Research

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Sympatry drives colour and song evolution in wood-warblers (Parulidae)

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Closely related species often exhibit similarities in appearance and behaviour, yet when related species exist in sympatry, signals may diverge to enhance species recognition. Prior comparative studies provided mixed support for this hypothesis, but the relationship between sympatry and signal divergence is likely nonlinear. Constraints on signal diversity may limit signal divergence, especially when large numbers of species are sympatric. We tested the effect of sympatric overlap on plumage colour and song divergence in wood-warblers (Parulidae), a speciose group with diverse visual and vocal signals. We also tested how number of sympatric species influences signal divergence. Allopatric species pairs had overall greater plumage and song divergence compared to sympatric species pairs. However, among sympatric species pairs, plumage divergence positively related to the degree of sympatric overlap in males and females, while male song bandwidth and syllable rate divergence negatively related to sympatric overlap. In addition, as the number of species in sympatry increased, average signal divergence among sympatric species decreased, which is likely due to constraints on warbler perceptual space and signal diversity. Our findings reveal that sympatry influences signal evolution in warblers, though not always as predicted, and that number of sympatric species can limit sympatry's influence on signal evolution.

1. Introduction

Animals exhibit an incredible diversity of communication signals. Many signals play a vital role in mate choice and intrasexual competition (hereafter: 'sexual signals'), and divergent sexual signals among species often serve as important indicators of species identity [1]. Traditional speciation models assume that sexual signal divergence occurs as a by-product of ecological adaptation or genetic drift [2], however, sexual signals can diverge under the direct influence of sexual selection. For example, sexual selection can promote signal divergence due to receivers favouring individuals with signals that are more effective in different environments, leading to the evolution of divergent signals, even among closely related species [3,4].

Signals are expected to diverge between closely related species with overlapping geographical ranges (e.g. figure 1a) to maintain reproductive isolation and prevent hybridization [2,5]. Prior studies have revealed that signals used for mate choice, and the preferences for those signals, are more divergent in areas where closely related species co-occur (i.e. sympatry) versus areas where each species occurs separately (i.e. allopatry) [6,7]. These studies provide evidence for sexual signals facilitating species identification of prospective mates, and thus maintaining reproductive isolation between sympatric species. Comparative studies offer limited additional evidence of sexual signal divergence for species recognition. For example, sympatric species exhibit greater divergence compared to allopatric species in song characteristics [8] and plumage colour [9] in birds. Similarly, the degree of sympatry between species
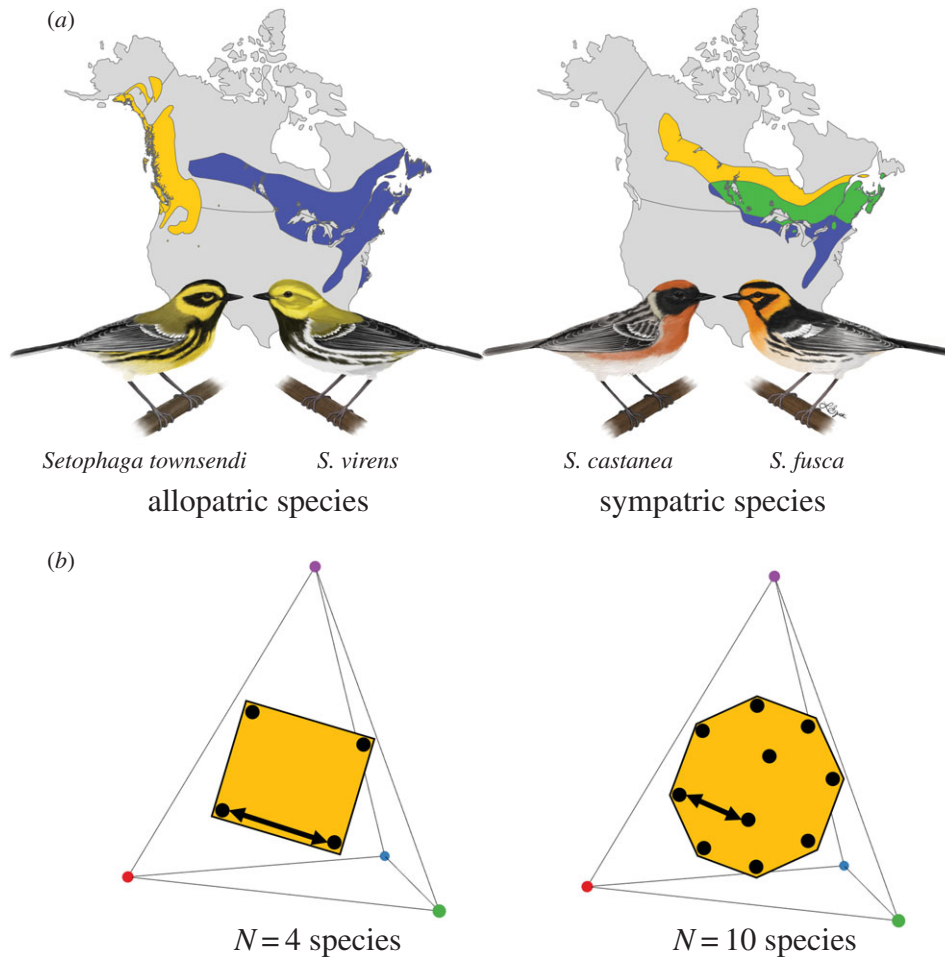
Figure 1. (a) Two example pairs of wood-warbler species, illustrating the effect of sympatry on sexual signal divergence. Male plumage colour divergence between the sympatric species (*Setophaga castanea* in gold; *S. fusca* in blue, range overlap in green) is much greater than between the allopatric species (*S. townsendi* in gold; *S. virens* in blue) in this example. (b) An illustration of how we predict the number of sympatric species to influence sexual signal divergence and sexual signal diversity. The tetrahedrons represent avian tetrachromatic perceptual space. The golden polygons represent the colour volume of sympatric species (black dots) and the arrows represent the colour divergence between those species. The two polygons (2D in the figure but 3D in analyses) represent our prediction for when a few species occur in sympatry ($N = 4$) versus several species ($N = 10$) and depicts how the number of sympatric species should increase the sympatric signal volume but will also decrease sympatric signal divergence. (Online version in colour.)

pairs correlates with plumage colour divergence in temperate birds in North America [10].

The effect of sympatry on sexual signal divergence should not always be linear. As more species co-occur, the degree to which sexual signals can diverge will be limited by the perceptual space and signal production mechanisms of those species. For example, colour perceptual space has a fixed size, based on the sensory capabilities of a given animal group (e.g. birds) [11]. As this colour space becomes increasingly crowded with more species, there should be a reduction in how much any particular species can simultaneously diverge in colour from multiple other species (figure 1b). Many animals are even further constrained by their available colour production mechanisms [12], with certain colour production mechanisms producing only a specific set of colours [13]. For instance, due to colour production limitations, birds can only produce a subset (ca 30%) of the colours they can perceive [12].

Other signals can also be limited by perceptual space or production mechanisms. Acoustic signals, for example, are also constrained by physiological and morphological limitations [14,15]. Body size constrains the frequencies produced by birds, especially at lower frequencies [15], and vocal tract morphology constrains vocal performance [14]. Outside of

signal production constraints, other factors can restrict sexual signal diversity, such as predation pressures [16]. Given these constraints, when many species co-occur in sympatry it should be difficult for each species to exhibit high signal divergence from all other species (figure 1b). Consequently, we predict that sympatry should favour signal divergence, but that the magnitude of signal divergence should decrease as the number of species in sympatry increases, due to bounded signal spaces becoming crowded.

In this study, we test the hypotheses that sympatry promotes sexual signal divergence, and that the number of species co-occurring in sympatry limits sexual signal divergence in wood-warblers (family: Parulidae), a widespread and speciose group of birds (figure 1). Wood-warblers exhibit tremendous variation in the degree of sympatric overlap (i.e. 1–70 species in sympatry), even among species within the same genus (e.g. *Setophaga*). Nevertheless, wood-warblers effectively maintain species integrity [17]. Wood-warblers also show little divergence in morphology (e.g. body size) [17], but exhibit remarkable diversification in sexual signals, including in plumage colour and song [18], which function in mate choice and intrasexual competition [19,20]. We predicted that plumage colour and song divergence would be greater in sympatric species and increase with the degree of

sympatric overlap. We also predicted that an increase in the number of sympatric species would decrease the average colour and song divergence among those sympatric species, due to wood-warbler signal space becoming too crowded. Sexual signals are also expected to diverge among species through a variety of other factors, such as genetic drift [19]. Therefore, we tested and controlled for the relationship between signal divergence and phylogenetic distance in our analyses. We also acknowledge the possibility that sexual signal divergence could facilitate sympatric overlap (i.e. the reverse of the above-described hypothesis), such that more-distinct species could remain reproductively isolated during secondary contact, while less-distinct species would have higher rates of hybridization due to lack of species recognition. While our analyses cannot completely distinguish between these alternatives, both hypotheses stem from the same underlying idea that signal divergence is important for species recognition to maintain reproductive isolation.

2. Methods

(a) Plumage colour analysis

We measured plumage reflectance from 818 museum specimens of 93 species (see electronic supplementary material, file S1 for specimen information), using established methods [20] (see electronic supplementary material, text S1 for details). Whenever possible, we measured five males and five females for each species. For each specimen, we measured the reflectance of 15 body regions: belly, breast, cheek (i.e. auricular), crown, eyebrow (i.e. supercilium), flank, mantle, nape, rump, inner tail (excluding outermost feathers), outer tail (the two outermost tail feathers), throat, undertail coverts (hereafter under-coverts), lower wing (i.e. primaries and secondaries) and upper wing (i.e. wing coverts).

We processed reflectance spectra in R [21] using the package *pavo* [22]. We averaged spectra such that each species was represented by one average spectrum per body region per sex. We converted the spectra into avian tetrachromatic colour space [11], using the average ultraviolet (UV) sensitive avian visual model [23] under an idealized light environment (i.e. we made no assumptions about each species' light environment), and calculated the absolute cone simulation values for each avian photoreceptor [22]. We used the absolute cone stimulation values to calculate the just-noticeable differences (JNDs) [24] for each colour patch between all species, and used these JND values as our measures of pairwise species colour distances (i.e. a separate species pairwise distance matrix per body region). To reduce the overall number of matrices (15 per sex) and therefore reduce the number of statistical tests we ran, we averaged the individual body region matrices into biologically relevant body region groups as follows: (i) head: cheek, crown, eyebrow, throat; (ii) upper-body: nape, mantle, rump; (iii) under-body: belly, breast, flank, under-covert; (iv) flight feathers: inner tail, outer tail, lower wing, upper wing. We also calculated an average JND-colour distance matrix for all body regions together (whole-body colour distance). We conducted these analyses separately for males and females. Finally, for our analyses testing the effect of number of sympatric species on colour divergence, we used the JND data to create a JND-colour-space [25], where all distances within this space are in units of JND and are perceptually equivalent [25].

(b) Song analysis

We obtained recordings of singing male warblers from public repositories and personal collections (see electronic supplementary

material, file S2 for recording information). We only tested male song because female song occurs in only a small subset of species ($n = 25$) [26]. For each species, we attempted to obtain five recordings that each contained one or more songs with high signal-to-noise ratio, no overlapping sounds, and no distortion, as determined aurally and by visual inspection of waveforms and spectrograms in Raven Pro software (v. 1.4; Cornell Lab of Ornithology, Ithaca, NY, USA). Multiple recordings of the same species were from different years or from locations at least 5 km apart to reduce the risk of including multiple recordings of the same individual, although these criteria were relaxed for rare and extinct species (e.g. *Vermivora bachmanii*). In total, we included 494 recordings from 102 species (only 10 species with fewer than five recordings). All recordings were converted to a standard format (WAVE format, 16-bit amplitude encoding, 44.1 kHz sampling rate) prior to analysis using Sample Manager software (v. 3.1; Audiofile Engineering, St Paul, MN, USA). Using Raven Pro, we measured duration, syllable rate, minimum frequency, maximum frequency, frequency range and entropy (a measure of tone purity or disorder) for the highest quality song per recording after each song was filtered with a 1.1-kHz high-pass filter and normalized to a peak amplitude of -1 dB (see electronic supplementary material, text S2 for full descriptions of each song variable). We then calculated the average of each song variable per species and calculated species pairwise differences for each song variable across species.

We used a cross-correlation approach to measure the overall song similarity of species pairs [8]. Cross-correlation values range from 0 (dissimilar songs) to 1 (identical songs), and incorporate both frequency and temporal aspects of song covariances [8] (see electronic supplementary material, text S2 for details). We used Raven Pro to calculate the song cross-correlation matrix using all individual songs, and then calculated an average value for each species dyad.

For our analyses testing the effect of number of sympatric species on song divergence, we created a 'song space' to calculate Euclidean distances between species for their songs. We used principal components (PCs) analyses on our six-song variables (see electronic supplementary material, table S1 for song variable correlations), resulting in three PCs with eigenvalues above 1.0, which we used as x, y, z axes for this song space (electronic supplementary material, table S2). Song space x (PC1) had positive loadings with minimum frequency, maximum frequency and song entropy; song space y (PC2) had positive loadings with song bandwidth and entropy and negative loadings with minimum frequency; and song space z (PC3) had positive loadings with duration and negative loadings with syllable rate (electronic supplementary material, table S2).

(c) Sympatry quantification

To quantify the degree of sympatric overlap within species dyads, we obtained digital polygons of warbler breeding ranges from BirdLife International and NatureServe [27] (datum: World Geodetic System 1984). Using the R package *rgdal* [28], we projected each breeding range using the Lambert azimuthal equal area projection (latitude at projection centre = 45°; longitude at projection centre = −100°; false northing = 0 m; false easting = 0 m), which converts locations on the surface of an ellipsoid into locations on a plane, without distorting the areas contained in each range. We used the R package *rgeos* [29] to calculate the degree of sympatric overlap for every species pair (hereafter 'degree of sympatric overlap'). We specifically calculated the proportion of species 1's breeding range that was overlapped by species 2's breeding range [30,31], and separately calculated the proportion of species 2's breeding range that was overlapped by species 1's breeding range, which allowed us to account for the asymmetry in the degree of sympatric overlap within a species

pair. For example, the Kirtland's warbler's (*Setophaga kirtlandii*) breeding range is completely overlapped by the American redstart's (*S. ruticilla*) breeding range, whereas less than 1% of the American redstart's breeding range is overlapped by the Kirtland's warbler's range.

(d) Statistical analyses

All statistical analyses were conducted in R [21], and we occasionally used natural log or square root transformations to meet statistical assumptions. We used a time-calibrated warbler phylogeny [32] and removed species with missing data using the R package *ape* [33]. To test for the effect of phylogenetic relatedness, we calculated patristic distances for each species pair (i.e. the sum of branch lengths between each pair of species in the trimmed phylogeny; hereafter 'phylogenetic distance').

We first tested whether colour and song divergence were greater in sympatric or allopatric species pairs. We categorized all species pairs with no sympatric overlap as allopatric and all species pairs with sympatric overlap greater than zero as sympatric. We then created linear mixed models with both sympatric categorization and phylogenetic distance as fixed effects predicting pairwise species colour or song distances, with each species in a pair as a separate random effect, using the R package *lme4* [34]. We also tested whether sympatric species pairs exhibited higher phylogenetic relatedness, with each species in a pair as a separate random effect, and found that allopatric species pairs exhibited higher phylogenetic distances ($t = -15.59$, $p < 0.001$). This result is not solely explained by deep phylogenetic relationships or biogeographic isolation, such as the allopatry between the North American *Oreothlypis* species and the South American *Myioborus* species, because an average of 65% of species pairs within genera and an average of 78% of species pairs within breeding continents were allopatric (electronic supplementary material, table S3).

We then removed all allopatric species pairs from the dataset and tested whether the degree of sympatric overlap predicted colour and song divergence in sympatric species pairs. Using only the sympatric species pairs, we created linear mixed models with degree of sympatric overlap and phylogenetic distance as fixed effects (these variables were not correlated in this dataset: $t = 0.17$, $p = 0.87$) predicting pairwise species colour or song distances, with each species in a pair as separate random effects. We accounted for a false discovery rate of these multiple comparisons within each set of models [35], following the recommendations of Nakagawa [36]. Our results were robust to this issue (electronic supplementary material, tables S4–S9). We also tested whether sympatric overlap predicted colour and song divergence using all species pairs, however, due to the large number of allopatric species pairs (e.g. 3043 of 4095 species pairs were allopatric in our male colour dataset), these results roughly mirrored the sympatric categorization results above (electronic supplementary material, tables S8 and S9).

We then tested whether the number of sympatric species predicted sympatric colour and song divergence through two sets of analyses. In our first analysis, we calculated the average colour or song difference between a given species and all its sympatric species; we refer to these values as 'sympatric colour distance' (electronic supplementary material, figure S1a) or 'sympatric song distance' (electronic supplementary material, figure S1b). For example, we identified every species that is sympatric with a particular warbler species (species A) and then calculated pairwise colour and song differences between 'species A' and each sympatric species (body regions separately for colour; electronic supplementary material, figure S1a,b). For colour, we measured the Euclidean distances between two species in JND-colour space (electronic supplementary material, figure S1a), and for song we used the Euclidean distances between two species in

the above-described song space (electronic supplementary material, figure S1b). However, when 'species A' exhibits a 90% range overlap with 'species B' and only a 10% range overlap with 'species C', we would expect 'species B' to have a stronger influence on plumage divergence for 'species A' compared to the influence of 'species C'. Therefore, we weighted each pairwise colour or song distance by the degree of sympatric overlap between the focal species and that sympatric species (e.g. 0.9 for the 'species A–B' pair and 0.1 for the 'species A–C' pair). We then averaged those weighted pairwise differences, such that there was a single, average sympatric colour and song distance (per body region and per sex for colour) between 'species A' and all the species it is sympatric with (electronic supplementary material, figure S1a,b). We then averaged each body region such that we had one average sympatric colour distance, per sex (electronic supplementary material, figure S1a). We used phylogenetic generalized least squares (PGLS) analyses on log-transformed values to test whether the number of sympatric species predicts sympatric colour or song distance, using the R package *caper* [37]. Finally, we re-ran these analyses without weighting each pairwise colour or song distance by the degree of sympatric overlap and obtained similar results (electronic supplementary material, table S10).

In our second analysis, we tested whether the number of sympatric species predicts sympatric colour and song diversity, which we measured as colour or song volume encompassed by sympatric species; we refer to these values as 'sympatric colour volume' (electronic supplementary material, figure S1c) and 'sympatric song volume' (electronic supplementary material, figure S1d). To calculate sympatric colour volume, we computed the smallest geometric shape (i.e. convex hull in three dimensions) that enclosed the colours across body regions for a particular species and its sympatric species in JND-colour space (electronic supplementary material, figure S1c), and we calculated the volume of that shape using the *convhulln* function in the R package *geometry* [38]. To calculate sympatric song volume, we computed the smallest geometric shape that enclosed the songs of a given species and its sympatric species in our song space (one average song per species), and calculated the shape's volume as we did for colour volume (electronic supplementary material, figure S1d). For song, when a given species occurred only with one or two other species in sympatry, we were unable to calculate a song volume ($n = 5$ species), but this was not an issue for colour volume because we calculated the volume using each body region (i.e. 15 colours per species). We calculated sympatric colour and song volume for each species for which we had both song and colour data ($n = 85$ species; sexes analysed separately for colour). We then used PGLS analyses on log-transformed values to test how the number of species that occur in sympatry predicts sympatric colour and song volume.

Our results for both sympatric signal distance and sympatric signal volume were robust to pseudoreplication as shown by re-running the above analyses using a randomized resampling technique repeated 10 000 times (electronic supplementary material, text S3 and table S11). Finally, we calculated the total colour and song volume for all wood-warblers and compared those volumes to the sympatric species colour and song volumes.

3. Results

(a) Colour and song divergence between allopatric and sympatric species pairs

Contrary to our predictions, allopatric wood-warbler species pairs had higher male and female plumage colour divergence than sympatric species pairs. Allopatric species pairs exhibited more divergent male and female whole-body coloration, head
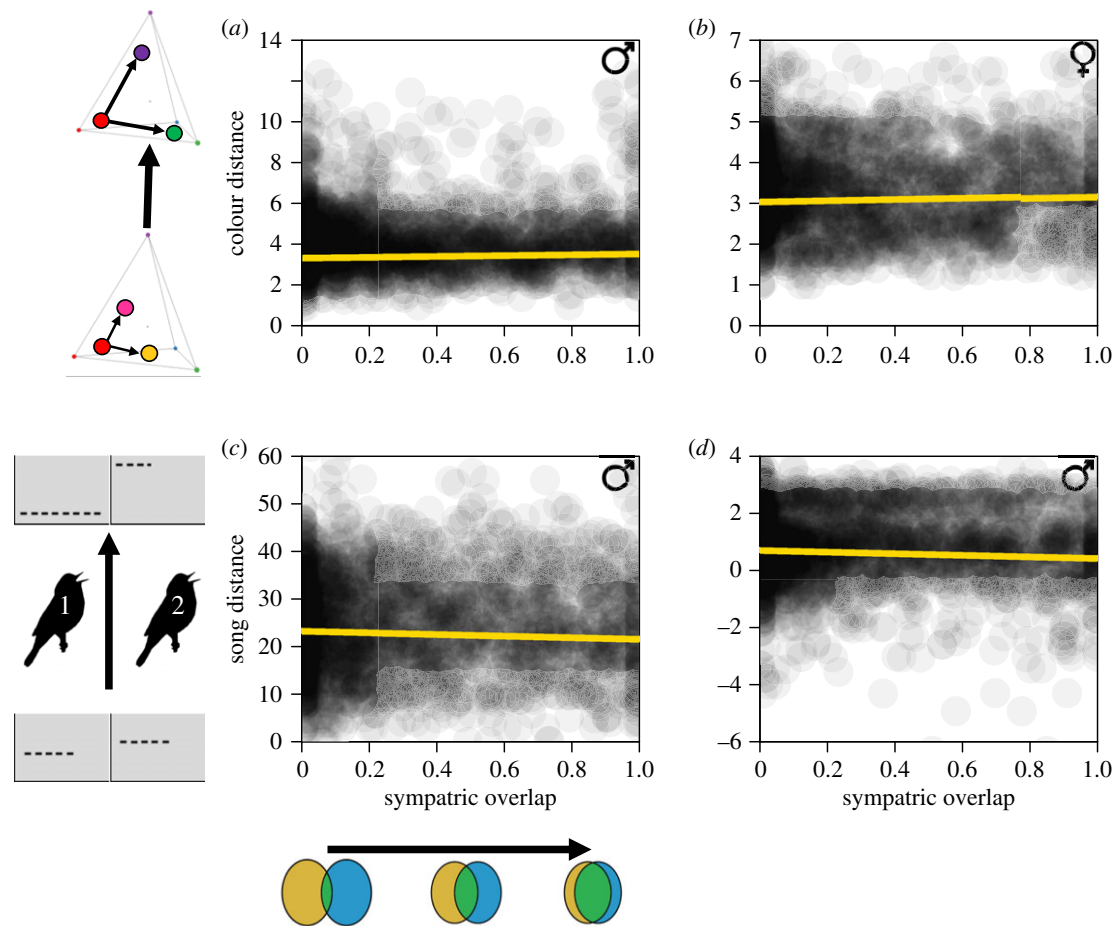
Figure 2. Degree of sympatric overlap predicts colour divergence in both male and female wood-warblers and song convergence in male wood-warblers, among sympatric species pairs only. As the degree of sympatric overlap between species increases, (a) male whole-body colour distance increases, (b) female whole-body colour distance increases, (c) male song bandwidth distance (square root transformed) decreases and (d) male song syllable rate distance (log transformed) decreases. Individual points (shaded grey circles) represent species pairs and the trendlines (gold lines) depict results from linear mixed models (electronic supplementary material, tables S6 and S7). Increasing colour distances are depicted in avian JND-colour space along the y-axis in (a), and example song differences are illustrated via pairs of sonograms that are increasingly different along the y-axis in (c). Finally, depictions of increasing sympatric overlap is under the x-axis in (c), showing two ranges (in yellow and blue) and their overlap (in green). (Online version in colour.)

coloration and under-body coloration (electronic supplementary material, table S4). Conversely, sympatric species pairs exhibited more divergent flight feather coloration in males only (electronic supplementary material, table S4). Male and female upper-body colour divergence and female flight feather colour divergence were not different between allopatric and sympatric species pairs (electronic supplementary material, table S4). All measures of male and female plumage colour divergence were positively related to phylogenetic distance (electronic supplementary material, table S4), such that more distantly related species pairs had more divergent coloration.

Allopatric species also had higher male song divergence than sympatric species pairs. Specifically, allopatric species pairs had more divergent song duration and minimum song frequencies, and less correlated songs (electronic supplementary material, table S5). Sympatric species pairs had more divergent song syllable rates (electronic supplementary material, table S5). Other male song variables were not different between allopatric and sympatric species pairs (electronic supplementary material, table S5). Song duration, minimum frequency, maximum frequency, entropy and syllable rate were positively related to phylogenetic distance (electronic supplementary material, table S5), such that more distantly related species pairs had more divergent songs. Male song bandwidth was not related to phylogenetic distance

(electronic supplementary material, table S5). Finally, male song cross-correlation was negatively related to phylogenetic distance (electronic supplementary material, table S5), such that more distantly related species pairs had more different songs (i.e. less correlated songs).

(b) Degree of sympatry and phylogenetic distance versus colour and song divergence in sympatric species

Across sympatric wood-warblers, both the degree of sympatry and phylogenetic distance predicted male and female plumage colour divergence. Species pairs with greater sympatric overlap exhibited more divergent male and female whole-body coloration (figure 2a,b), male head coloration and female under-body coloration (electronic supplementary material, table S6). Colour divergence in other body regions was not related to the degree of sympatry (electronic supplementary material, table S6). Additionally, all measures of male and female colour divergence, except for female head colour, were positively related to phylogenetic distance (electronic supplementary material, table S6).

Across sympatric wood-warblers, song divergence was negatively related to the degree of sympatry and positively
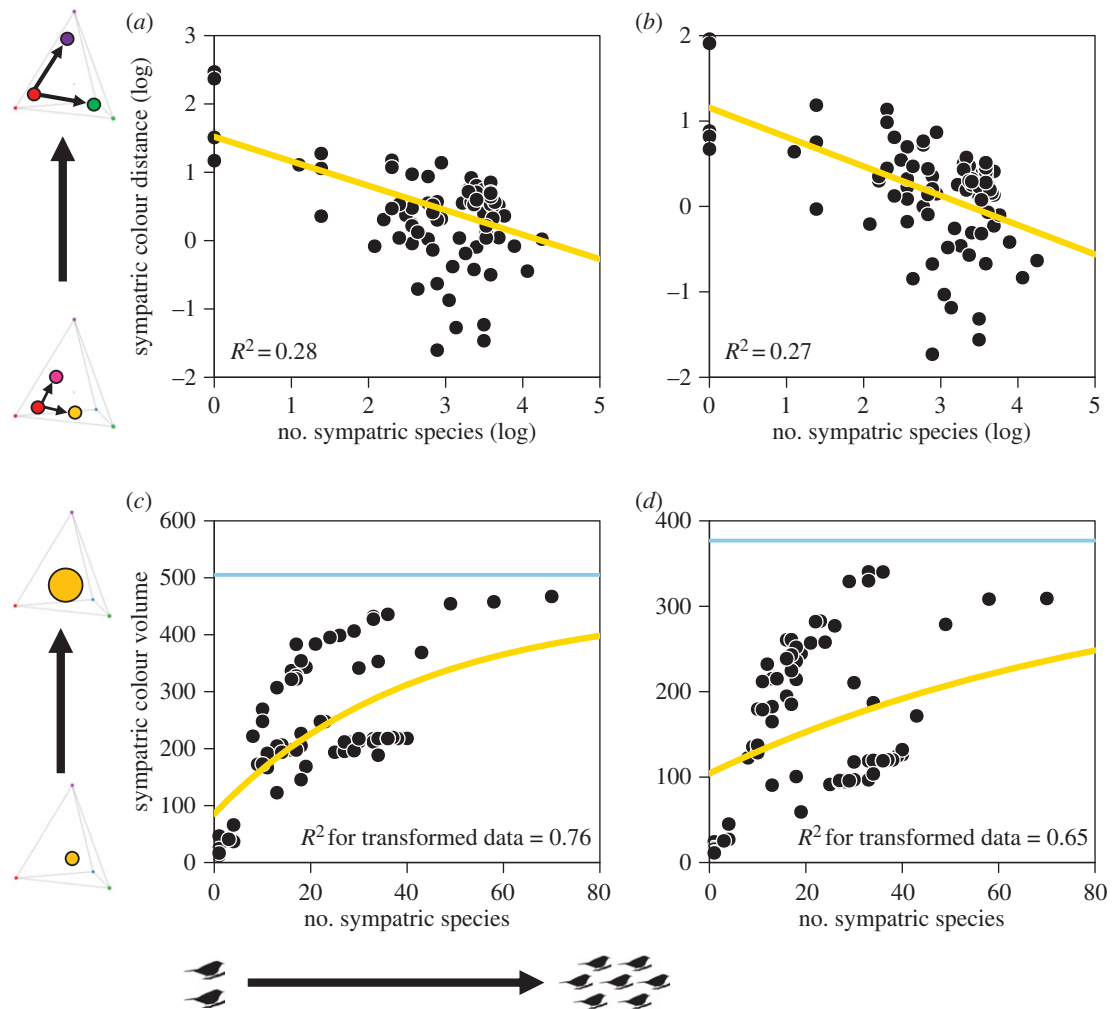
Figure 3. As the number of sympatric species increases, (a) male sympatric colour divergence decreases and (b) female sympatric colour divergence decreases. Additionally, (c) male sympatric colour volume and (d) female sympatric colour volume are positively related to the number of sympatric species. For (a) and (b), gold lines represent the trendline from PGLS analyses. The relationships between (c) male and (d) female sympatric colour volume and number of sympatric species (estimated by gold lines) depict the raw data to illustrate how as sympatric colour volume approaches the maximum warbler colour volume per sex (horizontal blue line), the relationship plateaus. The data were log transformed for PGLS analyses. Each point in (a) and (b) represents a sympatric colour distance (electronic supplementary material, figure S1a), while each point in (c) and (d) represents a sympatric colour volume (electronic supplementary material, figure S1c). The same example colour distances from figure 2a are depicted along the y-axis in (a). Increasing colour volumes depicted along the y-axis of (c). Number of sympatric species depicted by warbler silhouettes along x-axis of (c). (Online version in colour.)

related to phylogenetic distance. Species pairs with greater sympatric overlap had males with less divergent song bandwidth and syllable rate (electronic supplementary material, table S7; figure 2c,d). Other male song variables were not related to the degree of sympatry (electronic supplementary material, table S7). Male song minimum frequency, maximum frequency, bandwidth, entropy and syllable rate divergence were positively related to phylogenetic distance (electronic supplementary material, table S7). Male song duration was not related to phylogenetic distance (electronic supplementary material, table S7). Finally, male song cross-correlation was negatively related to phylogenetic distance (electronic supplementary material, table S7), such that more distantly related species pairs had more different songs.

(c) The influence of the number of sympatric species on sexual signal divergence and diversity

The number of sympatric species negatively predicted sexual signal divergence in wood-warblers. Specifically, male warbler sympatric colour distance was negatively related to the

number of sympatric species (PGLS: $F_{1,83} = 31.76$, $p < 0.001$, $\lambda = 0.59$), such that as the number of sympatric species increased, the average colour divergence between those species decreased (figure 3a). Similarly, female sympatric colour distance was negatively related to the number of sympatric species ($F_{1,83} = 29.99$, $p < 0.001$, $\lambda = 0.00$; figure 3b). Male sympatric song distance was negatively related to the number of sympatric species ($F_{1,83} = 5.37$, $p = 0.02$, $\lambda = 0.46$), such that as the number of sympatric species increased, the average song divergence between those species decreased (figure 4a), although this relationship was weaker than the plumage colour relationships.

Across wood-warblers, the number of sympatric species positively predicted sympatric colour volumes and song volumes. Male warbler sympatric colour volume was positively related to the number of sympatric species ($F_{1,83} = 268.6$, $p < 0.001$, $\lambda = 0.38$); that is, as the number of sympatric species increased, male colour volume of sympatric species also increased (figure 3c). Similarly, female warbler sympatric colour volume was positively related to the number of sympatric species ($F_{1,83} = 155.7$, $p < 0.001$, $\lambda = 0.69$). For both sexes, as
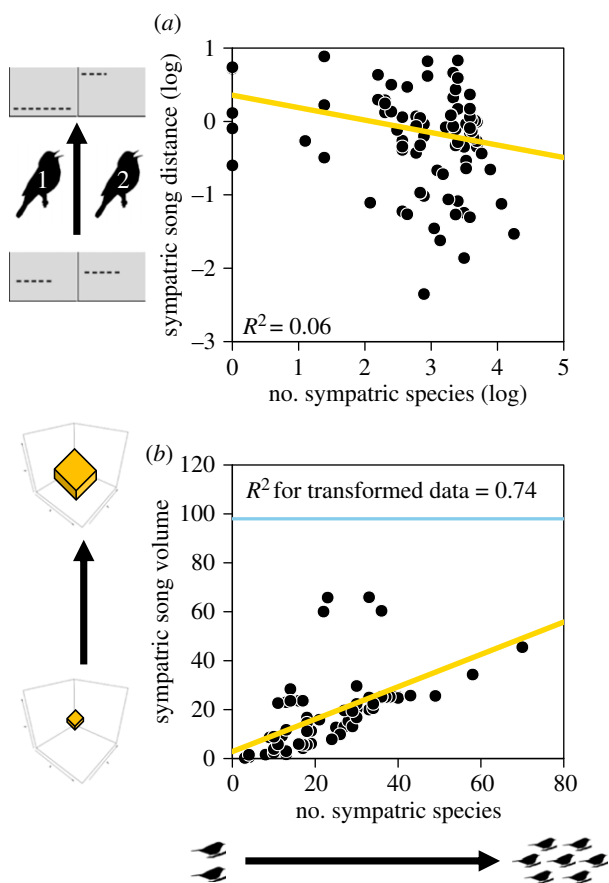
Figure 4. As the number of sympatric species increases, (a) male song divergence decreases. Additionally, (b) sympatric song volume for males is positively related to the number of sympatric species. For (a), gold lines represent the trendline from PGLS analyses. The relationship between sympatric song volume and number of sympatric species depicts the raw data and demonstrates how the relationship (estimated by the gold line) does not change as the sympatric song volume approaches the total warbler song volume (horizontal blue line). The data were log transformed for PGLS analyses. Each point in (a) represents a sympatric song distance (electronic supplementary material, figure S1b), while each point in (b) represents a sympatric song volume (electronic supplementary material, figure S1d). Example song differences are illustrated via pairs of sonograms that are increasingly different along the y-axis in (a). Increasing song volumes depicted along the y-axis of (b). Number of sympatric species depicted by silhouettes along the x-axis of (a). (Online version in colour.)

sympatric colour volume approached the total wood-warbler colour volume, the relationships between the number of sympatric species and sympatric colour volume plateaued (figure 3c,d). Male warbler sympatric song volume also had a positive relationship to the number of sympatric species ($F_{1,78} = 226.10$, $p < 0.001$, $\lambda = 0.56$); as the number of sympatric species increased, the male song volume of sympatric species increased. However, male warbler sympatric song volume did not exhibit the same logarithmic relationship as sympatric colour volume (figure 4b).

4. Discussion

Our findings suggest that sympatry influences signal evolution in wood-warblers. We found allopatric species have more divergent sexual signals than sympatric species. Among sympatric species, however, increased sympatric

overlap predicted increased divergence in male and female plumage coloration, but decreased divergence in male song. Additionally, our data demonstrate that the number of sympatric species constrains sexual signal divergence: higher numbers of sympatric species result in reduced average signal divergence. Overall, our results provide insight into the complex relationship between sympatry and signal evolution, and uniquely demonstrate that the number of species in sympatry impacts sexual signal evolution.

To our knowledge, we provide the first evidence for sexual signal evolution in multiple signal modalities related to sympatry, though not always in the predicted direction nor by the predicted evolutionary mechanisms. Our finding that the degree of sympatric overlap is positively related to plumage colour divergence among sympatric species expands upon the small number of comparative studies supporting the hypothesis that sympatry drives increases in sexual signal divergence [8–10]. While our analyses demonstrated that other factors, such as genetic drift, may contribute to colour divergence, it is not surprising that signal evolution should be driven by multiple selection pressures [19,20]. Therefore, the reported relationships between signal divergence and sympatric overlap demonstrate how sympatry, above and beyond other selection pressures, influences signal evolution, especially since sympatric overlap and phylogenetic distance were not related among sympatric species.

Interestingly, our comparison of plumage divergence between sympatric and allopatric species ran contrary to our predictions and other prior work within a species and across bird families [6,9]. Across all warblers, we found that allopatric species exhibited greater plumage divergence than sympatric species in both sexes. We speculate that these results demonstrate the effect of habitat divergence on plumage colour evolution. Species that occur in allopatry likely do not share similar habitats, while those within sympatry likely do (i.e. light environment, visual background, predatory species), though future work is needed to confirm this hypothesis. Therefore, species within a similar habitat should be selected to optimize colour signal conspicuousness, colour crypsis, or both in similar ways [3].

Our study also presents novel evidence that the number of sympatric species constrains the extent to which sympatry can drive signal divergence. In wood-warblers, as the number of sympatric species increased, sexual signal divergence between sympatric species decreased and for plumage coloration, we found this negative relationship to be explained by constraints on avian perceptual space and wood-warbler colour diversity. The types of colour production mechanisms found in wood-warblers typically produce a specific set of colours, such as the red to yellow continuum found in carotenoid pigments [13], which is likely a primary driver of limitations in wood-warbler colour diversity. However, even when considering all possible colour production types in birds, avian colour diversity only occupies a small fraction of avian colour space (i.e. ca 30% of the colours birds can see) [12]. Wood-warbler plumage coloration occupies only 3.7% of avian perceptual colour space, and therefore represents only 12.6% of total avian colour diversity (calculated from [12]). Thus, while the degree of sympatric overlap does drive increased male sexual signal divergence in wood-warblers, the number of sympatric species dampens the effect of sympatry on colour divergence such that the effect plateaus at high numbers of sympatric species. This dampening effect suggests that the

influence of sympatry on sexual signal divergence estimated in our study is likely conservative. Further, variation in the number of sympatric species could mask the influence of sympatry on sexual signal divergence in other studies (e.g. [39]). Therefore, we strongly recommend that future studies account for the number of species in sympatry when testing the effect of sympatry on signal divergence.

Our results for male song divergence were opposite to our predictions: among sympatric species pairs, those with greater sympatric overlap had less divergent song syllable rates and bandwidth. As with plumage divergence, however, song divergence was greater in allopatric species compared to sympatric species across all warbler species pairs. While song divergence, like colour, was also influenced by other factors like genetic drift, we posit that the negative relationship between sympatry and song divergence is a consequence of the acoustic adaptation hypothesis [40]. In other words, species that exhibit higher degrees of sympatric overlap likely occur in more similar habitats, and these habitats are driving song evolution so that songs are optimally transmitted within the local environment [40], though again, future work in wood-warblers is needed to confirm this idea. While we used a similar explanation to explain our colour divergence results between allopatric and sympatric species pairs, unlike colour, song divergence still exhibited a negative relationship with sympatric overlap, even among sympatric species pairs only. Therefore, we suspect that sympatry is not directly driving song evolution in wood-warblers but is indirectly related to song evolution due to shared habitats among sympatric species.

Further, as with our colour results, we found that the number of sympatric species is negatively related to song divergence, though the relationship was not as strong. Contrary to our colour results, however, we found no evidence that sympatric song volume approached the total song volume of wood-warblers. While some aspects of song are constrained by physiology or morphology [14,15], the temporal properties of songs add a dimension for song diversity not present in wood-warbler colour signals. Additionally, temporal song features might be less constrained than frequency characteristics, since some birds are known to take mini-breaths between syllables within a song, thereby allowing them to sing very long songs [41]. Therefore, further work is needed to understand why the number of sympatric species does still seem to limit song divergence.

Sexual signal diversity has long interested biologists, and speciation has often been implied as an important mechanism in this process [5]. Our results demonstrate a two-layered explanation for plumage colour evolution in wood-warblers: first, allopatric species are more colour divergent than sympatric species, likely due to habitat differences, and second, among sympatric species, sympatric overlap predicts plumage colour divergence. We also offer evidence that song evolution is likely driven by shared habitat occurring via sympatry. Our results also demonstrate that testing for a relationship between the degree of sympatric overlap and sexual signal divergence alone does not capture the entire picture, because the number of sympatric species can significantly limit sexual signal divergence. Therefore, we strongly encourage future work investigating the relationship between sympatry and signal divergence to also test the influence of the number of sympatric species, so that we can better understand how sympatry drives diversity in sexual signals.

References

1. West-Eberhard MJ. 1983 Sexual selection social competition and speciation. *Q. Rev. Biol.* **58**, 155–183.

2. Dobzhansky T. 1941 *Genetics and the origin of species*. 2nd edn. New York, NY: Columbia University Press.

3. Endler JA. 1992 Signals, signal conditions, and the direction of evolution. *Am. Nat.* **139**, S125–S153. (doi:10.1086/285308)

4. Marchetti K. 1993 Dark habitats and bright birds illustrate the role of the environment in species divergence. *Nature* **362**, 149–152.

5. Coyne JA, Orr HA. 2004 *Speciation*. Sunderland, MA: Sinaeur Associates, Inc.

6. Saetre GP, Moum T, Bures S, Kral M, Adamjan M, Moreno J. 1997 A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature* **387**, 589–592. (doi:10.1038/42451)

7. Höbel G, Gerhardt HC. 2003 Reproductive character displacement in the acoustic communication system of green tree frogs (*Hyla cinerea*). *Evolution* **57**, 894–904. (doi:10.1111/j.0014-3820.2003.tb00300.x)

8. Seddon N. 2005 Ecological adaptation and species recognition drives vocal evolution in neotropical suboscine birds. *Evolution* **59**, 200–215. (doi:10.1111/j.0014-3820.2005.tb00906.x)

9. Martin PR, Montgomerie R, Lougheed SC. 2015 Color patterns of closely related bird species are more divergent at intermediate levels of breeding-range sympatry. *Am. Nat.* **185**, 443–451. (doi:10.1086/680206)

10. Martin PR, Montgomerie R, Lougheed SC. 2010 Rapid sympatry explains greater color pattern divergence in high latitude birds. *Evolution* **64**, 336–347. (doi:10.1111/j.1558-5646.2009.00831.x)

11. Stoddard MC, Prum RO. 2008 Evolution of avian plumage color in a tetrahedral color space: a phylogenetic analysis of new world buntings. *Am. Nat.* **171**, 755–776. (doi:10.1086/587526)

12. Stoddard MC, Prum RO. 2011 How colorful are birds? Evolution of the avian plumage color gamut. *Behav. Ecol.* **22**, 1042–1052. (doi:10.1093/beheco/arr088)

13. Hill GE, Mcgraw KJ (eds) 2006 *Bird coloration volume 1: mechanisms and measurements*. Cambridge, MA: Harvard University Press.

14. Podos J. 2001 Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* **409**, 185–188. (doi:10.1038/35051570)

15. Martin JP, Doucet SM, Knox RC, Mennill DJ. 2011 Body size correlates negatively with the frequency of distress calls and songs of Neotropical birds. *J. Field Ornithol.* **82**, 259–268. (doi:10.1111/j.1557-9263.2011.00329.x)

16. Endler JA. 1980 Natural selection on color patterns in *Poecilia reticulata*. *Evolution* **34**, 76–91. (doi:10.2307/2408316)

17. Lovette IJ, Bermingham E. 1999 Explosive speciation in the New World *Dendroica* warblers. *Proc. R. Soc. Lond. B* **266**, 1629–1636.

18. Shutler D, Weatherhead P. 1990 Targets of sexual selection: song and plumage of wood warblers. *Evolution* **44**, 1967–1977.

19. Brakefield PM. 1990 Genetic drift and patterns of diversity among colour-polymorphic populations of the homopteran *Philaenus spumarius* in an island archipelago. *Biol. J. Linn. Soc.* **39**, 219–237. (doi:10.1111/j.1095-8312.1990.tb00513.x)

20. Simpson RK, Mistakidis AF, Doucet SM. 2020 Natural and sexual selection shape the evolution of colour and conspicuousness in North American wood-warblers (Parulidae). *Biol. J. Linn. Soc.* **130**, 89–100. (doi:10.1093/biolinnean/blaa015)

21. R Development Core Team. 2017 R: a language and environment for statistsical computing, version 3.4.1. Vienna, Austria: R Foundation for Statistical Computing. See http://www.R-project.org/.

22. Maia R, Gruson H, Endler JA, White TE. 2019 pavo 2: New tools for the spectral and spatial analysis of colour in R. *Methods Ecol. Evol.* **10**, 1097–1107. (doi:10.1111/2041-210X.13174)

23. Bloch NI. 2015 Evolution of opsin expression in birds driven by sexual selection and habitat. *Proc. R. Soc. B* **282**, 20142321. (doi:10.1098/rspb.2014.2321)

24. Vorobyev M, Osorio D. 1998 Receptor noise as a determinant of colour thresholds. *Proc. R. Soc. Lond. B* **265**, 351–358. (doi:10.1098/rspb.1998.0302)

25. Maia R, White TE. 2018 Comparing colors using visual models. *Behav. Ecol.* **29**, 649–659. (doi:10.1093/beheco/ary017)

26. Najar N, Benedict B. 2015 Female song in New World wood-warblers (Parulidae). *Front. Ecol. Evol.* **3**, 139. (doi:10.3389/fevo.2015.00139)

27. BirdLife International and NatureServe. 2012 Bird species distribution maps of the world. Cambridge, UK: BirdLife International and Arlington, VA: NatureServe.

28. Bivand R, Rundel C, Pebesma E, Stuetz R, Hufthammer KO, Giraudoux P, Davis M, Santilli S. 2020 Interface to Geometry Engine - Open Source ('GEOS'), version 0.5-5. See https://r-forge.r-project.org/projects/rgeos/.

29. Bivand R, Keitt T, Rowlingson B, Pebesma E, Summer M, Hijmans R, Rouault E, Warmerdam F, Ooms J, Rundel C. 2020 Bindings for the 'Geospatial' Data Abstraction Library, version 1.5-18. See http://rgdal.r-forge.r-project.org/.

30. Chesser RT, Zink RM. 1994 Modes of speciation in birds: a test of Lynch's method. *Evolution* **48**, 490. (doi:10.2307/2410107)

31. Barraclough TG, Vogler AP. 2000 Detecting the geographical pattern of speciation from species-level phylogenies. *Am. Nat.* **155**, 419–434. (doi:10.1086/303332)

32. Lovette IJ *et al*. 2010 A comprehensive multilocus phylogeny for the wood-warblers and a revised classification of the Parulidae (Aves). *Mol. Phylogenet. Evol.* **57**, 753–770. (doi:10.1016/j.ympev.2010.07.018)

33. Paradis E, Claude J, Strimmer K. 2004 APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290. (doi:10.1093/bioinformatics/btg412)

34. Bates D, Mächler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 51. (doi:10.18637/jss.v067.i01)

35. Benjamini Y, Hochberg Y. 1995 Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B.* **57**, 289–300. (doi:10.2307/2346101)

36. Nakagawa S. 2004 A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behav. Ecol.* **15**, 1044–1045. (doi:10.1093/beheco/arh107)

37. Orme D, Freckleton RP, Thomas GH, Petzoldt T, Fritz S, Issac N, Pearse W. 2013 caper: Comparative analysis of phylogenetics and evolution in R. version 0.5.2.

38. Habel K, Grasman R, Gramacy RB, Mozharovskyi P, Sterratt DC. 2019 geometry: mesh generation and surface tessellation. R package version 0.4.5.

39. McNaught MMK, Owens IPFI. 2002 Interspecific variation in plumage colour among birds: species recognition or light environment? *J. Evol. Biol.* **15**, 505–514. (doi:10.1046/j.1420-9101.2002.00431.x)

40. Ey E, Fischer J. 2009 The 'acoustic adaptation hypothesis'—a review of the evidence from birds, anurans and mammals. *Bioacoustics* **19**, 21–48. (doi:10.1080/09524622.2009.9753613)

41. Hartley RS, Suthers RA. 1989 Airflow and pressure during canary song: direct evidence for mini-breaths. *J. Comp. Physiol. A* **165**, 15–26. (doi:10.1007/BF00613795)

42. Simpson RK, Wilson DR, Mistakidis AF, Mennill DJ, Doucet SM. 2020 Sympatry drives colour and song evolution in wood-warblers (Parulidae). Dryad Digital Repository. (https://doi.org/10.5061/dryad.m63xsj410)