolve. To date, however, there have been mixed results when addressing this assertion: in some cases, song differences within species are correlated with genetic boundaries (Baker *et al.* 1982), whereas in other studies, the patterns are less clear (Soha *et al.* 2004). Thus, a paradox: why are song differences so easily diagnosed between related avian species, yet studies correlating song and genetic variation *within* species produce mixed results?

There an important geographic context to consider here, as well as the more general question as to whether cultural processes are acting similarly across different spatial scales. For instance, song dialects can occur over very small geographic scales and these presumably arose in the face of gene flow: at least six white-crowned sparrow dialects are contained within the Point Reyes peninsula of California

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Fig. 1 (A) A male white-crowned sparrow singing (photographed by Pete Seubert). (B–C) Song variants from Lipshutz *et al.* (2017) sampled from neighbouring populations. The panels show song spectrograms – visual representations of sound – with time on the horizontal axis, note frequency along the vertical axis and amplitude denoted by the darkness of the lines.

alone (<300 km^2; Kroodsma 2005). In contrast, the song differences observed over large geographic regions (sometimes called 'regiolects') more likely evolved during periods of geographic isolation (Fig. 2). Yet, even studies at this broad scale between regiolects, in situations where we expect strong concordance between songs and genes – because both have diverged to a high degree – we find conflicting patterns. This conflict is illustrated by past work comparing the distinct songs of white-crowned sparrow subspecies.

In western North America, there are two white-crowned sparrow subspecies – *Z. l. nuttalli* and *Z. l. pugetensis* – that differ subtly in morphometrics and plumage, but more strongly in several aspects of their songs. Early genetic analysis across the contact zone between these two subspecies did not find evidence of strong genetic differences. This was surprising, because if dialect boundaries also represent dispersal boundaries, genetic and song differences were expected to align. However, these analyses relied on low-resolution genetic markers, such as mitochondrial DNA or allozymes (Corbin & Wilkie 1988; Weckstein & Zink 2001). And this is one of the strengths of the new work presented by Lipshutz *et al.* (2017), where the authors bring genomic data to bear on the issue. Notably, Lipshutz *et al.* (2017) pair their powerful genetic assay with detailed song analyses and playback studies, which attempt to quantify song preferences.

A limitation of many bird song studies is that it is, in many cases, impossible to determine precisely what song characteristics birds prefer. Multivariate statistics are therefore a common tool: one can measure multiple correlated variables, transform them into a smaller number of axes explaining the most variation and hope that these quantitative metrics capture the qualitative differences in songs. This is precisely the approach applied by Lipshutz *et al.* (2017), and they were readily able to distinguish songs between the subspecies using several vocal characteristics (e.g. Fig. 1B and C).

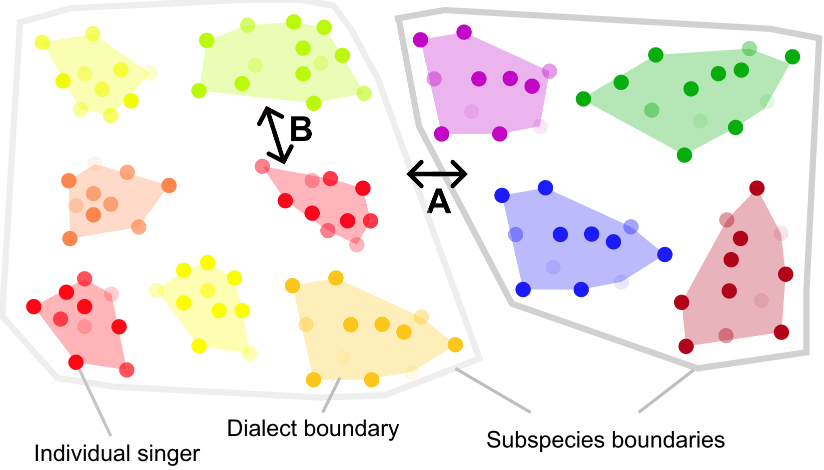

Fig. 2 A schematic of how song variation might be spatially partitioned within a songbird species. In this example, individual singers are represented by the coloured points – indicated by the filled shading of each point – that differ subtly within dialects, but differ strongly between dialects, as shown by the coloured polygons. These dialects make-up the larger milieu of song variation observed between subspecies, which sometimes exhibit strong song differences over larger spatial scales, termed 'regiolects' (represented by the darker/colder colours on the right versus lighter/warmer colours on the left). Presumably, the processes generating divergence between dialects (e.g. across the boundaries denoted at 'B') are similar to those generating divergence – and possibly reproductive isolation – between subspecies (e.g. across boundaries denoted at 'A'), although evidence to date has been mixed. Lipshutz *et al.* (2017) used genomic and behavioural assays to make comparisons analogous to those across 'A'. Additional work using more sensitive genomic methods will hopefully resolve the generation, maintenance and evolutionary influence of small-scale dialect boundaries.

Lipshutz *et al.* (2017) then used reduced-representation genome sequencing to characterize the genomic background of the subspecies. With the resolving power of thousands of markers, the subspecies now showed clear geographic structure, grouping into two distinct genetic clusters. The populations also exhibited a north–south clinal pattern of admixture, which is expected under a scenario of allopatric divergence followed by secondary contact and hybridization (a scenario the authors explicitly tested for and confirm in their study).

The most important measure here, and arguably the most difficult to quantify, was how the birds responded to the different songs from the two subspecies. For this, the authors used song playback trials of sparrows in the wild. This kind of playback method involves finding a focal individual – usually a male singing and defending his territory – and simulating a territory intrusion. This is carried out by positioning a portable speaker within the male's territory, playing a prerecorded vocalization and then observing the bird's response. Usually this response includes aggressive wing flashes, quick bursts of flight and flying close to the speaker (or some combination therein). It is important to note that these studies are usually focused on males. This might seem like an odd approach, given that knowing the preference of females is key to this kind of mate choice trial. As it is with many playback studies, however, Lipshutz *et al.* (2017) rely on the indirect responses of males to draw their conclusions – a logistical trade-off – mostly because females can be cryptic and difficult to find and observe.

The playbacks to the sparrows revealed several significant findings. First, birds from the ends of the study transect – outside the zone of admixture – had reduced aggression to heterotypic songs as compared to songs they were more familiar with. This suggests that the song differences have diverged enough between the subspecies that these differences are a premating reproductive isolating barrier. Second, in the contact zone, birds tended to be less discerning of the different song types, responding aggressively to both types. This is consistent with birds in the hybrid zone learning multiple song types, which could facilitate territory defence against a range of intruders with different songs. Finally, they found that an individual's response could be predicted by comparing its own song to the song used in the playback stimuli: a song similar to its own produced a more aggressive response, and vice versa. Taken together, these findings support a central role of song in isolating these taxa.

Is this new research beginning to resolve the paradox noted above, where studies found clear behavioural differences between species based on song, but less clear patterns within species? In many ways, they are: these new studies demonstrate that subspecies (and closely related species, e.g. Kenyon *et al.* 2017) are altering their responses based on population-specific vocalizations and Lipshutz *et al.* (2017), in particular, highlight how song differences might drive premating isolation early in the speciation process.

There are, however, still questions about how song dialects function across much smaller spatial scales, although this may again be a matter of genetic resolution. The genomic data necessary to quantify dispersal at this restricted scale would need to be very high. While this may have seemed unimaginable thirty years ago, analyses across very small spatial scales and at this genomic magnitude are now within the realm of possibility. For instance, Chen *et al.* (2016) used genomic data to identify the fine dispersal movements – on a scale of tens of kilometres – of highly related Florida Scrub Jays. Therefore, it seems reasonable to envision an analogous genomic study of white-crowned sparrows, but across multiple dialect boundaries and on a very small scale. Indeed, as Lipshutz *et al.* (2017) demonstrate, even after decades of study, it appears that the songs of the white-crowned sparrow will continue to provide fruitful material for addressing consequential questions about cultural evolution and the evolution of reproductive isolation in birds.

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