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Recurrent selection explains parallel evolution of genomic regions of high relative but low absolute differentiation in a ring species

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Abstract

Recent technological developments allow investigation of the repeatability of evolution at the genomic level. Such investigation is particularly powerful when applied to a ring species, in which spatial variation represents changes during the evolution of two species from one. We examined genomic variation among three subspecies of the greenish warbler ring species, using genotypes at 13 013 950 nucleotide sites along a new greenish warbler consensus genome assembly. Genomic regions of low within-group variation are remarkably consistent between the three populations. These regions show high relative differentiation but low absolute differentiation between populations. Comparisons with outgroup species show the locations of these peaks of relative differentiation are not well explained by phylogenetically conserved variation in recombination rates or selection. These patterns are consistent with a model in which selection in an ancestral form has reduced variation at some parts of the genome, and those same regions experience recurrent selection that subsequently reduces variation within each subspecies. The degree of heterogeneity in nucleotide diversity is greater than explained by models of background selection, but is consistent with selective sweeps. Given the evidence that greenish warblers have had both population differentiation for a long period of time and periods of gene flow between those populations, we propose that some genomic regions underwent selective sweeps over a broad geographic area followed by within-population selection-induced reductions in variation. An important implication of this 'sweep-before-differentiation' model is that genomic regions of high relative differentiation may have moved among populations more recently than other genomic regions.

Keywords: genomic differentiation, greenish warbler, linked selection, *Phylloscopus trochiloides*, selective sweep, speciation

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Introduction

The question of 'How repeatable is evolution?' has captured the interest and imagination of generations of scientists and motivated much empirical and theoretical research (e.g. Gould 1990; Travisano *et al.* 1995; Wichman *et al.* 1999; Wood *et al.* 2005; Conte *et al.* 2012; Meyer *et al.* 2012; Renaut *et al.* 2014; Bauer & Gokhale 2015; Pereira *et al.* 2016; Renaut & Dion-Côté 2016).

Thought experiments and empirical investigations of this question have mostly focused on phenotypic patterns and/or genetic changes in particular genes of interest, and answers appear to depend on the timescale considered. Considering long timescales in the history of life, Gould (1990) wrote that 'any replay of the tape would lead evolution down a pathway radically different from the road actually taken'. On a much shorter time frame, Lenski and others (e.g. Meyer *et al.* 2010) have shown strikingly parallel evolution in replicate laboratory populations of bacteria. On intermediate timescales, groups such as stickleback fish (Colosimo *et al.* 2005), sunflowers (Renaut *et al.* 2014) and copepods (Pereira *et al.* 2016) show strong patterns of parallel evolutionary responses to similar environments.

The rapid development of genomic technology now allows expansion of investigations of the repeatability of evolution to DNA sequences across the whole-genome scale (Lobkovsky & Koonin 2012). When an ancestral species evolves into several differentiated descendent populations, do similar regions of the genome appear to play a key role in differentiation? Alternatively, are the patterns highly unrelated, with little similarity between daughter populations in the regions that display differentiation? Investigations of such questions are most powerful when they involve more than just two populations. Here, we investigate patterns of genomic differentiation in a ring species (Mayr 1942; Cain 1954; Irwin *et al.* 2001c; Irwin & Wake 2016), a situation that allows comparison of the structuring of genomic differentiation at a range of spatial and temporal scales. In a ring species, two terminal forms are reproductively isolated (to a large degree) where they co-occur, but these forms are connected by a long chain of populations encircling an uninhabited area; through this chain, there is a gradient in phenotypic and genetic traits and little if any reproductive isolation. We examine patterns of genomic differentiation between the two terminal forms as well as between each of them and a population halfway along the chain connecting them, and we ask how similar the patterns of differentiation are between the three comparisons.

The ring species under investigation is the greenish warbler (*Phylloscopus trochiloides*) species complex (Fig. 1), which consists of two forms breeding in Siberia (*P. t. viridanus* in the west and *P. t. plumbeitarsus* in the east) and a connecting chain of three subspecies to the south that form a gradient around the uninhabited Tibetan Plateau (*P. t. ludlowi* in the western Himalayas, *P. t. trochiloides* in the central and eastern Himalayas and *P. t. obscuratus* in central China; Fig. 1; Ticehurst 1938; Mayr 1942). Previous genetic and phenotypic analyses (Irwin 2000, 2012; Irwin *et al.* 2001b, 2005, 2008; Alcaide *et al.* 2014) have indicated that there is strong (but not complete) reproductive isolation between *viridanus* and *plumbeitarsus* where they meet in central Siberia, whereas around the southern ring there is little reproductive isolation, although there are indications of some past phases of geographic separation followed by secondary contact. The geographic history of the complex is likely very complicated, given the Pleistocene history of many phases of glaciation cycles, but it is clear from the genetic and phenotypic data that west Siberian *viridanus* expanded into Siberia from

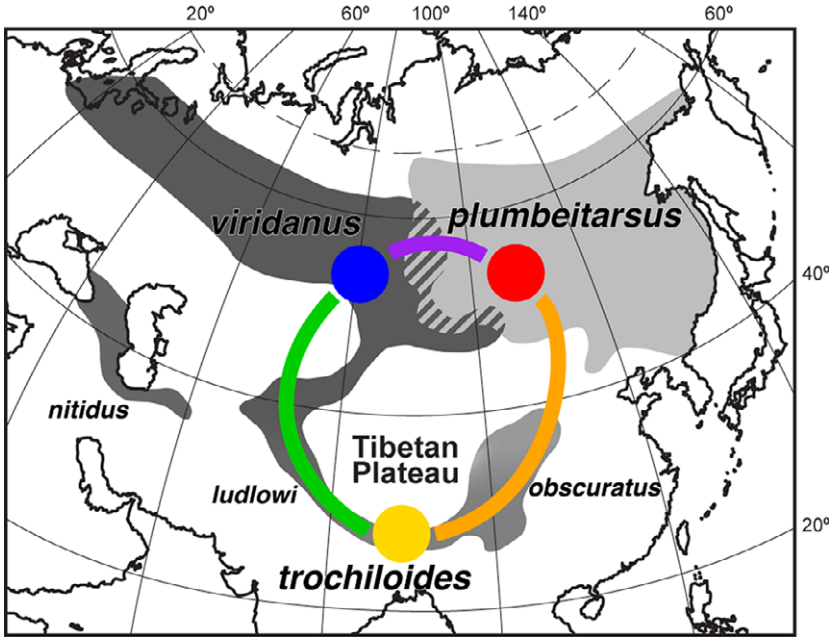

Fig. 1 Map of the greenish warbler range, indicating the three main populations under study, and the comparisons between them. The gradient around the greenish warbler ring is shown in shades of grey, and colours indicate the populations (circles) and population comparisons (lines) shown in subsequent figures (note the green and orange line colours represent population comparisons, not other populations as in previous publications). Names of subspecies are indicated, with the three being compared in larger font.

central Asia (i.e. from the western side of the current ring) and east Siberian *plumbeitarsus* expanded into Siberia from eastern Asia (i.e. from the east side of the current ring).

Phenotypic variation around the ring indicates that there has been a combination of parallel and divergent evolution in different traits during the two northward expansions. Body size, seasonal migration distance and song length have evolved in parallel, with the two Siberian forms (*viridanus* and *plumbeitarsus*) showing strong similarity to each other and both differing in the same way from the southern forms; parallel evolution in these traits is likely due to parallel shifts in habitat and other environmental characteristics during the two northward expansions. In contrast, plumage patterning, migratory routes and song and call structure have evolved strong differences between the Siberian forms. Some of these differences (e.g. plumage and structure of vocalizations) are likely due to the complexities of sexual and social selection in causing highly stochastic patterns of evolution (Irwin 2000, 2012; Irwin *et al.* 2001b, 2008). Hence, based on phenotypic patterns, we have reason to expect some parallel and some divergent selection on the genome.

A number of studies of genomic differentiation between pairs of populations have observed distinct chromosomal regions with much higher relative differentiation (i.e. F_{ST}) than most of the genome (e.g. flycatchers, Ellegren *et al.* 2012; Burri *et al.* 2015; mosquitos, Turner *et al.* 2005; rabbits, Carneiro *et al.* 2014; mice, Harr 2006; butterflies, Nadeau *et al.* 2012); these regions have been referred to as 'genomic islands of speciation', 'genomic islands of differentiation' and 'genomic islands of divergence'. Two primary explanations have been given for such regions (Nachman & Payseur 2012; Cruickshank & Hahn 2014; Fig. 2A and B). First, in a context of speciation with gene flow (Fig. 2A), the islands of high relative differentiation form because they contain loci involved in reproductive isolation (i.e. 'speciation genes') within the hybrid zone between the two populations, causing those loci to have low gene flow between the two populations compared to other parts of the genome (Wu 2001; Nosil *et al.* 2009; Feder & Nosil 2010; Nosil & Feder 2012; Via 2012). Loci in high physical linkage with those speciation genes undergo hitchhiking with the speciation genes, such that an island of differentiation forms, facilitating the build-up of linked loci that contribute further to reproductive isolation. Second, in a model of selection in allopatry (Fig. 2B), islands of high relative differentiation are not directly caused by loci causing reproductive isolation when gene flow is occurring, but rather by loci under directional and/or background selection in one or both populations (Noor & Bennett 2009; Turner & Hahn 2010; Nachman & Payseur 2012; Cruickshank & Hahn 2014; Delmore *et al.* 2015). The selection causes reduced within-population variation at the selected locus as well as areas in close physical linkage; reduced within-population variation tends to be accompanied by greater relative differentiation between populations, as relative differentiation is generally estimated (approximately) as a ratio of between-group nucleotide differentiation to

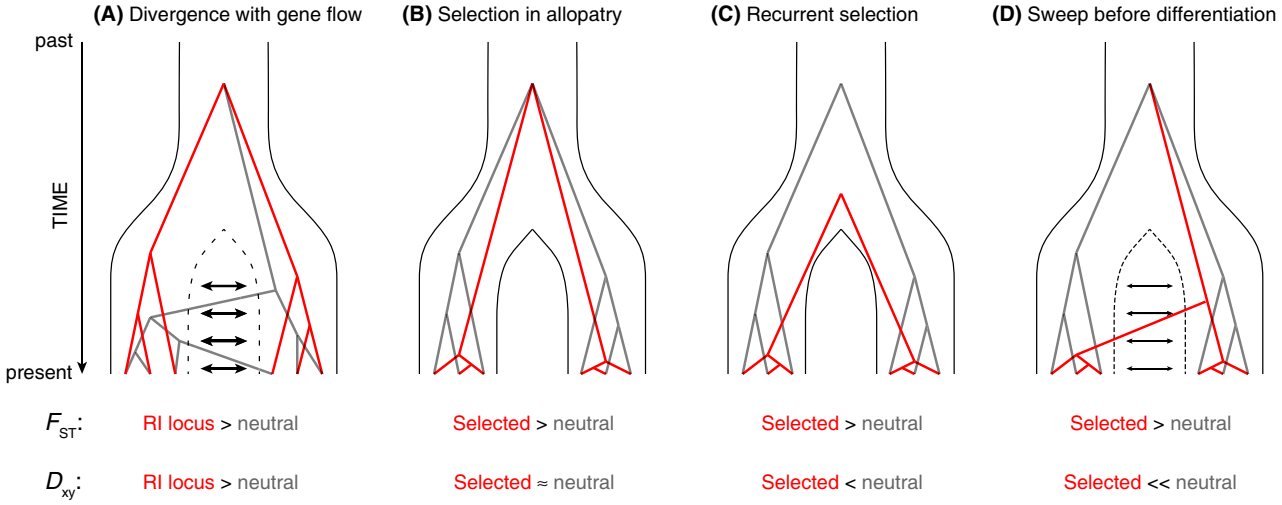

Fig. 2 Depictions of four explanations of how genomic areas of high relative differentiation develop, along with comparisons of the expected levels of relative (F_{ST}) and absolute (D_{xy}) differentiation between selected and neutral parts of the genome. Each panel depicts a history in which a single ancestral population split into two daughter taxa. In A and D there is some amount of migration between the two populations, whereas in B and C there is not. In each scenario, example genealogies of six individuals (three in each taxon) are shown for a selected genomic region (in red; in A, 'RI locus' refers to a locus causing reproductive isolation) and a neutral locus (in grey).

the total nucleotide variation (the sum of between-group and within-group nucleotide variation).

In both models, close physical linkage of genes plays an important role, because linkage reduces recombination between selected loci and nearby neutral loci, preserving the association between a particular set of alleles (Feder & Nosil 2010; Nachman & Payseur 2012). Hence, we expect a relationship between islands of differentiation and areas of low recombination, as observed in sunflowers (Renaut *et al.* 2013) and flycatchers (Burri *et al.* 2015). Just as recombination rates vary across the genome, intensity of selection is expected to vary across the genome, and the combined effect of recombination and selection together influences the amount of heterogeneity in differentiation across the genome (Nachman & Payseur 2012; Renaut *et al.* 2013; Burri *et al.* 2015).

Nachman & Payseur (2012) and Cruickshank & Hahn (2014) proposed a way to distinguish the standard speciation-with-gene-flow model and the selection-in-allopatry model for the formation of peaks of high relative differentiation, by examining patterns of absolute nucleotide differentiation (i.e. D_{xy}) between groups, rather than focusing primarily on patterns of relative differentiation (i.e. F_{ST}). Under the standard speciation-with-gene-flow model (Fig. 2A), D_{xy} is expected to be high in the islands of high F_{ST}, because reproductive isolation due to loci in those regions prevents those regions from flowing between populations, whereas the rest of the genome flows between populations, eventually reducing D_{xy} as variation is stochastically lost through drift. In contrast, under a selection-in-allopatry model (Fig. 2B), absolute differentiation is not expected to be high where relative differentiation is high; rather, increased relative differentiation in areas with loci under selection is due solely to decreased within-group variation (i.e. π). Hence, in our comparisons of patterns of genomic differentiation around the greenish warbler ring, we compare patterns of F_{ST} as well as D_{xy} and π.

Our focus here is on describing and understanding the causes of patterns of genomic heterogeneity in nucleotide diversity and differentiation among three populations of greenish warblers, rather than on questions of overall relationships of populations around the ring, which was described in previous work (e.g. Alcaide *et al.* 2014). We ask several specific questions. First, are there distinct genomic regions of high relative differentiation between populations? Second, are regions of differentiation located in similar regions of the genome in the three pairwise population comparisons? Given that there are two distinct south-to-north geographic clines in greenish warblers (west and east of the Tibetan Plateau), we can compare the patterns of genomic differentiation that have occurred along each. Third, do regions of high relative differentiation display high absolute differentiation (consistent with the standard speciation-with-gene-flow model for the formation of such high-F_{ST} regions)? Alternatively, is high relative differentiation due entirely to low within-group variation (consistent with the selection-in-allopatry model)? To explore whether patterns of nucleotide variation across the genome may be explained in part by variation in mutation rate or recombination rate, we also analyse genomic variation in four outgroup taxa. Finally, we ask whether the Z chromosome (a sex chromosome) shows different levels of within- and between-population variation compared to autosomes, as theory and previous observational studies in other systems suggest that sex chromosomes may differentiate faster and play an especially important role in speciation (Charlesworth *et al.* 1987; Ellegren *et al.* 2012; reviewed by Oyler-McCance *et al.* 2015).

Material and methods

Sampling

We used DNA extracts obtained from blood samples of wild-caught birds. These included 135 samples broadly distributed around the greenish warbler ring; broad genomic relationships among these were previously summarized in Alcaide *et al.* (2014). We also included in the present study four samples of other species related to greenish warblers (*Seicercus whistleri*, *Phylloscopus fuscatus*, *Phylloscopus inornatus* and *Phylloscopus humei*). Of these, *S. whistleri* is most closely related to greenish warblers (despite having a different genus name), and the other three species are in a different clade, with *P. inornatus* and *P. humei* being closely related sister taxa (Johansson *et al.* 2007; Price 2010).

Building a greenish warbler consensus reference genome

For the purpose of mapping GBS (Genotype-By-Sequencing; Elshire *et al.* 2011) reads to a reference genome, we wished to construct a reference genome based on variation throughout the greenish warbler ring, such that our mapping of genetic variation would not be biased towards any one part of the ring. Hence, we chose one individual from each of the three most divergent subspecies around the ring (*viridanus*, bird TL2; *trochiloides*, LN10; and *plumbeitarsus*, BK2), conducted whole-genome shotgun sequencing and de novo assembly on each and then constructed a consensus reference sequence based on those three individuals. Each of the three whole-genome sequencing libraries (one for each reference individual) were run within one lane of an

Illumina HiSeq 2000 automated sequencer at the Next-Gen Sequencing Facility of the Biodiversity Research Centre (University of British Columbia, Vancouver, Canada), producing 26.8 billion base pairs (Gbp) of sequence for *viridanus* (21X coverage assuming a genome size of 1.3 Gbp), 43.2 Gbp for *trochiloides* (33X) and 42.0 Gbp for *plumbeitarsus* (32X).

We trimmed and removed duplicates from each library using TRIMMOMATIC (version 0.32; Bolger *et al.* 2014), using the settings 'TRAILING:3 SLIDINGWINDOW:4:10 MINLEN:30' and FASTUNIQ (version 1.1, default settings, Xu *et al.* 2012), respectively. The Avian Genome Consortium (Zhang *et al.* 2014) recently assembled genomes for 48 birds using SOAPDENOVO (version 1.05, kmer of 27, Luo *et al.* 2012). We used the same settings to obtain de novo assemblies for each of our three greenish warblers ($k = 27$, $d = 1$ and $M = 3$; https://github.com/gigascience/paper-zhang2014/tree/master/Genome_assembly/SOAPdenovo).

Summary statistics for the resulting assemblies are presented in Table S1 (Supporting information). The length of each assembly is similar to other avian genome assemblies (between 1 and 1.2 Gbp; Ellegren 2013). Faircloth *et al.* (2012) identified two sets of ultraconserved elements (UCEs) using whole-genome alignments for the chicken, anole and zebra finch. The first set included 5561 elements; the second was limited to UCEs with higher coverage and included 2560 elements. We aligned these sequences to each genome using NCBI's BLASTn. Results are shown in Table S1 (Supporting information) and show that all assemblies had at least 91% of the first set and 99.5% of the second set.

To order the scaffolds in each de novo assembly and organize into putative chromosomes, we used BWA-MEM (version 0.7.10) to align scaffolds to the repeat-masked version of the zebra finch genome assembly (version 3.2.4; Warren *et al.* 2010), resulting in a high fraction of scaffolds mapping (TL2: 78.4%; LN10: 82.4%; BK2: 80.2%). Given the high synteny of the avian genome between chicken and zebra finch (Warren *et al.* 2010; Ellegren 2013; Kawakami *et al.* 2014), we make the assumption that synteny is also high between zebra finch and the warblers studied here. We imported these alignments into GENEIOUS (version 9), which was used to construct the reference sequence for each chromosome as follows. For each individual, we annotated all regions of the zebra finch reference that had no coverage in the greenish warbler sequences, and then we extracted the consensus sequence, using a consensus threshold of 0% (fewest ambiguities) and with 'If no coverage call Ref' checked. This resulted in a reference genome for each individual, consisting of greenish warbler sequence where there was coverage, and zebra finch sequence where there was not coverage (but with those no-coverage regions annotated). We used the Mauve plug-in (with the ProgressiveMauve algorithm on default settings, except with 'Assume collinear genomes' checked; Darling *et al.* 2010) to align the three individual references to each other, one chromosome at a time. This Mauve alignment was then extracted, and then all regions within each reference sequence that had no greenish warbler sequence coverage (i.e. those annotated as a no-coverage region) were converted to missing bases. We then trimmed the ends of the chromosome alignments consisting of only missing bases, and then extracted the consensus of these three sequences (using consensus threshold of 0%, with 'ignore gaps' checked, and 'If no coverage call N'; these settings meant that the majority base among the three sequences was used; if no majority, a base was randomly chosen from those present; if none present, 'N' was used). This procedure resulted in a reference sequence for each chromosome consisting only of consensus greenish warbler sequence, but of similar length as the zebra finch reference chromosome.

For the great majority of chromosomal regions, the alignment steps in the above procedure appeared to work very well, but for a few small regions (Table S2, Supporting information) the initial alignments in the Mauve step appeared poor and too long, with many large gaps inserted in each sequence such that very little sequence in each individual actually aligned to sequence in other individuals. For these regions, an additional alignment step was added, involving realigning that small region with customized parameters (e.g. adjusting the 'seed weight' in Mauve; Table S2, Supporting information). In every case, a parameter set was found that resulted in a good (and much shorter) alignment.

The consensus greenish warbler genome includes 31 chromosomes or chromosome fragments together totalling 1.05 Gbp in length (we included only whole chromosomes, leaving out sequences aligning to the 'unknown' and 'random' parts of the zebra finch assembly), with 18.4% N's and 40.7% GC content (compared to 9.8% N's and 41.3% GC in the zebra finch assembly).

Mapping of GBS reads and genotyping

Raw GBS reads from Alcaide *et al.* (2014) were reanalysed using an entirely distinct bioinformatic pipeline. These reads were produced by paired-end Illumina sequencing of two libraries, the first ('Plate 1') containing 96 samples broadly distributed around the greenish warbler ring, and the second ('Plate 2') containing 70 samples, 65 of which (39 adults, 25 chicks and 1 duplicate sample for control purposes) were from a single research site (Keylong; site code PA) along the

southwest side of the ring (the other 5 were outgroup species, only 4 of which produced good sequence; see 'Groups in each analysis' below). Alcaide *et al.* (2014) describes details of library preparation and numbers of GBS reads produced (briefly, approximately 3.3 million reads per individual in the first library, and 4.5 million in the second library).

We demultiplexed reads according to the in-line GBS barcode using a custom Perl script that separated reads, removed barcode and adaptor sequence and removed sequences shorter than 30 bp in length. The demultiplexing script allowed no mismatches in the barcode sequence. Reads were then trimmed for base quality using TRIMMOMATIC-0.32 (with options TRAILING:3 SLIDINGWINDOW:4:10 MINLEN:30). We used BWA-MEM (Li & Durbin 2009) on default settings to align trimmed reads to our greenish warbler consensus genome, and the programs PICARD (http://broadinstitute.github.io/picard/) and SAMTOOLS (Li *et al.* 2009) were used to produce BAM files containing the alignments. The program GATK (McKenna *et al.* 2010) was then used to realign reads around indels (using the tools *RealignerTargetCreator*, followed by *IndelRealigner*) and then call genotypes (*HaplotypeCaller*, with options '–emitRefConfidence GVCF –max_alternate_alleles 2 -variant_index_type LINEAR -variant_index_parameter 128000'), resulting in a GVCF file for each individual. Genotyping information from all individuals within an analysis was then combined into a single file for each chromosome using the GATK command *GenotypeGVCFs*, with the option '-allSites' used such that genotypes at both variant and invariant sites were retained, and the option '-L' used to specify the chromosome (data were separated by chromosome at this point, in order to reduce file size and facilitate downstream computational efficiency).

A variety of analyses limited to different sets of individuals were then performed, with the GATK command *SelectVariants* used to choose information for each set of individuals. We then used a combination of VCFTOOLS (Danecek *et al.* 2011) and custom-written scripts to apply a series of filters to determine which sites were included in the analysis: first, indels and SNPs with more than two alleles were removed, to avoid the complicating effects of such variants on the calculation of differentiation statistics. Second, we removed sites where more than 40% of individuals had missing genotypes, to restrict the analysis to sites with data from a substantial portion of individuals. Third, we removed sites with MQ < 20, to avoid poorly mapped reads. Fourth, we removed sites with heterozygosity above 60%, to avoid paralogs. We converted the resulting vcf file to a matrix of genotypes of each individual at each site.

Illustration of genomic relationships around the ring

We used principal coordinates analysis (PCA; using custom scripts in R (R Core Team 2014) version 3.1.2, employing the 'pca' command [from the PCAMETHODS package; Stacklies *et al.* 2007] with method 'svdImpute' to account for missing genotypes) to summarize and visualize genomic relationships among all greenish warbler individuals. We first filtered out any individuals that were missing genotypes at more than 25% of the SNPs in the analysis (this applied to four individuals: AA8, DA6, Liz5118 and Liz5195). We centred but did not scale genotypic values, thereby ensuring that each nucleotide mismatch had equal weighting in the PCA (this means that more variable SNPs have larger influence).

Estimation of differentiation statistics across the genome

A custom script in R was used to estimate summary differentiation statistics and to produce graphs of variation across the genome. First, for each nucleotide site we calculated allele frequencies for each group of individuals defined in an analysis. We used these frequencies to calculate, for each site, both within- and between-group average pairwise differentiation between two individuals. Within-group nucleotide differentiation (π) was calculated as $2p(1-p)$, where p is the frequency of one of the alleles (each nucleotide had either 1 [invariant] or 2 alleles); thus, π ranges from zero to 0.5. Between-group nucleotide differentiation (D_{xy}) was calculated as $p_1(1-p_2) + p_2(1-p_1)$, where p_1 is the frequency of a given allele in the first group and p_2 is the frequency of that allele in the second group; D_{xy} ranges from zero to one.

For sites that were variable, we estimated nucleotide-specific F_{ST} according to Weir & Cockerham's (1984) equation for $\hat{\theta}$ (top of their page 1363). This method assumes random mating within populations, and corrects for two types of sampling bias due to limited sample size: that due to limited sample size of individuals within groups and that due to sampling a limited number of populations out of all possible replicate populations (both real and imagined, under the same evolutionary parameters that the real sampled populations evolved). We used this method to estimate F_{ST} for each nucleotide for each pair of populations included in an analysis, and also to estimate F_{ST} among all groups in an analysis. For sample sizes, we used the numbers of individuals successfully genotyped at that specific nucleotide site in that specific population.

Given our focus on patterns of differentiation across the genome rather than at individual nucleotide sites,

we also calculated averages of these statistics on windows across each chromosome. To ensure that summary statistics for each window were not influenced by differing sample sizes of nucleotide sites within each window, windows were defined based on a fixed number of sites (5000 or 10 000 nucleotides depending on the analysis; see next section) for which we had good genotypic information (i.e. they survived the filtering process described above), rather than a fixed window size across the reference genome. Windows were aligned starting from the side of each chromosome corresponding to the beginning side of that chromosome in the zebra finch genome (i.e. the left side in figures), and summary statistics were not calculated for incomplete window fragments on the other (right) side.

For each window, we calculated mean π for each group and mean D_{xy} for each pair of groups; these statistics incorporate information from both variant and invariant sites. We also estimated multilocus F_{ST} for each window by summing the numerators of the $\hat{\theta}$ equation across sites and then dividing by the sum of the denominators of the same equation across sites (Weir & Cockerham 1984).

Two analyses: greenish warblers and outgroup species

Our study employed two major analyses of differentiation within and between populations. In the first, we included 15 individuals from each of the three major greenish warbler taxa (*viridanus*, *trochiloides*, *plumbeitarsus*; Table S3, Supporting information). Individuals were chosen for inclusion in these groups prior to examining the genotypic data; they were individuals with high sequence coverage that well represented the core population of that taxon (i.e. far from known hybrid zones, and avoiding those individuals identified by Alcaide *et al.* (2014) as having some chromosome fragments from other taxa). We view this sampling procedure as appropriate because we wanted our analysis to represent differentiation between the core populations of each taxon.

In the second analysis, we included one individual from each of nine taxa (five greenish warblers, of which four are analysed here; and four outgroup species; Table S4, Supporting information). While this is certainly a small sample of each taxon, note (i) that each individual is diploid and thus contains one of each chromosome from each parent, meaning the sample size of chromosomes of each taxon is two, and (ii) a windowed analysis summarizes patterns at thousands of nucleotides in each window, reducing the impact of sampling error on windowed averages. However, because of the increased sampling error compared to the 15-individuals-per-taxon analysis above, and because heterozygosity of individuals tends to be underestimated using low-coverage sequencing data, the magnitude of the π and F_{ST} statistics should not be compared directly between the two analyses. Nevertheless, overall patterns of variation within and among greenish warbler populations were very similar across chromosomes in the two analyses, indicating that our comparisons with outgroup species are also valid, and data from one individual per taxon were sufficient to recover strong correlations in within-group variation observed across the genome between *viridanus*, *trochiloides* and *viridanus* using larger sample sizes (see Results).

Window size was set at 5000 nucleotide sites in the first (45-sample) analysis (corresponding to an average of 144 SNPs per window), and 10 000 in the second (9-sample) analysis (due to the greater influence of noise in the second).

Results

Mapping of GBS reads to the reference greenish warbler genome resulted in the identification of 580 356 single nucleotide polymorphisms among 135 greenish warbler samples. Whole-genome relationships, as summarized using principal components analysis (PCA; Fig. S1, Supporting information), show the pattern expected based on previous research (Alcaide *et al.* 2014; see also Bradburd *et al.* 2016), of two highly distinct Siberian forms (*viridanus* and *plumbeitarsus*) and a progression of genomic signatures through the ring of populations to the south. Note that Alcaide *et al.* (2014) summarized variation in the same GBS reads, but used an entirely distinct bioinformatics pipeline and based their PCA on only 2334 SNPs due to very restrictive filtering; the fact that the current study recovers similar patterns using much less restrictive filtering and approximately 250 times the number of SNPs gives strong confidence in the inferred relationships. We use these overall genomic relationships as a backdrop to explore patterns of variation in relative and absolute differentiation across the genome.

We first examined differentiation between three major geographic groups around the ring: *viridanus* in west Siberia, *trochiloides* in the south and *plumbeitarsus* in east Siberia. To ensure that variation in sample size did not influence comparisons of patterns among these taxa, we chose 15 individuals of each taxon to include in an analysis of between-group relative nucleotide differentiation (F_{ST}), between-group absolute nucleotide differentiation (D_{xy}) and within-group nucleotide variation (π). To estimate absolute nucleotide differentiation, we used our GBS reads to identify invariant nucleotide sites as well as variant ones, resulting in a data set of

12 639 111 invariant and 374 839 variant nucleotide sites among our 45 samples in the analysis, and calculated statistics in windows across each chromosome. We show results first for a single chromosome (Fig. 3), and then for the entire genome (Fig. 4).

Relative differentiation shows tremendous variability across the genome, with most chromosomes having one to several distinct islands of high F_{ST} compared to a background of much lower F_{ST} elsewhere. Remarkably, the locations of peaks of high F_{ST} are highly similar in all pairwise comparisons among the three taxa (Figs 3–5; see statistical tests in caption of Fig. 5). The Z chromosome on average shows much higher levels of F_{ST} than the autosomes (e.g. mean F_{ST} among windows in

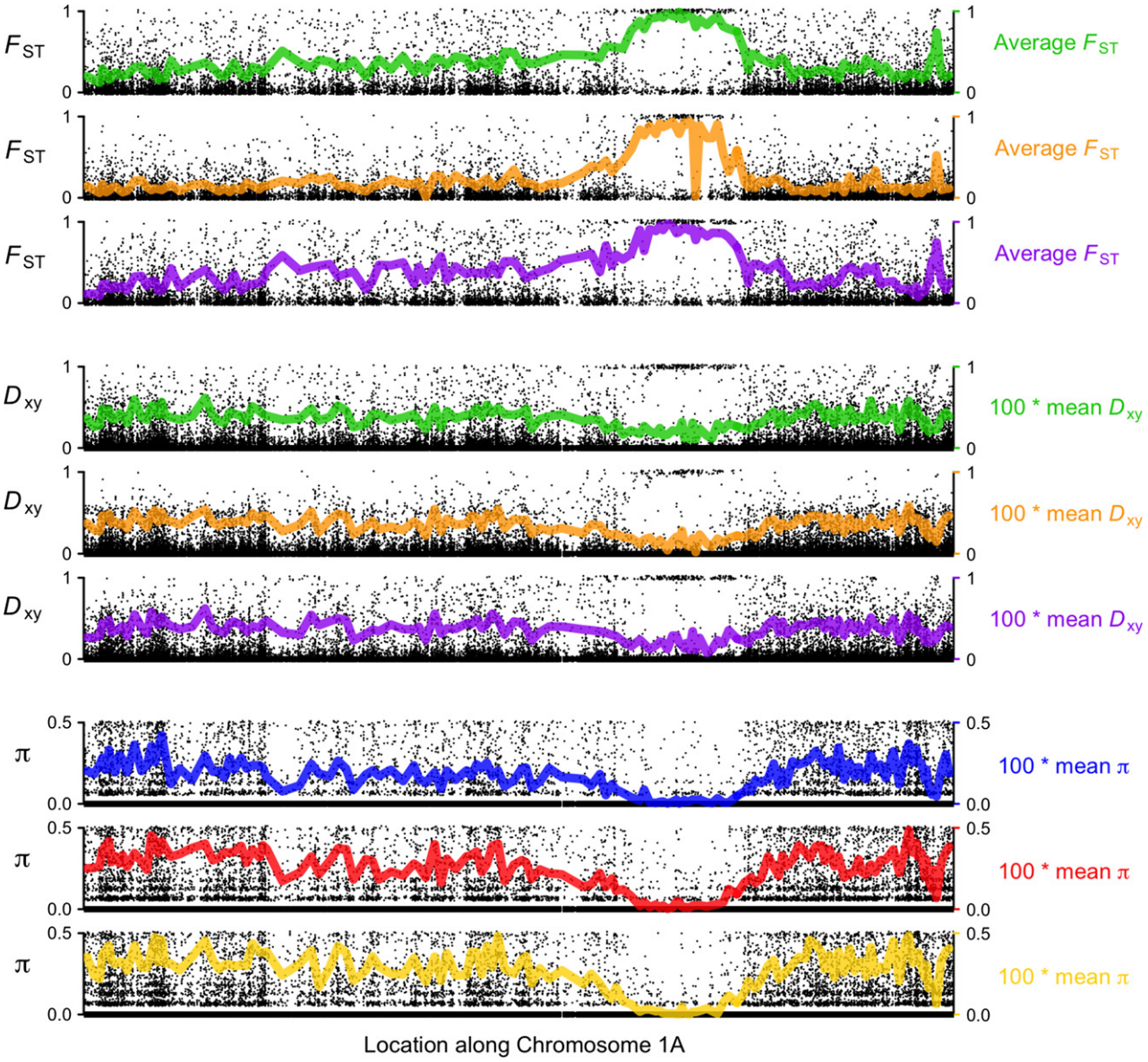

Fig. 3 Nucleotide differentiation across chromosome 1A shows a region of consistently strong relative differentiation (F_{ST}, top) and low absolute differentiation (D_{xy}, middle) between greenish warbler populations, as well as extremely low within-population variation (π, bottom). Each graph shows per-nucleotide statistics for 647 363 nucleotides, 24 764 of which are variable among a data set of 15 *viridanus*, 15 *trochiloides* and 15 *plumbeitarsus*, and coloured lines show windowed averages (5000 nucleotide sites per window; averages for D_{xy} and π are multiplied by 100, to better use the vertical axis). The top three graphs show F_{ST} (only defined for variable markers) between *trochiloides* and *viridanus* (top, green), *trochiloides* and *plumbeitarsus* (middle, orange) and *viridanus* and *plumbeitarsus* (bottom, purple). The middle three graphs show D_{xy}, using the same population comparisons as above. The lower three graphs show π within *viridanus* (top, blue), *plumbeitarsus* (middle, red) and *trochiloides* (bottom, yellow). Note that a small amount of jittering (2.5% of each vertical axis) was added to individual nucleotide statistics (i.e. the black dots). This chromosome is 76 983 199 bp long.

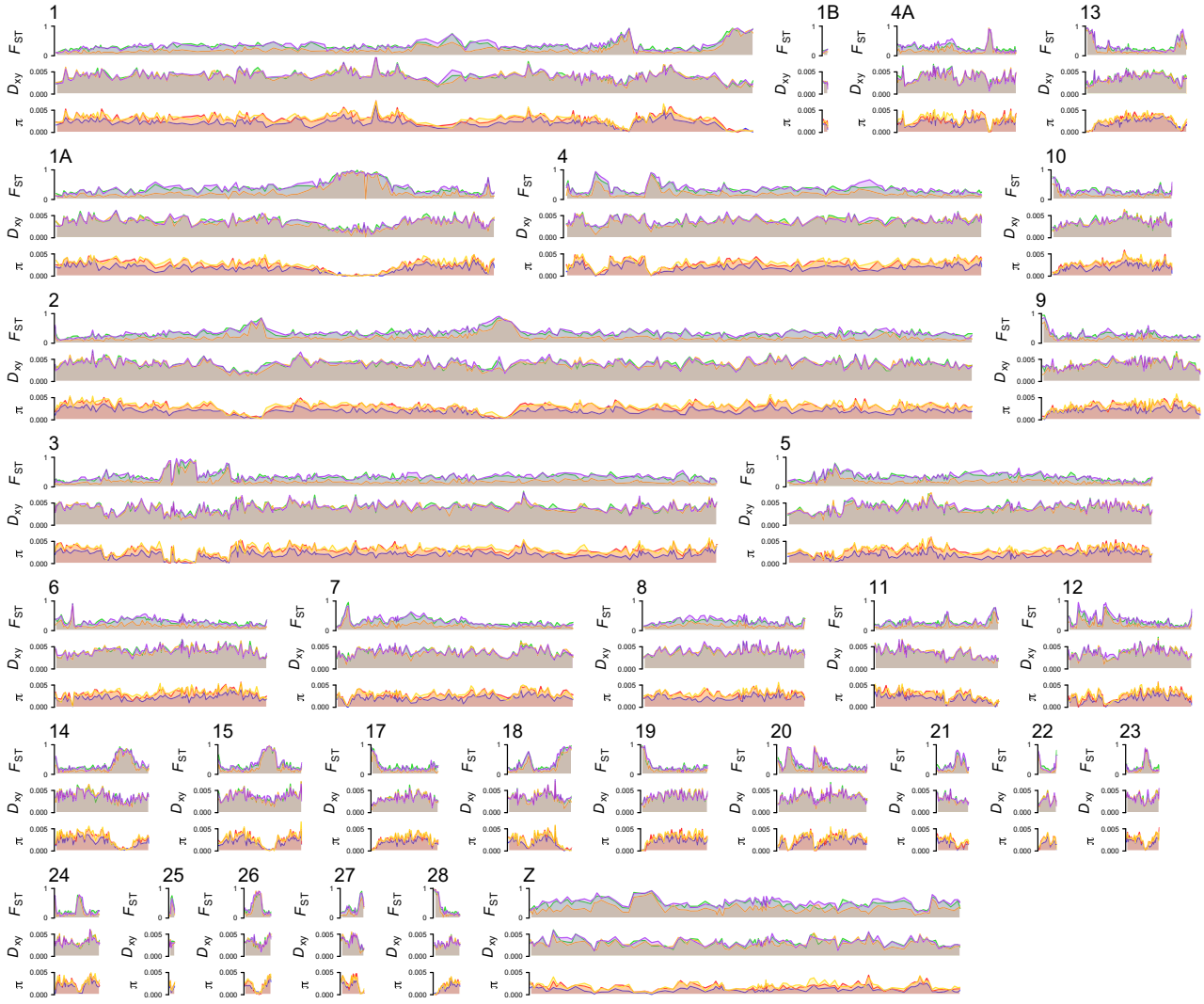

Fig. 4 Nucleotide differentiation shows consistent patterns of strong genomic structuring within and between greenish warbler populations. For each chromosome, graphs show variation in per-window relative nucleotide differentiation (F_{ST}, top), absolute nucleotide differentiation (D_{xy}, middle) and within-group nucleotide variation (π, bottom), using the same colours (3 often overlapping lines for each small plot) for particular population comparisons (for F_{ST} and D_{xy}) and populations (for π) as in Figs 1 and 3.

the comparison of *viridanus* and *plumbeitarsus*: 0.33 for autosomes vs. 0.52 for Z chromosome; Welch's *t*-test: $t = -12.1$, df = 115.9, $P < 10^{-15}$); for this reason, we focus on autosomes first, and return to the Z chromosome later.

Surprisingly, regions of high relative differentiation usually have low absolute differentiation (D_{xy}; Figs 3, 4 and 6), and this is consistent among all comparisons (Spearman rank correlations: *trochiloides* vs. *viridanus*: $P < 9.4*10^{-9}$; *trochiloides* vs. *plumbeitarsus*: $P < 10^{-15}$; *viridanus* vs. *plumbeitarsus*: $P = 0.0096$; $n = 2486$ windows). This is partly explained by the remarkably low within-group nucleotide diversity (π) in these genomic regions (Fig. 7). Regions of low π have strikingly similar locations in all three taxa (Figs 3, 4 and 8a). Moreover, these regions have much lower within-group diversity than would be proportional to the reduced between-group absolute differentiation alone: in Fig. 8b, we show that regions with a low ratio of π to D_{xy}, which we call 'standardized nucleotide diversity', are consistent among all three greenish warbler taxa. Hence, the regions of high F_{ST} have lower within- and between-group absolute variation than the genomic background, but the ratio of within- to between-group absolute variation is especially low. The fact that these regions are consistent among all three taxa is an indication that common processes in the different greenish warbler populations have influenced these patterns. It should be noted that the three pairwise F_{ST} comparisons share some statistical nonindependence, as each comparison

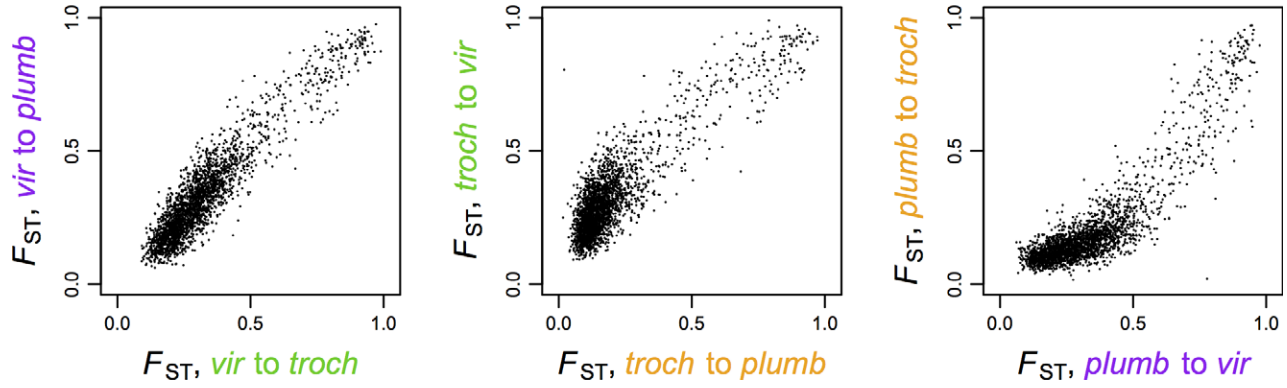

Fig. 5 Per-window relative differentiation (F_{ST}) between each pair of greenish warbler populations is strongly correlated with that between each of the other pairs. Each graph is a bivariate plot of F_{ST} between one pair of populations vs. F_{ST} between a second pair, with each dot representing one of 2486 windows across the autosomal genome, each consisting of 5000 nucleotide sites. Within each comparison, there is a dense low-F_{ST} cluster containing the great majority of windows, and a long string of higher-F_{ST} windows. These tend to have high F_{ST} in all three comparisons (Spearman rank correlations: *vir* to *troch* vs. *vir* to *plumb*: $r_s = 0.876$; *troch* to *plumb* vs. *troch* to *vir*: $r_s = 0.697$; *plumb* to *vir* vs. *plumb* to *troch*: $r_s = 0.760$; for each, $P < 10^{-15}$).

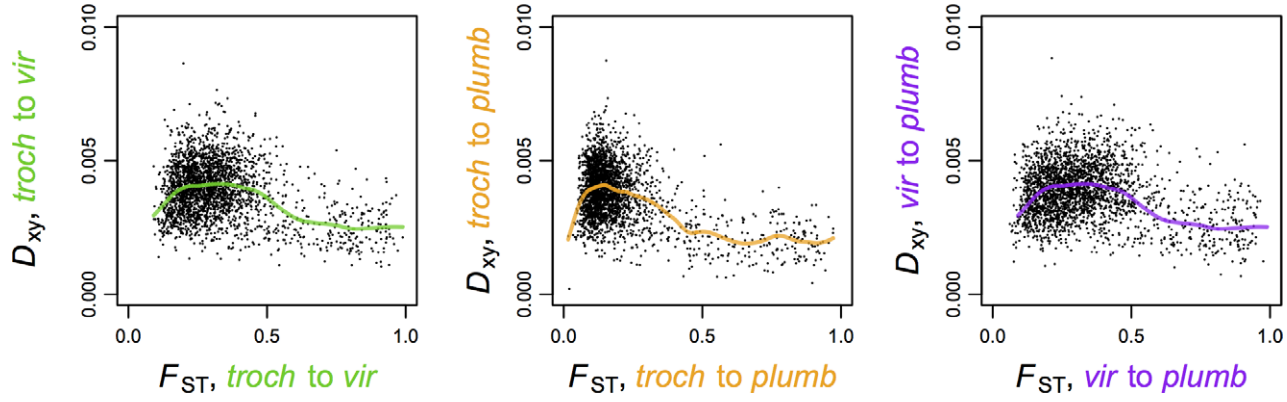

Fig. 6 Genomic areas with high relative differentiation (F_{ST}) tend to have low absolute nucleotide differentiation (D_{xy}). Each graph shows D_{xy} vs. F_{ST} between two greenish warbler subspecies. Each dot represents a single autosomal window containing 5000 nucleotide sites. Each comparison shows a similar pattern of a small subset of the 2486 autosomal windows deviating strongly from the majority, having large relative differentiation (right side of graph) and low absolute variation (low on the graph). Coloured lines show the cubic splines fit of D_{xy} to F_{ST} (with smoothing parameter equal to one, using the 'smooth.spline' function in R). The correlation among all windows is significantly negative in each pair of populations (Spearman rank correlation: *troch* to *vir*, $r_s = -0.115$, $P = 9.4*10^{-9}$; *troch* to *plumb*, $r_s = -0.239$, $P < 10^{-15}$; *vir* to *plumb*, $r_s = -0.052$, $P < 9.6*10^{-3}$).

of two population pairs involves a shared population (e.g. comparisons A-B and B-C both involve population B). Nonetheless, measurements of π apply to a single population and hence do not suffer from this potential problem; the strong similarities among populations in patterns of variation in π across the genome largely explain the strong similarities in F_{ST} among population comparisons.

We considered how variation in mutation rates across the genome might affect these patterns. Regions with reduced mutation rates are expected to show lower between-group absolute differentiation and within-group variation, assuming all else is equal. The effect should be similar (i.e. proportional) on both D_{xy} and π; hence, variation in mutation rate alone among genomic regions would not explain the association between high F_{ST} and low D_{xy}.

Nevertheless, to explore whether differences in mutation rate across the genome might be partly responsible for the shared patterns of variation in D_{xy} among greenish warbler population comparisons (and likewise in shared patterns in π), we compared patterns in greenish warblers with those of four other related species in the same family of Phylloscopidae. To ensure no effects of differing sample sizes on our conclusions, we conducted an analysis using just a single individual from

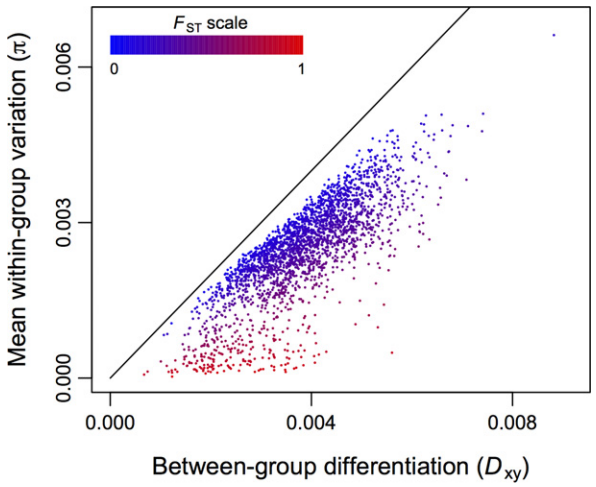

Fig. 7 Autosomal windows with high relative differentiation (F_{ST}; illustrated with increasing red colour, whereas blue indicates low F_{ST}) tend to have low between-group absolute differentiation (D_{xy}) and exceptionally low average within-group variation (π). This graph shows the comparison of *viridanus* to *plumbeitarsus* (15 individuals each), but all other comparisons of greenish warbler populations show similar patterns. The diagonal line shows the 1:1 relationship that would be expected if within-group variation matched between-group differentiation (i.e. with no population differentiation).

each of nine taxa (four more distantly related species of Phylloscopidae warblers, plus five greenish warbler subspecies, of which results are presented here for four [*viridanus*, *trochiloides*, *obscuratus* and *plumbeitarsus*]; see Methods for details and justification of why a single sample per taxon is sufficient for this analysis). This analysis identified 11 055 883 invariant and 448 392 variant nucleotide sites among the nine taxa.

We reasoned that if areas of consistently low D_{xy} between pairs of greenish warblers are largely explained by consistently low mutation rates in those regions over evolutionary time, then we would see a strong correlation across the genome between D_{xy} between greenish warbler populations and D_{xy} between distantly related species. We see evidence for only a weak correlation. For example, D_{xy} between *Phylloscopus fuscatus* and *Seicercus whistleri*, two of the most distantly related species in our study, shows only weak correlation with D_{xy} between greenish warbler subspecies (e.g. compared to *viridanus–trochiloides*: $r = 0.14$, $P = 4.7*10^{-6}$), explaining less than 2% of the variation (Fig. S2, Supporting information). In contrast, the correlation in D_{xy} between different pairs of greenish warbler populations is dramatically higher (e.g. *viridanus–trochiloides* compared to *obscuratus–plumbeitarsus*: $r = 0.68$, $P < 10^{-15}$), explaining 47% of the variation (Fig. S2, Supporting information). We conclude that the strikingly similar patterns of variation across the greenish warbler genome in different populations cannot be explained as a result of phylogenetically stable differences in mutation rate across the genome.

Given the strong genomic patterns observed within the greenish warbler complex (genomic regions of consistently low π, moderately low D_{xy} and high F_{ST}), we asked whether another species complex within the same genus displayed similar patterns. *Phylloscopus inornatus* and *humei* are sister species that were only recently recognized to be distinct (Irwin *et al.* 2001a). Like the greenish warblers, these two sister species show a strong correlation among genomic regions in within-taxon diversity (π; Fig. 9). However, there is only a weak correlation in π between the two species complexes across genomic windows (Fig. 9), and when this within-taxon diversity is standardized by the between-taxon differentiation (D_{xy}) within each species complex (thereby controlling for the fact that each taxon necessarily tends to have less diversity at each window than the entire species complex has at that window), the correlation is even weaker (Fig. S3, Supporting information), explaining only 2.2% of the variation. These results suggest that the two species complexes differ strongly in the genomic positions where factors such as low recombination rate and strong selection have caused unusually low within-group variation. Despite this difference, both complexes show similar patterns of regions of high F_{ST} having moderate or low D_{xy} and exceedingly low π (Fig. S4, Supporting information).

Turning to the Z chromosome, recall from above that this sex chromosome shows higher average F_{ST} between greenish warbler populations than the autosomal genome does. To investigate whether this could be due in part to a higher rate of substitution on the Z chromosome, we compared absolute differentiation in the Z and the autosomes between distant species (between *fuscatus* and *whistleri*; and between *trochiloides* and *whistleri*). Results in both cases showed quite similar distributions of D_{xy} in the two chromosome classes (Fig. S5, Supporting information); the first species pair showed no significant difference between the distribution of D_{xy} in the Z and autosomes (*t*-test; $t = -1.66$, df = 1133, $P = 0.096$), and the second rather surprisingly showed a slightly lower mean D_{xy} in the Z (0.0136) than in the autosomes (0.0148) ($t = 2.84$, df = 1133, $P = 0.005$), suggesting a slightly lower substitution rate in the Z.

A graph of absolute differentiation between *viridanus* and *plumbeitarsus* vs. mean within-group variation (Fig. 10) shows similar patterns for the Z chromosome as other high-F_{ST} regions of the genome: D_{xy} and π across the Z chromosome are both significantly low compared to most of the autosomal genome (see stats in caption of Fig. 10). Despite this overall pattern, no windows on the Z chromosome reach the exceedingly

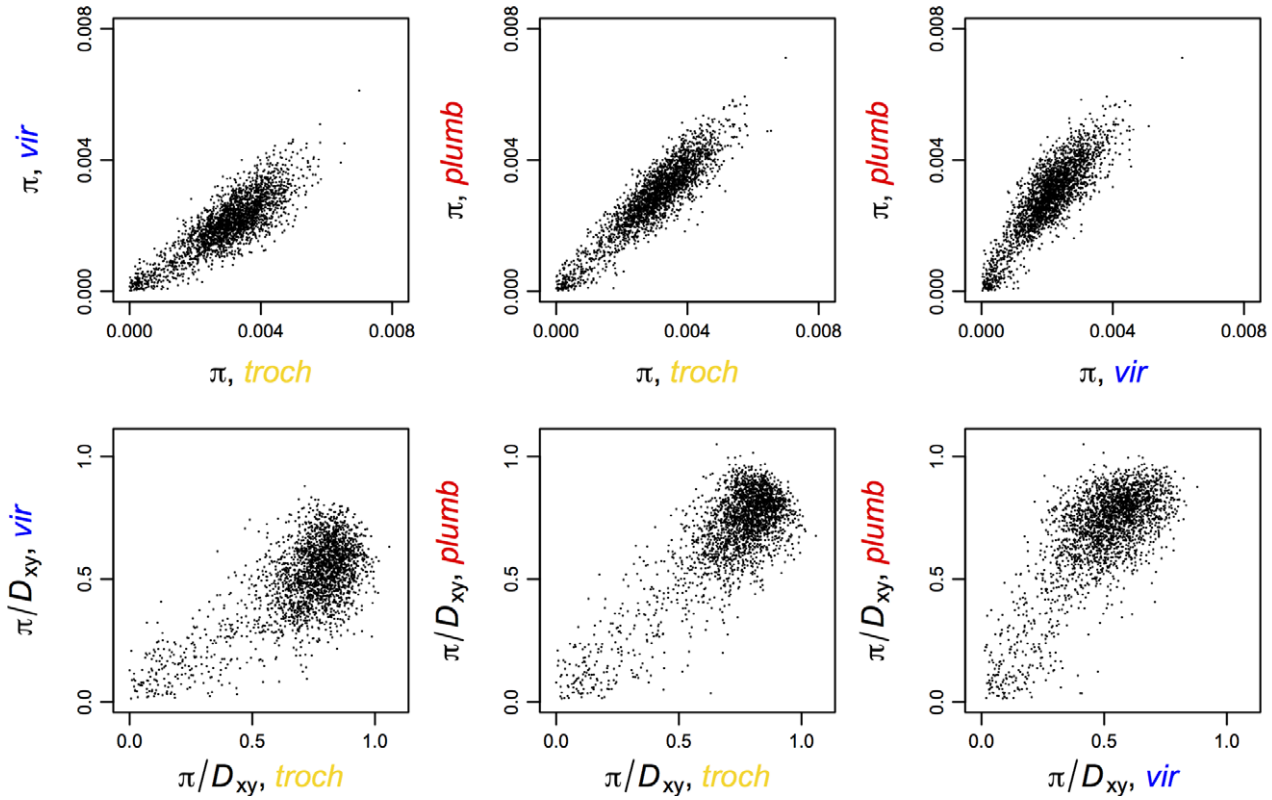

Fig. 8 The three major greenish warbler taxa show consistent pattern of which windows show low within-group nucleotide diversity (π; top row of graphs), even when standardized by between-group absolute differentiation (i.e. the ratio of π to maximum between-group nucleotide diversity; bottom row). Each plot shows the relationship among autosomal windows of π of one taxon to that of another (top), or windowed within-group nucleotide variation (π) divided by the maximum between-group nucleotide differentiation (D_{xy}) out of all three comparisons (bottom). Each dot represents a single autosomal window. Relationships are strong and highly significant (Pearson correlation test, with df = 2484: *troch* vs. *vir*: $r = 0.840$ [top] and 0.691 [bottom]; *troch* vs. *plumb*: $r = 0.907$ and 0.805; *vir* vs. *plumb*: $r = 0.854$ and 0.732; for each, $P < 10^{-15}$). This analysis is based on 15 individuals per group.

low levels of π seen in some parts of the autosomal genome (Fig. 10).

Discussion

Prior knowledge regarding phenotypic variation among greenish warblers led us to expect some combination of parallel and nonparallel patterns of genomic change among greenish warbler populations. But given that even parallel phenotypic changes can in theory be brought about through different changes at the level of the genome, we began this study with the expectation that patterns of genomic differentiation would be highly idiosyncratic, with each population showing its own peculiar patterns in terms of which genomic regions show reduced or inflated within- and between-group variation. In striking contrast to this expectation, regions with reduced within-group nucleotide diversity (π) are remarkably similar in the three focal populations, and these regions are of high between-group relative differentiation (F_{ST}) between all pairs of populations. Moreover, these regions of high F_{ST} tend to have surprisingly low absolute differentiation (D_{xy}) between populations. Overall, these patterns indicate remarkable commonalities in the genomic regions that are subject to recurrent selection in diverse populations of greenish warblers.

By examining how these patterns relate to those within and between more distantly related species of warblers, we have been able to ask whether they may partially be due to factors that are structured across the genome in a relatively constant way over broad spans of evolutionary time. For instance, recombination rate (Renaut *et al.* 2013; Burri *et al.* 2015) and mutation rate might be expected to vary among different parts of the genome (due to structures such as centromeres and telomeres) in a relatively consistent way across a phylogeny. Likewise, the intensity of selection (background or directional) might vary consistently across the genome over broad spans of evolutionary time. If the

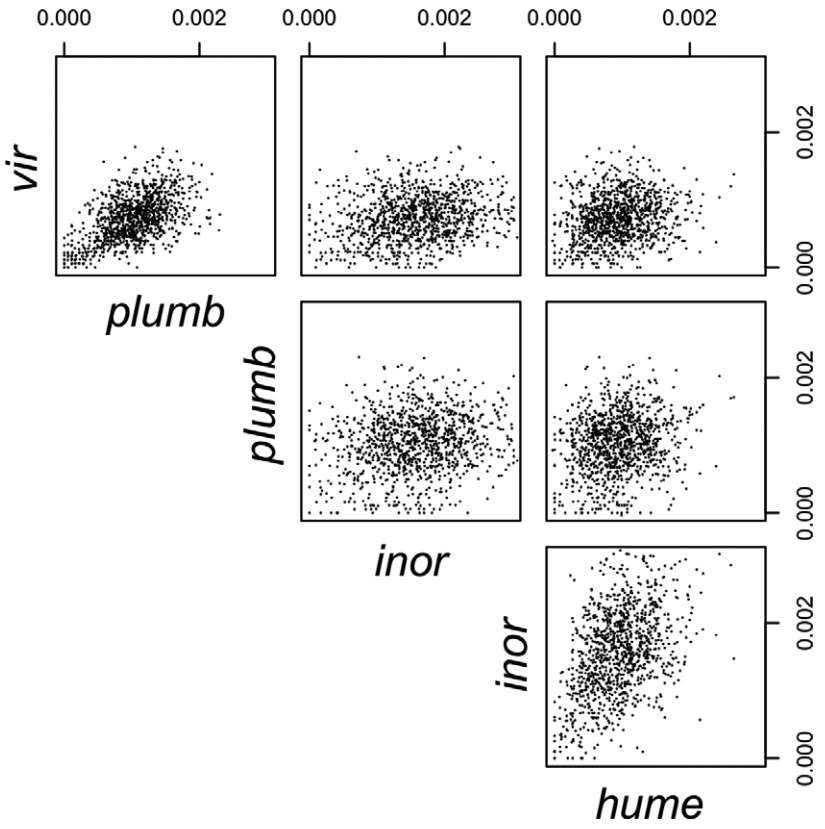

Fig. 9 Within-group nucleotide variation (π) per window is highly correlated between taxa within the greenish warbler complex (and also within the *inornatus*/*humei* complex), but only weakly correlated between these groups. Based on one individual per taxon, correlations of within-group nucleotide variation (π) among autosomal windows are moderately high within each species complex (e.g. *viridanus* vs. *plumbeitarsus*: $r = 0.539$, $P < 10^{-15}$; *inornatus* vs. *humei*: $r = 0.413$, $P < 10^{-15}$; in each, df = 1088) but much lower between these complexes (e.g. *viridanus* vs. *inornatus*: $r = 0.189$, $P = 3.3*10^{-10}$; the other three comparisons have similar correlations). Note that for *inornatus*, a few windows have π values that are slightly too large to be shown on these plots.

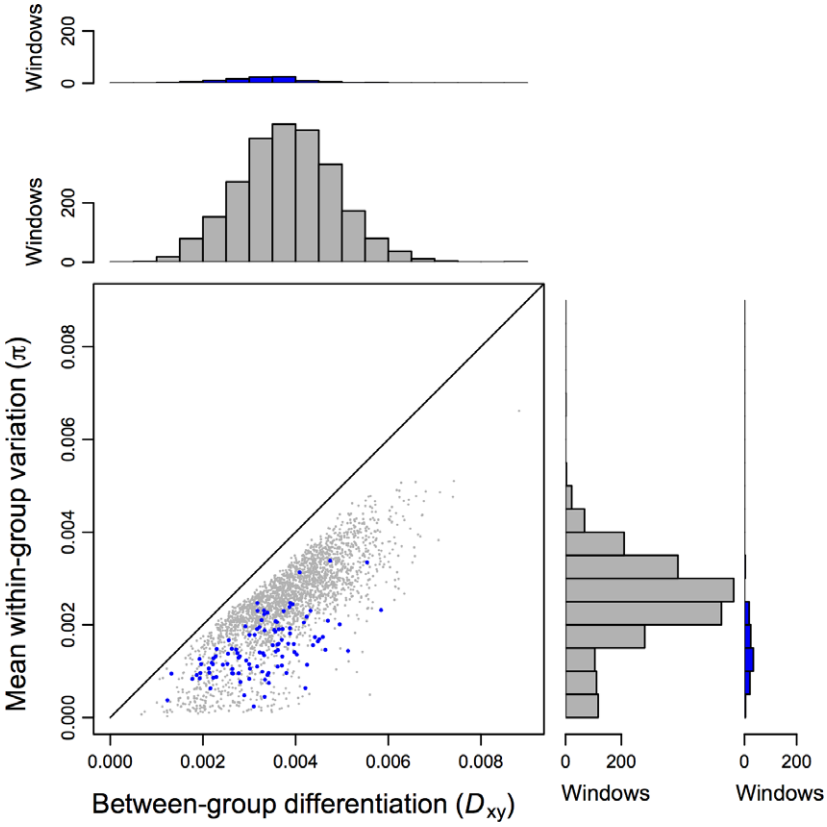

Fig. 10 The Z chromosome (blue) differs strongly from the rest of the genome (grey) in the distribution of absolute nucleotide differentiation (D_{xy}) and mean within-group nucleotide differentiation (π). This figure shows the comparison of *viridanus* to *plumbeitarsus* (15 individuals each), with each dot representing a single window of 5000 nucleotide sites; all other comparisons of greenish warbler populations show similar patterns. The diagonal line shows the 1:1 relationship that would be expected if within-group variation matched between-group differentiation (i.e. with no population differentiation). Histograms along each axis show that the Z chromosome has lower D_{xy} and π than the rest of the genome (Welch's *t*-test; D_{xy}: $t = 5.90$, df = 115.2, $P = 3.7*10^{-8}$; π: $t = 15.68$, df = 124.6, $P < 10^{-15}$).

phylogenetically conserved components of such factors have a large influence on patterns of variation in π and F_{ST}, we would expect similar patterns of variation in these statistics across the genomes of greenish warblers and outgroup species. In contrast to this expectation, we find only weak (e.g. 1–5% variation explained) or no correlation between the structure of variation in greenish warblers and those within or between outgroup species. We conclude from this that the component of variation in these factors that is relatively constant over evolutionary time explains little of the genomic structuring of differentiation in greenish warblers. However, variation in these factors that is more localized in the phylogeny (i.e. confined to just the greenish warbler complex, due to rapid change in these factors over evolutionary time) could play an important role.

Although the location of regions of especially low within-group variation differs between the greenish warbler complex and the *P. inornatus/humei* complex, the relationships between within-group variation, between-group absolute differentiation and between-group relative differentiation are remarkably similar. In both cases, regions of high F_{ST} tend to have moderate or low D_{xy} and very low π. These similarities point to common causal processes, although the genomic locations at which those processes are focused differ in the two species complexes.

To build an understanding of what processes may lead to the observed patterns, consider that we can view nucleotide differentiation between a pair of individuals as an estimate of the relative age of their common ancestor (Zeng & Corcoran 2015), also known as their relative coalescence time (to truly estimate time, we would need to know the mutation rate, but assuming the rate is constant allows us to estimate relative coalescence times; Fig. 2). Thus, the regions of high F_{ST} have very short within-group coalescence times (following from their very low π) and moderately short between-group coalescence times (following from their low D_{xy}). The ratio of these between- to within-group coalescence times (D_{xy}/mean π) is high, consistent with the high relative differentiation (F_{ST}). The rest of the genome (areas of low F_{ST}) have long coalescence times, both between- and within-population, and the ratio between those times is much closer to one, implying that within-group common ancestors tend to be almost as old as between-group common ancestors.

Hence, the genomic regions of high F_{ST} tend to be those where all greenish warblers share a common ancestor unusually recently, and where all individuals within a specific subspecies of greenish warbler are even more closely related. Nachman & Payseur (2012) and Cruickshank & Hahn (2014) proposed an explanation for such regions of low D_{xy} and high F_{ST} between two taxa: selection (directional and/or background) in the common ancestral taxon reduced variation in those regions of the genome, and subsequent selection in both daughter taxa reduced the within-group variation even proportionally more (Fig. 2C). Delmore *et al.* (2015) proposed a related explanation: that certain regions experienced selective sweeps that passed between geographic races within a geographically variable species complex, reducing variation in those regions dramatically compared to the rest of the genome, and recurrent selection in those regions then reduced within-group variation even more (Fig. 2D). Both models can be reasonably considered with respect to greenish warblers.

Evaluation of whether regions of low D_{xy} result from reduced variation in the common ancestor or more recent selective sweeps depends somewhat on evaluating the relative importance of background selection (due to deleterious mutations; Charlesworth *et al.* 1993) and positive selection (due to new beneficial mutations) in contributing to these patterns. Both selective forces can cause reductions in diversity in a panmictic common ancestor (Cruickshank & Hahn 2014), but only positive selection leads to selective sweeps over broad geographically structured species. Zeng & Charlesworth (2011) presented a detailed analysis of a variety of background selection scenarios, using both structured coalescent models and forward-in-time simulations; they obtained values of $B(T_2)$, a measure of the diversity-reducing effect of background selection, ranging from 0.37 to 0.82 depending on the parameters used, meaning that the largest effect estimated was that background selection resulted in 37% of the diversity expected under pure neutrality, such that variability in nucleotide diversity (π) between different parts of the genome would vary at most approximately 2.7-fold (i.e. 1/0.37). In contrast, within each of the greenish warbler taxa, nucleotide diversity varies between different genomic windows to a much greater degree (Figs. 3, 4, 7 and 8): for example, in the comparison of *trochiloides* to *viridanus*, mean π is 5.2-fold smaller (19%) in high-F_{ST} ($F_{ST} > 0.6$) windows than in other windows ($F_{ST} < 0.6$), and 14.8-fold smaller (6.7%) in very high-F_{ST} ($F_{ST} > 0.9$) windows than in low-F_{ST} windows ($F_{ST} < 0.6$). Although more modelling is needed to fully explore all the possible impacts of background selection (Zeng & Charlesworth 2011; see also Zeng & Corcoran 2015, which shows modest effects of background selection on π, D_{xy} and F_{ST}), we conclude that modelling to date does not support background selection as being responsible for the very large heterogeneity in nucleotide variation across the genome of greenish warblers, although it almost certainly plays some role in these patterns.

Given the above, we suggest that beneficial mutations followed by selective sweeps are likely involved in

generating patterns of variation in greenish warbler genomes. Such sweeps appear to have occurred within each subspecies (explaining regions of low π) as well as more deeply in time (explaining regions of low D_{xy}), either in a panmictic common ancestor or across a geographically variable ancestor. Given the evidence for long-standing geographic structure in greenish warblers (e.g. deep mitochondrial phylogeny with monophyletic differences on the order of 1.0–1.5 million years between the groups examined here, phenotypic and genomic differentiation, large geographic range over a geographically complex continent) and the evidence for some current gene flow throughout the whole species complex (Irwin *et al.* 2001b, 2005; Alcaide *et al.* 2014), we think that a model in which selective sweeps moved throughout a geographically structured ancestor is most parsimonious. This could perhaps be investigated further using the genomic variation along with inferred mutation rates to infer the age of a putative panmictic common ancestor, and then examining whether the distribution of coalescent times for windows across the genome (as inferred from D_{xy}) is consistent with coalescence of all windows in that common ancestor (i.e. the model in Fig. 2C is predicted to produce a tighter range of coalescence times than the model in Fig. 2D). Such an analysis would need to make a variety of assumptions and is beyond the scope of this study, but we reason that such a model of coalescence in a panmictic common ancestor is difficult to reconcile with the broad distribution of observed D_{xy} values (Fig. 7), as the common ancestor would need to be simultaneously very recent and very large. This reasoning leads us to invoke selective sweeps through a geographically structured ancestor as the most parsimonious history for regions with the lowest D_{xy} values.

We propose that the results are most consistent with a model in which recurrent selection, gene flow and partial reproductive isolation likely play important roles. In this combined model, gene flow among geographically differentiated populations allows global selective sweeps to occur at specific regions where globally favourable mutations have arisen (for an example of such a sweep of a genomic region between two mosquito species, see Norris *et al.* 2015; see Staubach *et al.* 2012 for evidence of sweeps between subspecies of House Mouse). When a genomic region undergoes a geographically global selective sweep, both between-population and within-population variation in that region become greatly reduced compared to the genomic background. Subsequent mutation and selection within each population can then reduce the within-group variation at those regions even more, resulting in increased relative differentiation between populations. If the fixation of these subsequent mutations is due in part to local adaptation that differs between populations, these regions might then play a role in (partial) reproductive isolation by causing reduced fitness in hybrids. If so, regions that play a role in reproductive isolation (i.e. contain 'speciation genes') do not tend to have high absolute differentiation – this is because they in fact have a more recent common ancestor than the rest of the genome.

We call this model the 'sweep-before-differentiation' model (Fig. 2D) for the formation of peaks of high relative differentiation. It is particularly applicable to situations in which there is low and/or intermittent gene flow between populations, in contrast with other models in which gene flow is either high (Fig. 2A) or zero (Fig. 2B). The low gene flow prevents complete homogenization of neutral or locally adapted parts of the genome, but it also facilitates the spread of globally advantageous mutations. The genomic regions that undergo global sweeps can subsequently diverge through selection within each local population, possibly leading to those regions causing low fitness in hybrids. This model is completely compatible with the idea that the effects of selection are strongest when it occurs on (multiple linked) genes in areas of low recombination (Noor & Bennett 2009; Nachman & Payseur 2012; Renaut *et al.* 2013; Burri *et al.* 2015).

This sweep-before-differentiation model for the formation of peaks of high relative differentiation incorporates the somewhat counterintuitive idea that individuals within a species complex tend to be more closely related in the differentiation peaks than they are elsewhere in the genome (Delmore *et al.* 2015). Because of the historical emphasis in the literature on relative differentiation (F_{ST}) being positively related to gene flow between populations (reviewed by Whitlock & McCauley 1999), such peaks have often been interpreted as regions that are more distantly related between populations compared to elsewhere in the genome. However, most of the theory relating F_{ST} to gene flow is based on assumptions of selective neutrality. When selection plays an important role, the relationship between F_{ST} and gene flow can be much more complex (Whitlock & McCauley 1999). The inverse relationship between D_{xy} and F_{ST} observed in greenish warblers (and other systems; Cruickshank & Hahn 2014) suggest that regions of high F_{ST} are areas that share a more recent common ancestor than the rest of the genome, which may share high levels of variation due to shared ancestral polymorphism and/or recent gene flow.

The sweep-before-differentiation model incorporates a mixture of elements from other models for the formation of genomic islands of differentiation. Like the divergence-with-gene-flow model (Wu 2001; Nosil *et al.* 2009), it envisions gene flow as playing a role during

the process of differentiation; however, unlike the standard divergence-with-gene-flow model, the crucial role for gene flow is in allowing global selective sweeps; it is these regions that tend to then become differentiated (either with or without gene flow). Like the selection-in-allopatry model (Noor & Bennett 2009; Nachman & Payseur 2012; Cruickshank & Hahn 2014), differentiation can occur via selection in allopatry (but in this case following a phase of gene-flow-mediated sweeps). We note that Cruickshank & Hahn (2014) proposed that a pattern of low or moderate D_{xy} in regions of high F_{ST} peaks should be viewed as evidence against the divergence-with-gene-flow model; however, if recurrent selection in the common ancestor (Cruickshank & Hahn 2014) or through geographic sweeps (Delmore *et al.* 2015) has reduced variation in those parts of the genome likely to undergo differential selection, then a pattern of low D_{xy} in F_{ST} peaks is expected even under the standard divergence-with-gene-flow model.

It should be noted that much of the logic above and in most other discussions of models for the formation of islands of differentiation (e.g. Cruickshank & Hahn 2014) depends on the assumption that mutation rate is approximately constant across the genome, as this allows D_{xy} and π to be taken as approximately proportional to mean coalescence time of pairs of individuals. We specifically examined this assumption by asking whether regions of low D_{xy} between greenish warbler populations also have low D_{xy} between outgroup species, and found that there was only a very weak correlation (2%), providing little evidence that regions of low D_{xy} can be explained primarily as a result of phylogenetically conserved low mutation rate. However, if mutation rate evolves more quickly, such that greenish warblers have a substantially different mutation rate landscape than the outgroup species, then one alternative scenario remains possible: if regions of low recombination tend to also have low mutation rate, linked selection on them might produce regions with low D_{xy} and high F_{ST}. This scenario does not require global sweeps to explain the pattern, but it does require selection and low recombination jointly reducing variation on the same genomic regions in multiple populations. We feel the 'sweep-before-differentiation' model is more parsimonious for the data at hand than this alternative model that depends on strong associations of recombination landscapes, mutation landscapes and selection among different greenish warbler populations, while at the same time having these factors evolve rapidly enough to be very different than in outgroup species. Future analyses should examine both possibilities in more detail.

A large literature has discussed the commonly observed pattern of greater F_{ST} on the Z chromosome than on autosomes (Charlesworth *et al.* 1987; Ellegren *et al.* 2012; reviewed by Oyler-McCance *et al.* 2015). We note that our results are not consistent with one of the commonly proposed explanations – that there is a higher mutation rate (and therefore substitution rate) on the Z chromosome, due to the higher proportion of time that Z chromosomes occur in males (where mutation rates have been proposed to be higher). Rather, our results indicate a similar distribution of D_{xy} on the Z chromosome and on autosomes between distant species of warbler, and a slightly lower distribution of D_{xy} on the Z chromosome than on autosomes between greenish warblers. The latter observation, along with the very low π on the Z chromosome (and concomitantly high F_{ST}) is consistent with the well-known hypothesis (Charlesworth *et al.* 1987; Ellegren *et al.* 2012; Oyler-McCance *et al.* 2015) that the Z chromosome is particularly prone to recurrent selective episodes. In this respect, the entire Z chromosome displays similar characteristics of differentiation as the 'islands of relative differentiation' in the autosomal genome, suggesting the Z chromosome may also be subject to geographic sweeps followed by local reductions of variation. The lower effective population size of the Z compared to autosomes (Charlesworth *et al.* 1987) and an expected lower recombination rate because it is hemizygous in females (Qvarnström & Bailey 2009) could also contribute to these patterns.

We note that these inferences could not have been made without decomposing relative nucleotide differentiation (F_{ST}) into components of between-group absolute nucleotide differentiation (D_{xy}) and within-group nucleotide differentiation (π). We have followed the lead of Nachman & Payseur (2012) and Cruickshank & Hahn (2014) here, and we reiterate their call for close examination of these statistics in analyses of population differentiation and its causes. Furthermore, we emphasize that great insight can be gained from plotting the relationships between these variables across the genome. In particular, we suggest that researchers regularly plot the relationship (among genomic windows) between absolute differentiation and average within-group variation (Fig. 7). In the case of no selection and panmixia (i.e. high migration between populations), points should be clustered near the 1:1 line (i.e. variation within each group is similar to variation between). Population differentiation will move the genome away from (to the right of) this line, as between-group variation becomes greater than within-group variation. If differentiation is due only to mutation and lack of gene flow (i.e. without selection), the points will move gradually away from the 1:1 line. If selection reduces within-population variation in some parts of the genome, these regions will move steadily lower on the graph, and the position of those regions

along the horizontal axis shows whether those regions have low absolute differentiation (indicating ancestral reductions in diversity or geographic sweeps) or high absolute differentiation (suggesting those parts may harbour ancient reproductive-isolation genes). The plot can also be used to examine the properties of regions of high relative differentiation, by colouring points according to F_{ST} (Fig. 7).

In conclusion, we see remarkable similarities in the genomic patterns of variation within and between three phenotypically divergent populations within the greenish warbler ring species. Comparisons with outgroup species suggest these similarities are not well explained by phylogenetically conserved differences in mutation rate or recombination rate across the genome (at least in terms of the components of variation in those factors that stay relatively consistent over moderately long evolutionary time). In particular, the differences observed between the greenish warbler complex and the *Phylloscopus inornatus/humei* complex in genomic locations of relative divergence cast doubt on the hypothesis that regions of high relative differentiation in these warblers correspond to genomic structural features such as centromeres or telomeres (e.g. Ellegren *et al.* 2012), unless those features have largely changed location in the approximately 12 million years of evolution since these two species complexes shared a common ancestor (Johansson *et al.* 2007; Price 2010). We conclude that the patterns are supportive of linked selection impacting most strongly on the same portions of the genome within each population of greenish warblers, but that these locations differ among outgroup species groups separated by more evolutionary time. We note however that the evidence for selection acting on similar regions does not indicate that selection pressures are identical in the three populations; rather, it simply indicates that selection is operating on traits encoded by similar regions. Even selection on the same gene could be in differing directions in two populations (e.g. selection for larger wing bars in one place, and smaller in another) while having a similar effect in causing reduced within-group variation. Given the strong evidence for certain phenotypes typically being under selection in warbler populations (e.g. beak size and shape, wing bars, migratory route and distance, singing behaviour; Richman & Price 1992; Irwin 2000; Irwin & Irwin 2005; Price 2008; Tietze *et al.* 2015; Delmore *et al.* 2016), it is reasonable that certain genomic regions also are consistently under more intense selection than others. The evidence that these regions have lower between-group coalescence times suggests that there have been selective sweeps in some of these regions throughout the species complex. The possibility that regions of high relative differentiation have experienced more recent gene flow than other genomic regions has received very little previous consideration, and could dramatically alter our understanding of the process of genomic differentiation during speciation. Altogether, the results of this study point to a remarkable level of repeatability of patterns of selection and genomic differentiation within a widespread and geographically variable species complex.

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D.E.I., M.A. and J.H.I. designed the research; M.A. conducted the laboratory work; K.E.D. assembled the individual genomes; K.E.D. and G.L.O. assisted with the bioinformatics pipeline; D.E.I. produced the consensus genome, wrote the R scripts, conducted the analysis of GBS reads and wrote the manuscript with input from all authors.

Data accessibility

Whole-genome shotgun sequencing reads have been deposited at NCBI SRA under the accessions SRX472921 (*viridanus*), SRX1625194 (*trochiloides*) and SRX1630030 (*plumbeitarsus*).

The three whole-genome shotgun assemblies have been deposited at DDBJ/ENA/GenBank under the accessions LXPA00000000 (*viridanus*), LXOZ00000000 (*trochiloides*) and LXOY00000000 (*plumbeitarsus*).

GBS reads have been deposited at NCBI SRA under the accession SRX473141; within this accession are two runs: SRR1176844 contains the reads from Plate 1, and SRR1177796 contains the reads from Plate 2 (for samples in each plate, see Methods, under the heading 'Mapping of GBS reads and genotyping'; or see script file referred to below).

The consensus genome, scripts containing code for all steps in the analysis and production of figures, and files containing processed data at several stages (vcf files containing called genotypes, R input files, files containing per-locus statistics and files containing windowed statistics) have been deposited in the Dryad

Digital Repository (doi:10.5061/dryad.t951d). Included is a file containing custom-built functions which may be of use in other analyses. We have also made the R files available at a GitHub site: https://github.com/darreni/GW_genomics.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Summary statistics for de novo genome assemblies obtained with SOAPdenovo, including the number of ultra-conserved elements (UCEs) from Faircloth *et al*. (2012) that aligned to each assembly.

Table S2 Locations of regions that were locally re-aligned with Mauve (Darling *et al*. 2010).

Table S3 Individuals used in the study.

Fig. S1 Genomic relationships around the ring of greenish warblers.

Fig. S2 Levels of absolute differentiation (D_{xy}) across genomic windows are highly correlated between comparisons of pairs of greenish warbler populations (e.g., *viridanus-trochiloides* vs. *obscuratus-plumbeitarsus*, shown here), but only weakly correlated between each pair of greenish warbler populations and the distantly related species comparison *Phylloscopus fuscatus – Seicercus whistleri*.

Fig. S3 The ratio of within- to between-group nucleotide variation per window is only weakly correlated between the greenish warbler complex and the *inornatus/humei* complex.

Fig. S4 Analyses of two phylogenetically distant taxon pairs of *Phylloscopus* warblers (left: *humei/inornatus*; right: *viridanus/plumbeitarsus*) shows that in both, autosomal windows with high relative differentiation (F_{ST}; illustrated with increasing red color, whereas blue indicates low F_{ST}) tend to have moderate or low between-group absolute differentiation (D_{xy}) and exceptionally low average within-group variation (π).

Fig. S5 The distribution of absolute nucleotide differentiation (D_{xy}) is similar in the Z chromosome (blue) and the autosomal genome (grey) in comparisons between distantly related species (left: *fuscatus* and *whistleri*; right: *trochiloides* and *whistleri*).