

## Supplemental Material

### Linked selection and recombination rate variation drive the evolution of the genomic landscape of differentiation across the speciation continuum of *Ficedula* flycatchers

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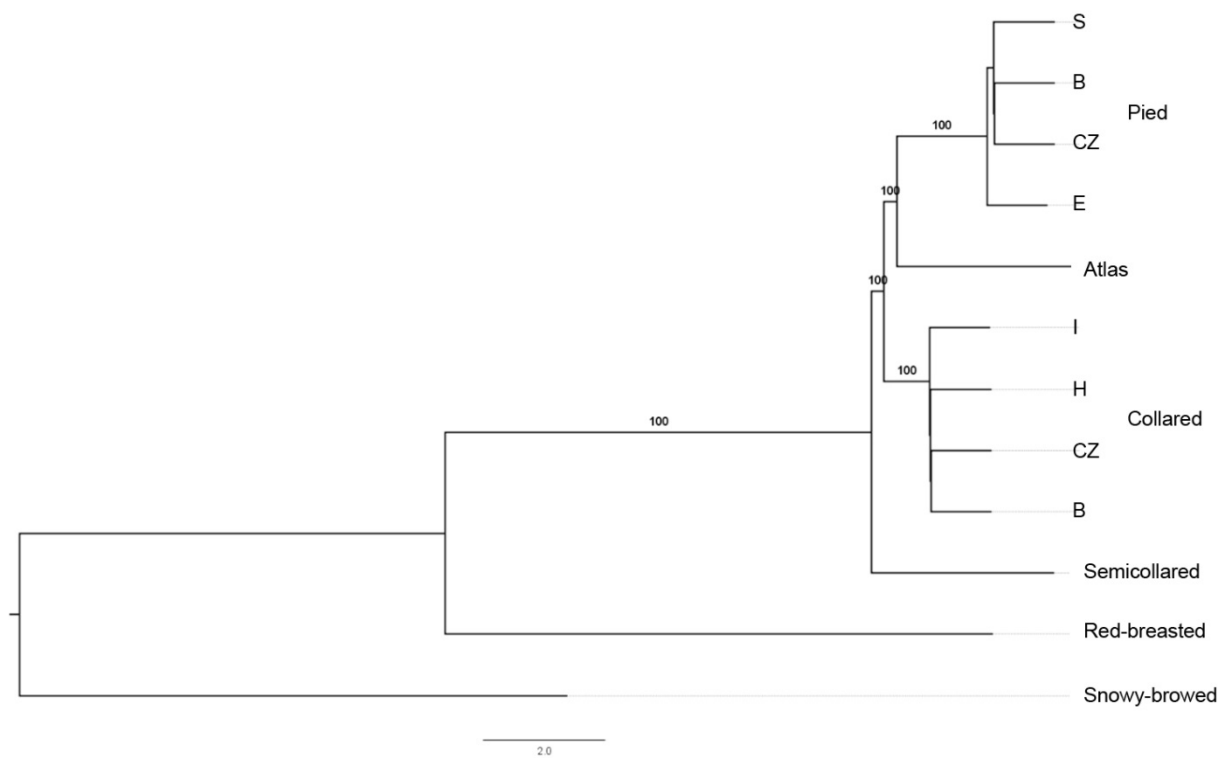
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[reto.burri@ebc.uu.se](mailto:reto.burri@ebc.uu.se), Tel +46 70 534 00 71, Fax +46 18 471 63 10 (RB)

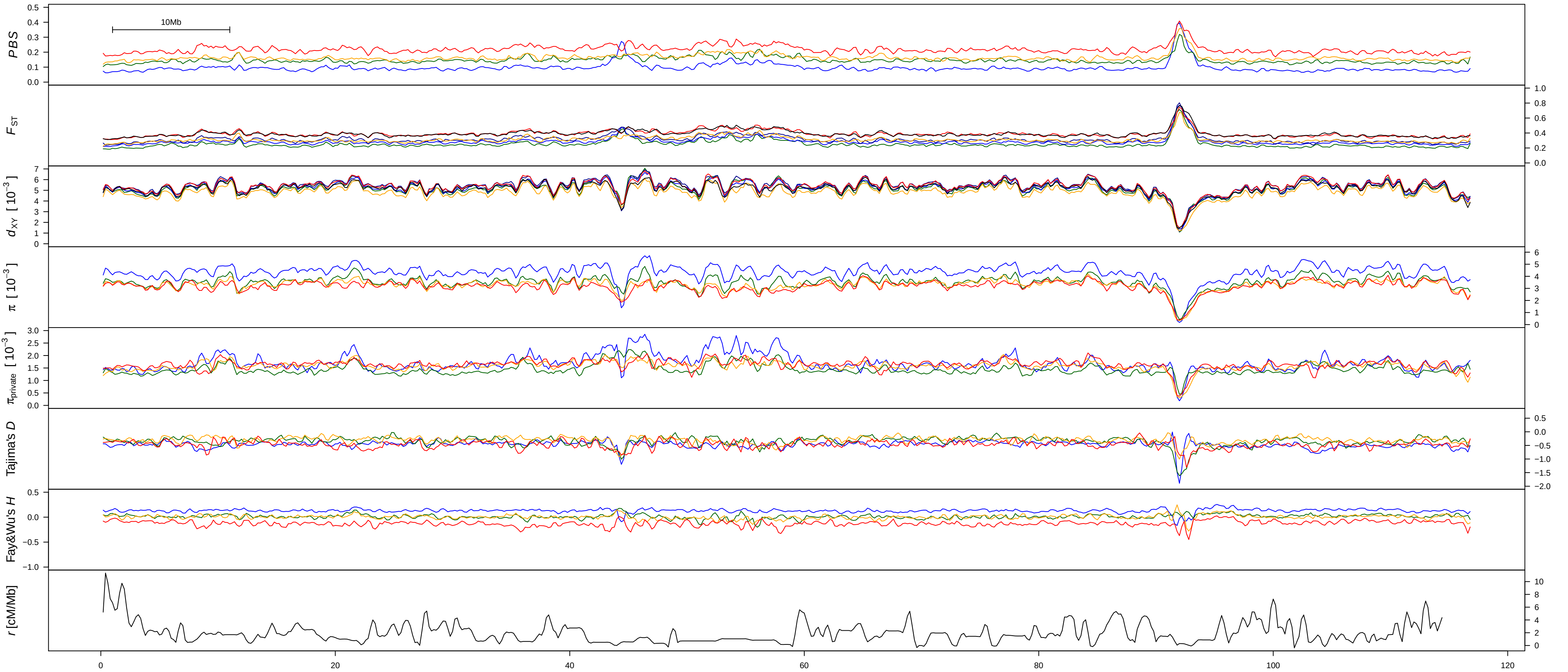
[hans.ellegren@ebc.uu.se](mailto:hans.ellegren@ebc.uu.se), Tel +46 18 471 64 60, Fax +46 18 471 63 10 (HE)

**Supplemental Figure S1.** Species tree topology as inferred from the distribution of gene trees under the multispecies coalescent. Branch lengths are depicted in units of  $4N_e$  generations, and external branch lengths are not estimated. Numbers on internal branches show bootstrap support values in percent from 1,000 bootstrap replicates.

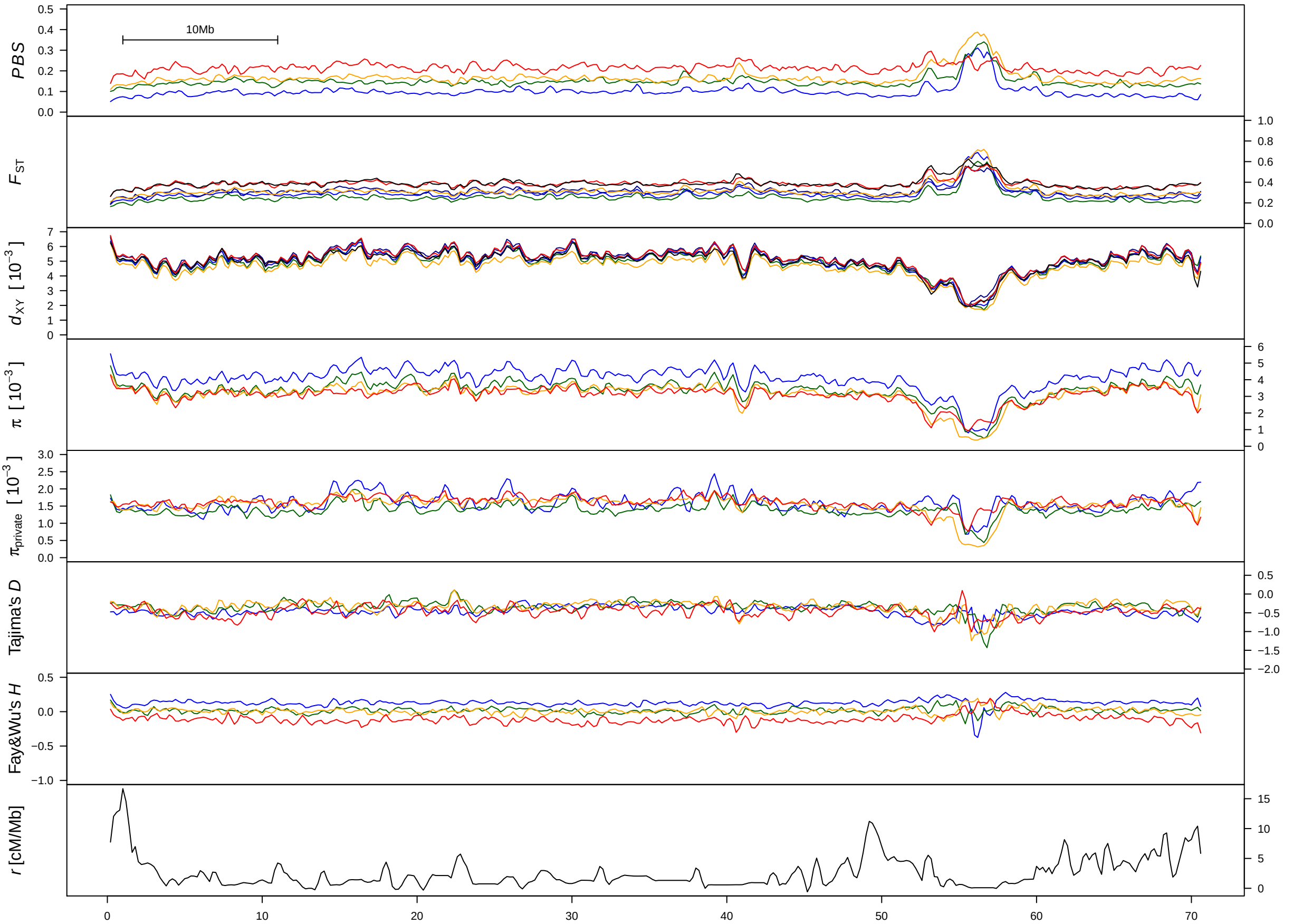


**Supplemental Figure S2.** Population genomic parameters along the genome. Color codes, species-specific parameters: blue, collared; green, pied; orange, Atlas; red, semicollared.  $F_{ST}$  and  $d_{xy}$ : green, collared-pied; light blue, collared-Atlas; blue, collared-semicollared; orange, pied-Atlas; red, pied-semicollared; black, Atlas-semicollared. Chromosome 25 has been omitted due to its short length.

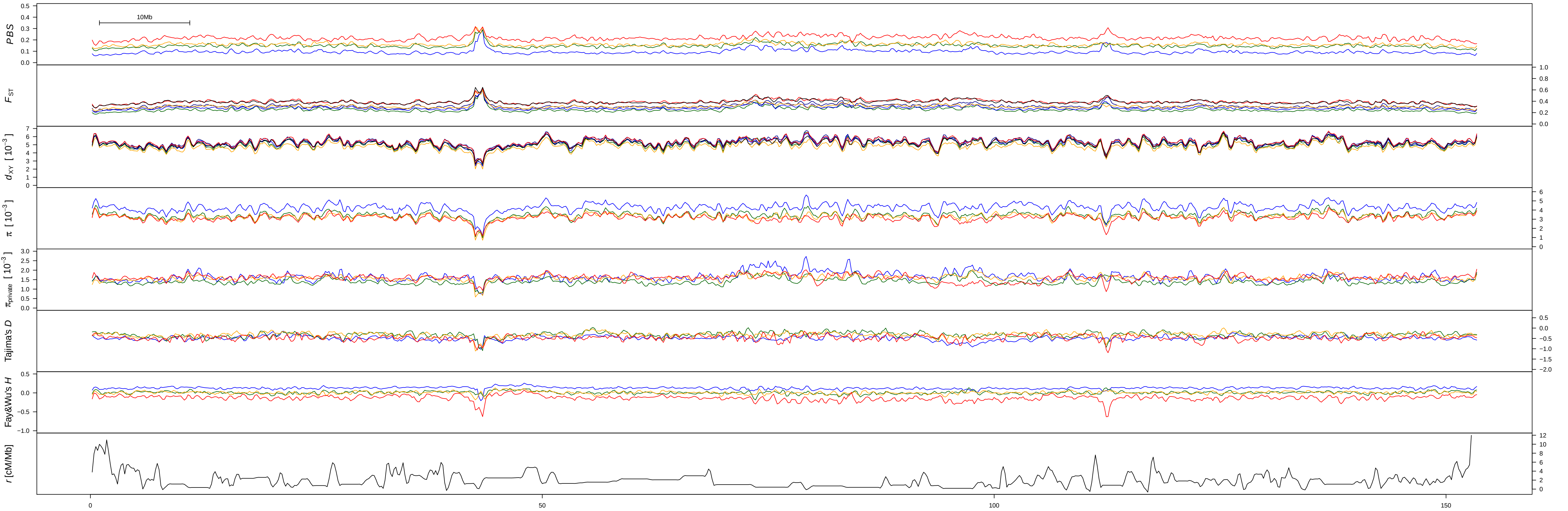
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# Chr1A

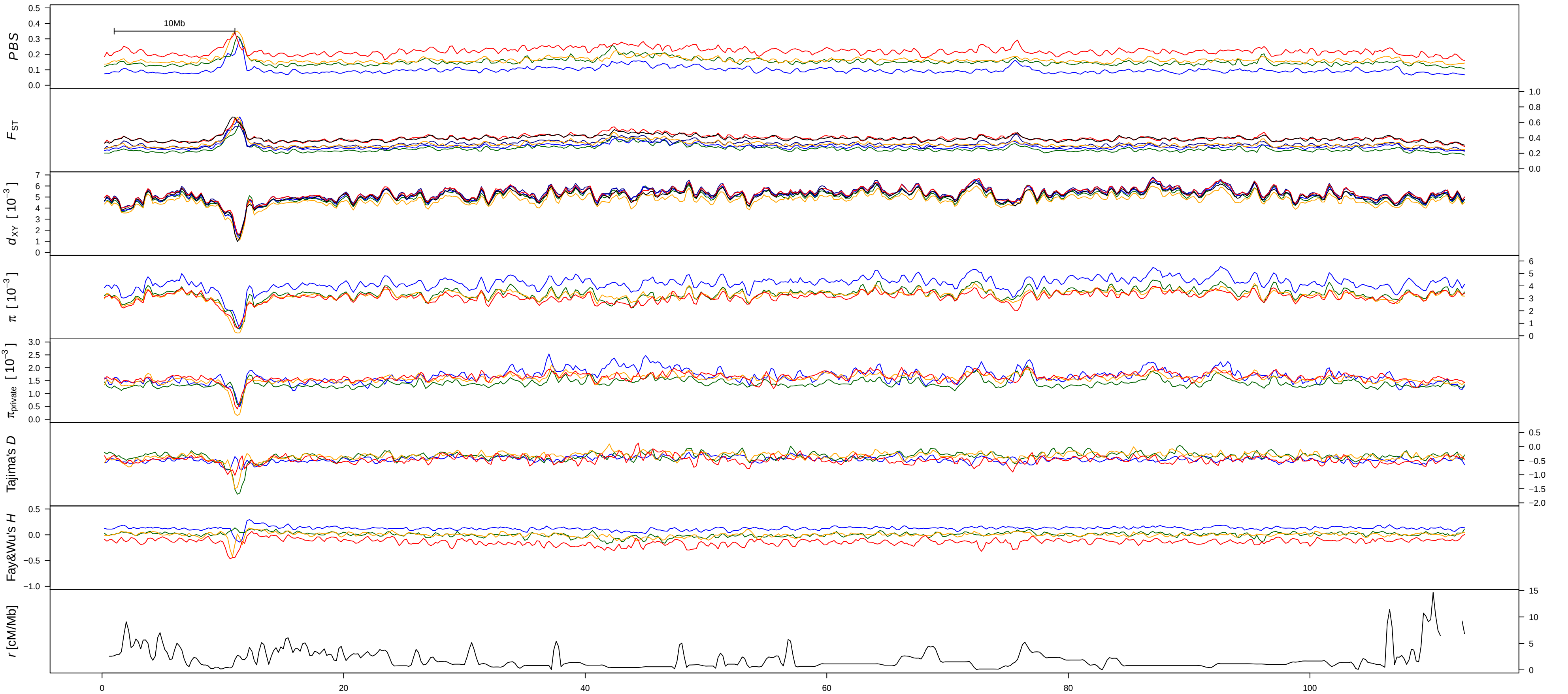


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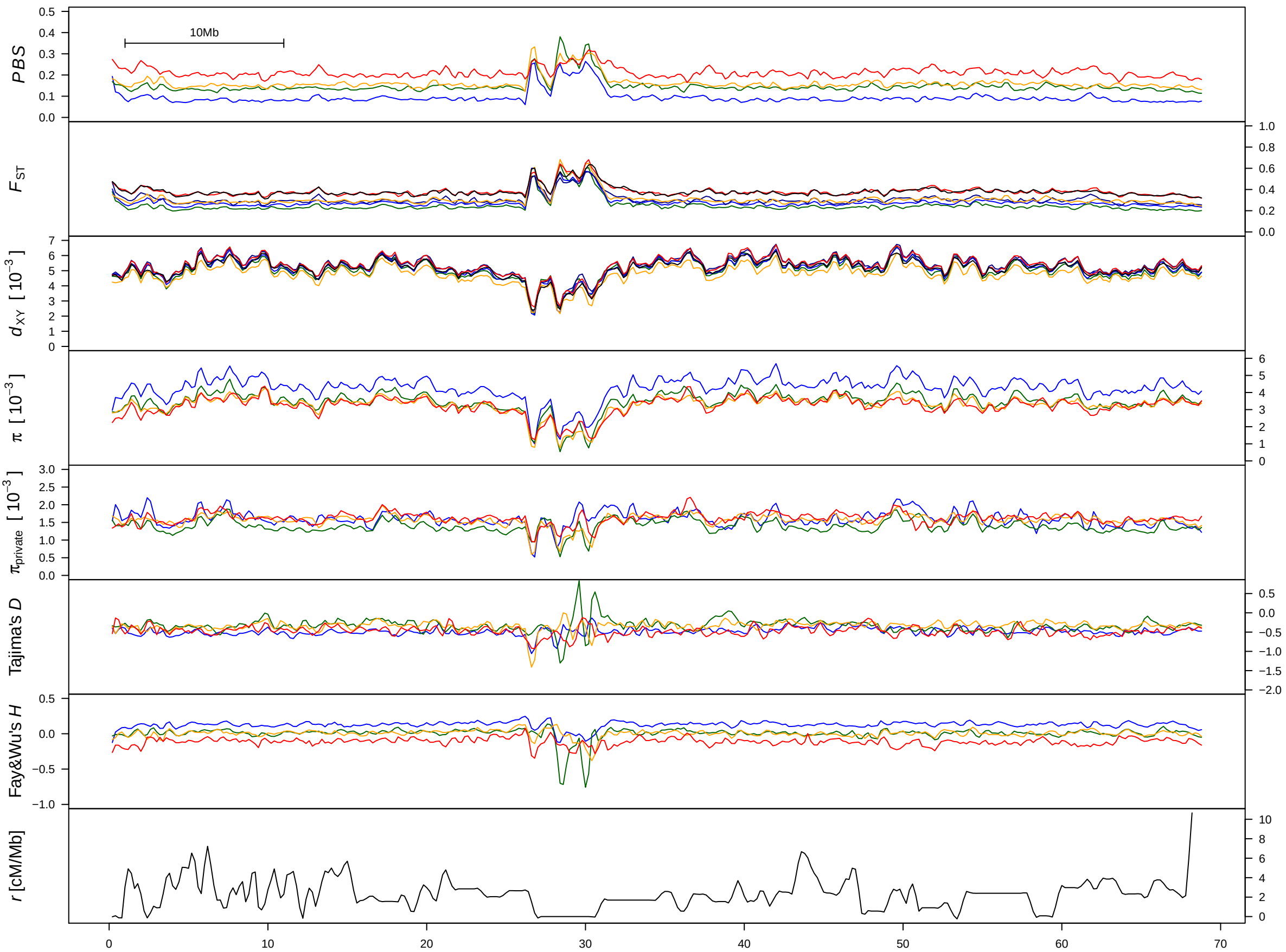




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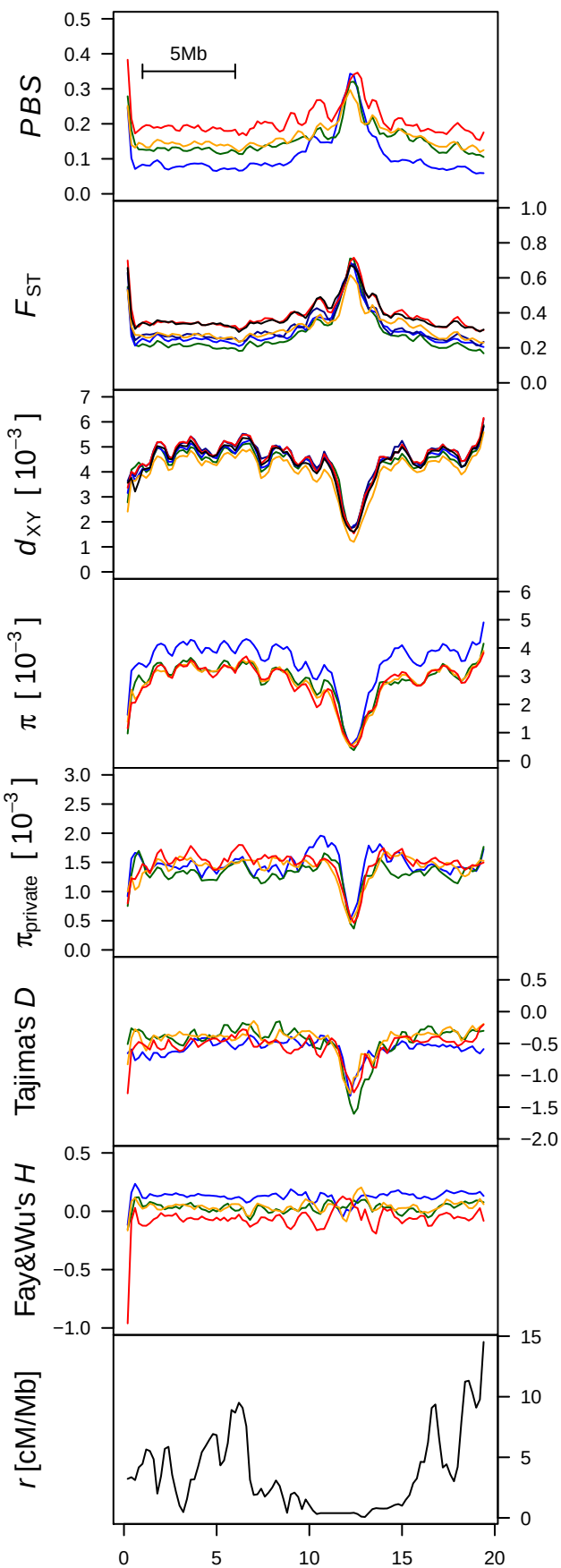


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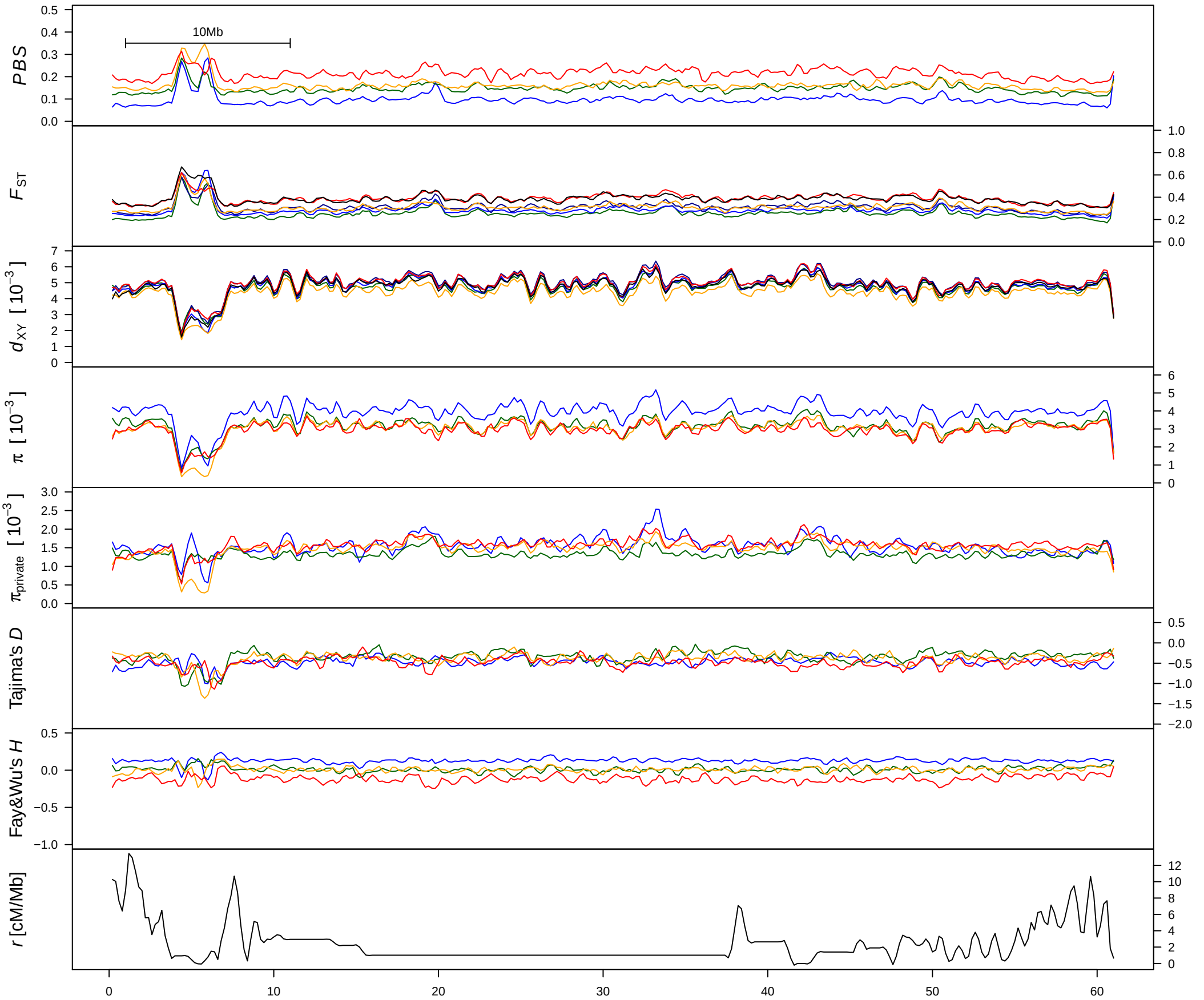




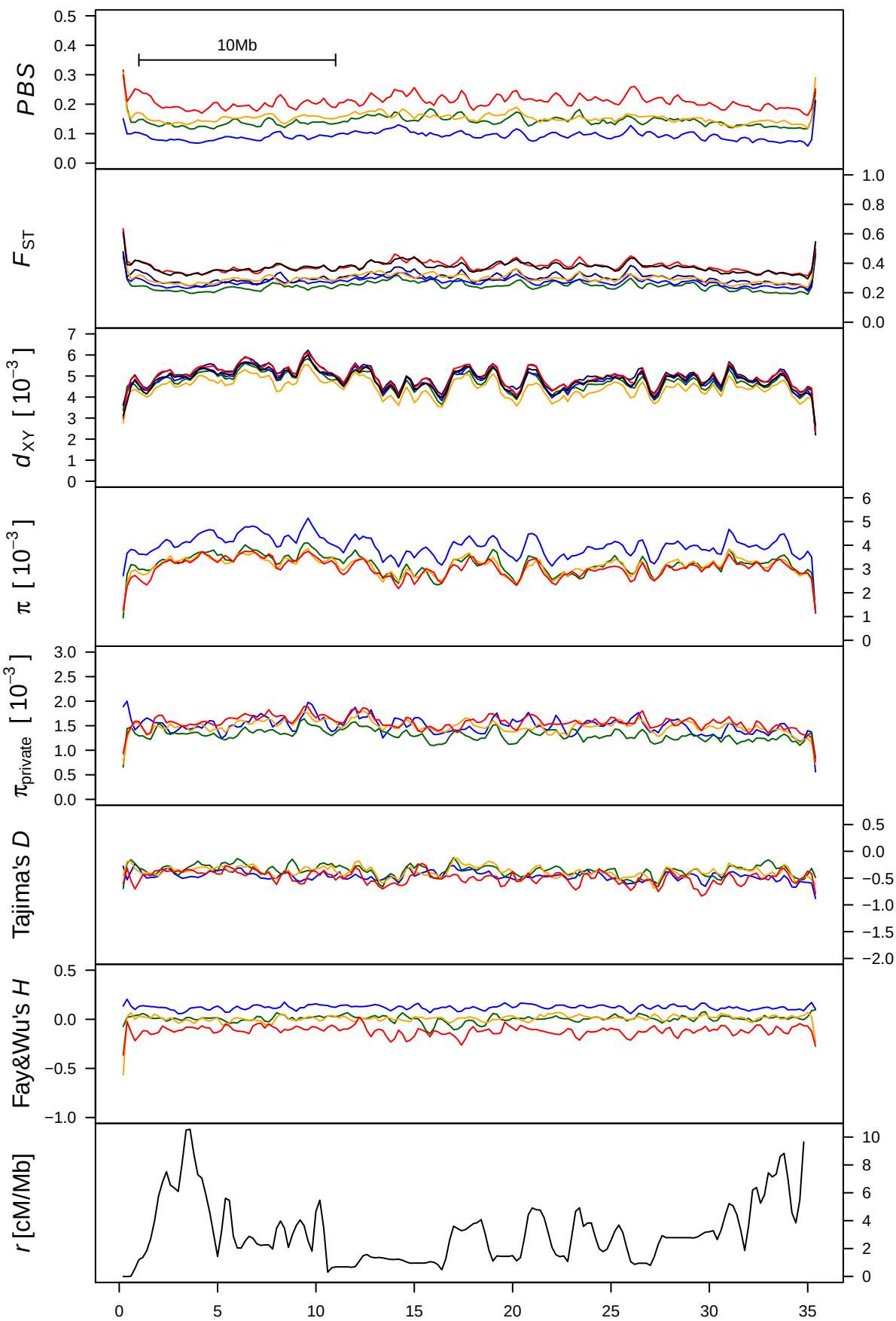
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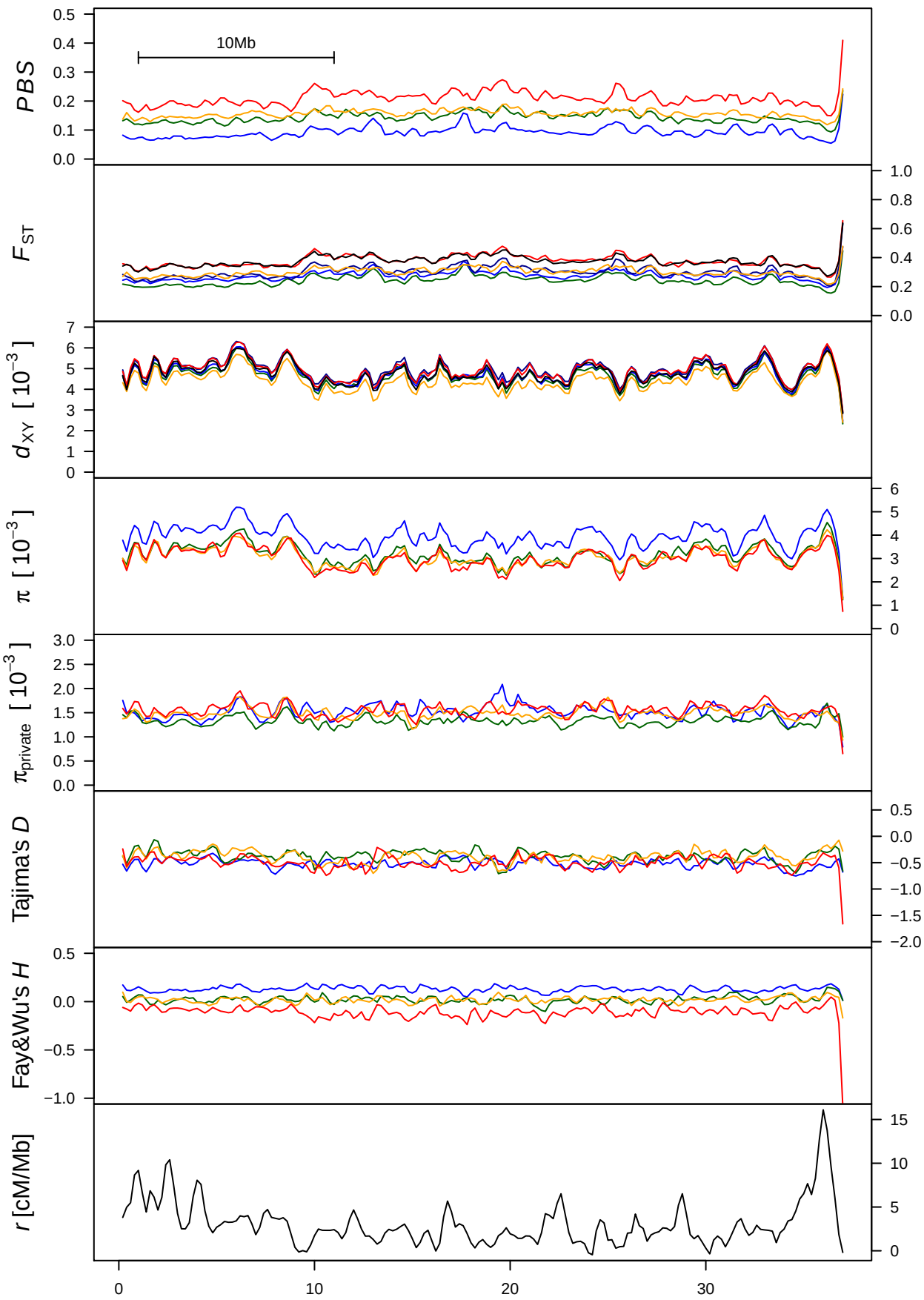
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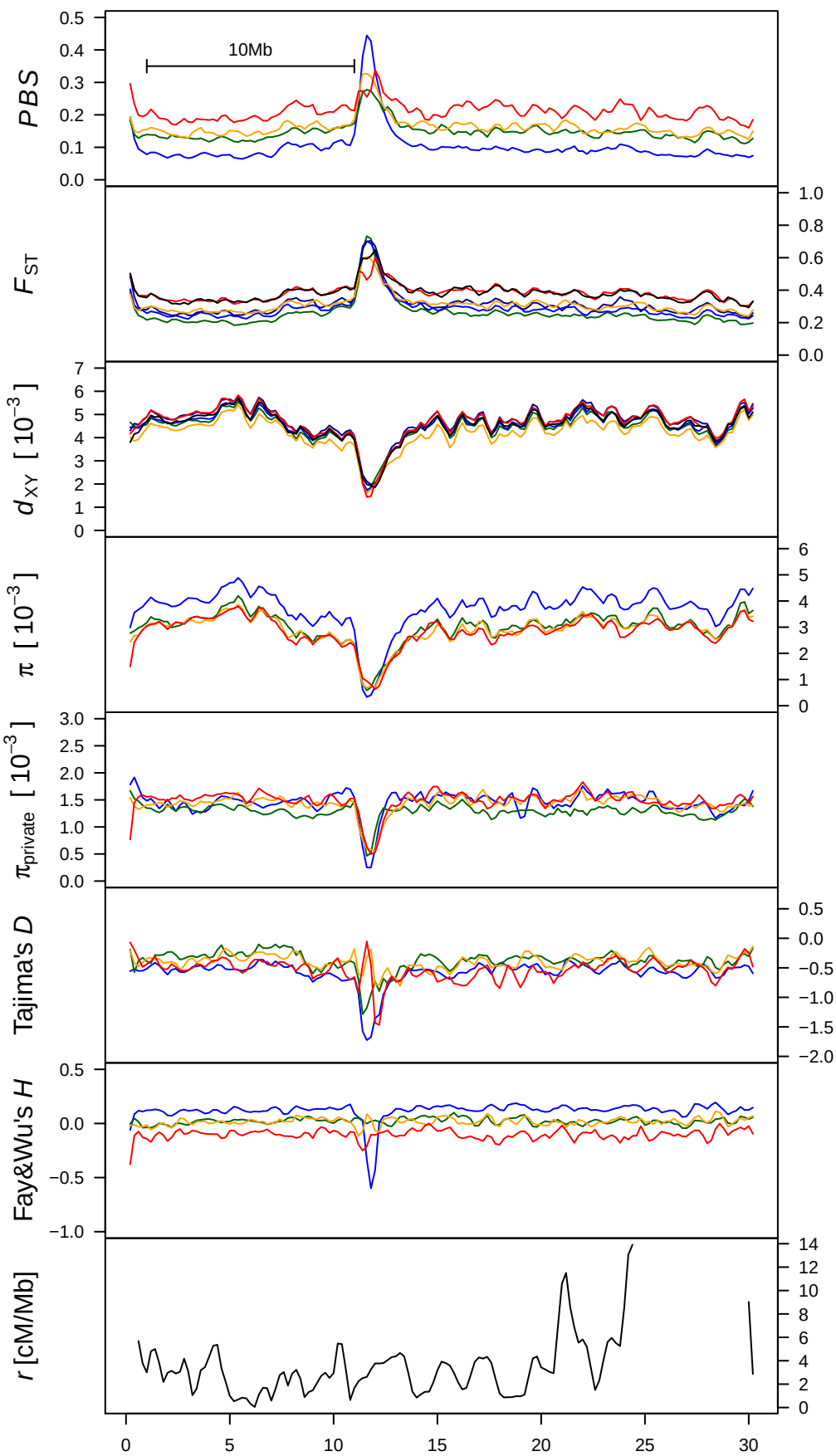
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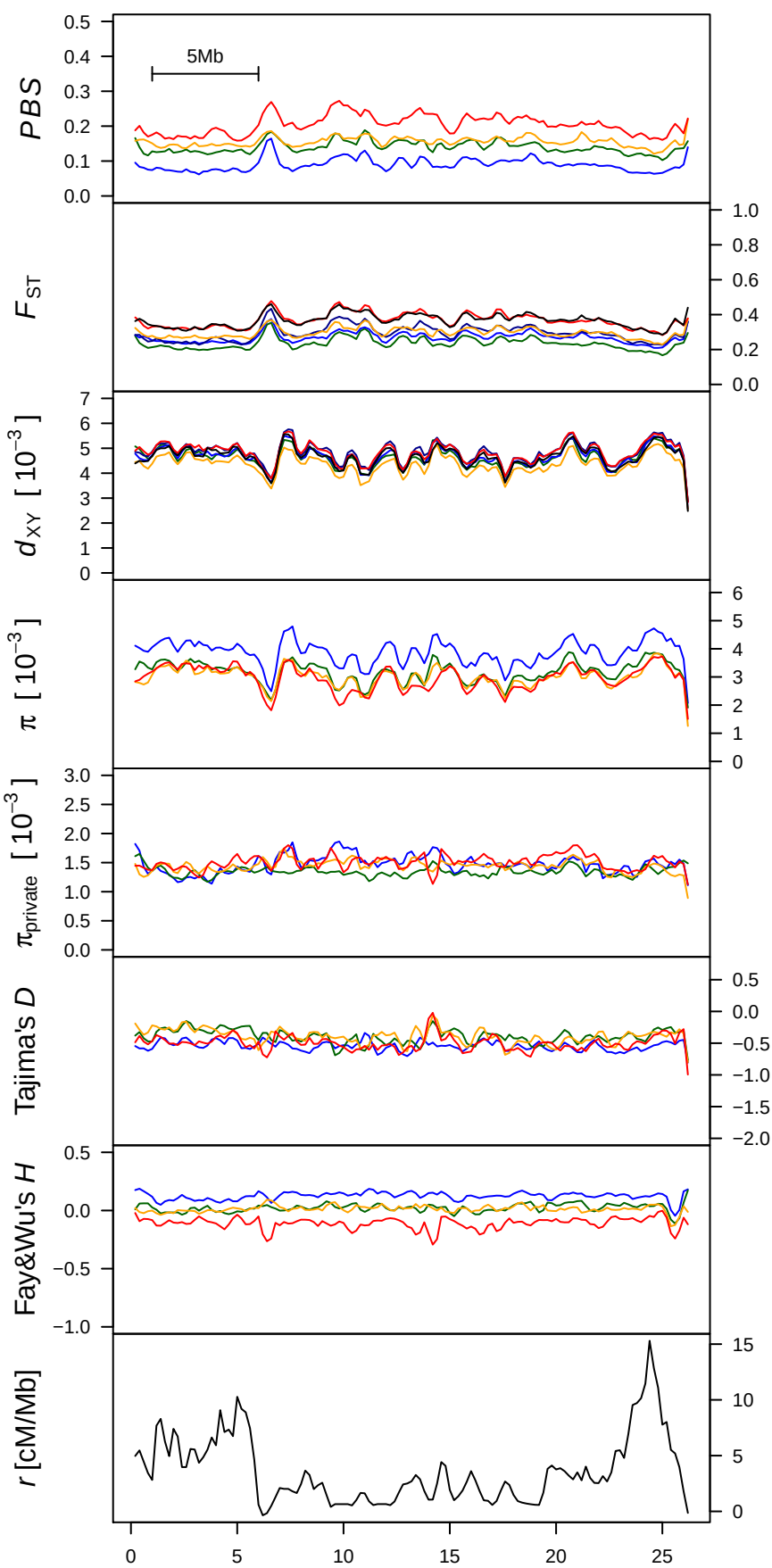


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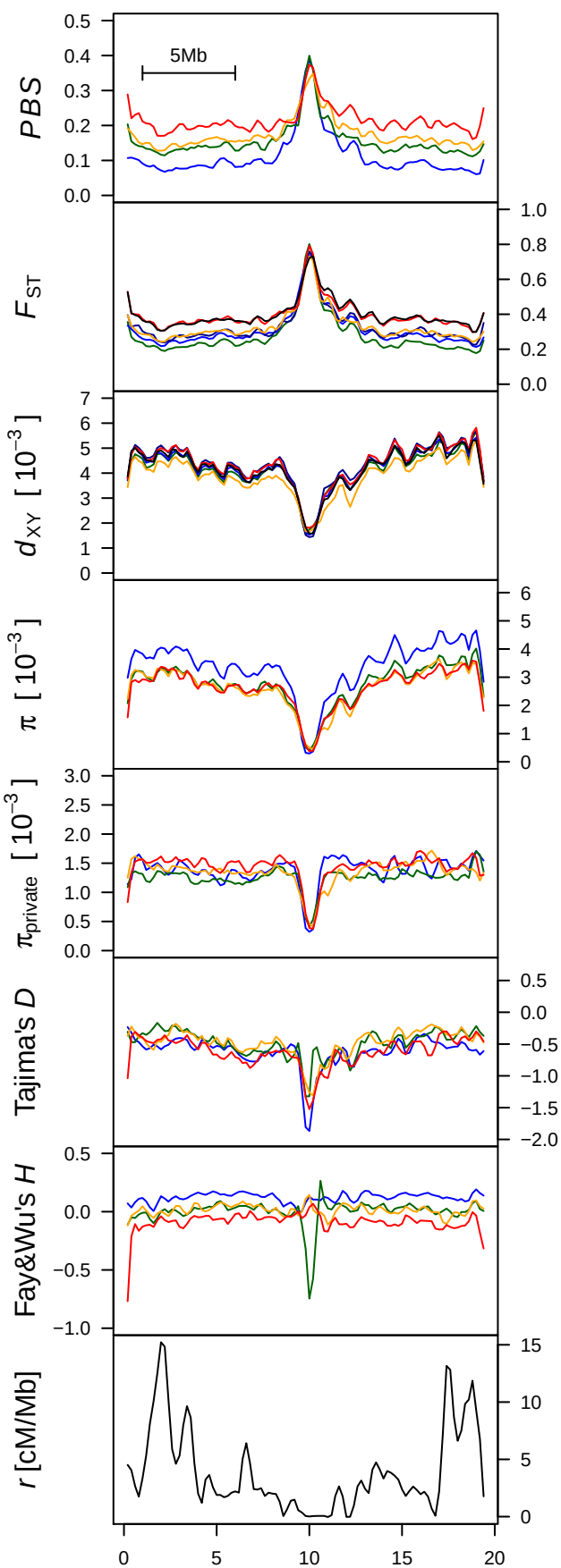




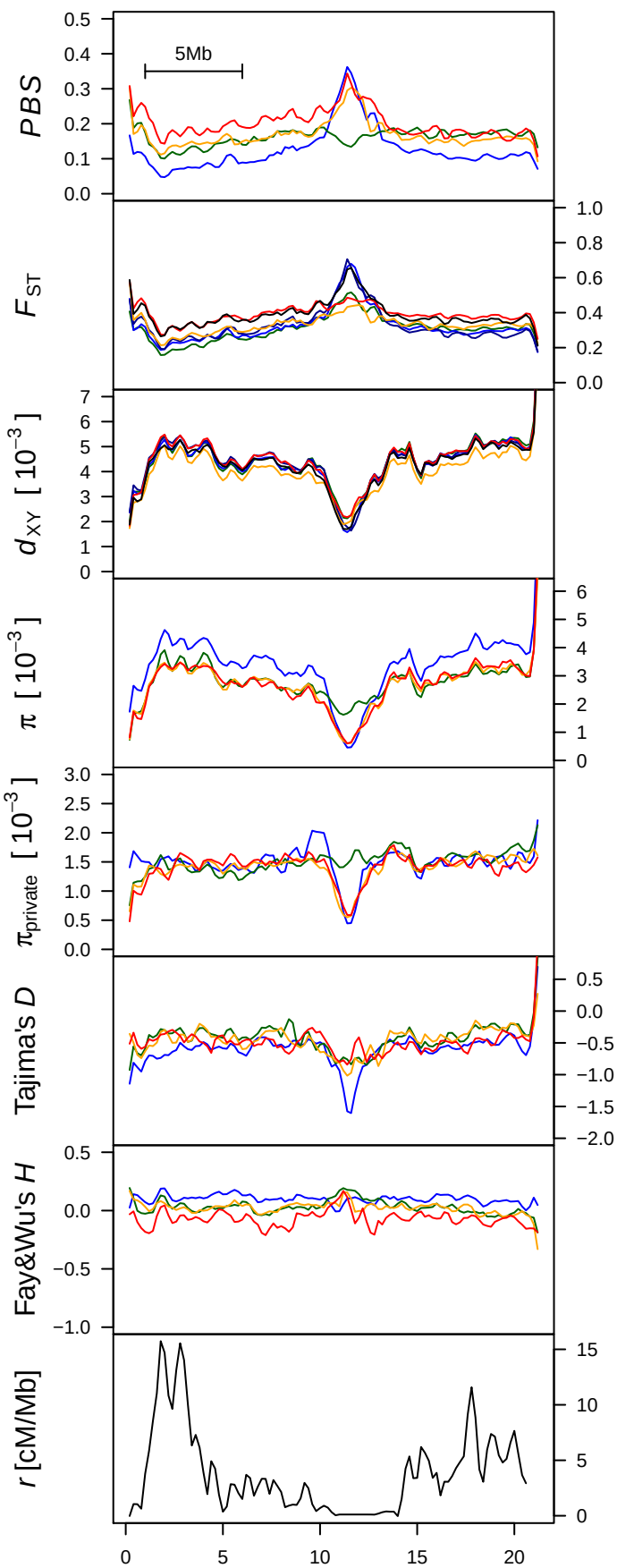
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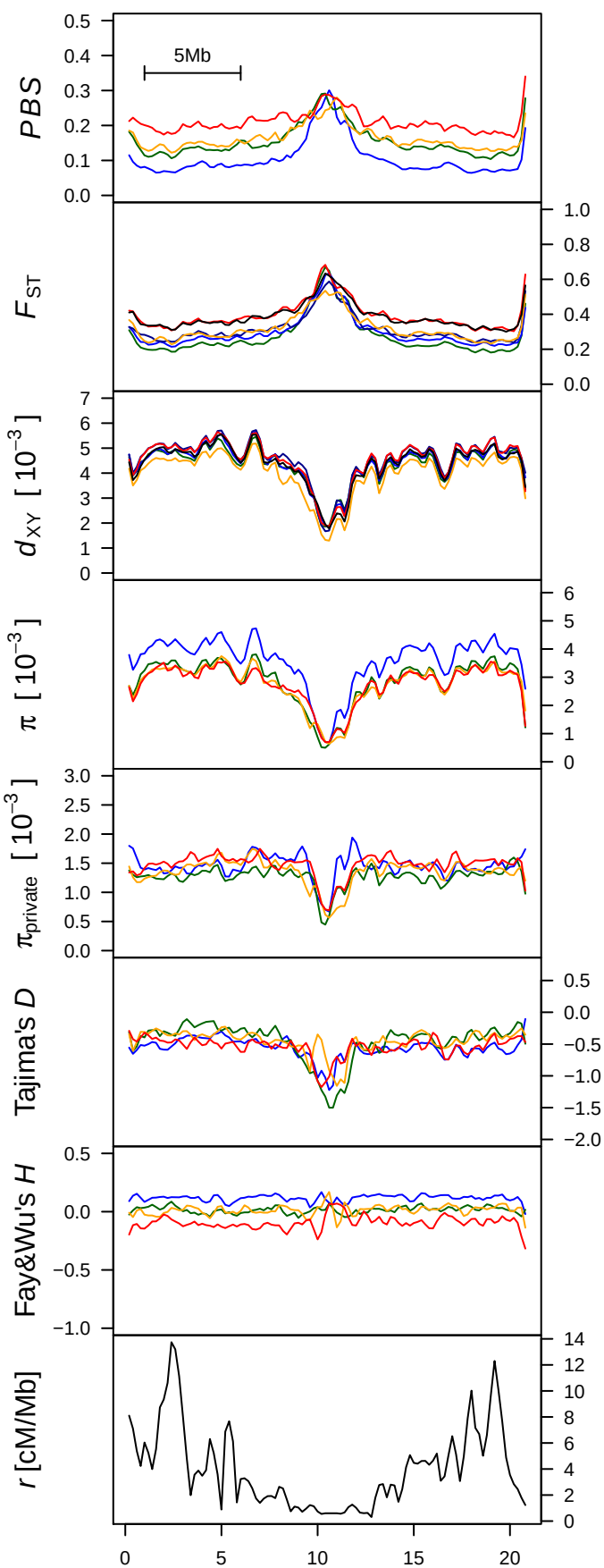
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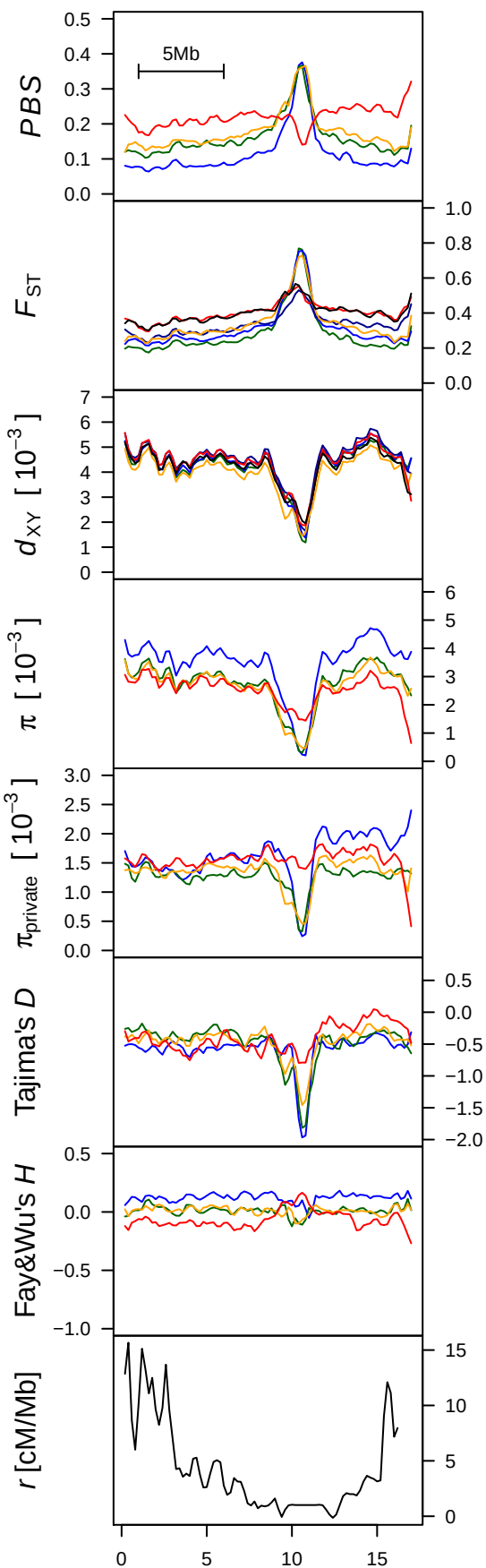
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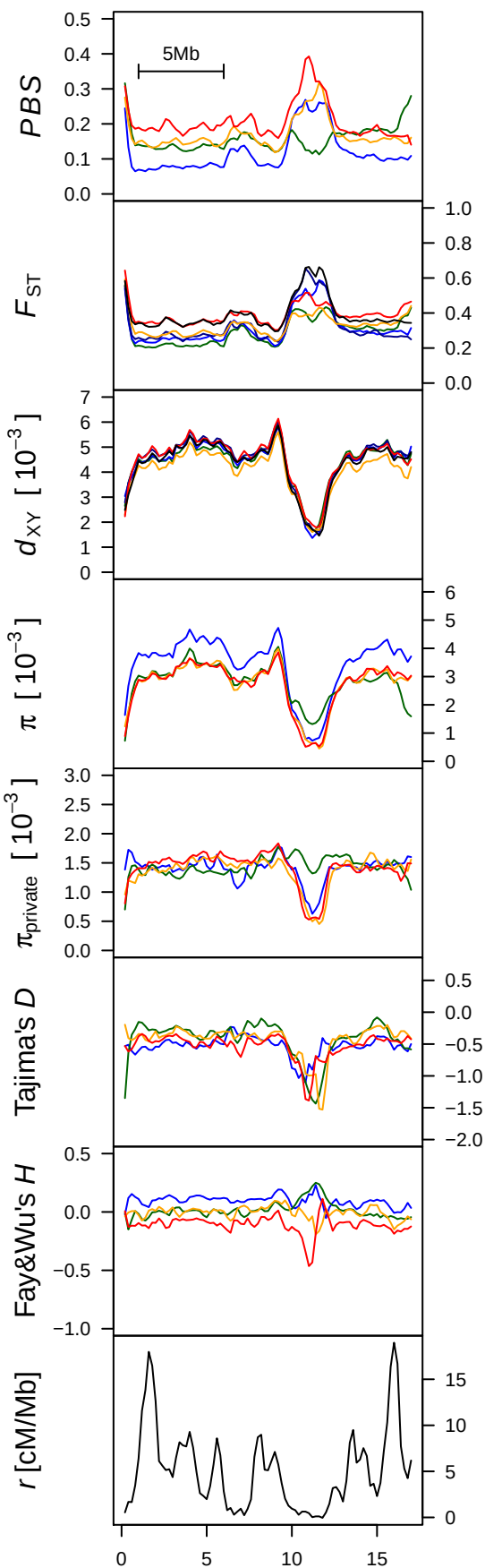
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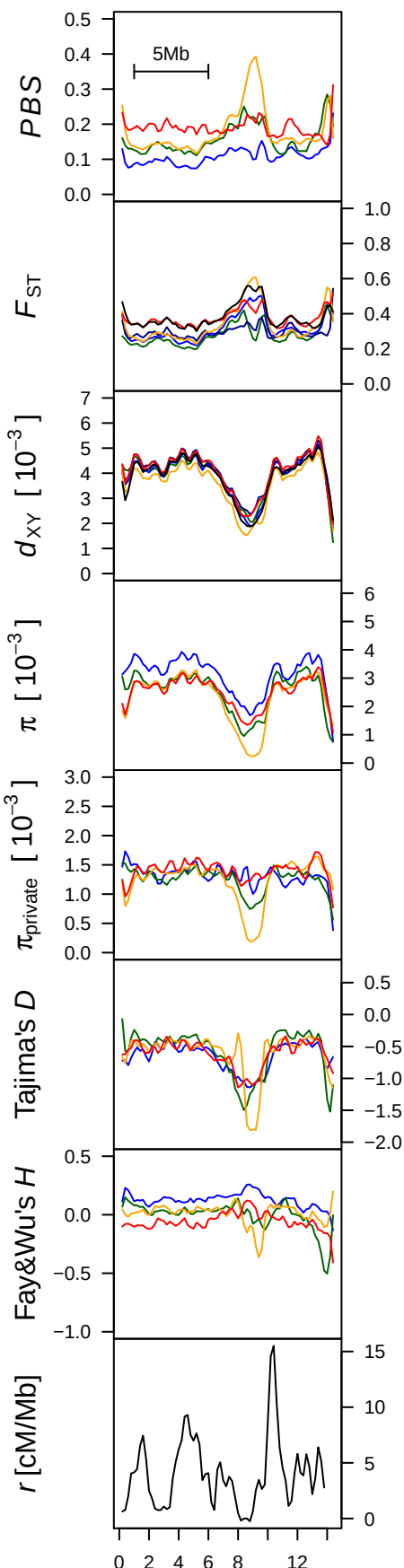
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# Chr14

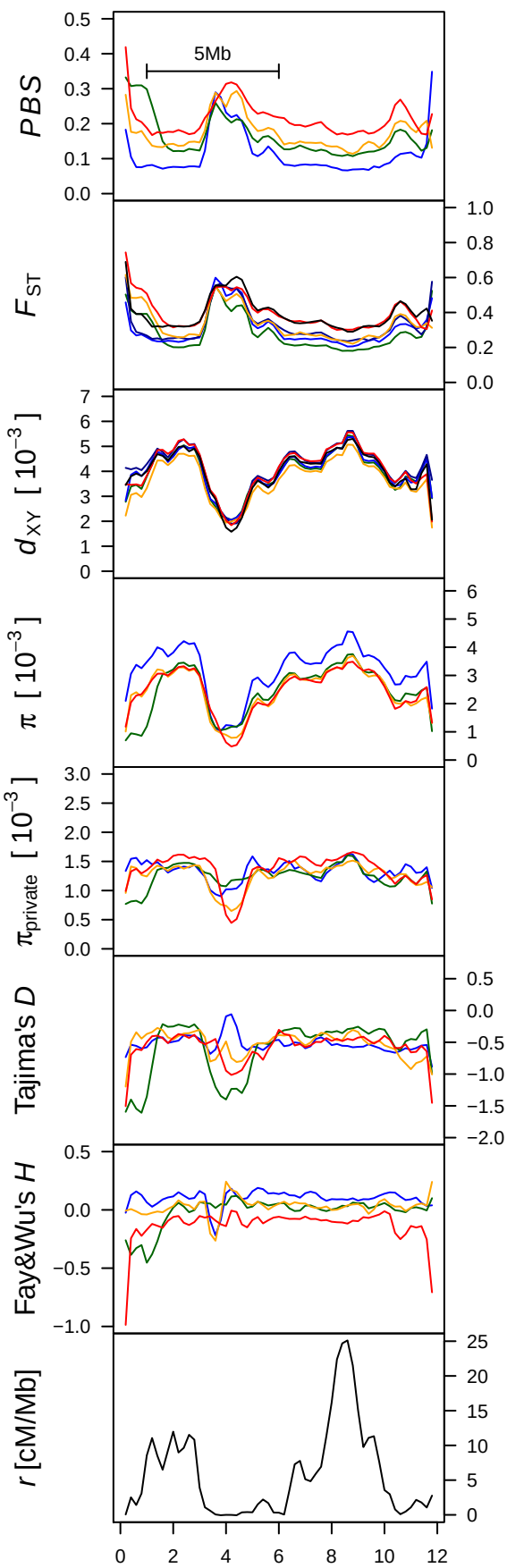


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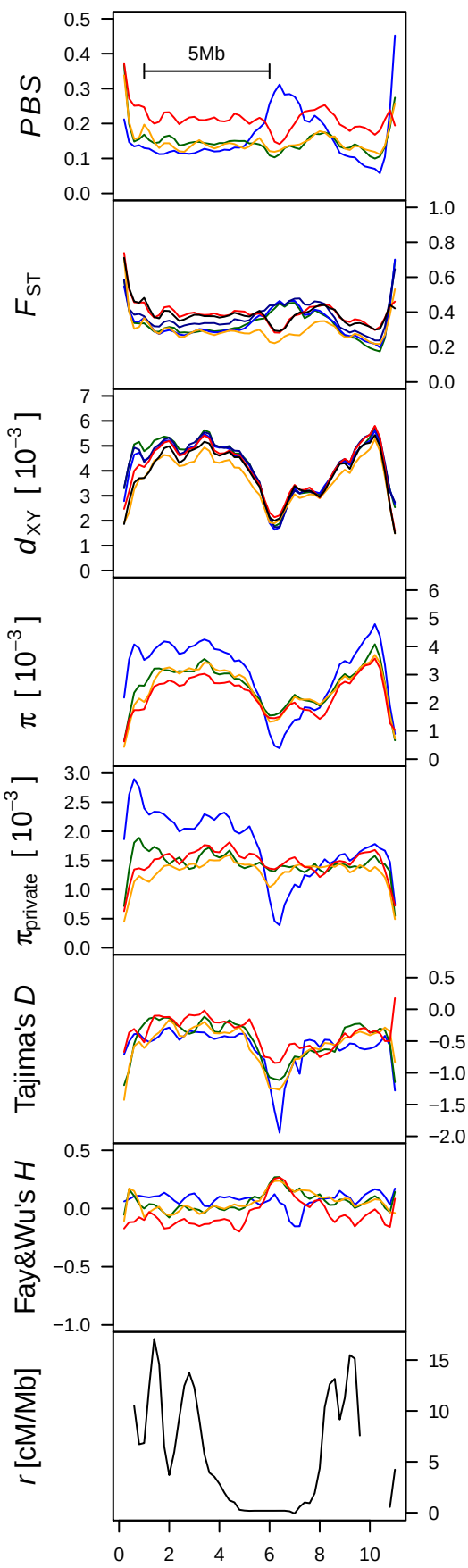




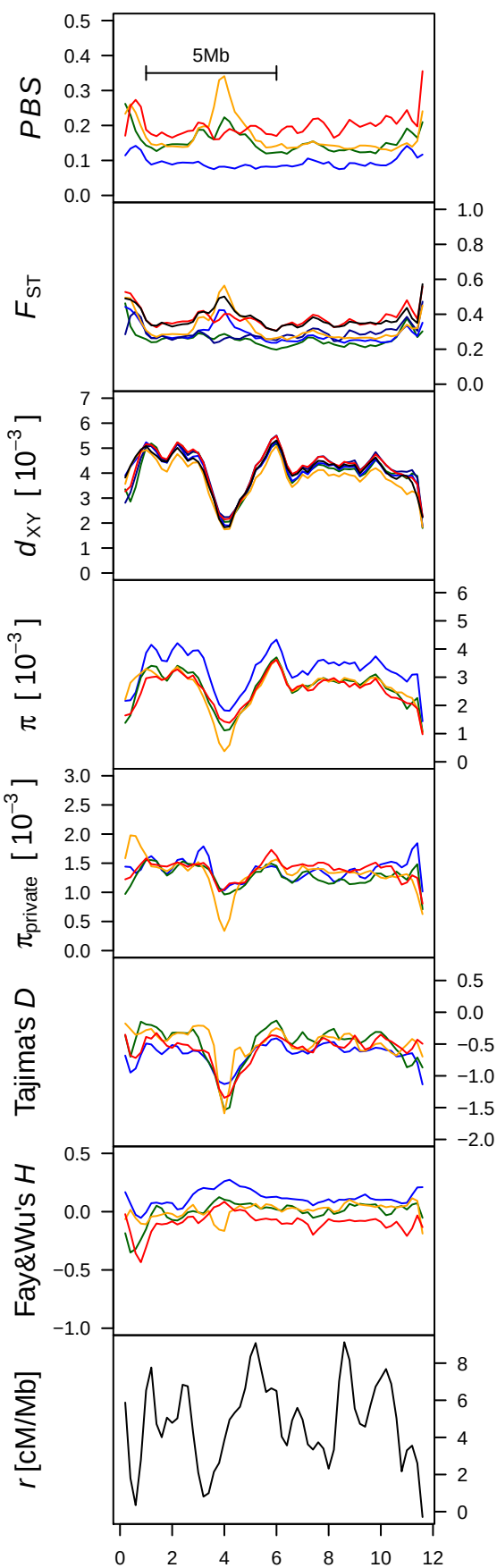
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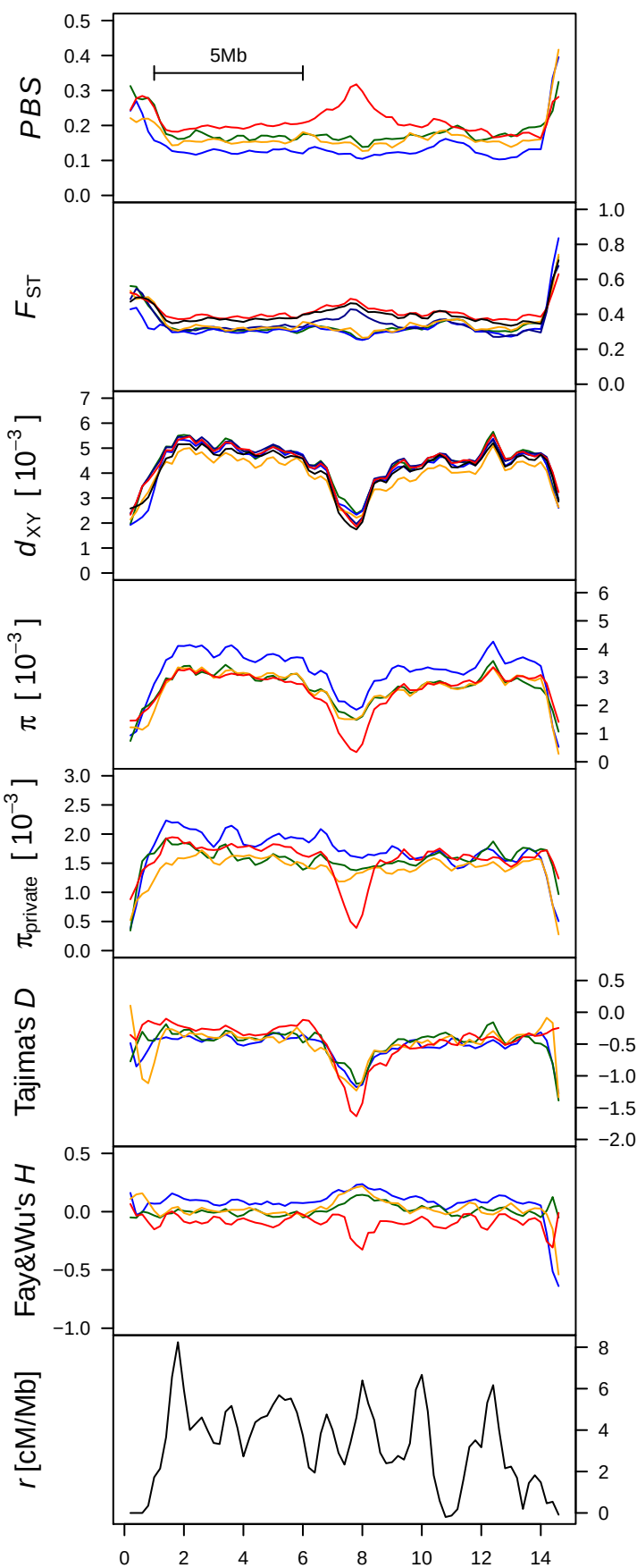
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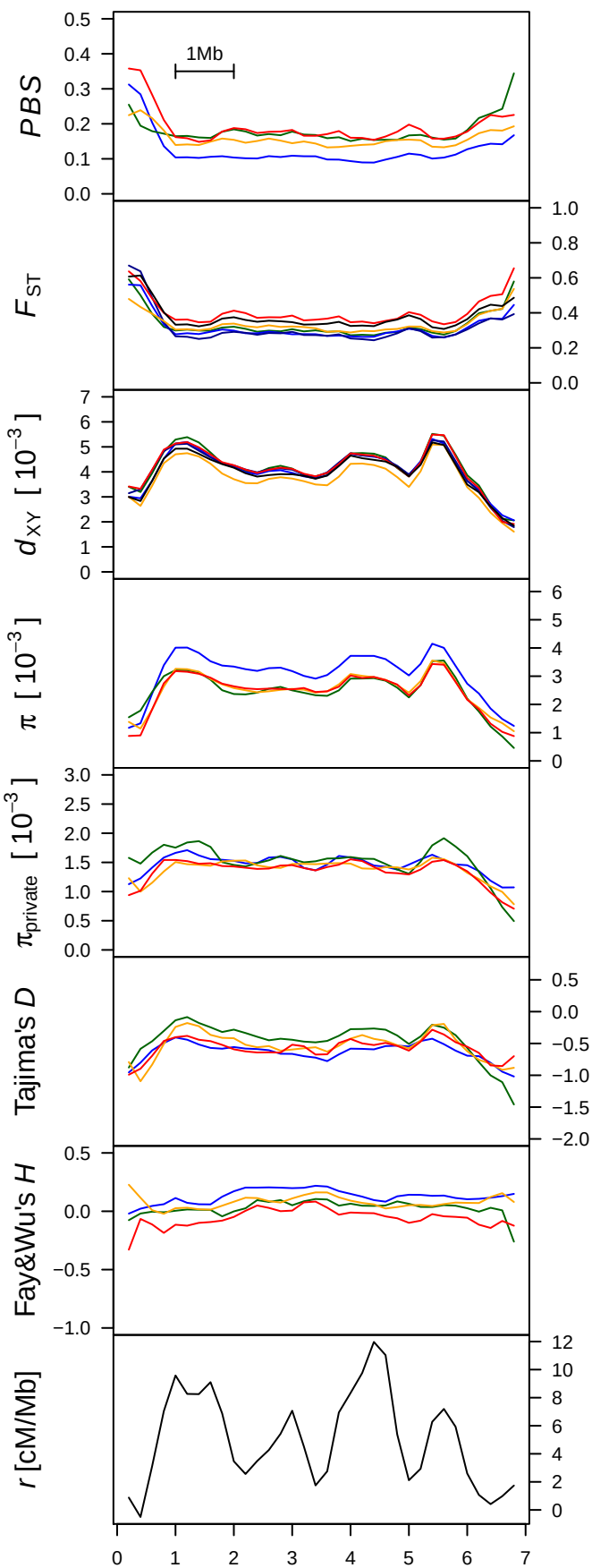
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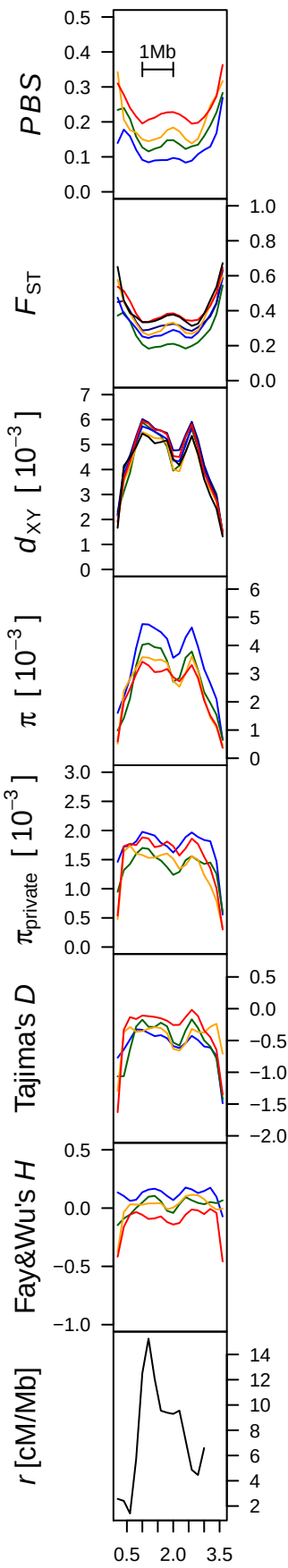
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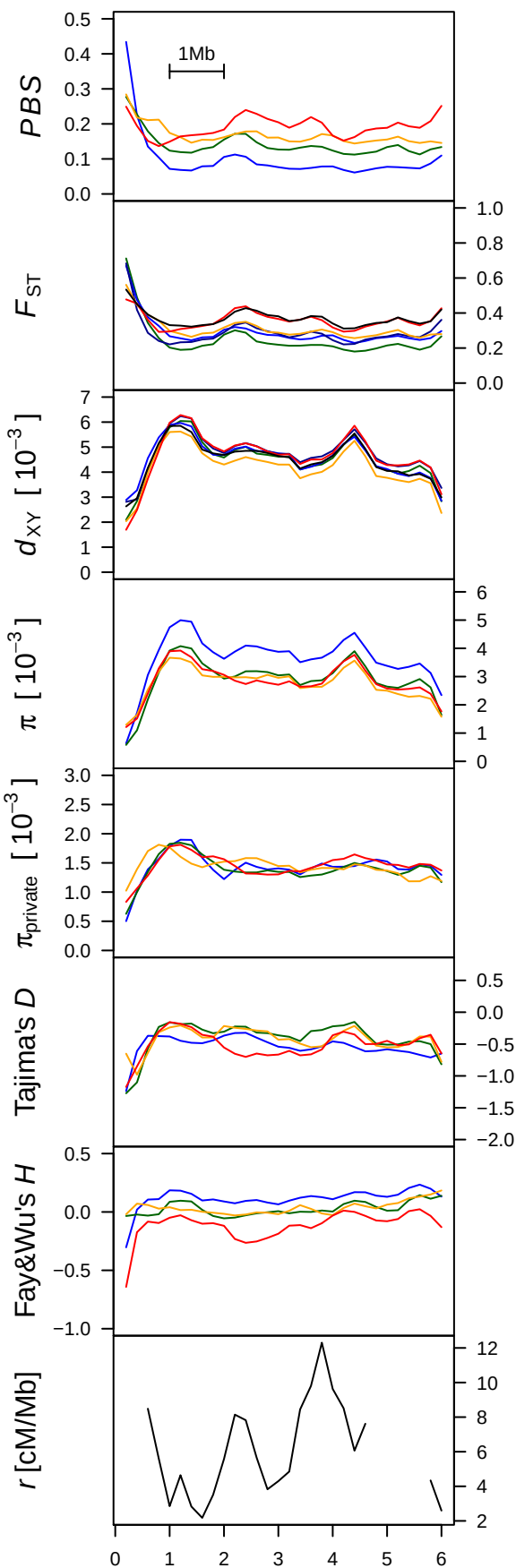
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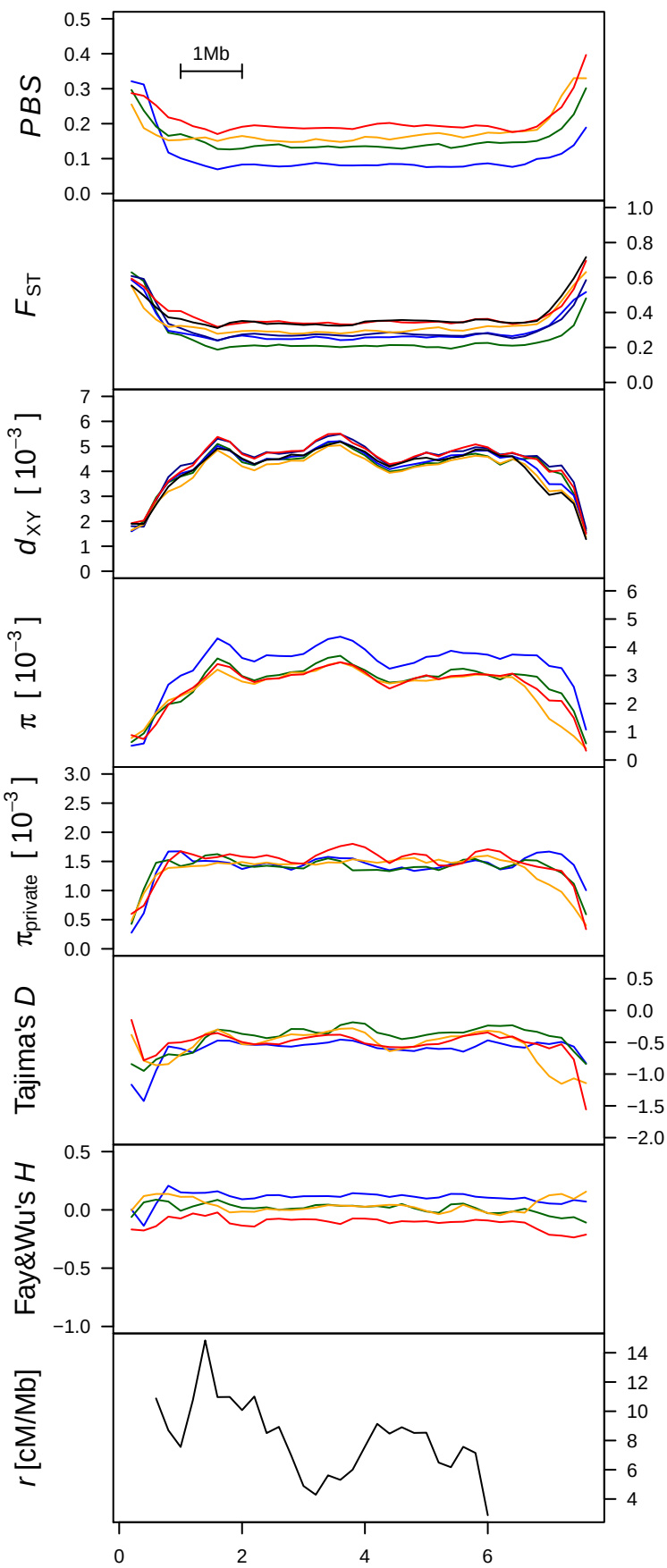
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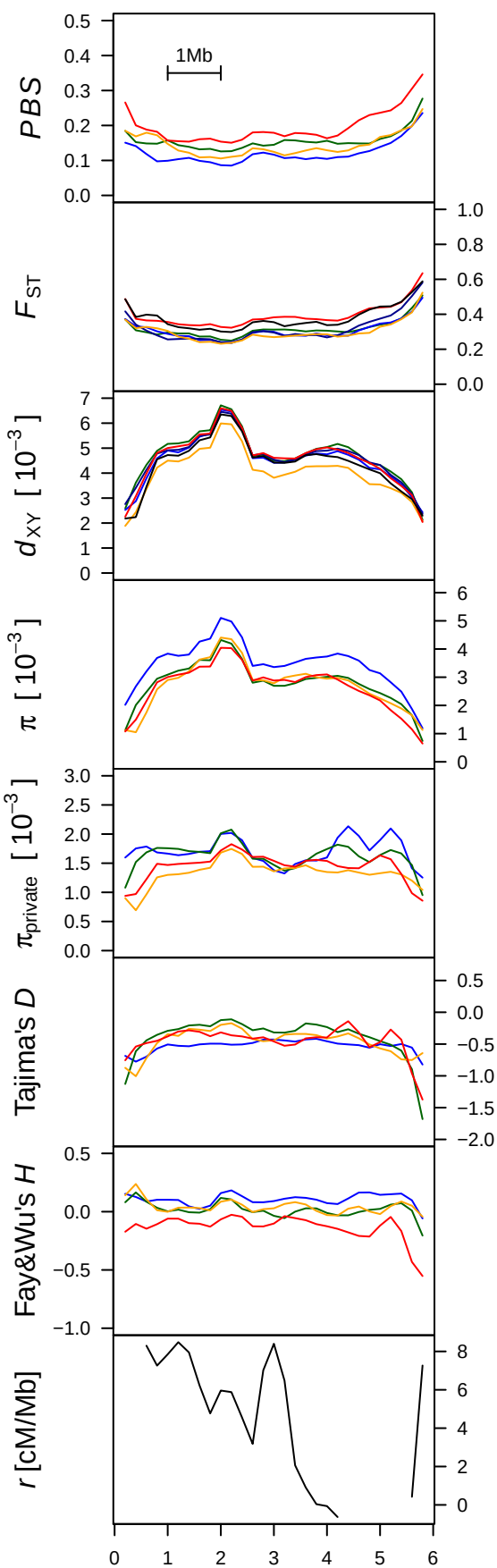


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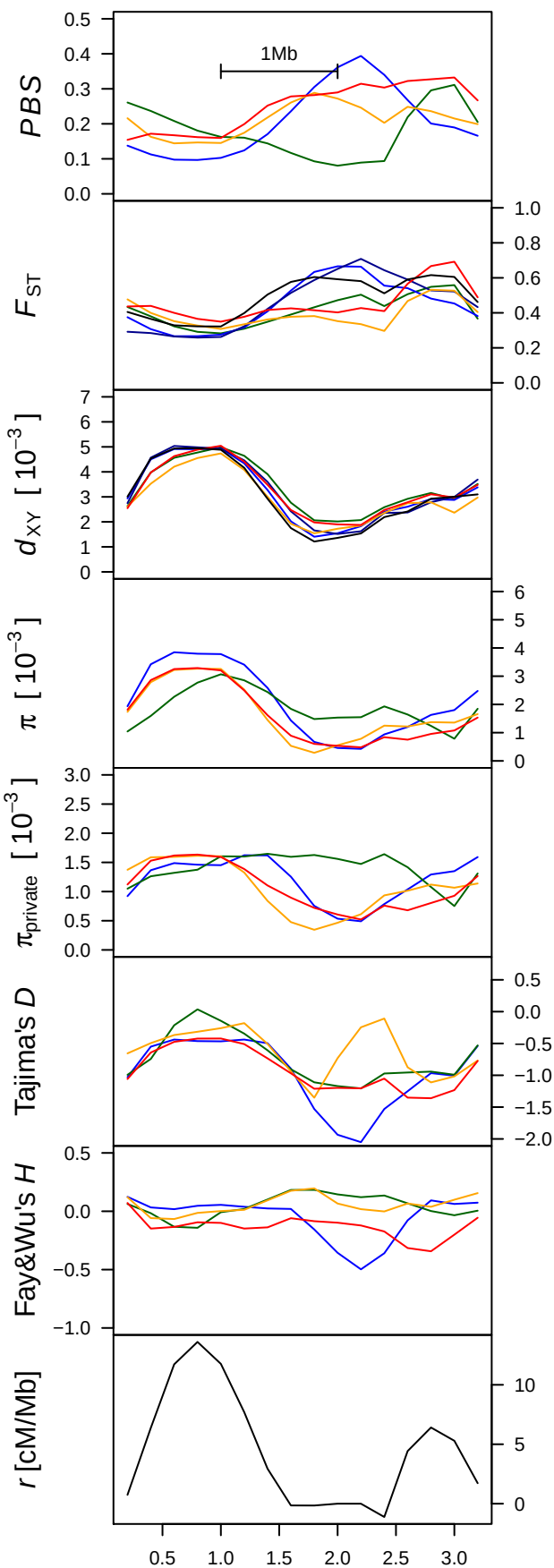




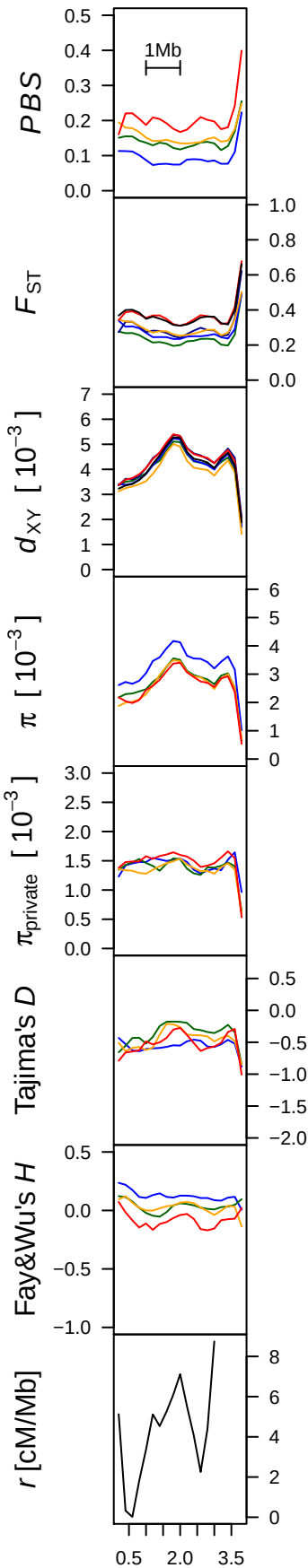
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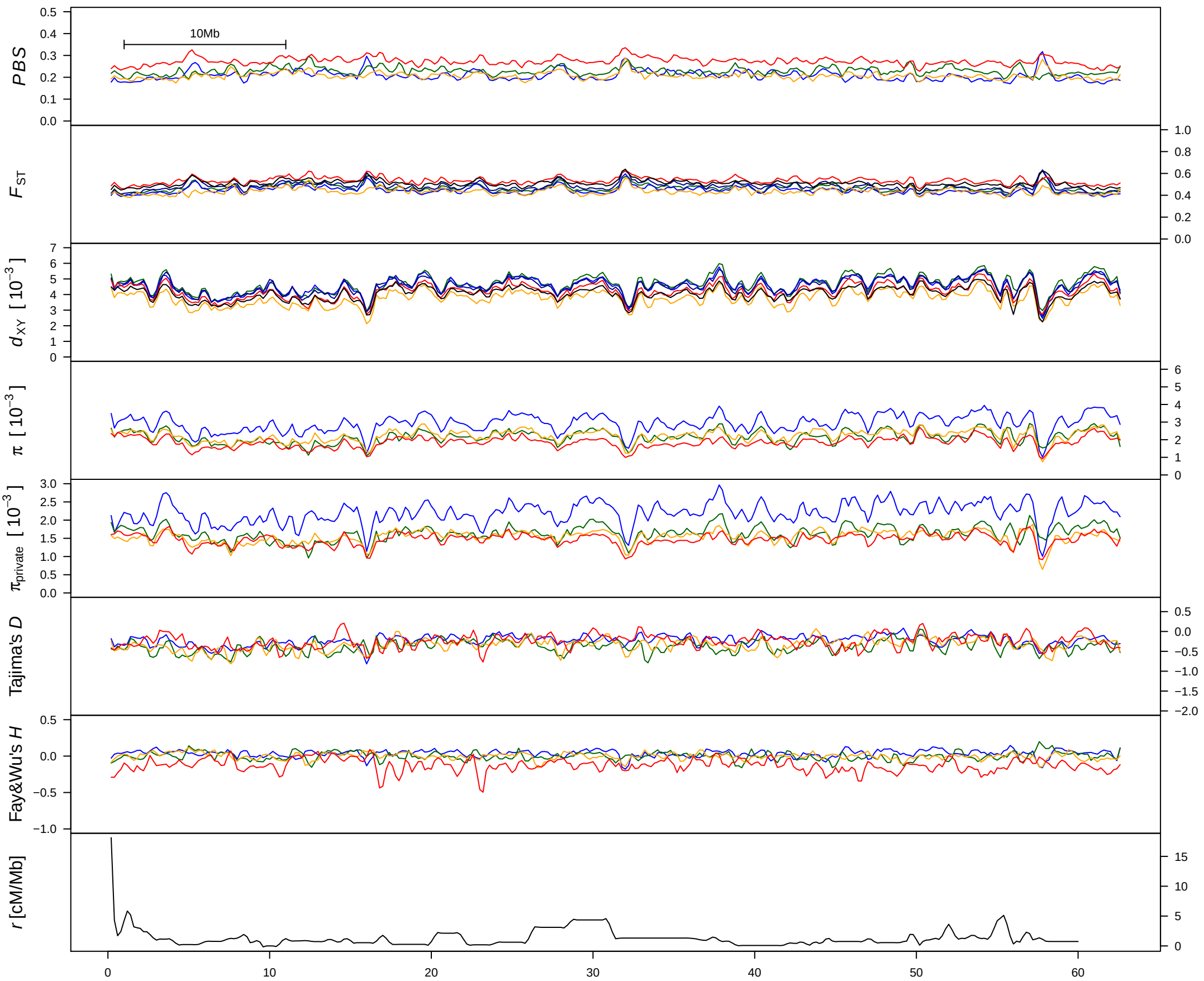
# Chr27



# Chr28



# ChrZ



**Supplemental Figure S3.** Differentiation across the speciation continuum. Pairwise estimates of  $F_{ST}$  within collared flycatcher (cyan, Italy-Hungary; blue, Italy-Baltic) and between collared flycatcher and pied (green), Atlas (orange), semicollared (red), red-breasted (dark-red), and snowy-browed flycatcher (black). As only one individual of red-breasted (dark-red) and snowy-browed flycatcher was sequenced, all pair-wise comparisons are based on one individual per species only. Parameter estimates were not smoothed.

Chr1

10Mb

$F_{ST}$

1.0

0.8

0.6

0.4

0.2

0.0

0

20

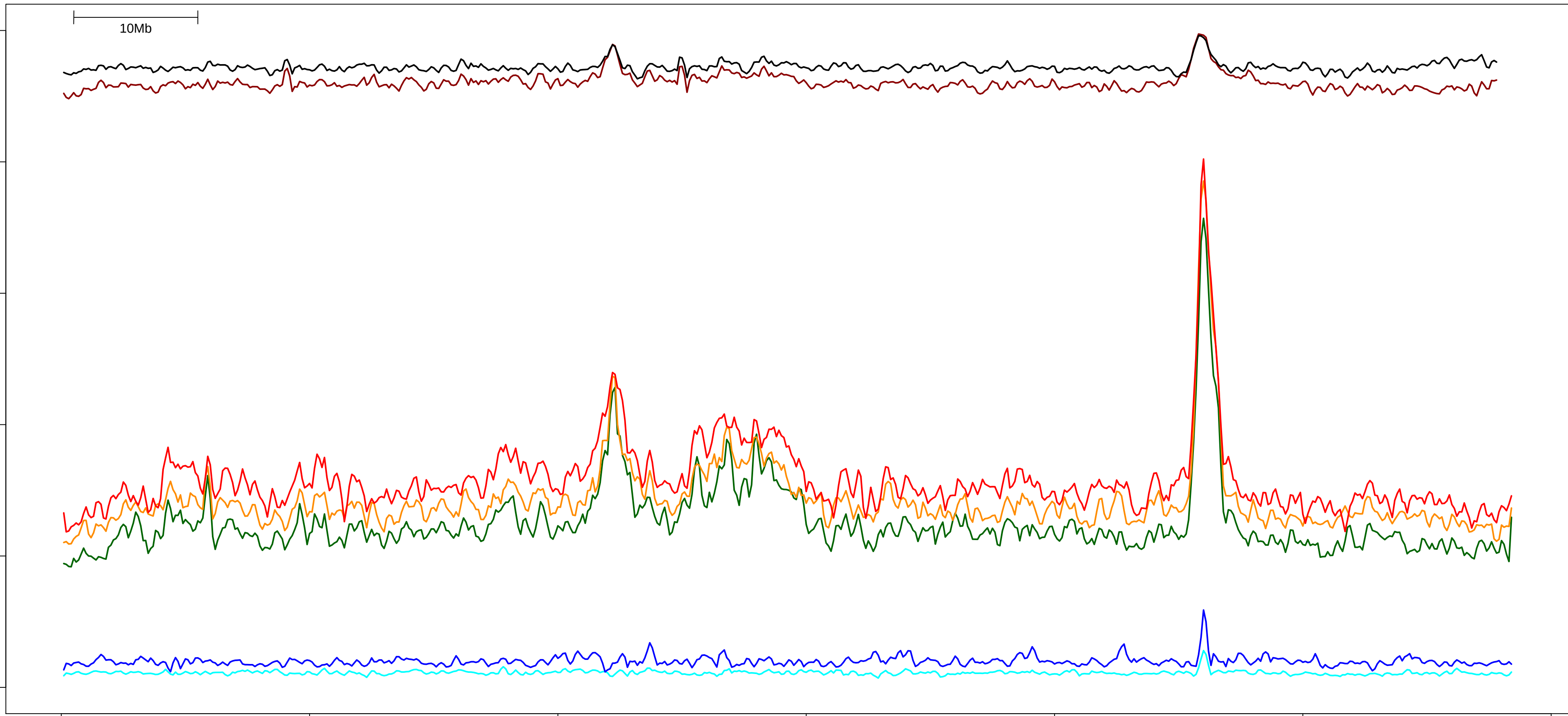
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60

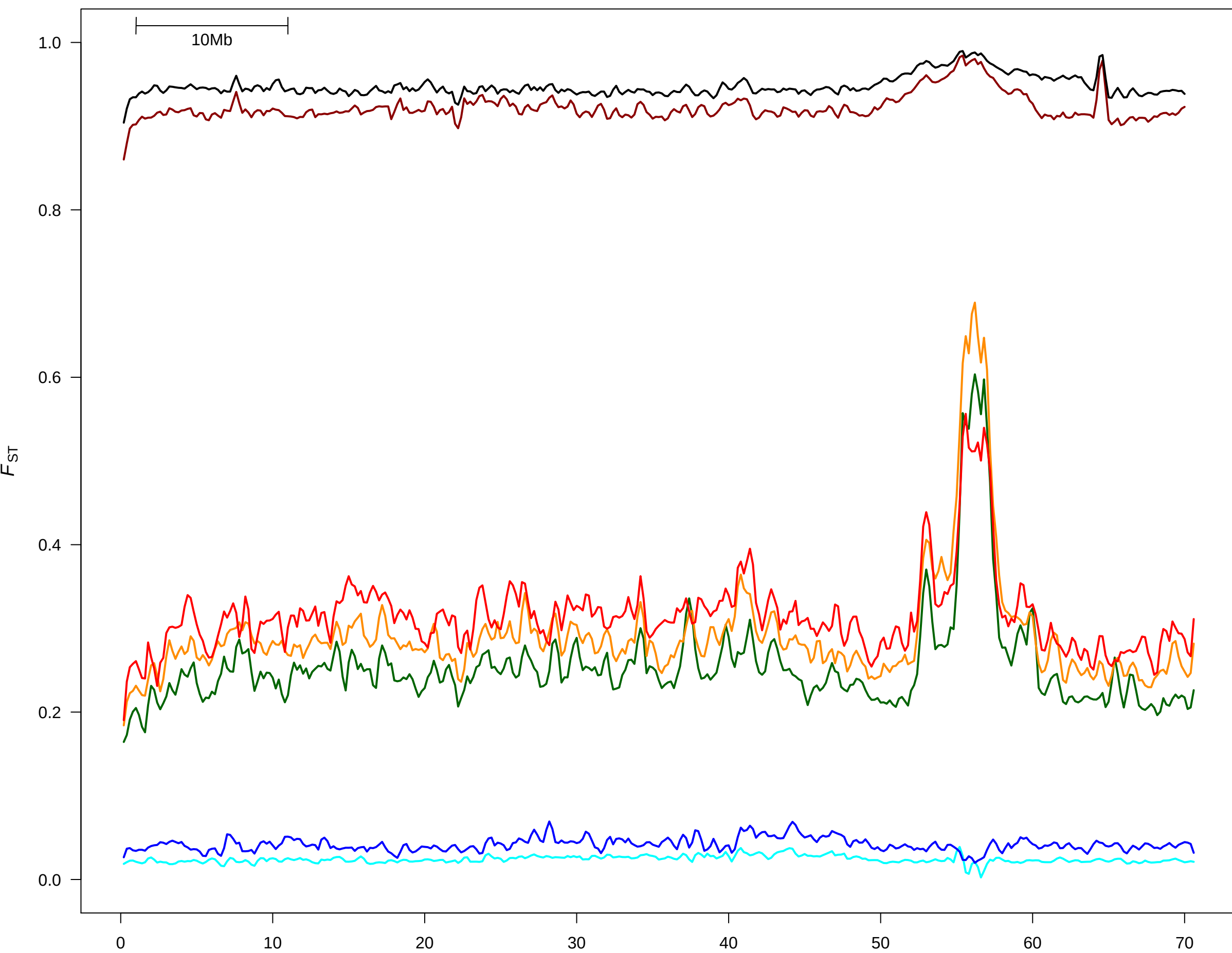
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100

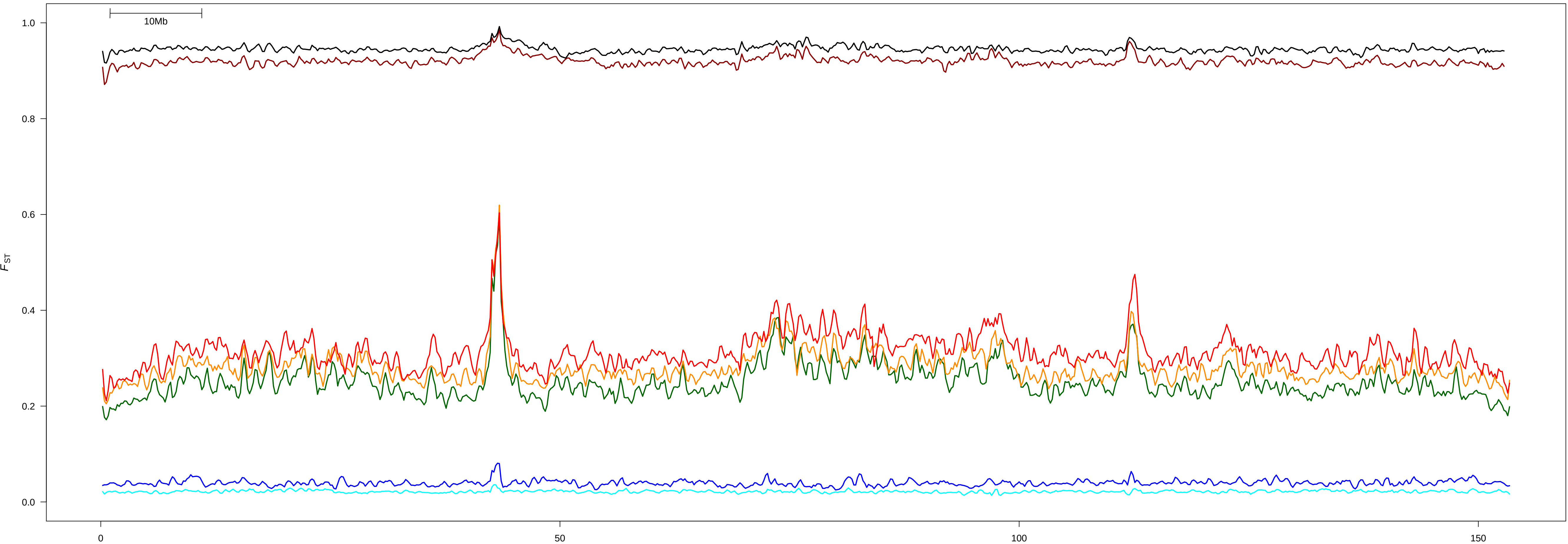
120



Chr1A

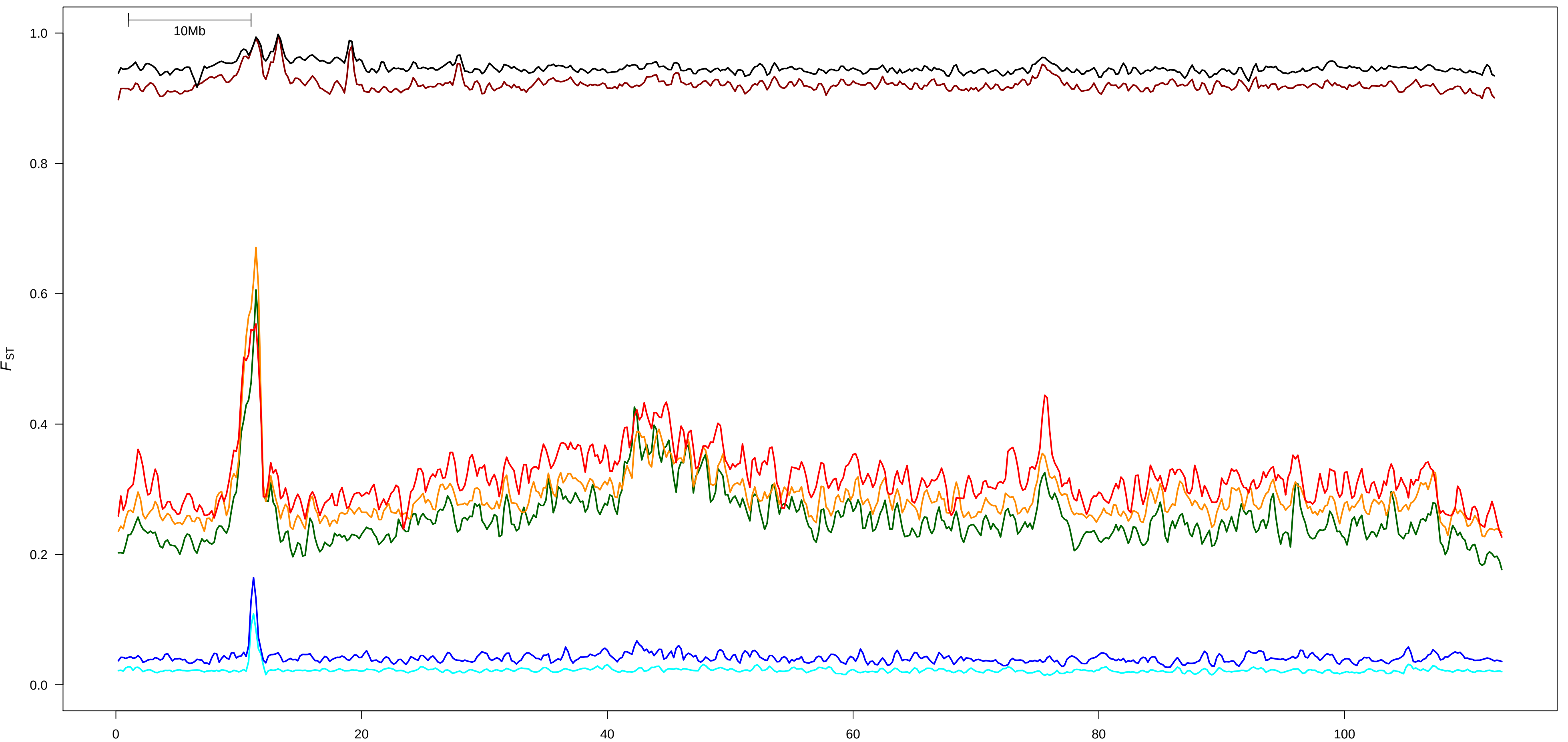


Chr2

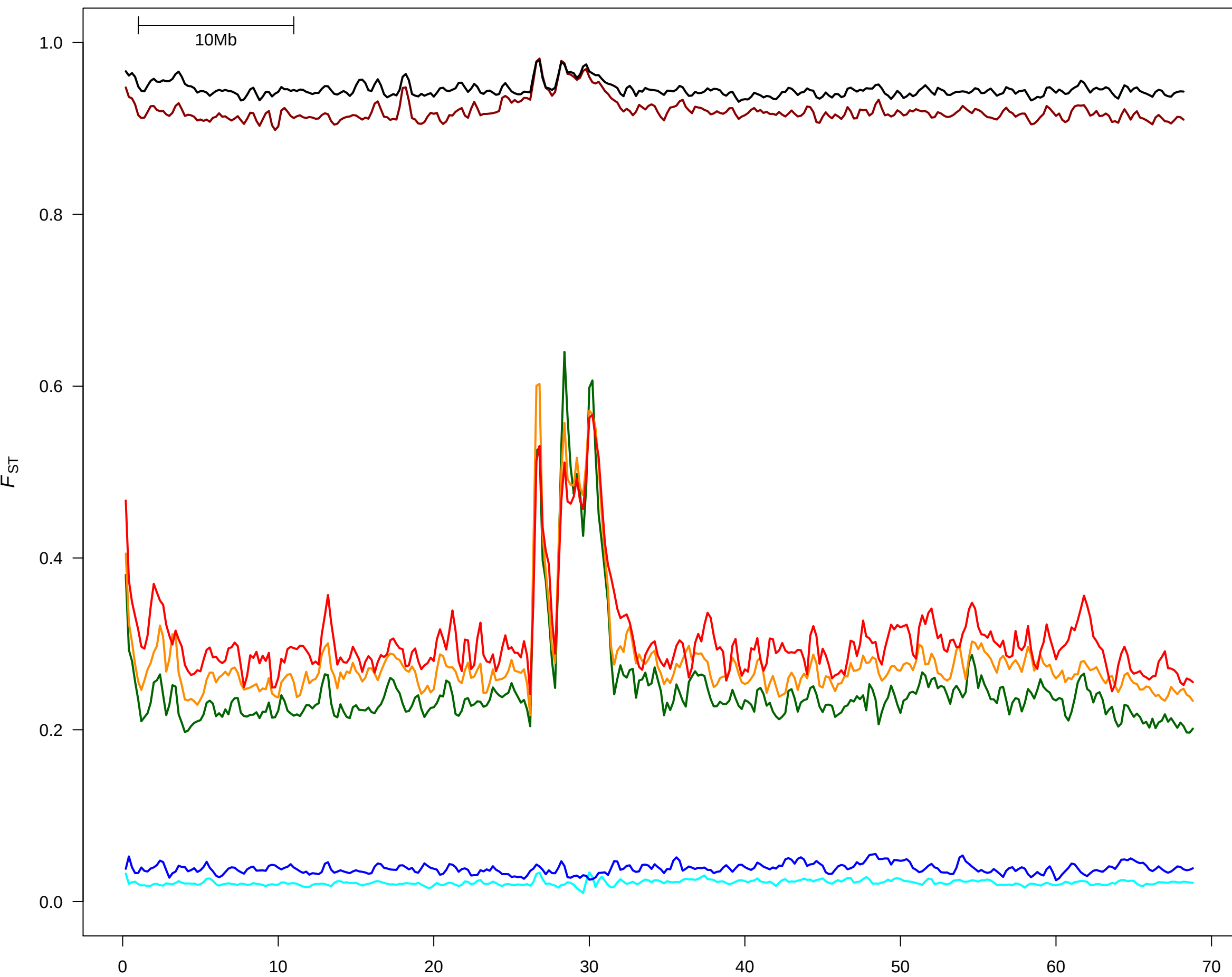




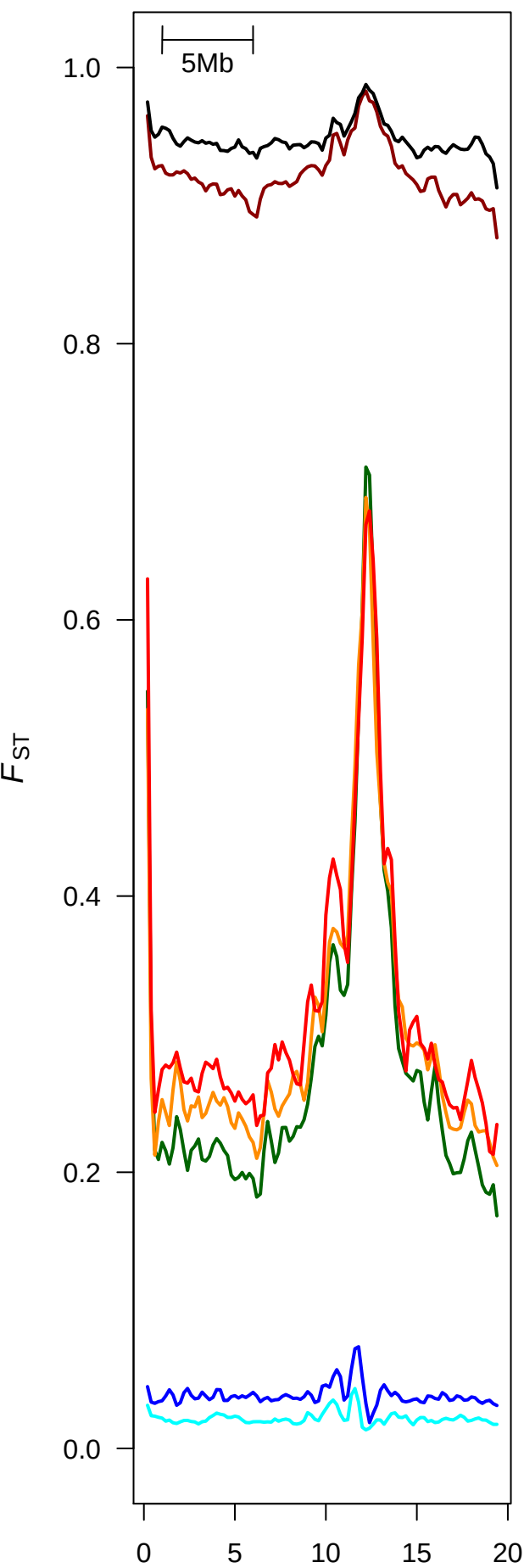
Chr3



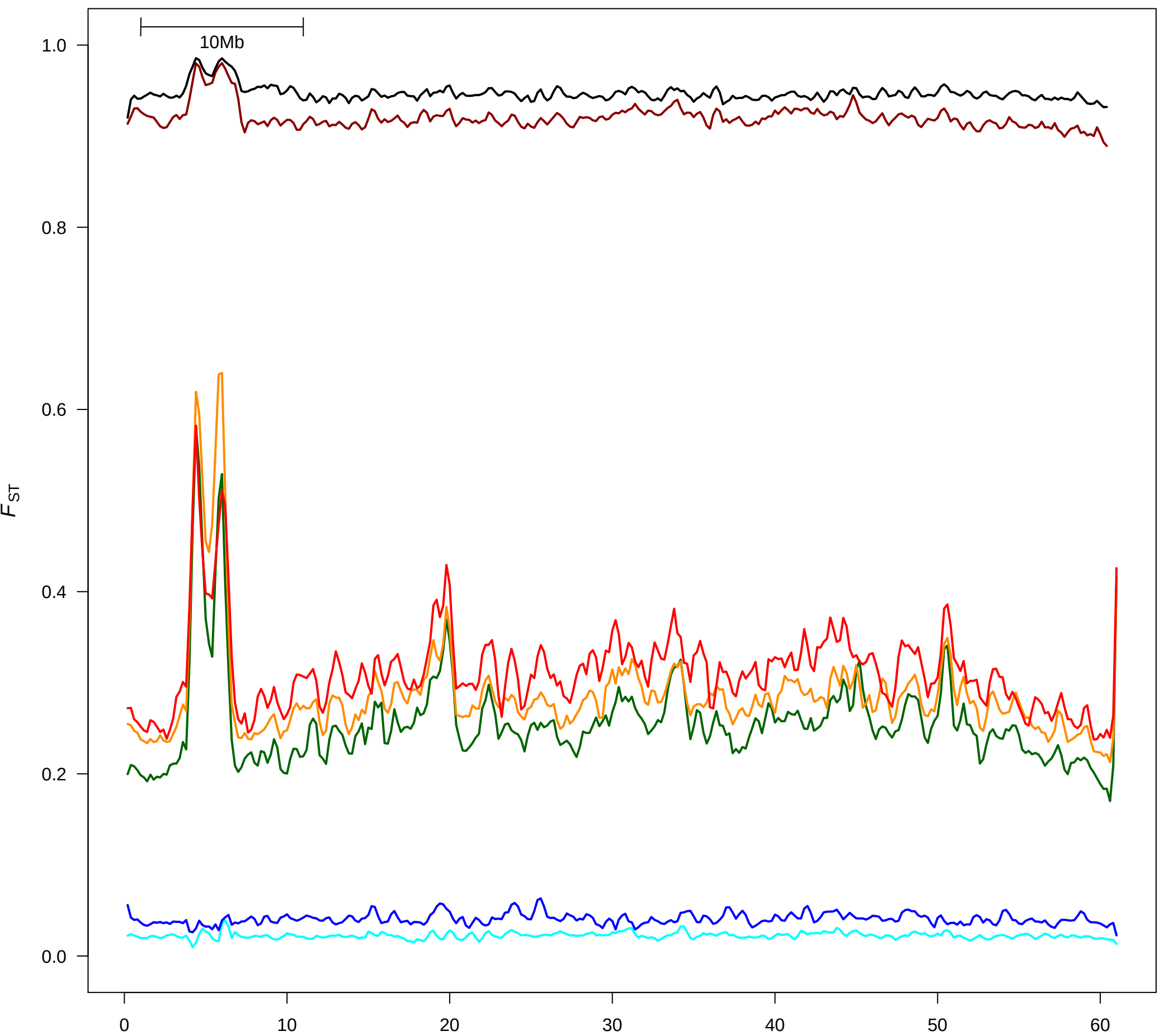
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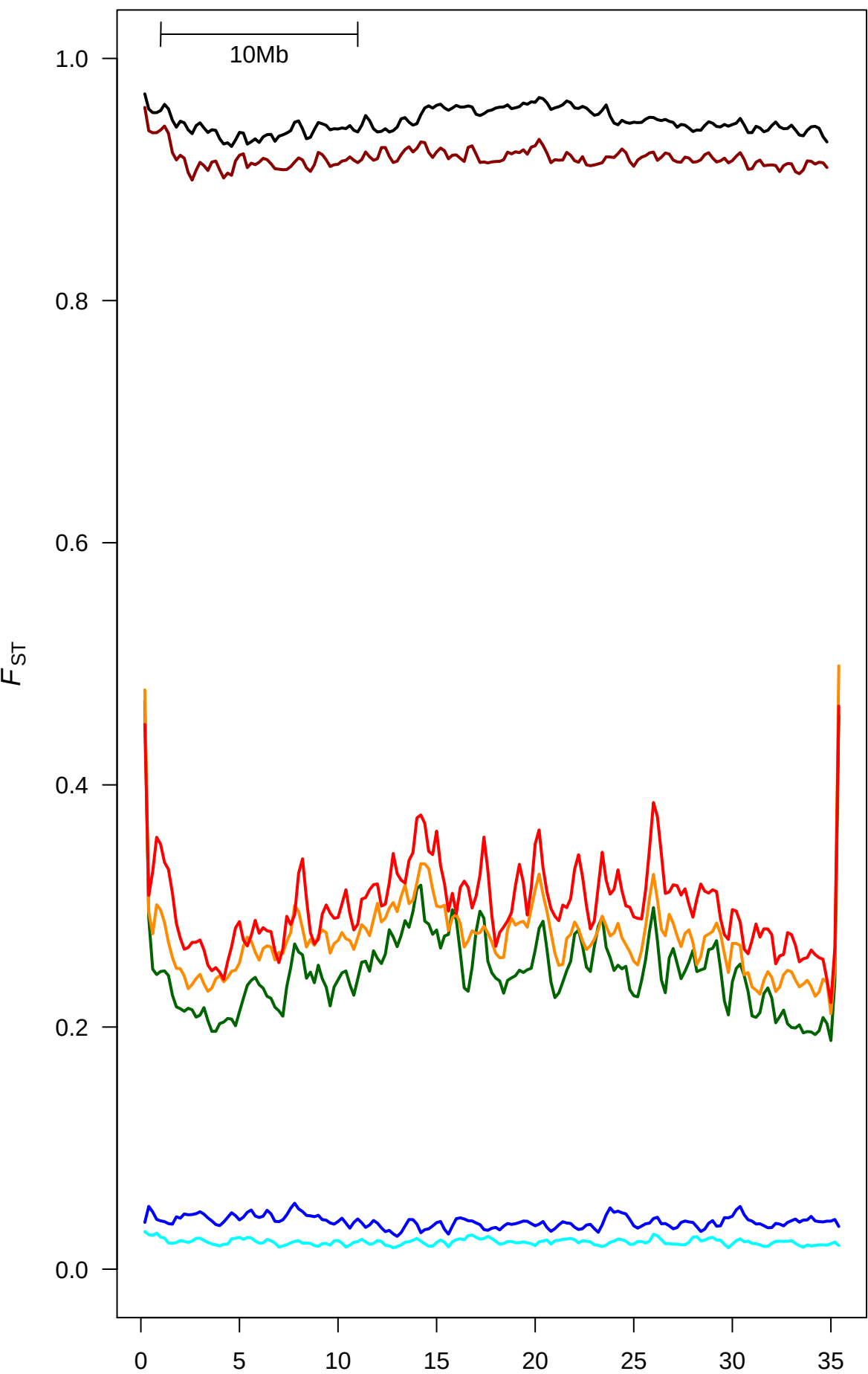
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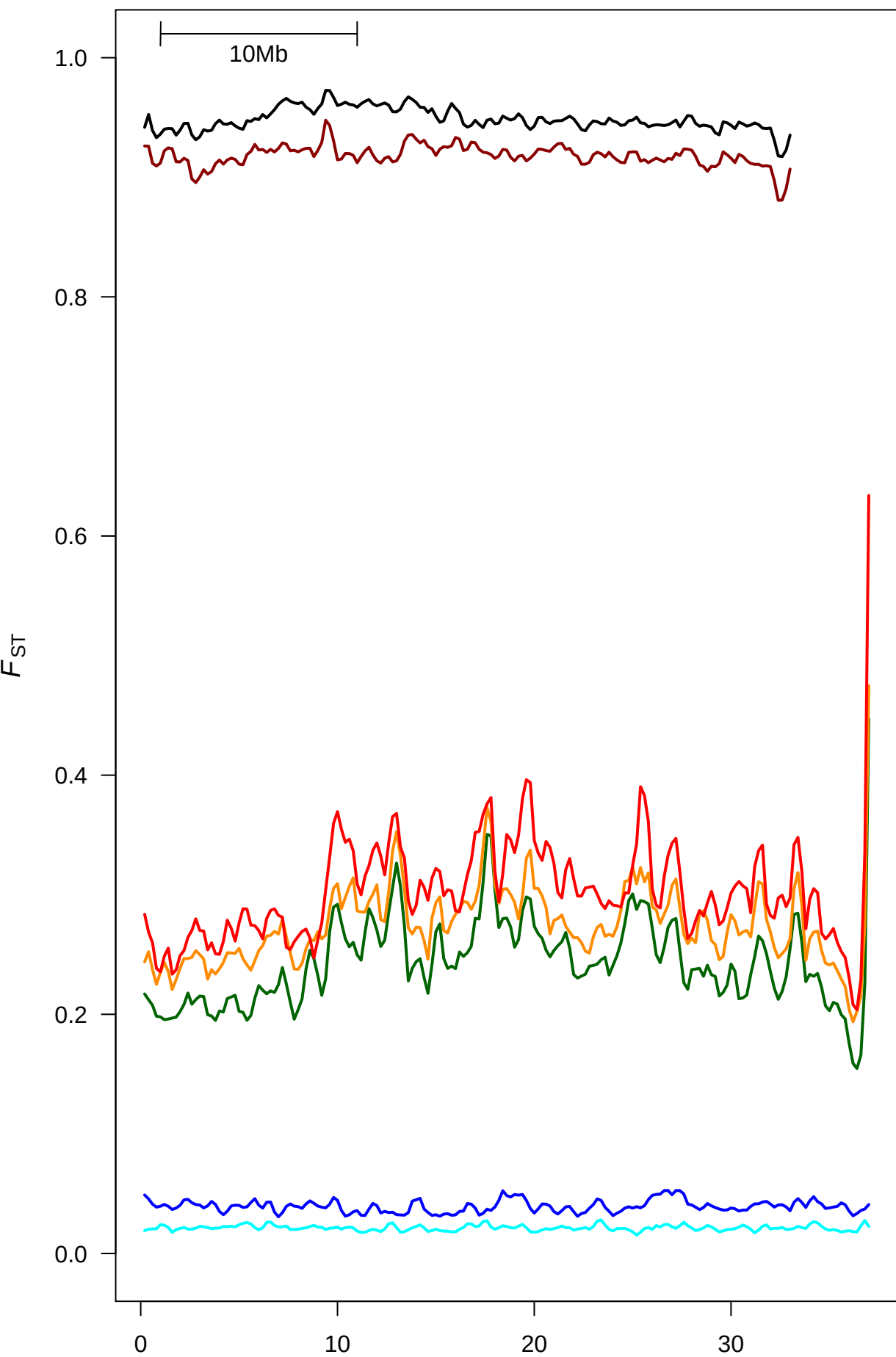
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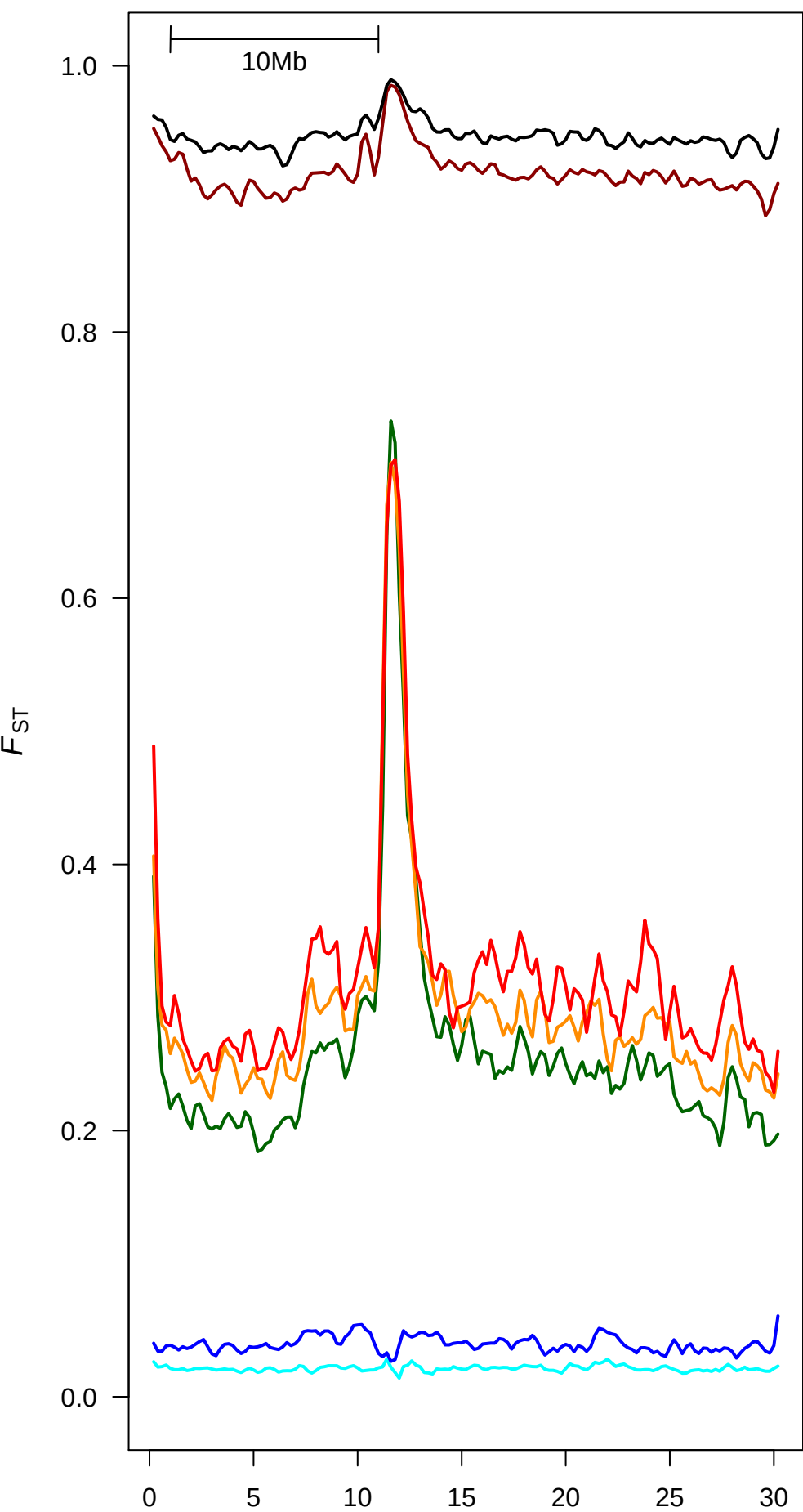
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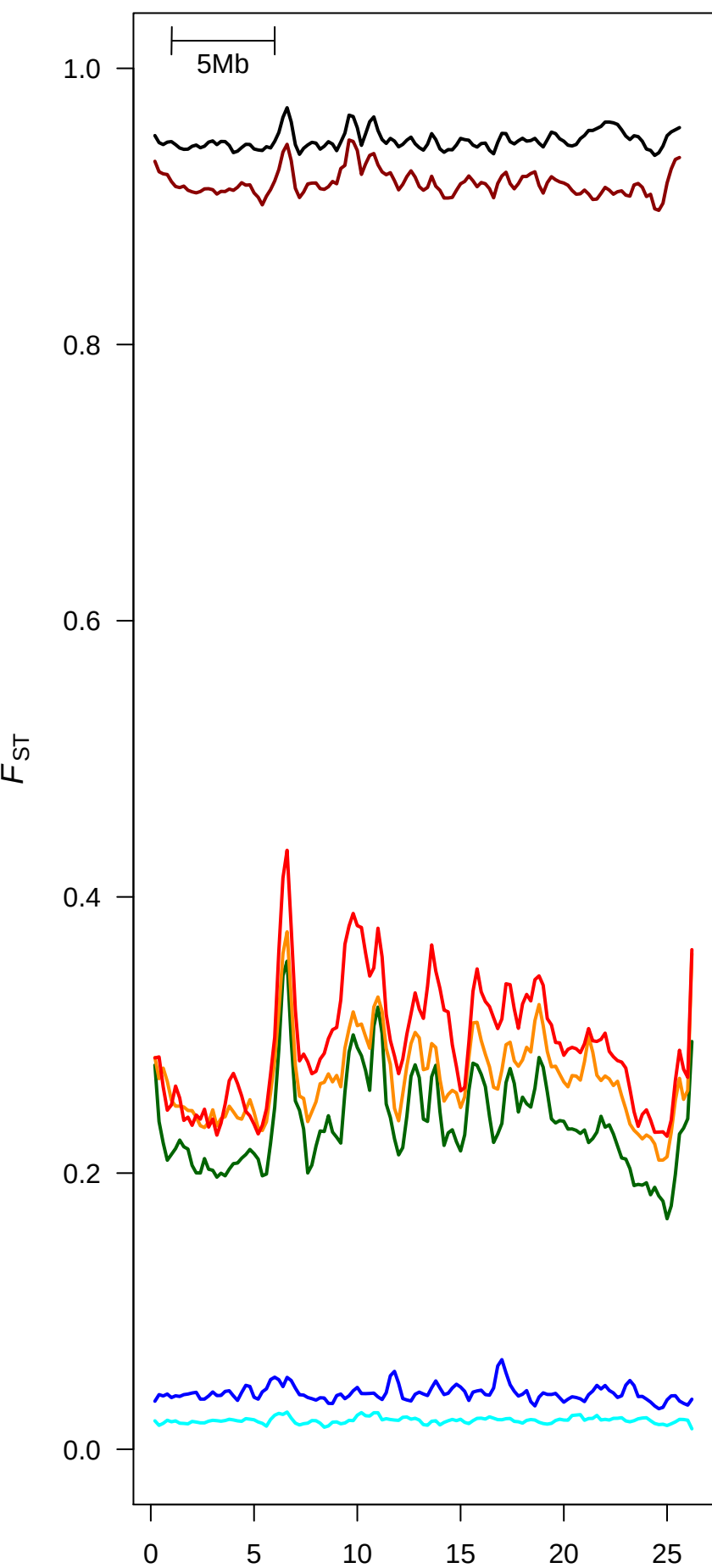
Chr7



Chr8

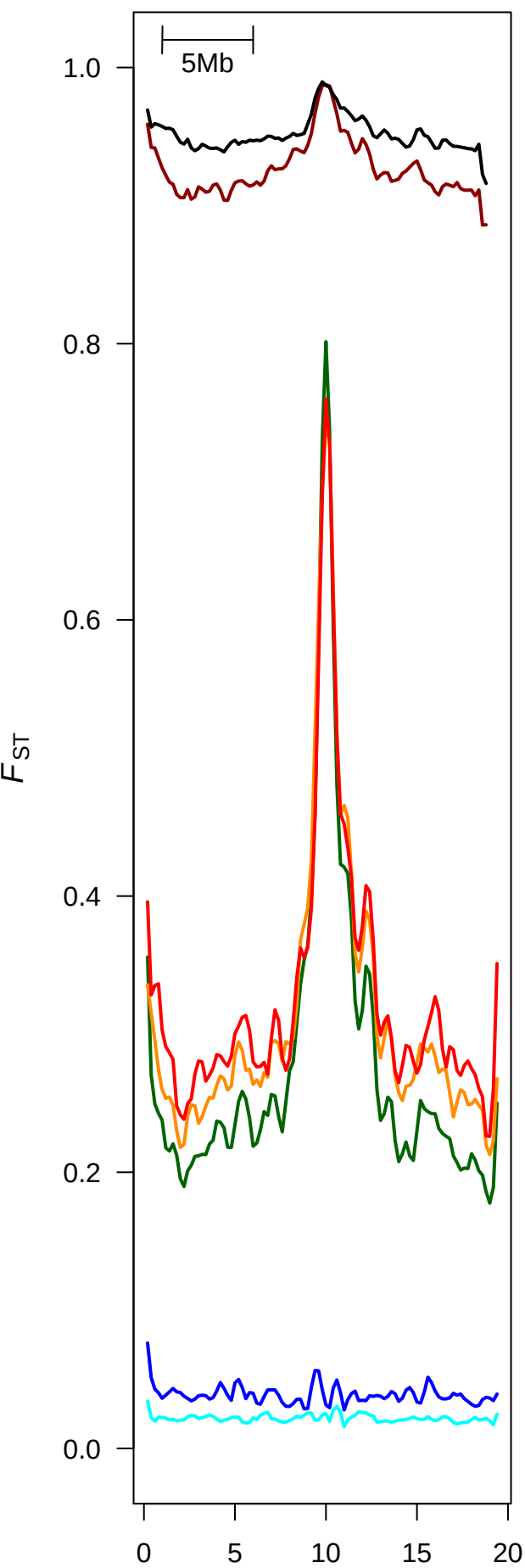


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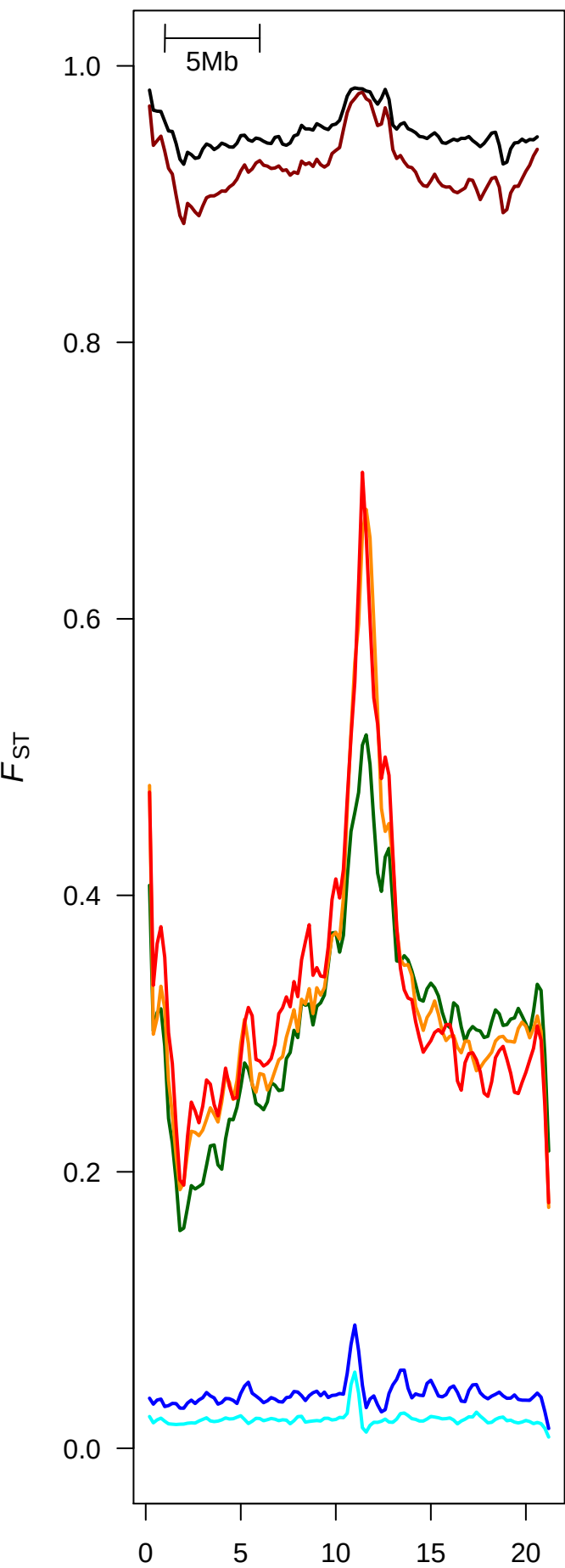




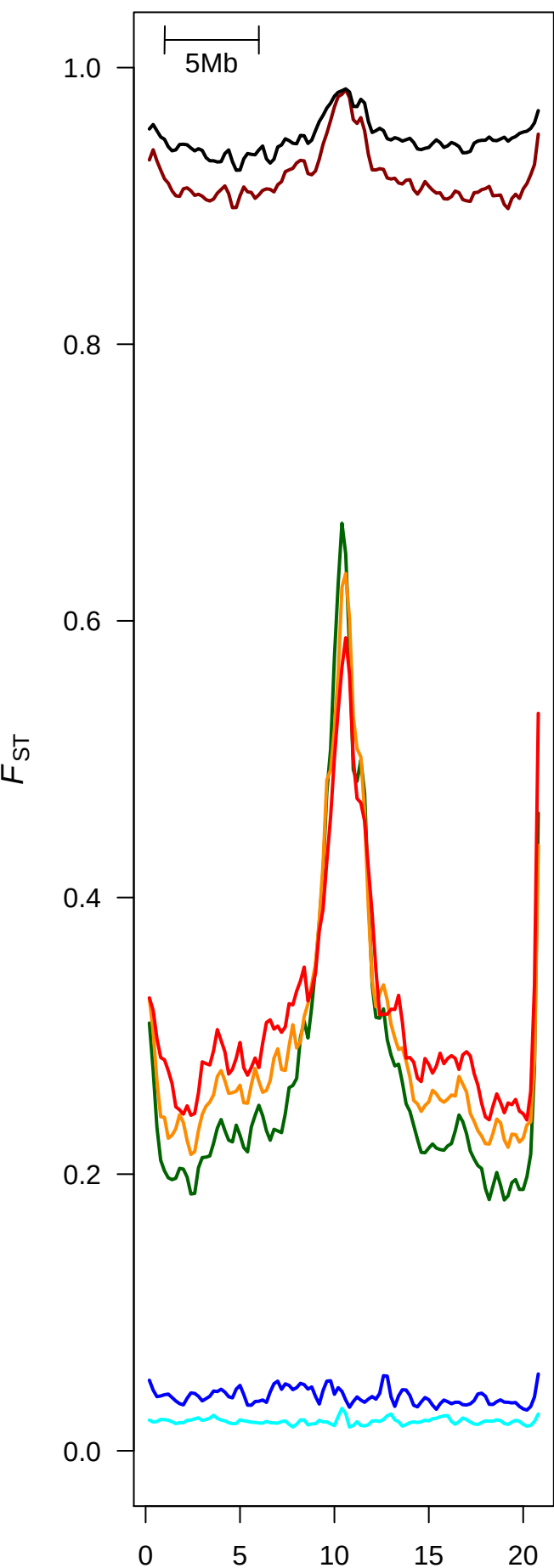
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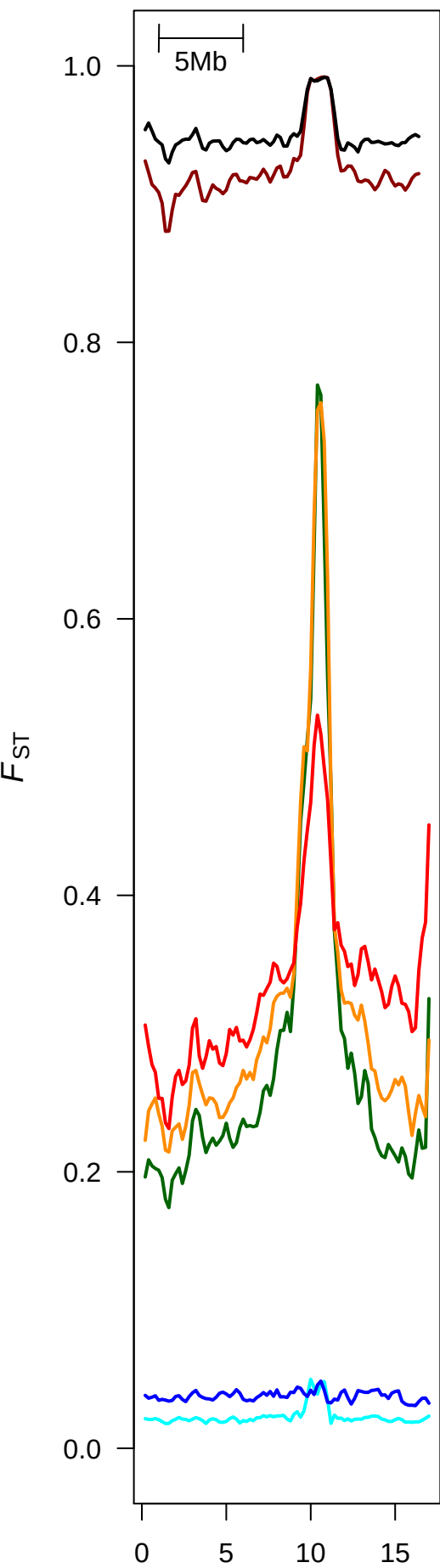
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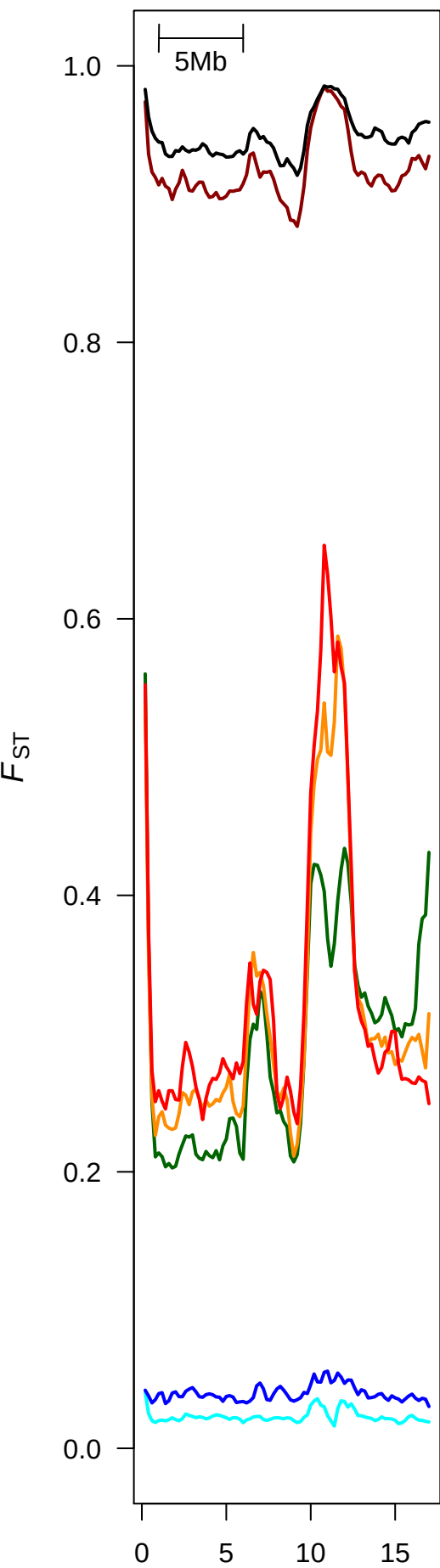
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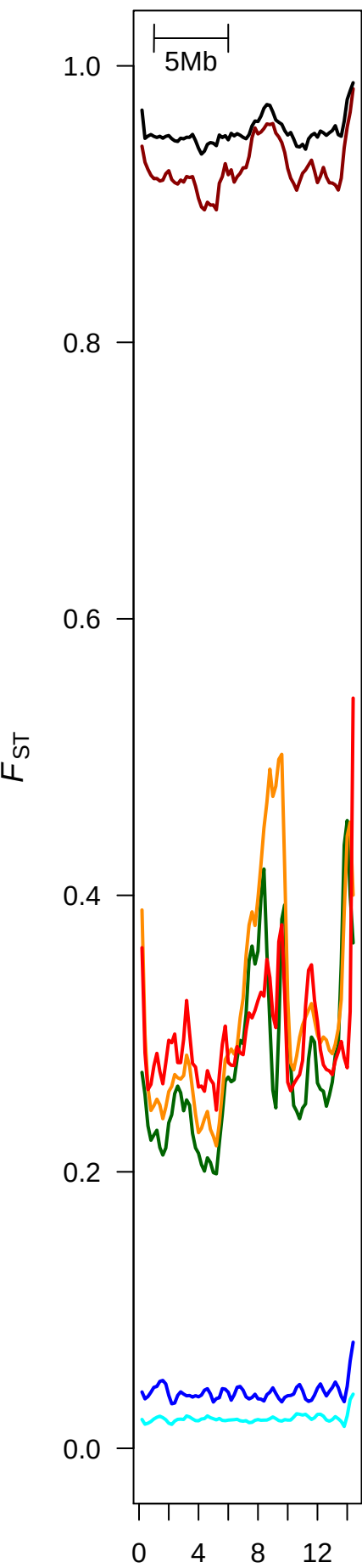
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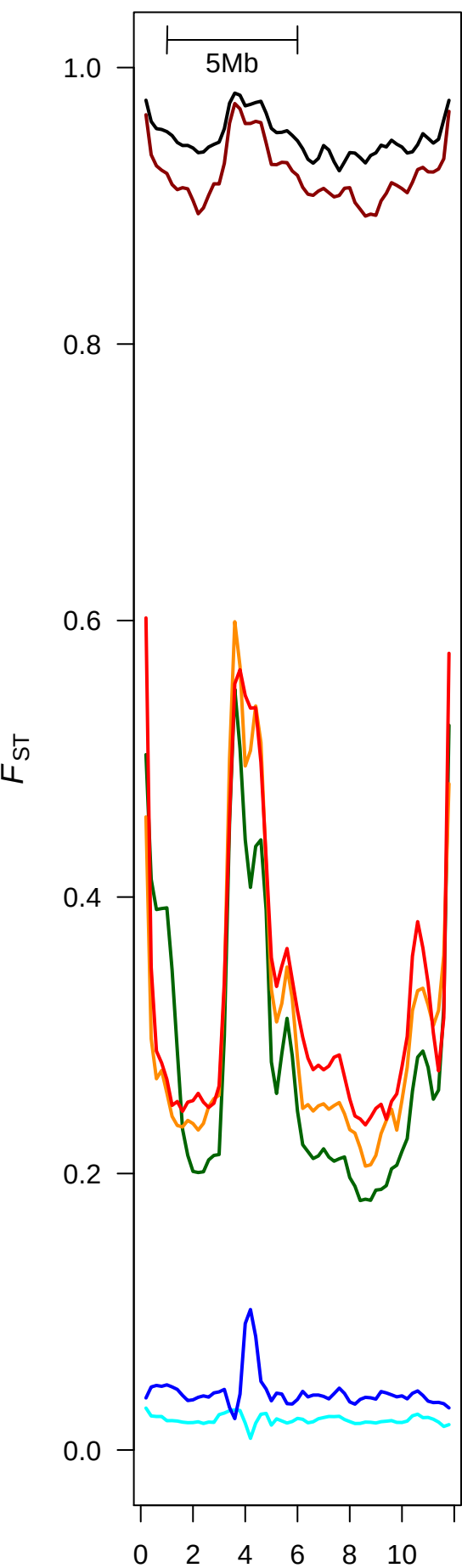
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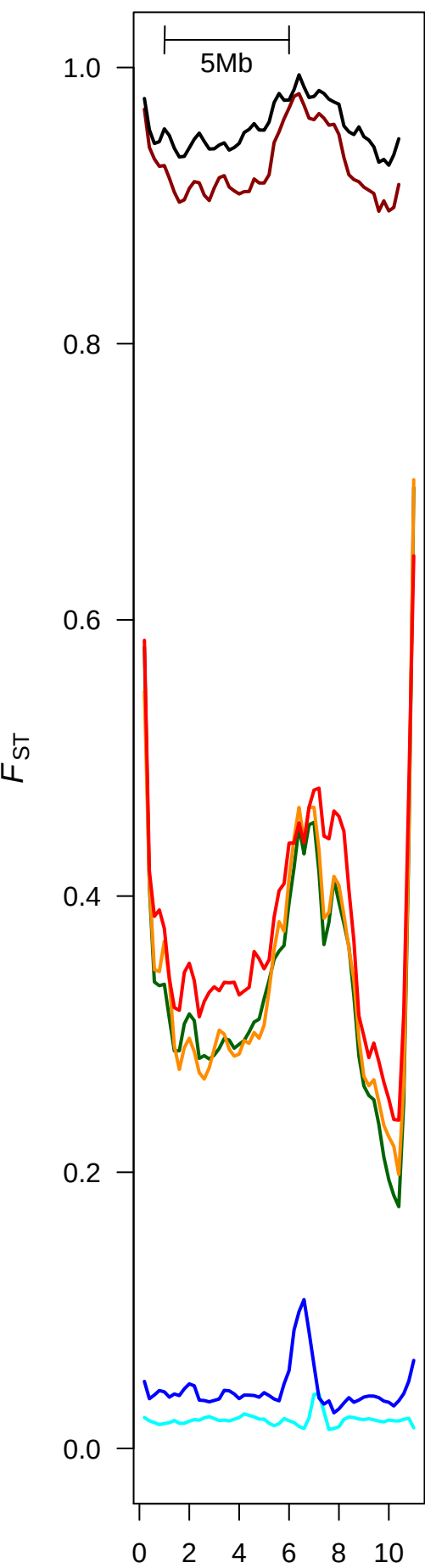
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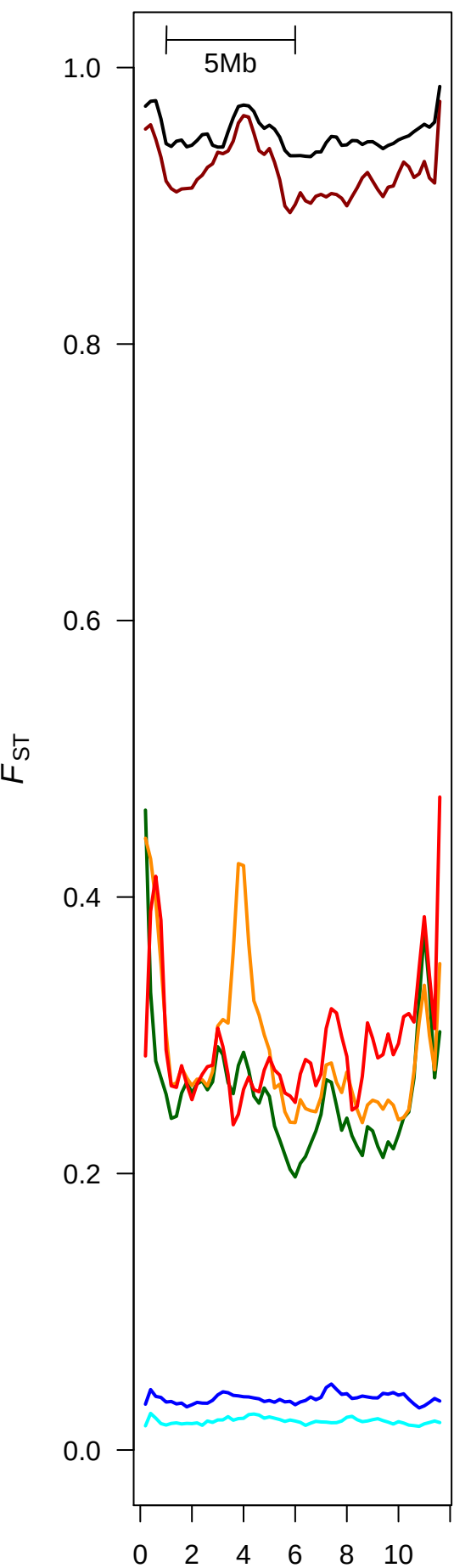


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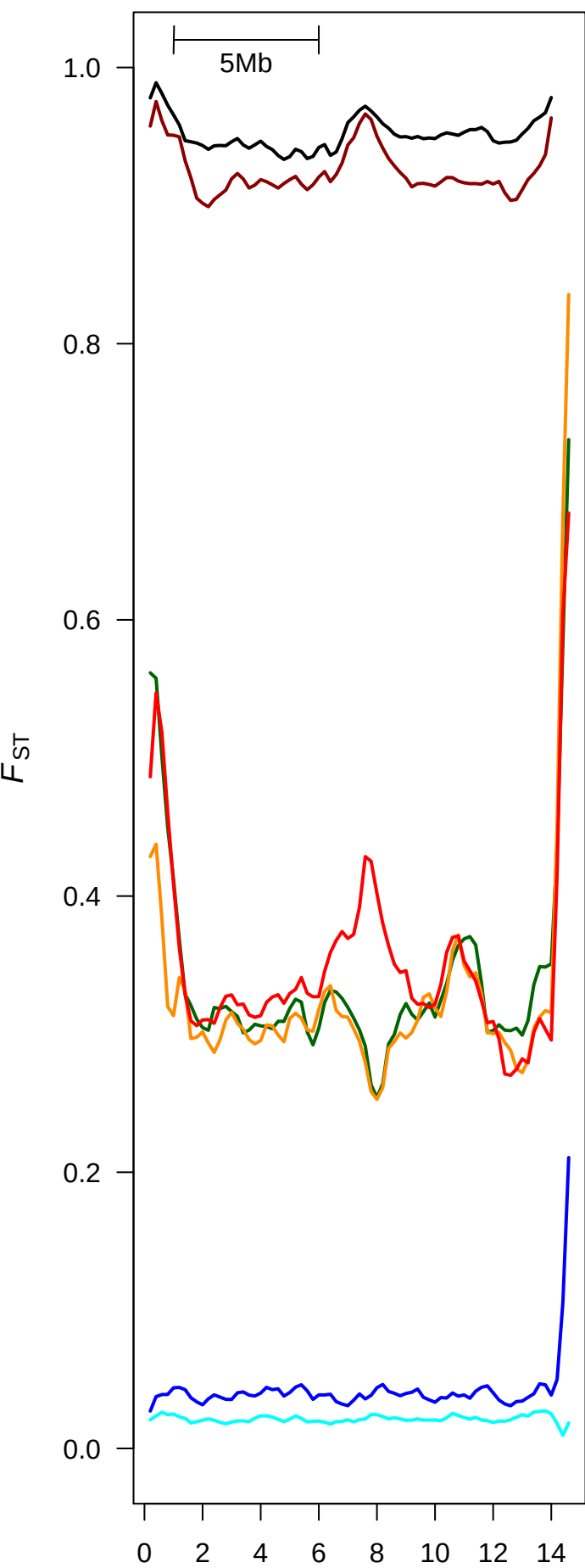




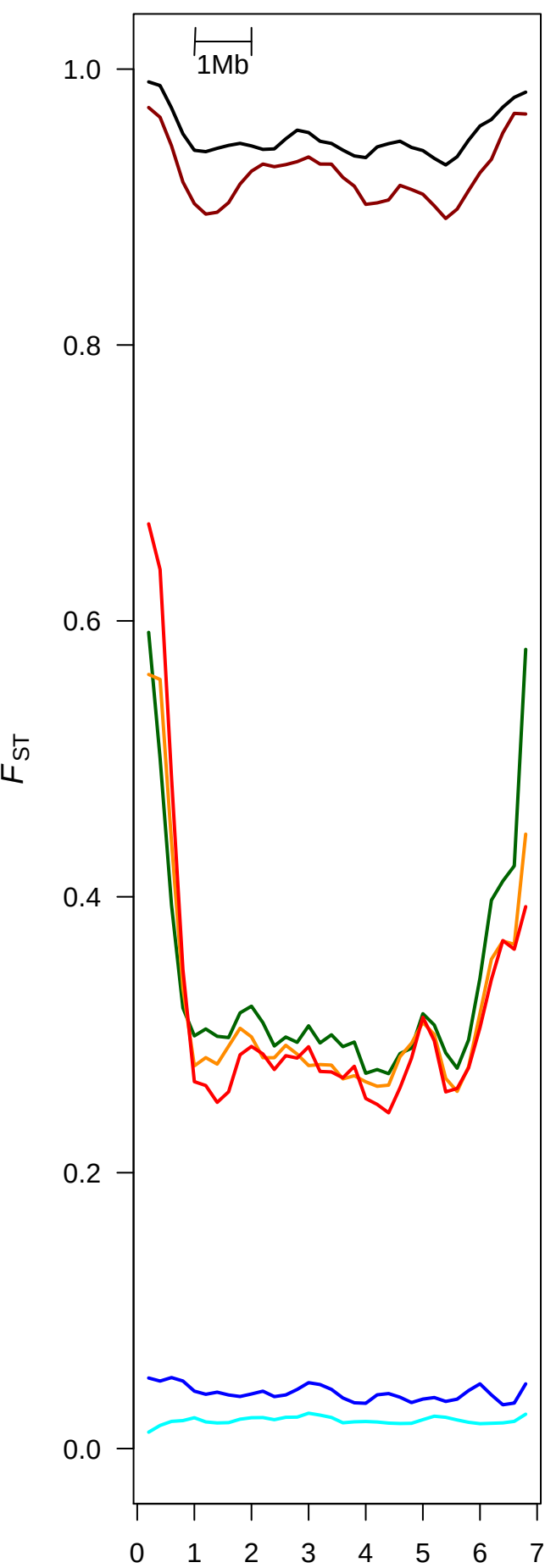
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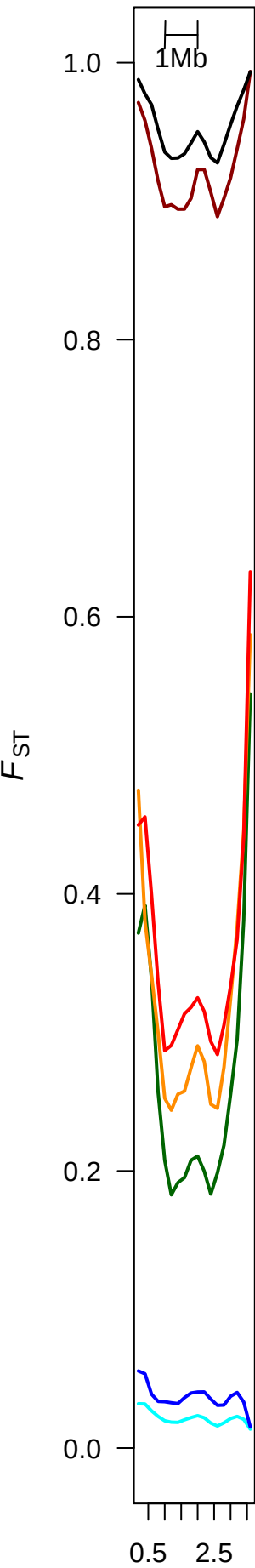
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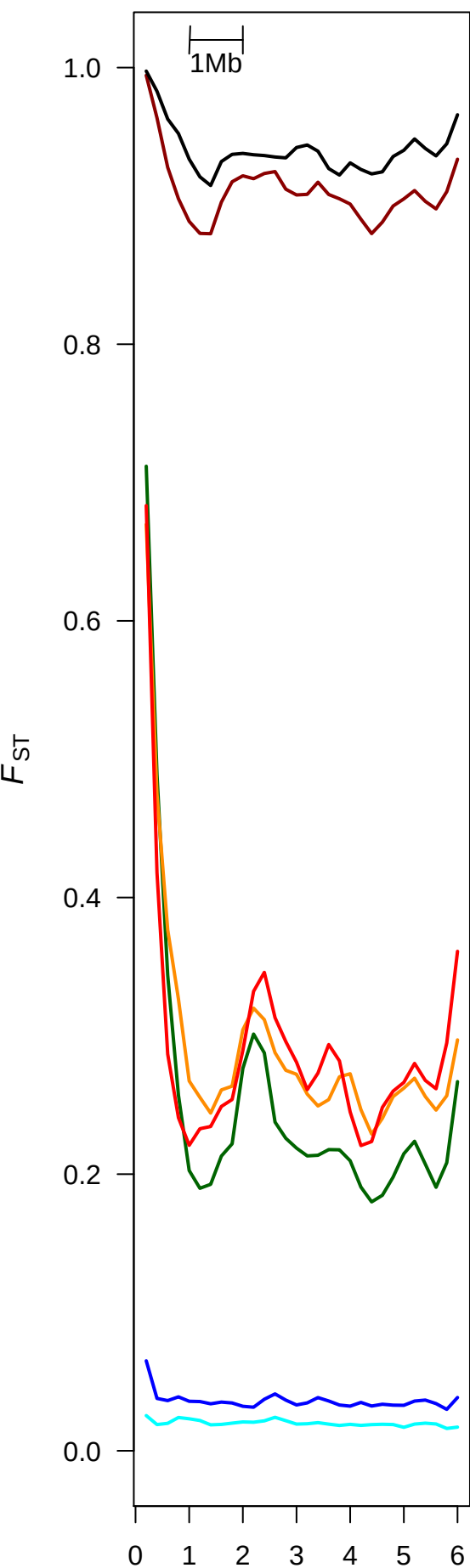
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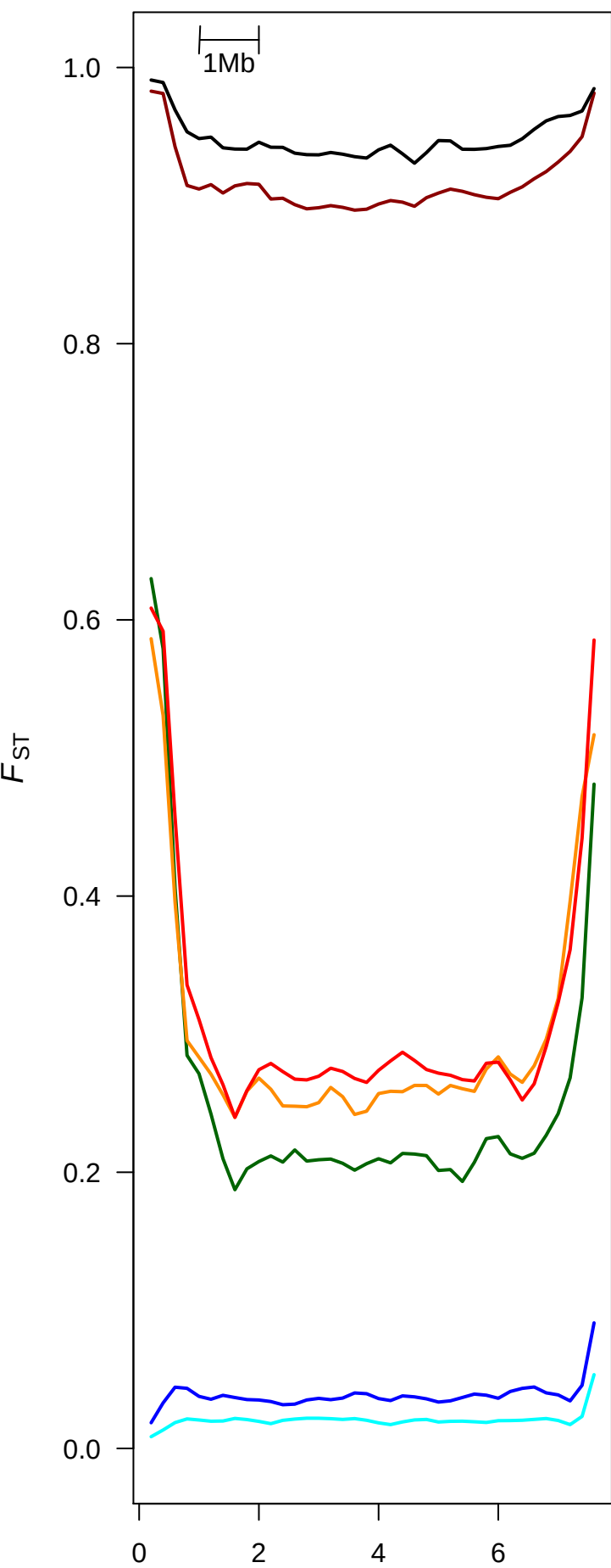
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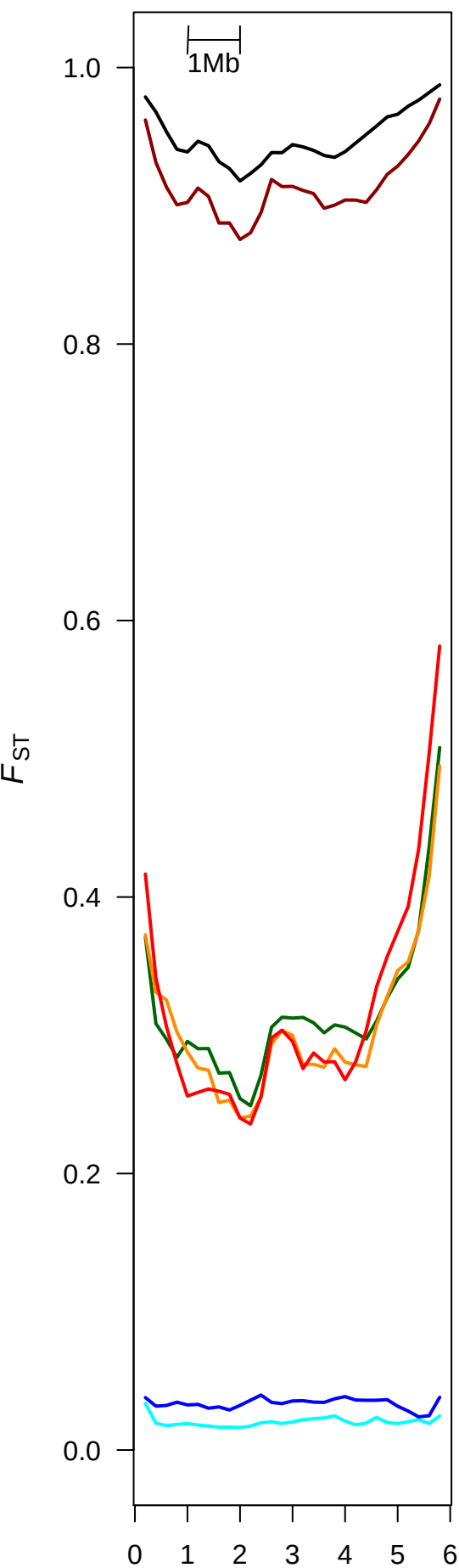
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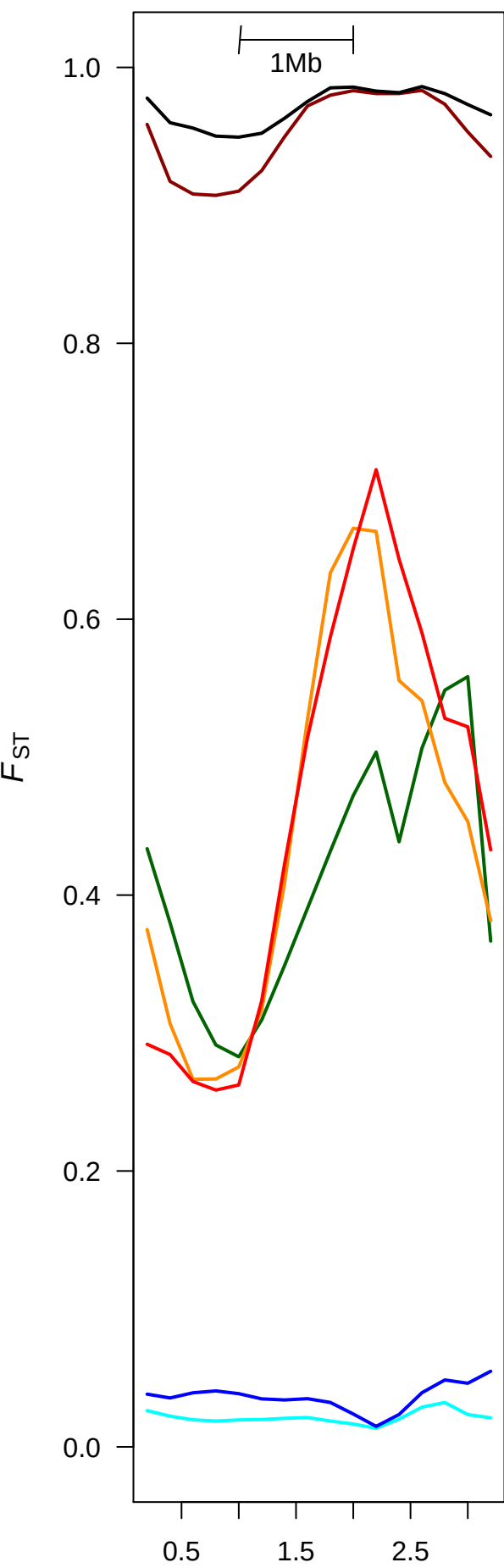
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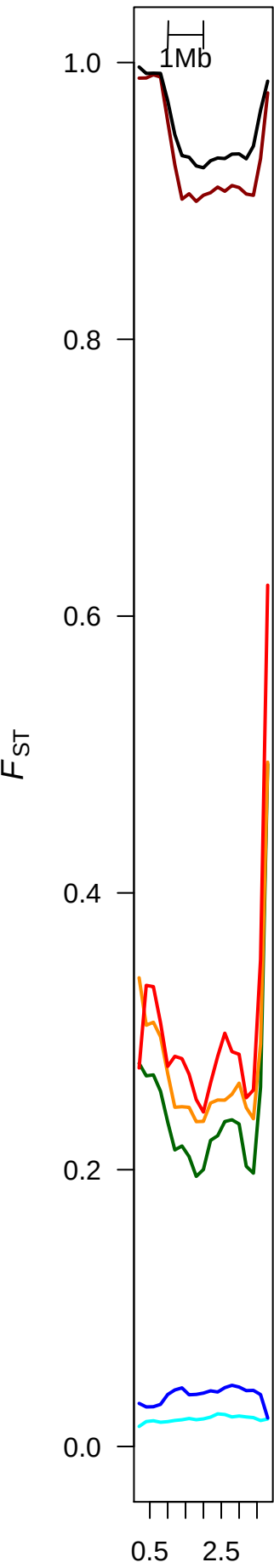


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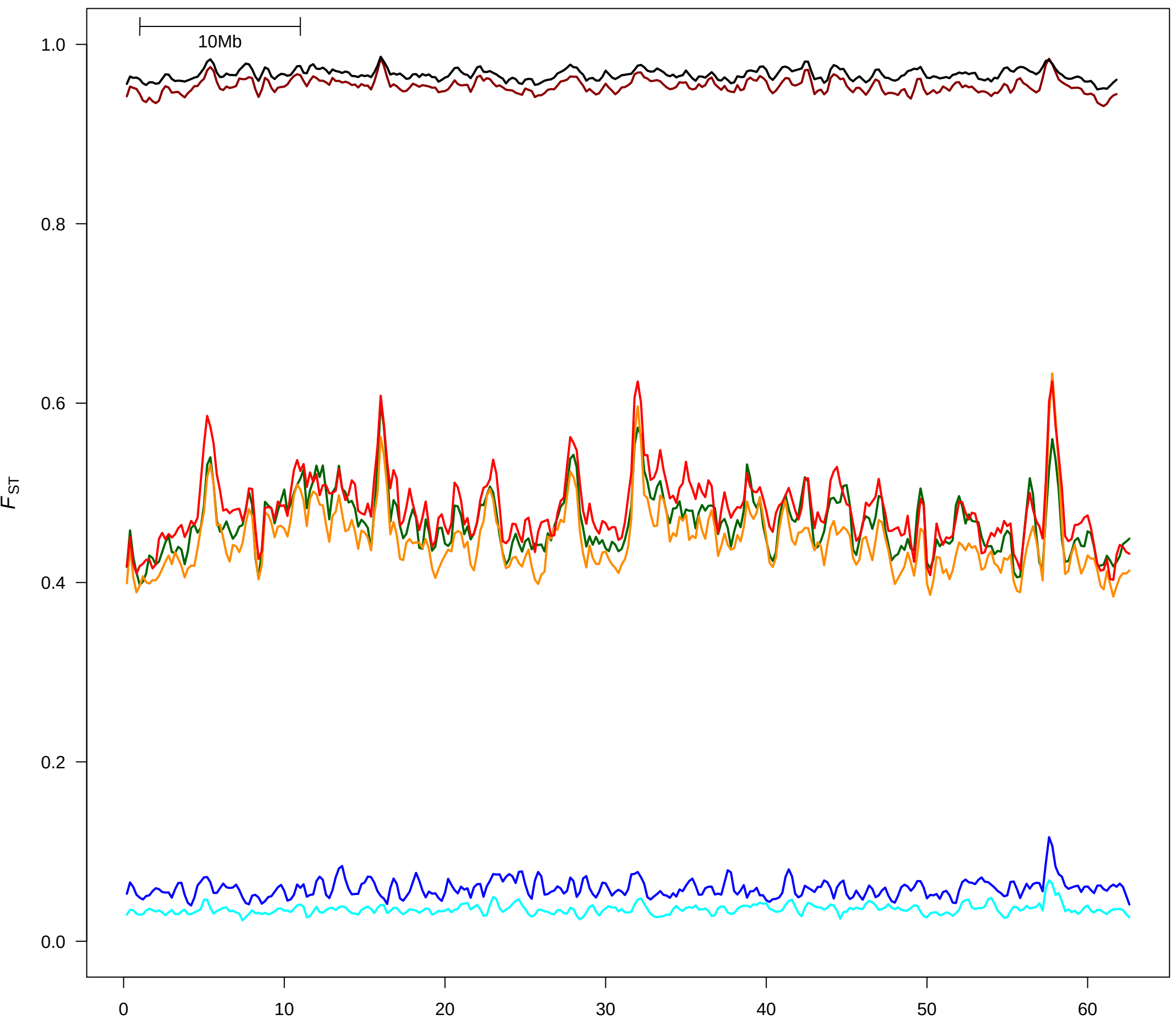




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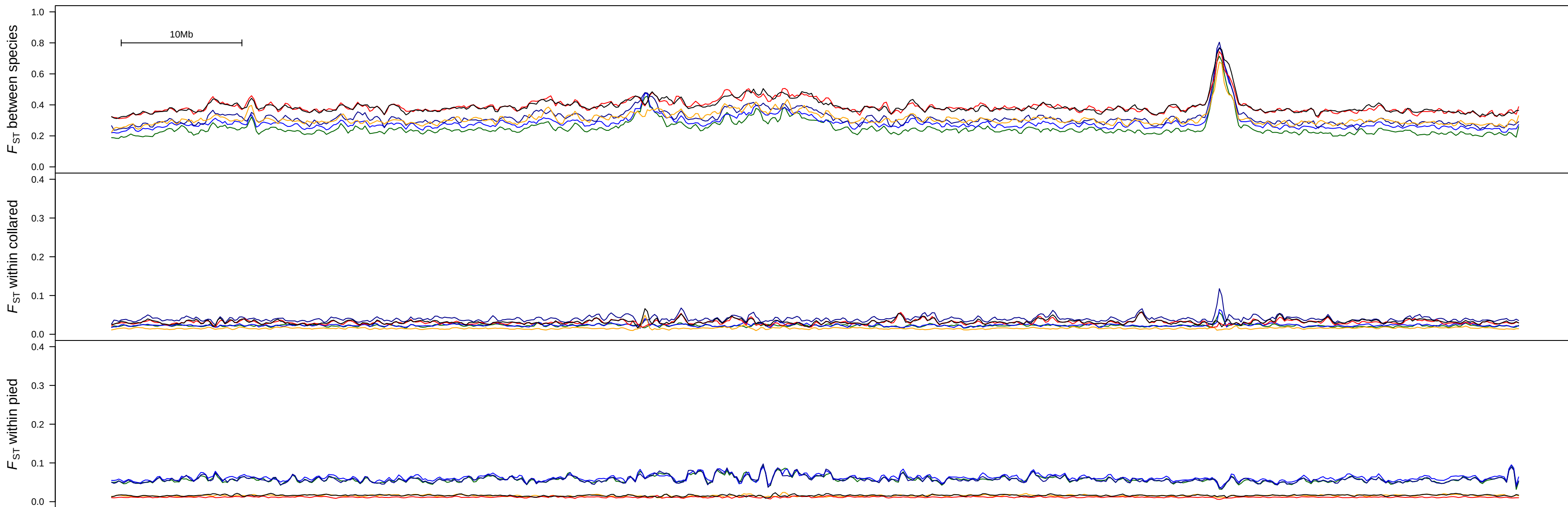


ChrZ

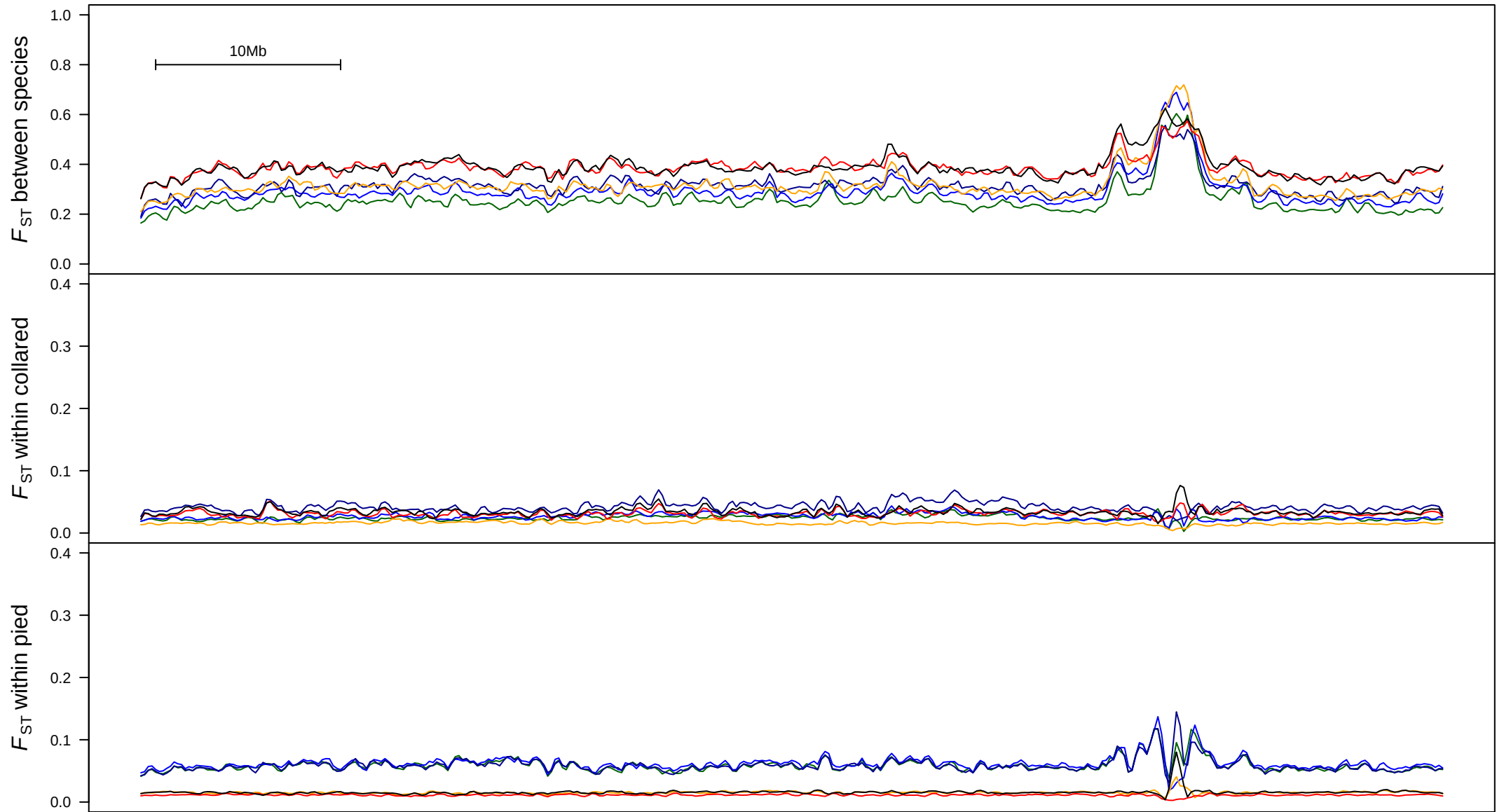


**Supplemental Figure S4.** Differentiation between species and between populations within collared and pied flycatcher, respectively. Color codes, between species: green, collared-pied; light blue, collared-Atlas; blue, collared-semicollared; orange, pied-Atlas; red, pied-semicollared; black, Atlas-semicollared. Within collared flycatcher: green, Italy-Hungary; light blue, Italy-Czech Republic; blue, Italy-Baltic; orange, Hungary-Czech Republic; red, Hungary-Baltic; black, Czech Republic-Baltic. Within pied flycatcher: green, Spain-Sweden; light blue, Spain-Czech Republic; blue, Spain-Baltic; orange, Sweden-Czech Republic; red, Sweden-Baltic; black, Czech Republic-Baltic. See **Supplemental Figure S2** for details.

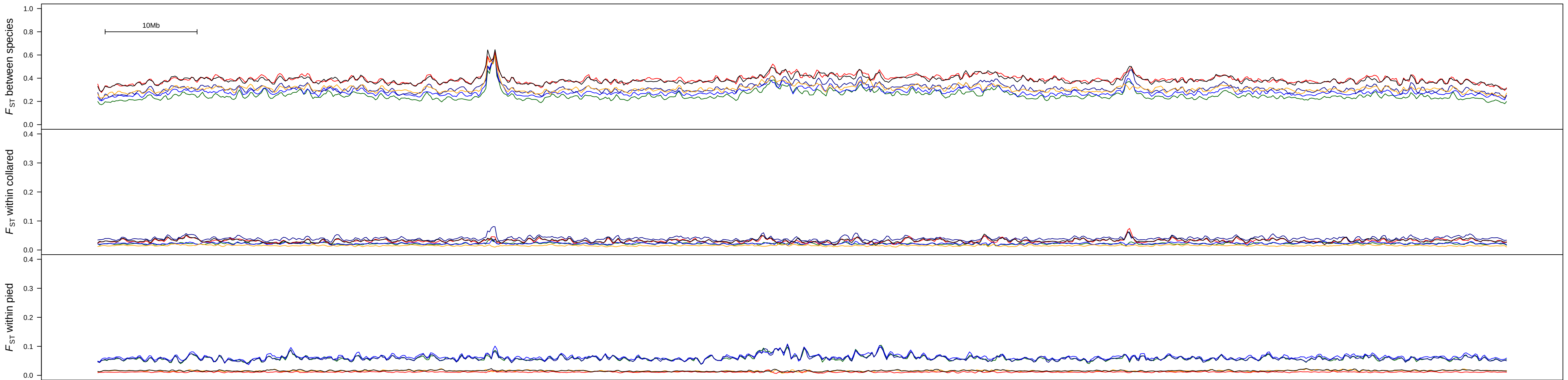
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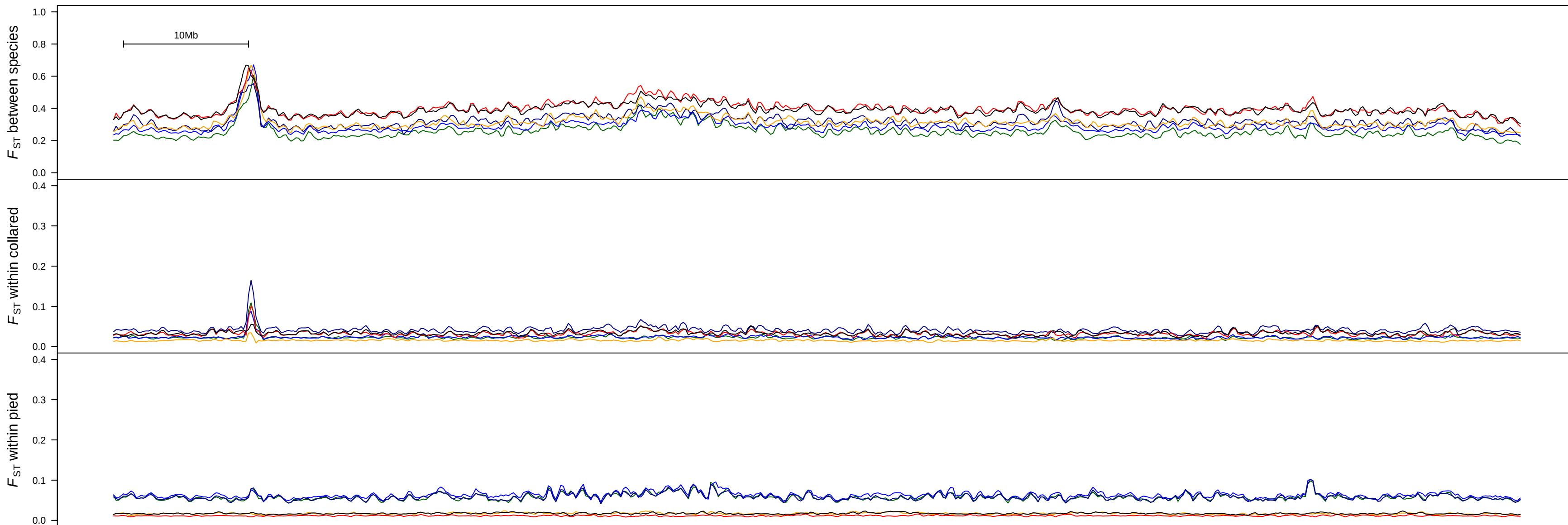
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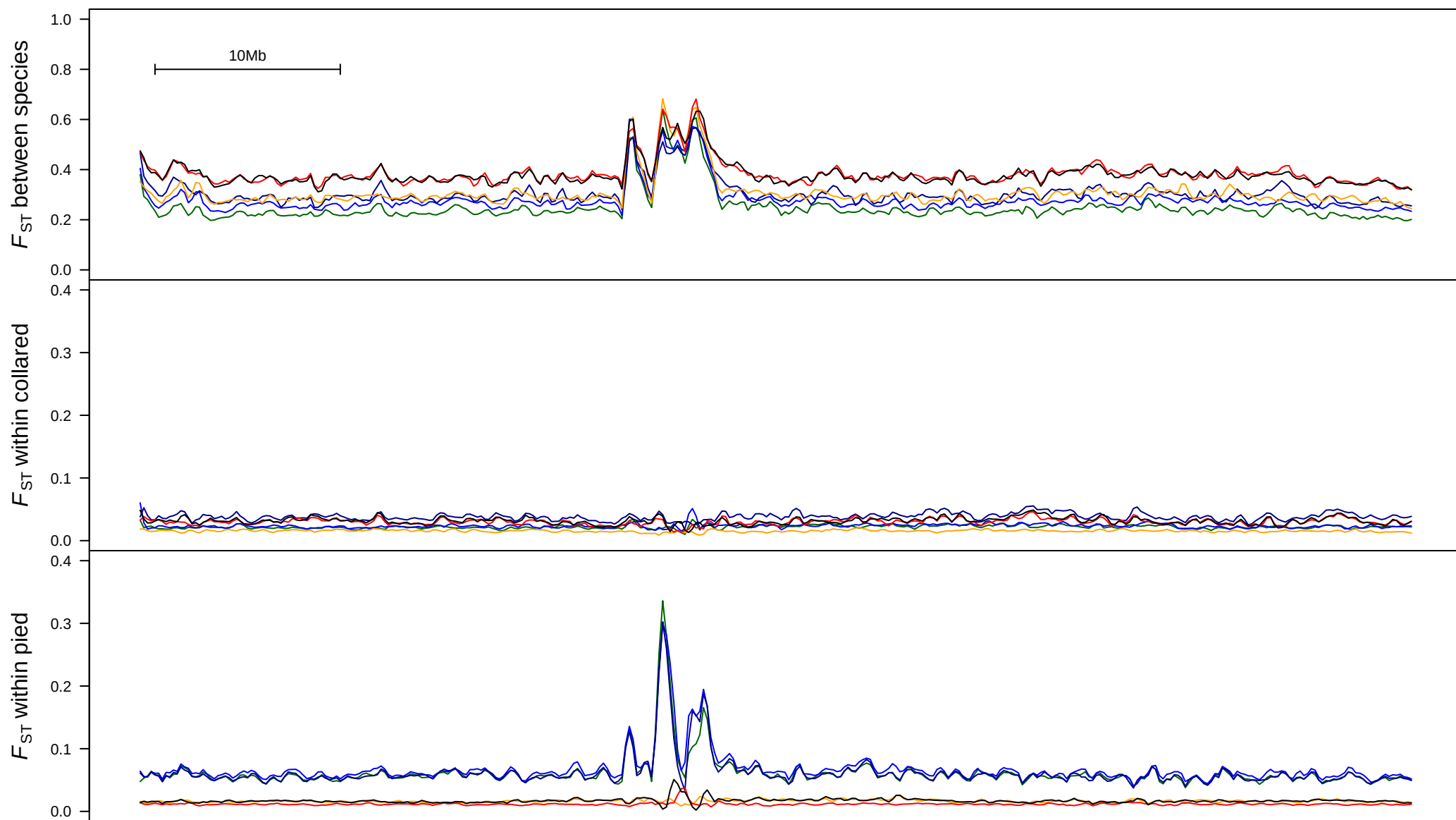
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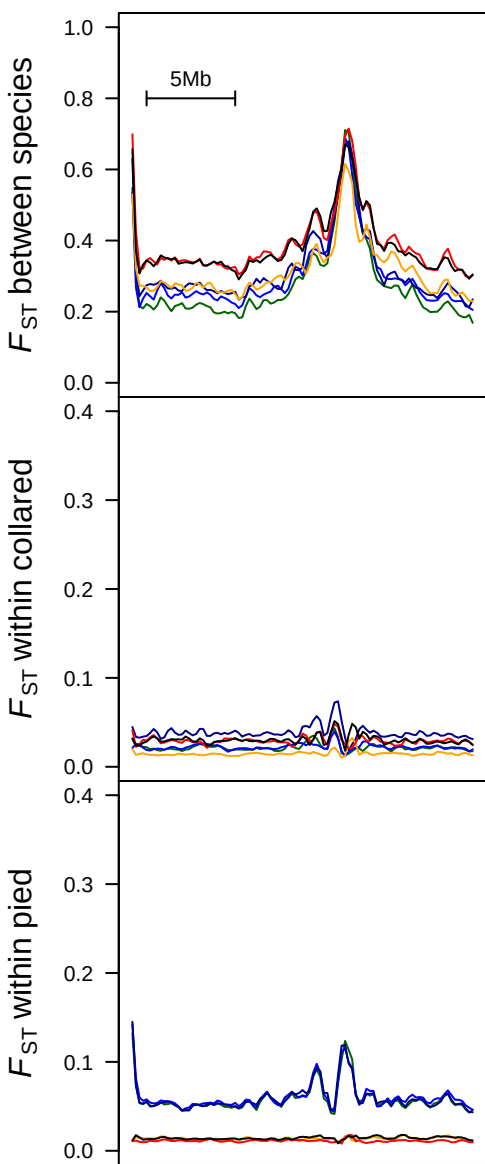


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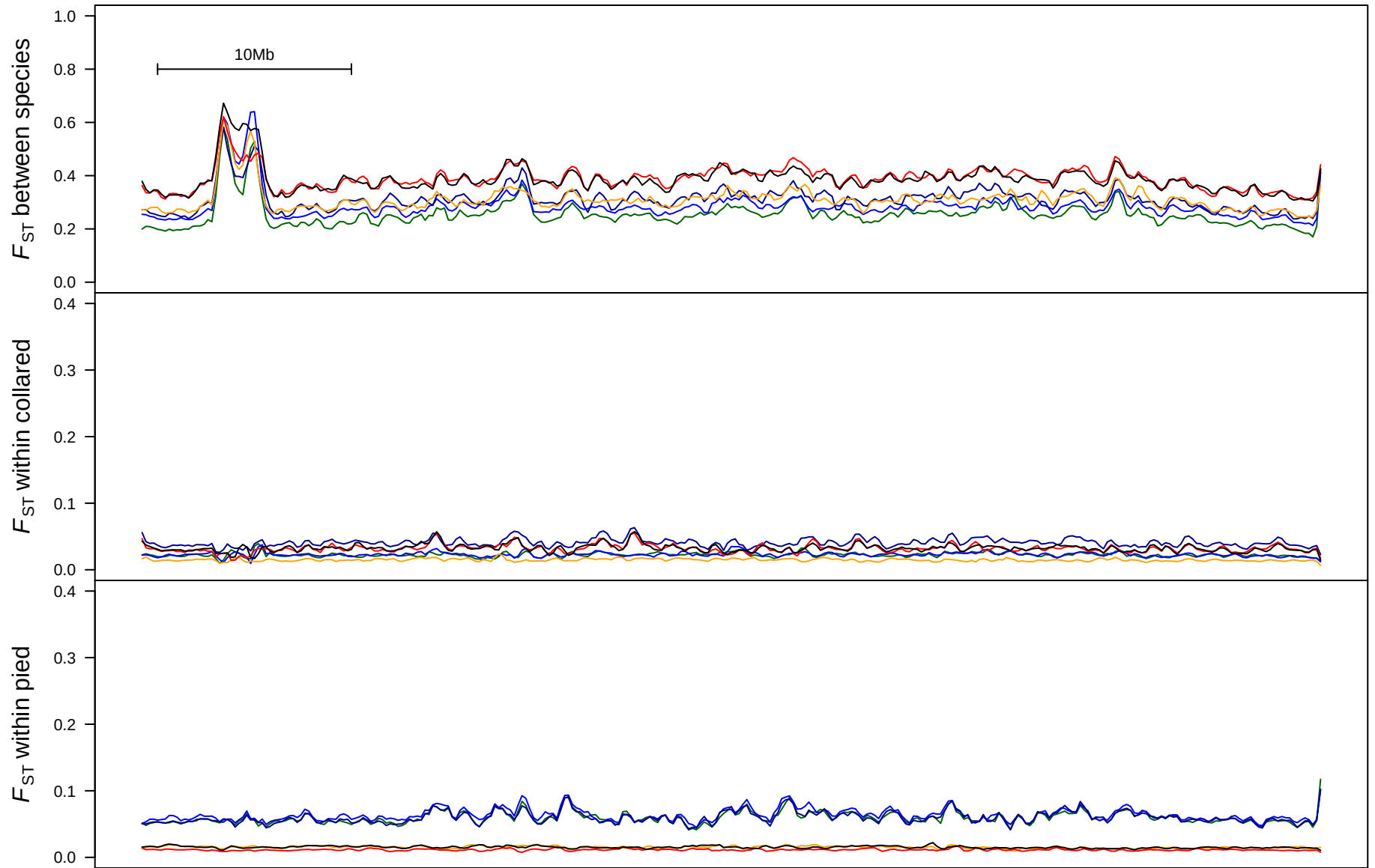




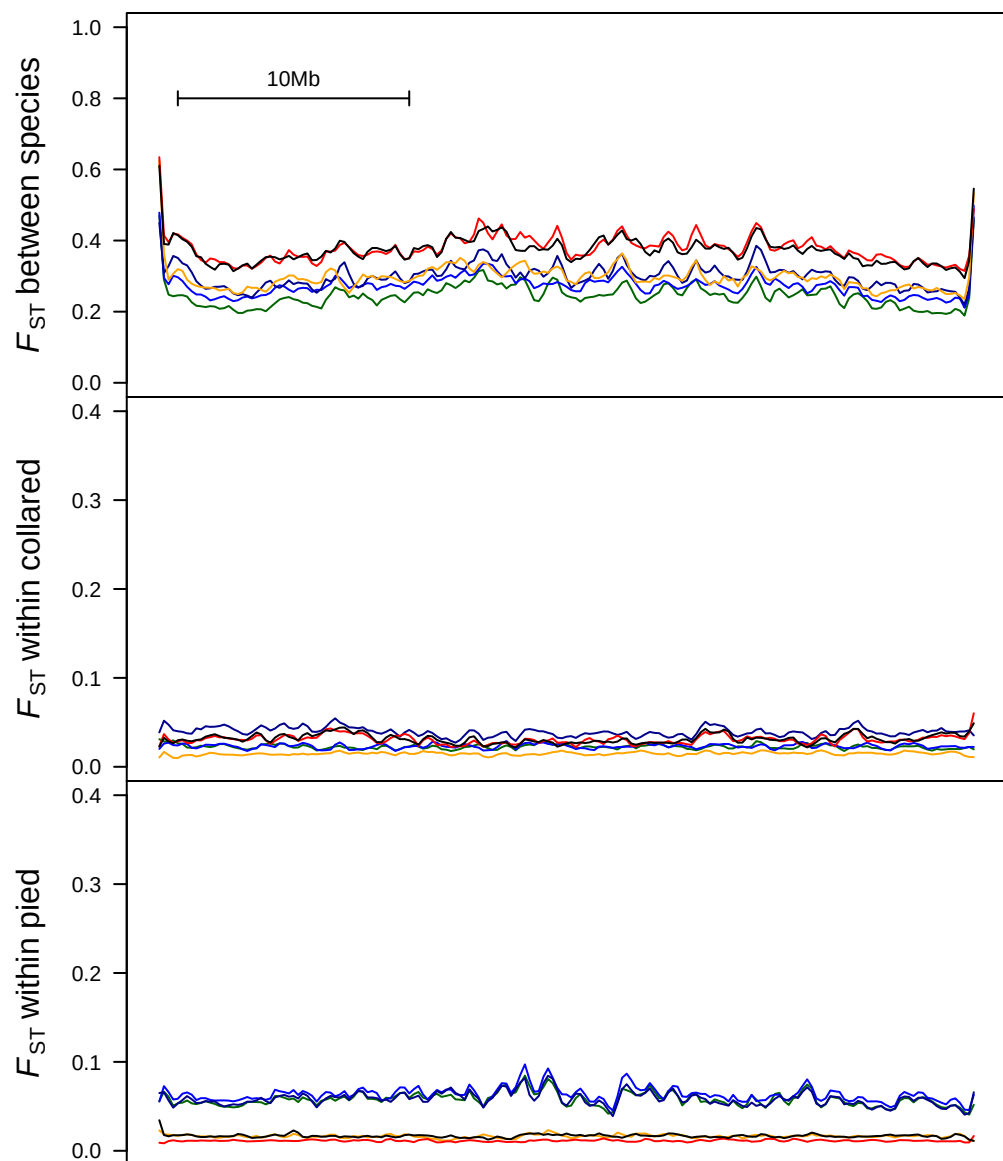
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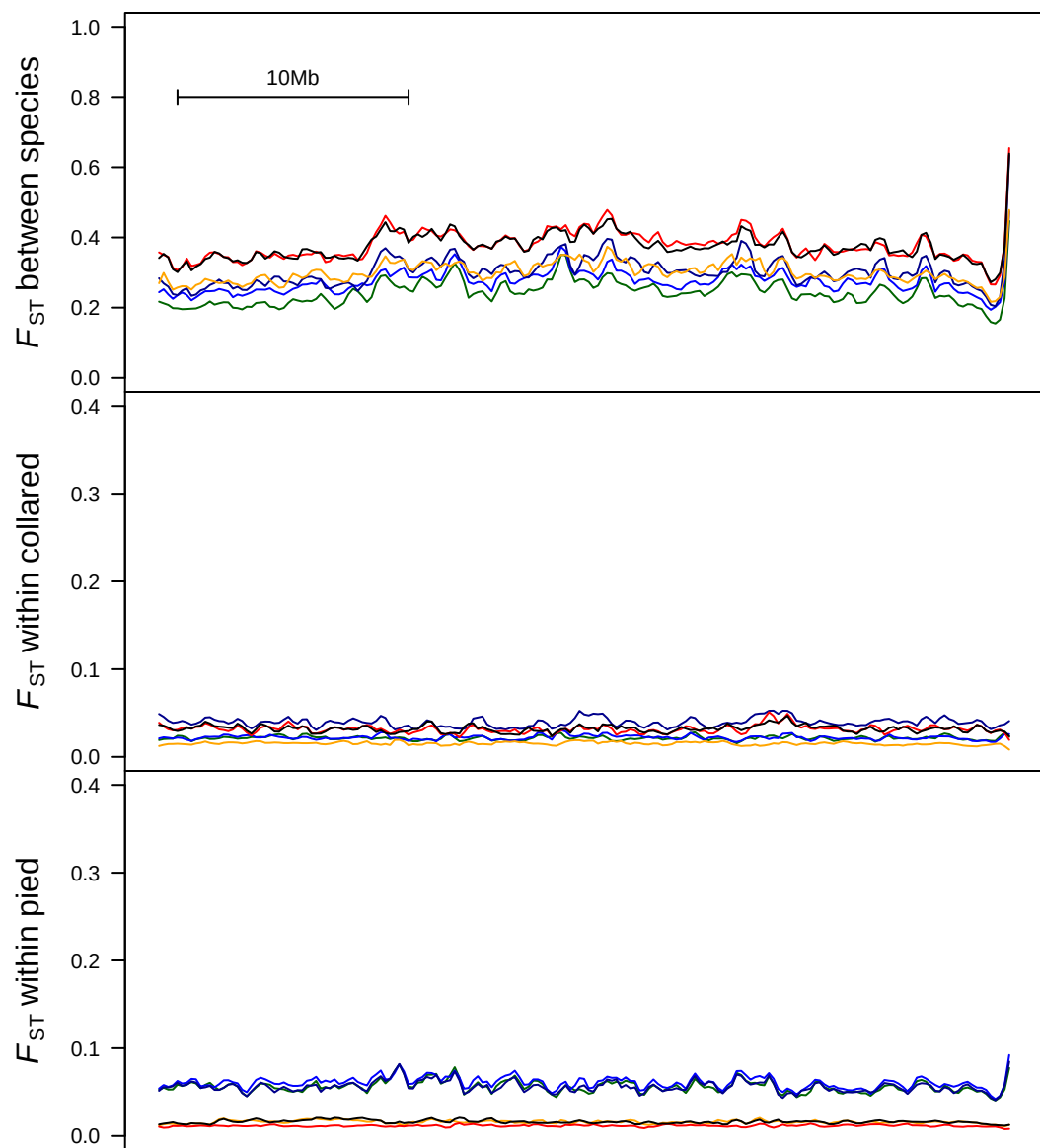
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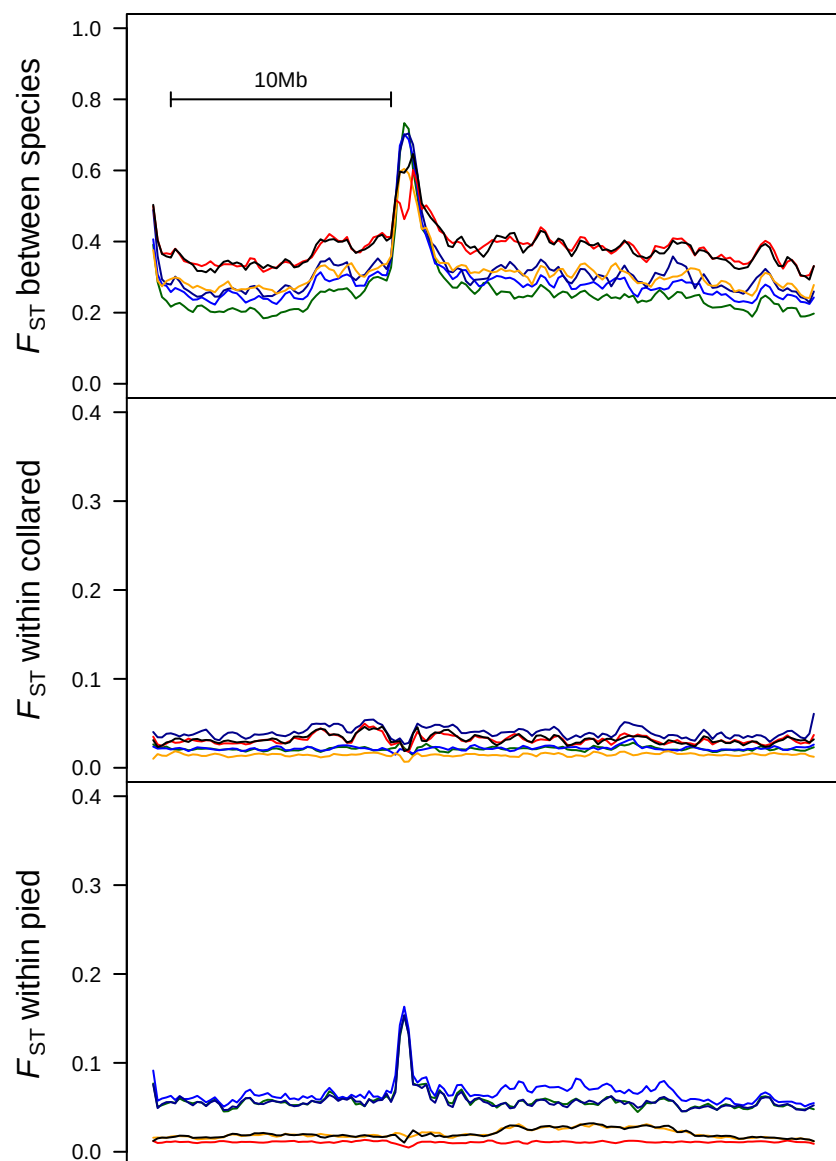
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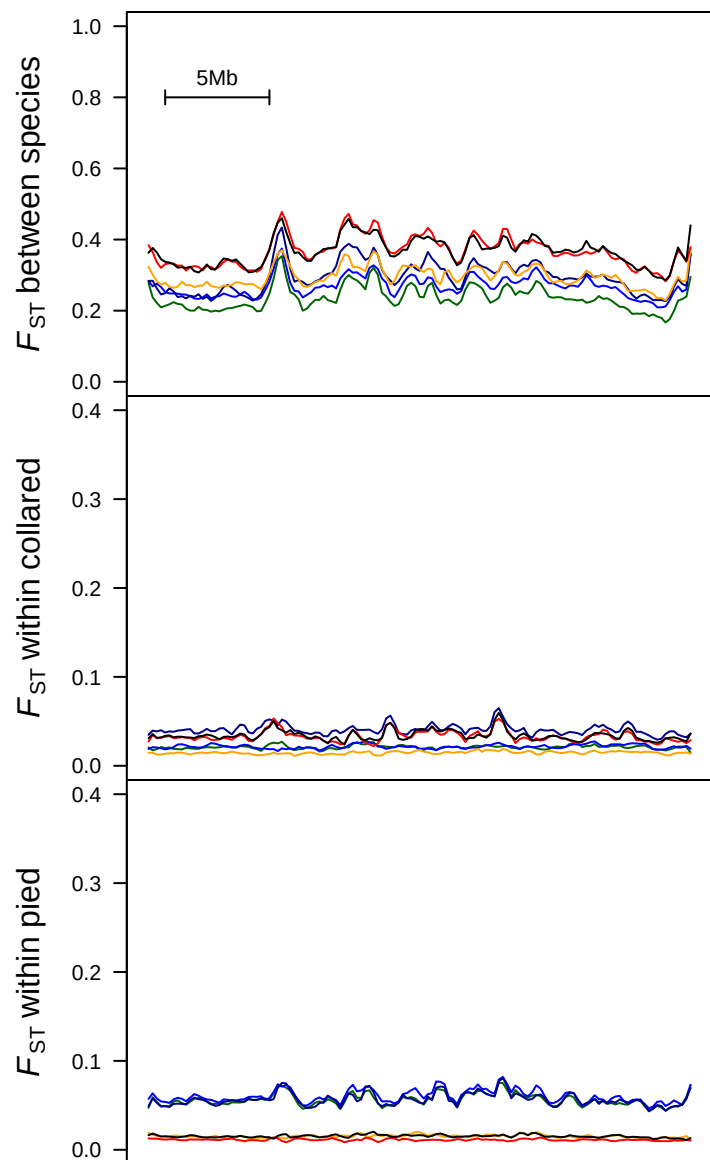
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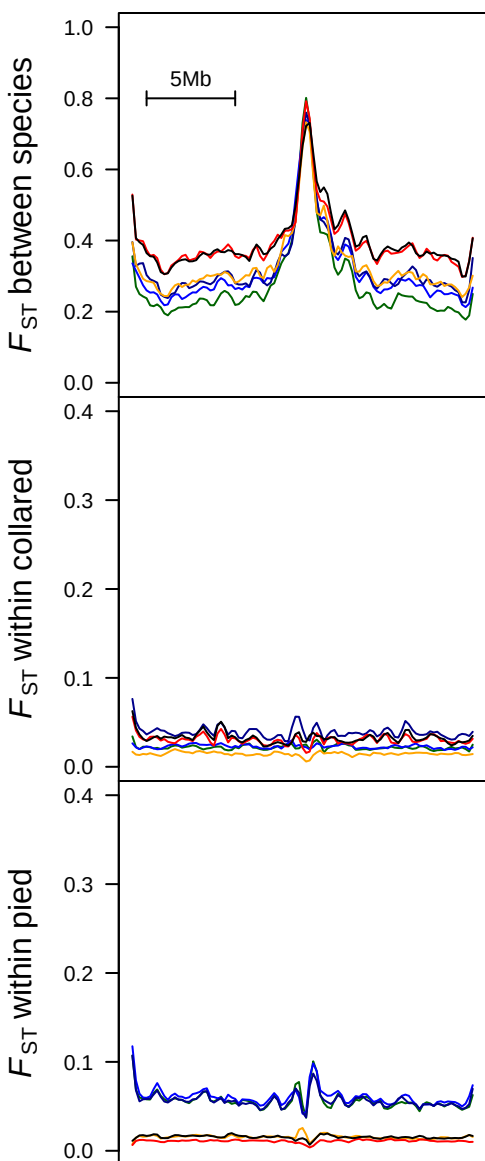
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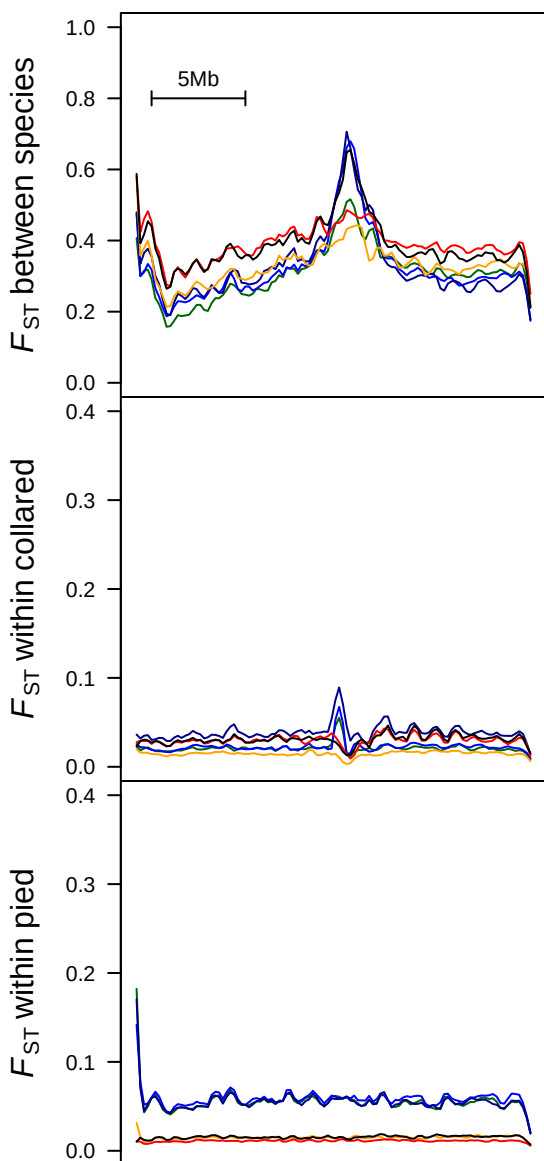
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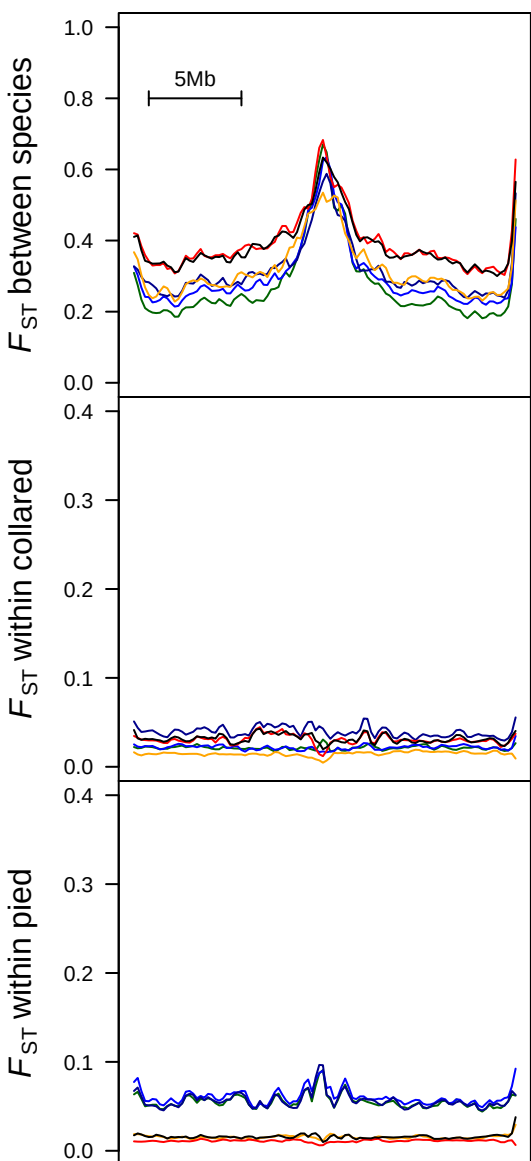


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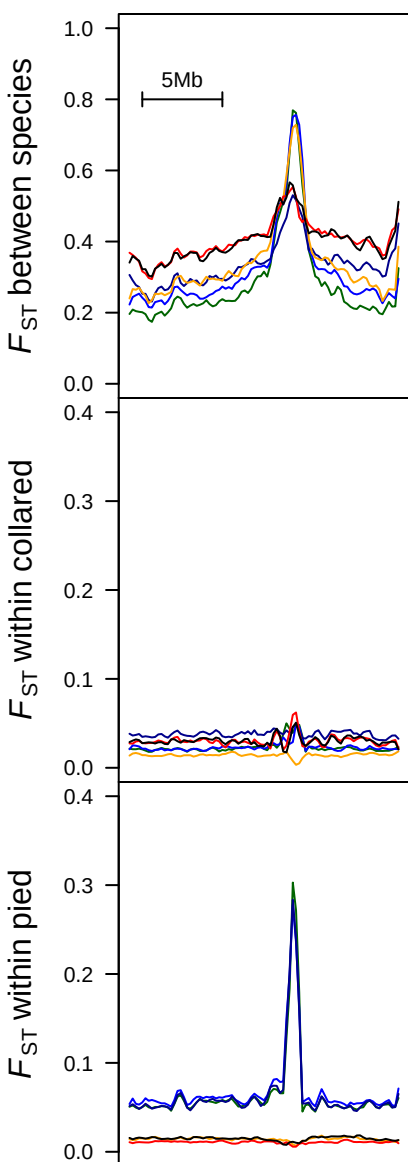




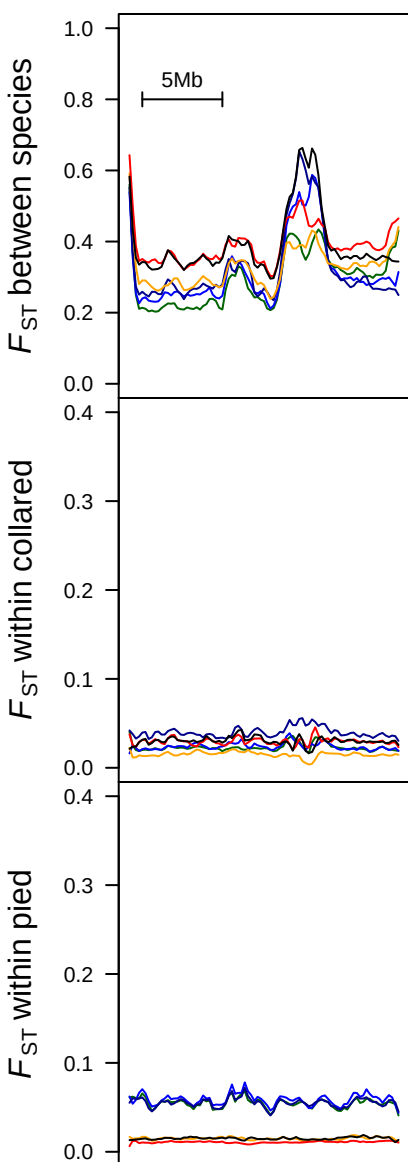
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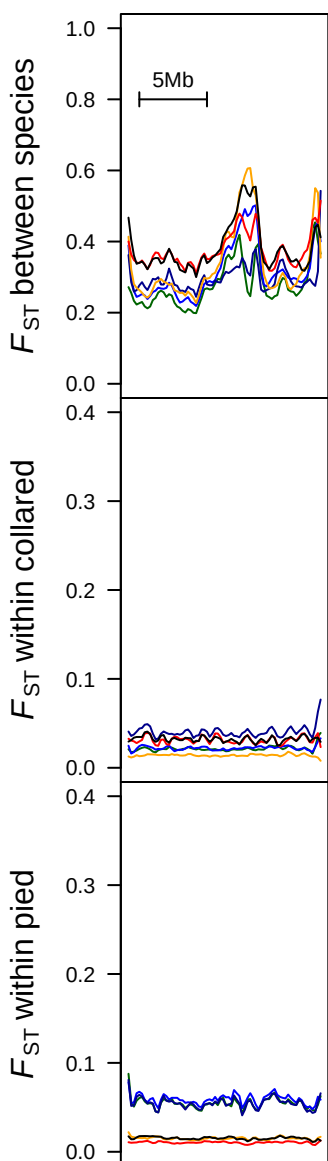
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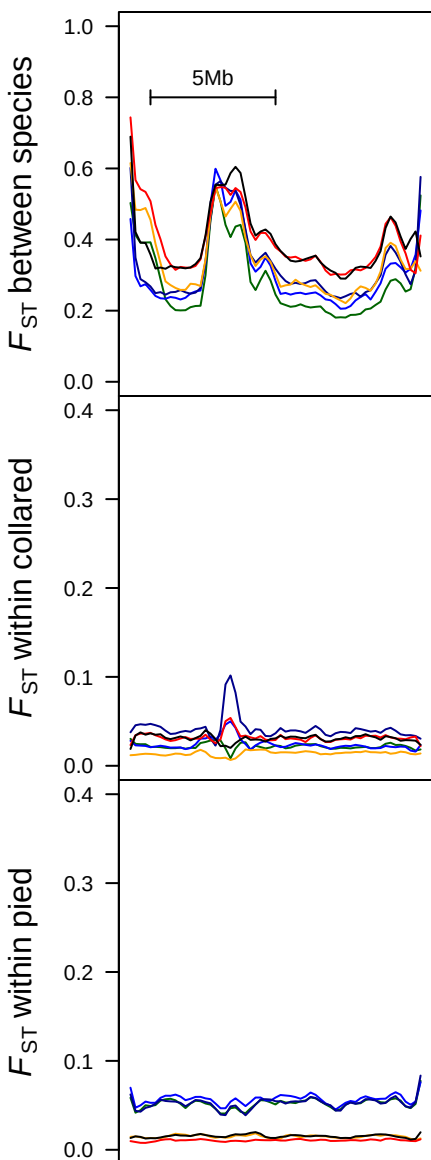
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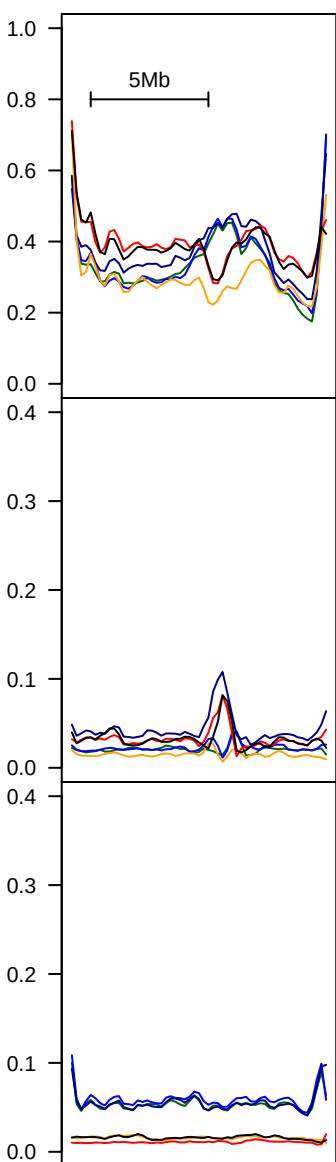
# Chr15



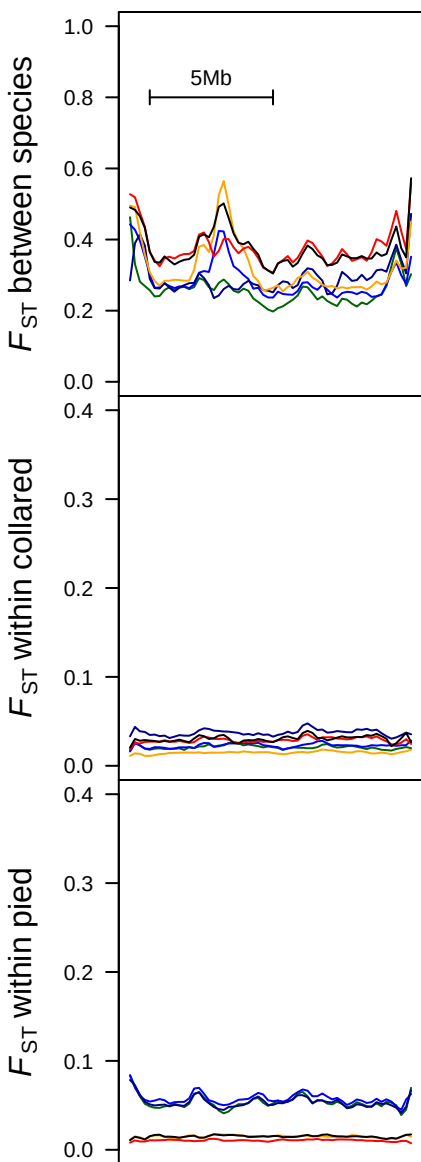
# Chr17



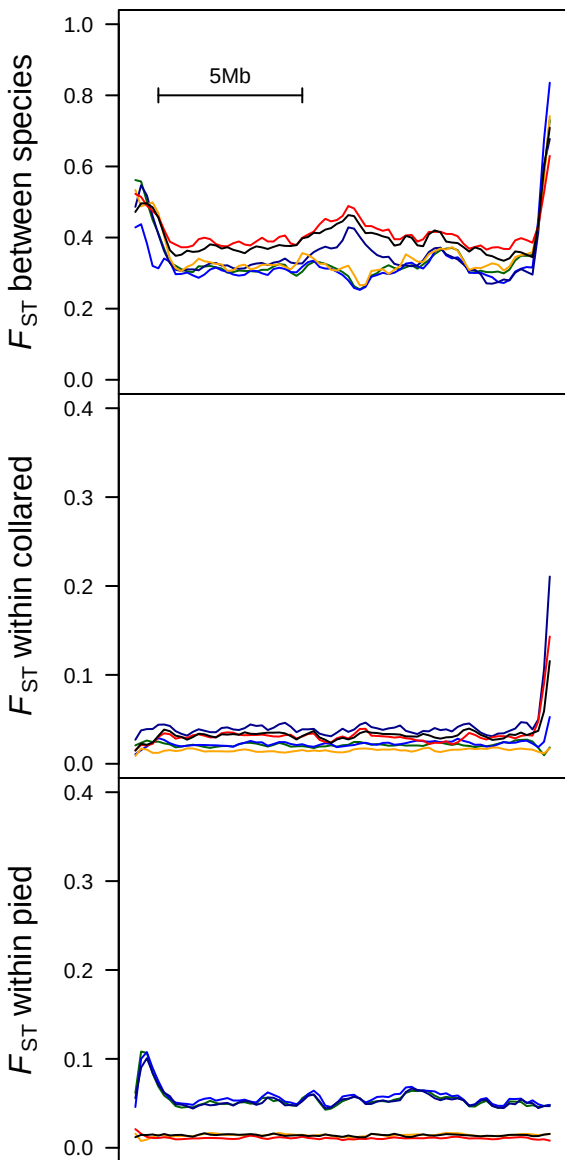
# Chr18



# Chr19

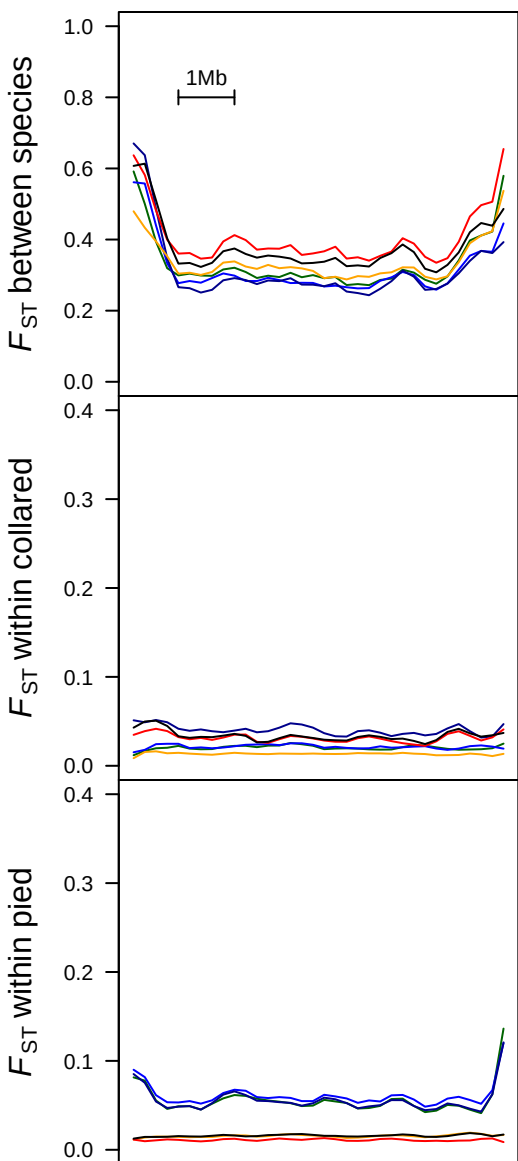


# Chr20





# Chr21

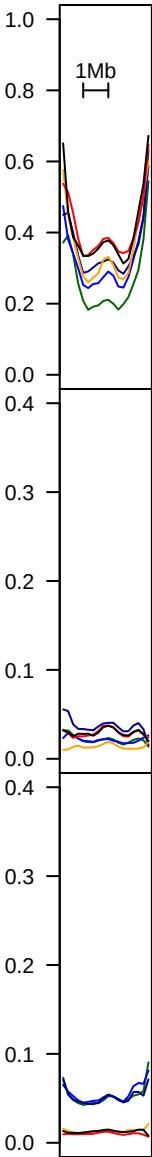


# Chr22

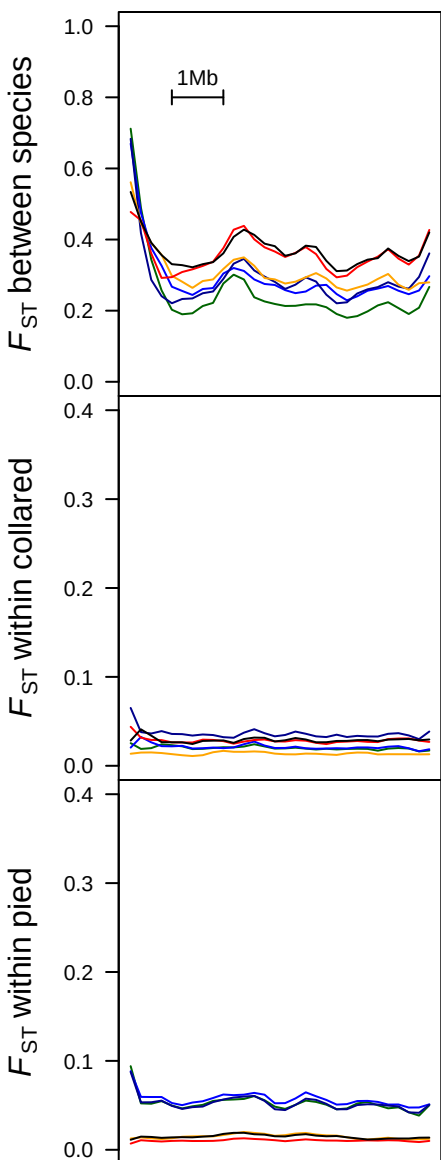
$F_{ST}$  between species

$F_{ST}$  within collared

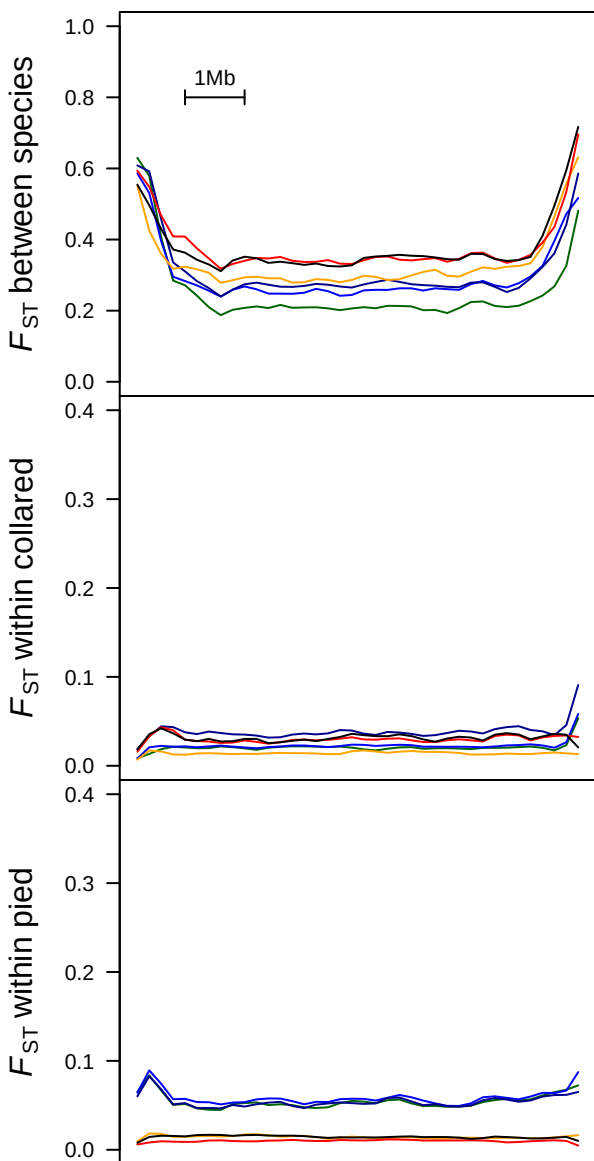
$F_{ST}$  within pied



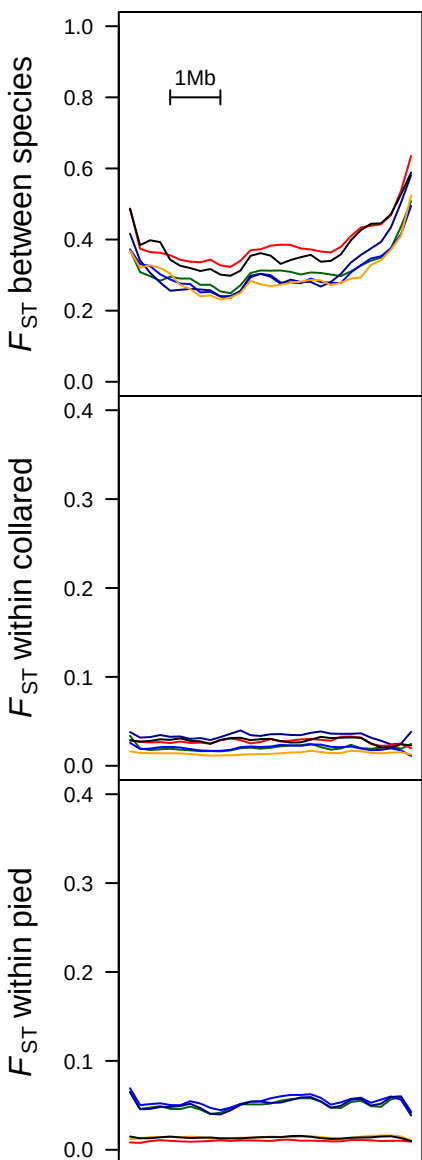
# Chr23



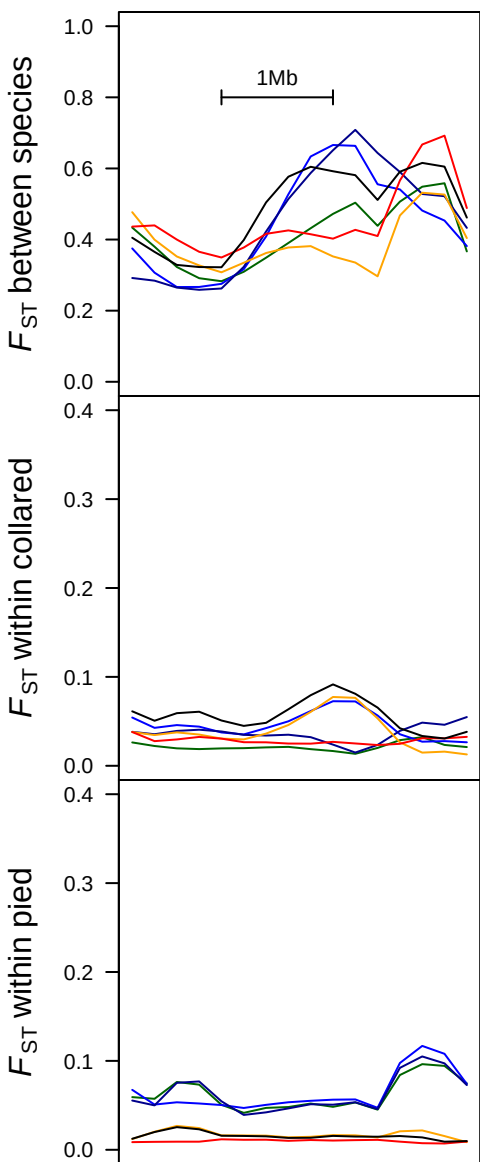
# Chr24



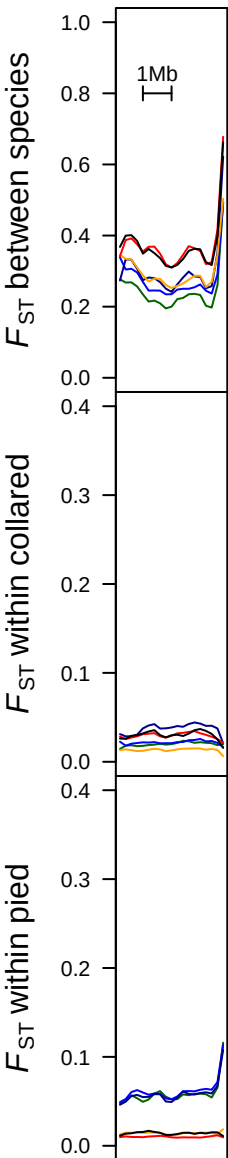
# Chr26



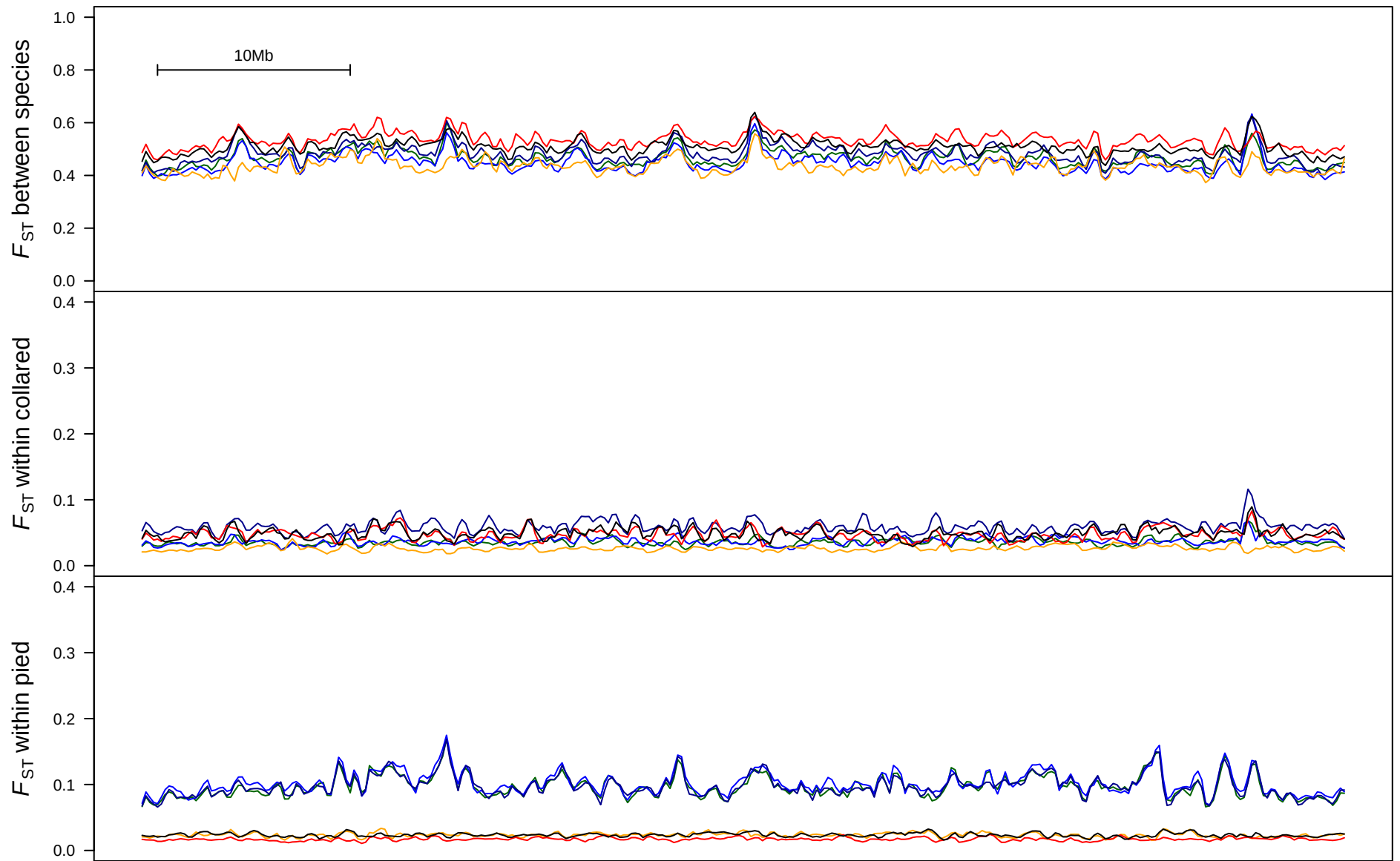
# Chr27



# Chr28



# ChrZ





**Supplemental Table S1.** Sampling information.

| Common Name             | Latin Name             | Population     | Location          | N  |
|-------------------------|------------------------|----------------|-------------------|----|
| Collared flycatcher     | <i>F. albicollis</i>   | Italy          | Terminillo        | 20 |
|                         |                        | Hungary        | Pilis             | 20 |
|                         |                        | Czech Republic | Hynkov            | 20 |
|                         |                        | Baltic         | Öland             | 19 |
| Pied flycatcher         | <i>F. hypoleuca</i>    | Spain          | Lozoya, Valsain   | 20 |
|                         |                        | Sweden         | Uppsala           | 20 |
|                         |                        | Czech Republic | Karlova, Studánka | 20 |
|                         |                        | Baltic         | Öland             | 19 |
| Atlas flycatcher        | <i>F. speculigera</i>  | Morocco        | Ifrane            | 20 |
| Semicollared flycatcher | <i>F. semitorquata</i> | Bulgaria       | Kamchia           | 20 |
| Red-breasted flycatcher | <i>F. parva</i>        | Sweden         | -                 | 1  |
| Snowy-browed flycatcher | <i>F. hyperythra</i>   | Indonesia      | -                 | 1  |

**Supplemental Table S2.** Sequencing coverage, mapping success, and number of valid and variable sites.

| Species      | Population | Coverage <sup>1)</sup> | % Mapped<br>Reads <sup>1)</sup> | No. Valid<br>Sites <sup>2)</sup> | No. SNP<br>ANGSD <sup>3)</sup> | No. SNP<br>GATK <sup>4)</sup> |
|--------------|------------|------------------------|---------------------------------|----------------------------------|--------------------------------|-------------------------------|
| Collared     | Italy      | 13.9 ± 3.8             | 94.6 ± 1.0                      | 885,879,957                      | 13,518,938                     | 15,174,516                    |
|              | Hungary    | 13.4 ± 3.7             | 95.8 ± 0.7                      | 885,904,921                      | 13,229,027                     | 13,911,690                    |
|              | Czech      | 15.0 ± 3.5             | 95.3 ± 1.5                      | 885,870,434                      | 13,235,654                     | 15,936,762                    |
|              | Rep.       |                        |                                 |                                  |                                |                               |
| Pied         | Baltic     | 15.8 ± 4.8             | 89.9 ± 4.9                      | 885,851,144                      | 12,611,012                     | 12,723,411                    |
|              | Spain      | 13.0 ± 4.7             | 96.0 ± 0.9                      | 885,495,216                      | 11,197,959                     | 12,276,100                    |
|              | Sweden     | 16.0 ± 4.6             | 90.9 ± 3.1                      | 885,510,043                      | 10,464,435                     | 13,320,084                    |
|              | Czech      | 14.4 ± 3.4             | 93.4 ± 1.3                      | 885,489,919                      | 10,646,332                     | 11,740,351                    |
| Atlas        | Rep.       |                        |                                 |                                  |                                |                               |
|              | Baltic     | 12.9 ± 3.5             | 95.1 ± 0.7                      | 885,494,916                      | 10,363,846                     | 14,162,290                    |
|              | Morocco    | 20.9 ± 3.4             | 95.2 ± 0.8                      | 885,605,525                      | 10,644,398                     | 13,560,614                    |
|              |            |                        |                                 |                                  |                                |                               |
| Semicollared | Bulgaria   | 12.3 ± 3.8             | 95.3 ± 0.7                      | 885,669,808                      | 10,630,802                     | 14,556,488                    |
| Red-breasted | Sweden     | 13.5                   | 83.6                            | NA                               | NA                             | 14,367,678                    |
| Snowy-browed | Indonesia  | 7.8                    | 72.6                            | NA                               | NA                             | 21,024,170                    |

<sup>1)</sup> Mean ± standard deviation. <sup>2)</sup> Number of sites passing filtering criteria in ANGSD. <sup>3)</sup> Number of variable sites output by ANGSD. <sup>4)</sup> Number of sites with non-reference variants contained in the 90% and 99% VQSR tranches in GATK. Note that the numbers of SNPs include invariant sites within the focal species.

**Supplemental Table S3.** Genetic diversity in the genomic background and differentiation islands.  $\pi$ , average nucleotide diversity;  $\pi_{\text{private}}$ , average nucleotide diversity estimated from private alleles. Mean of the minimum value in each island is provided in parentheses.

| Region     | Diversity              | Collared               | Pied                   | Atlas                  | Semicollared           |
|------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Background | $\pi$                  | $3.97 \cdot 10^{-3}$   | $3.20 \cdot 10^{-3}$   | $3.07 \cdot 10^{-3}$   | $2.97 \cdot 10^{-3}$   |
|            | $\pi_{\text{private}}$ | $1.62 \cdot 10^{-3}$   | $1.40 \cdot 10^{-3}$   | $1.52 \cdot 10^{-3}$   | $1.57 \cdot 10^{-3}$   |
| Islands    | $\pi$                  | $2.53 \cdot 10^{-3}$   | $1.96 \cdot 10^{-3}$   | $1.76 \cdot 10^{-3}$   | $1.77 \cdot 10^{-3}$   |
|            |                        | $(1.02 \cdot 10^{-3})$ | $(1.08 \cdot 10^{-3})$ | $(1.00 \cdot 10^{-3})$ | $(1.03 \cdot 10^{-3})$ |
|            | $\pi_{\text{private}}$ | $1.54 \cdot 10^{-3}$   | $1.27 \cdot 10^{-3}$   | $1.14 \cdot 10^{-3}$   | $1.26 \cdot 10^{-3}$   |
|            |                        | $(0.93 \cdot 10^{-3})$ | $(0.79 \cdot 10^{-3})$ | $(0.71 \cdot 10^{-3})$ | $(0.70 \cdot 10^{-3})$ |

**Supplemental Table S4.** ABBA-BABA tests for gene flow. Populations/species among which the test indicates gene flow are highlighted in bold.

| 1. Inner          | 2. Inner              | 1. Outgroup           | Mean(D) | SE(D)  | p-value    |
|-------------------|-----------------------|-----------------------|---------|--------|------------|
| collared Italy    | collared CZ           | pied CZ               | 0.0010  | 0.0010 | 0.3344     |
| pied Spain        | pied CZ               | collared CZ           | 0.0004  | 0.0005 | 0.4186     |
| <b>pied Spain</b> | Atlas                 | <b>collared Italy</b> | -0.1648 | 0.0027 | $<10^{-4}$ |
| <b>pied Spain</b> | Atlas                 | <b>semicollared</b>   | -0.0108 | 0.0016 | $<10^{-4}$ |
| pied Spain        | <b>collared Italy</b> | <b>semicollared</b>   | 0.1162  | 0.0018 | $<10^{-4}$ |
| Atlas             | <b>collared Italy</b> | <b>semicollared</b>   | 0.1242  | 0.0016 | $<10^{-4}$ |

**Supplemental Table S5.** Mean sequence divergence ( $d_{xy}$ ) for background and island regions.

Mean of the minimum values in each island is provided in parentheses.

| Comparison                         | Background           | Islands                                       |
|------------------------------------|----------------------|-----------------------------------------------|
| Collared – Pied flycatcher         | $4.83 \cdot 10^{-3}$ | $3.72 \cdot 10^{-3}$ ( $2.72 \cdot 10^{-3}$ ) |
| Collared - Atlas flycatcher        | $4.89 \cdot 10^{-3}$ | $3.65 \cdot 10^{-3}$ ( $2.76 \cdot 10^{-3}$ ) |
| Collared - Semicollared flycatcher | $5.05 \cdot 10^{-3}$ | $3.83 \cdot 10^{-3}$ ( $2.89 \cdot 10^{-3}$ ) |
| Pied - Atlas flycatcher            | $4.49 \cdot 10^{-3}$ | $3.24 \cdot 10^{-3}$ ( $2.41 \cdot 10^{-3}$ ) |
| Pied - Semicollared flycatcher     | $5.01 \cdot 10^{-3}$ | $3.71 \cdot 10^{-3}$ ( $2.74 \cdot 10^{-3}$ ) |
| Atlas - Semicollared flycatcher    | $4.84 \cdot 10^{-3}$ | $3.51 \cdot 10^{-3}$ ( $2.66 \cdot 10^{-3}$ ) |

**Supplemental Table S6.** Mean relative node depths (RND) for background and island regions. Mean of the minimum values in each island is provided in parentheses.

| Comparison                         | Background | Islands       |
|------------------------------------|------------|---------------|
| Collared – Pied flycatcher         | 0.197      | 0.145 (0.102) |
| Collared - Atlas flycatcher        | 0.199      | 0.141 (0.102) |
| Collared - Semicollared flycatcher | 0.206      | 0.148 (0.107) |
| Pied - Atlas flycatcher            | 0.184      | 0.128 (0.090) |
| Pied - Semicollared flycatcher     | 0.205      | 0.146 (0.103) |
| Atlas - Semicollared flycatcher    | 0.200      | 0.138 (0.101) |

**Supplemental Table S7.** Tajima's  $D$  in the genomic background and differentiation islands.

| Species                 | % islands with<br>reduced $D$ <sup>1)</sup> | Mean $D$<br>background | Mean average $D$<br>in islands |
|-------------------------|---------------------------------------------|------------------------|--------------------------------|
| Collared flycatcher     | 17.6                                        | -0.480                 | -0.632                         |
| Pied flycatcher         | 28.6                                        | -0.360                 | -0.665                         |
| Atlas flycatcher        | 11.7                                        | -0.362                 | -0.58                          |
| Semicollared flycatcher | 13.9                                        | -0.464                 | -0.659                         |

<sup>1)</sup> Multiple testing adjustment using sequential Bonferroni correction.

**Supplemental Table S8.** Fay and Wu's  $H$  in the genomic background and differentiation islands.

| Species                 | % islands with<br>reduced $H$ <sup>1)</sup> | Mean $H$<br>background | Mean average $H$<br>in islands |
|-------------------------|---------------------------------------------|------------------------|--------------------------------|
| Collared flycatcher     | 5.9                                         | 0.120                  | -0.060                         |
| Pied flycatcher         | 2.9                                         | 0.014                  | -0.015                         |
| Atlas flycatcher        | 5.9                                         | 0.012                  | -0.002                         |
| Semicollared flycatcher | 5.6                                         | -0.112                 | -0.165                         |

<sup>1)</sup> Multiple testing adjustment using sequential Bonferroni correction.