Additional file 3: Comparative study of isoRelate and hmmIBD

Methods

Summary of files and variables used

The following summarizes results generated from a comparative study of hmmIBD and isoRelate (Henden L, et al. BioRxiv. 2016). Analyses were based on data generated by artificial recombination (details below). The steps, data and scripts required to reproduce this study are as follows.

1. Download the hmmIBD_benchmark repository from https://github.com/artaylor85/hmmIBD_benchmark and unzip the pf3k_data directory.
2. Install hmmIBD following instructions at https://github.com/glipsnort/hmmIBD/releases (v2.0.0).
3. Install isoRelate following instructions at https://github.com/bahlolab/isoRelate/releases (results here based on v0.1.0 installed Aug 9th 2017).
4. Set working directory to this source file location.
5. Run `Simulate_chimeric_genotypes.R`.
6. Run `Run_isolate_hmmIBD.R`.
7. Run `Post_process_results.R`.
8. Run/knit this file.

Simulation of artificially recombined data

We used artificially recombined data to compare results generated under hmmIBD and isoRelate to a known truth that was not generated under either model. Artificially recombined data were based on the MalariaGen Pf3k samples, pilot release 5.0 (https://www.malariagen.net/projects/pf3k). These data were filtered prior to their use in this comparative study, leaving only single nucleotide polymorphisms (SNPs) in the accessible genome (as defined by Manske M, et al. Nature 2012), and those with a high probability of being monogenomic (as defined by DEploid from Zhu SJ, Almagro-garcia J, Mcvean G. BioRxiv. 2017). The filtered data can be found in `pf3k_data`. Using `Simulate_chimeric_genotypes.R` we:

1. Extracted samples from sites with 100 or more samples (Thies, Kassena, Pursat).
2. For each site, removed multiallelic SNPs (unsupported by isoRelate) and those with minor allele frequency ≤ 0.01, leaving 57307, 41992, 69438 SNPs per sample from Kassena, Pursat, Thies, respectively.
3. Calculated and saved allele frequencies and data sets based on the unrecombined data to ensure frequencies were not based on chimeric samples.
4. For each pairwise comparison within a site, calculated the average identity-by-state, IBS (one minus the genome-wide average SNP difference), and plotted.
5. Extracted unrelated pairs (those with IBS < 1 percentile of the empirical IBS distribution).
6. Artificially recombined each unrelated sample pair to create a "chimeric child". Recombination was simulated by sampling crossover positions (in base pairs, bp) from an exponential distribution with mean equal to the recombination rate in Morgans, M, per bp (see `functions.R`).
7. Recorded the parent of each DNA segment in each chimeric child, and plotted the number of crossovers per chromosome averaged over all the chimeric children per site.

In addition to the above steps, we generated an erroneous copy of each parent and chimeric child. More specifically, for each SNP with probability equal to the genotyping error 0.005, the copied allele was replaced by its biallelic counterpart.

Experiments to evaluate timing

Timing experiments were performed on the first 50 samples per site (including unrecombined parents and non-erroneous chimeric children), and repeated 3 times on a MacBook Air laptop with 1.7 GHz Intel Core i7 processor using the parameter values listed in the table below. Some of the parameter values differ to the defaults provided in order to more closely match the two methods.

Table 1: Specified parameter values. NA denotes not applicable. †In isoRelate, the "recombination rate" is a function of distance in M. The equivalent fixed rate in M/bp in hmmIBD was thus based on the empirical relationship between positions in bp and centimorgans provided in the png_pedmap data set of the isoRelate package.

Parameter	isoRelate	hmmIBD
genotyping error	0.005	0.001
recombination rate	5.83e-07 M/bp†	5.83e-07 M/bp
minimum no. SNPs per segment	0	NA
minimum length (bp) per segment	0	NA
Minimum marker spacing (bp)	NA	0
Minimum informative sites per genome	NA	0

Experiments to evaluate inference

For each site, IBD segments between 50 "chimeric children" and each of their two parents were inferred under isoRelate and hmmIBD using the parameter values listed in the table above. Accuracy, sensitivity and specificity were calculated as follows, where for a given pairwise comparison and SNP, a true positive is an IBD observation given an IBD state, and a true negative is a not IBD (nIBD) observation given a nIBD state,

$$\text{Accuracy} = \frac{\sum \text{ True positive} + \sum \text{ True negative}}{\text{Number of SNPs}}, \tag{1}$$

$$\text{Sensitivity} = \frac{\sum \text{ True positive}}{\sum \text{ IBD states}}, \tag{2}$$

$$\text{Specificity} = \frac{\sum \text{ True negative}}{\sum \text{ nIBD states}}. \tag{3}$$

We also compared estimates of the numbers of generations inferred under isoRelate and hmmIBD, and the proportion simulated IBD with the posterior probability of IBD inferred under hmmIBD (the latter was not directly available under v0.1.0 of isoRelate).

To investigate the impact of genotyping error, the entire process was then repeated for the erroneous copies of 50 chimeric children and their parents. We expect error to introduce small and incorrectly inferred segments into otherwise correctly inferred segments of DNA that are both IBD and not. Concomitantly, we expect error to spuriously increase estimates of numbers of generations since most recent common ancestors.

Results

Timing

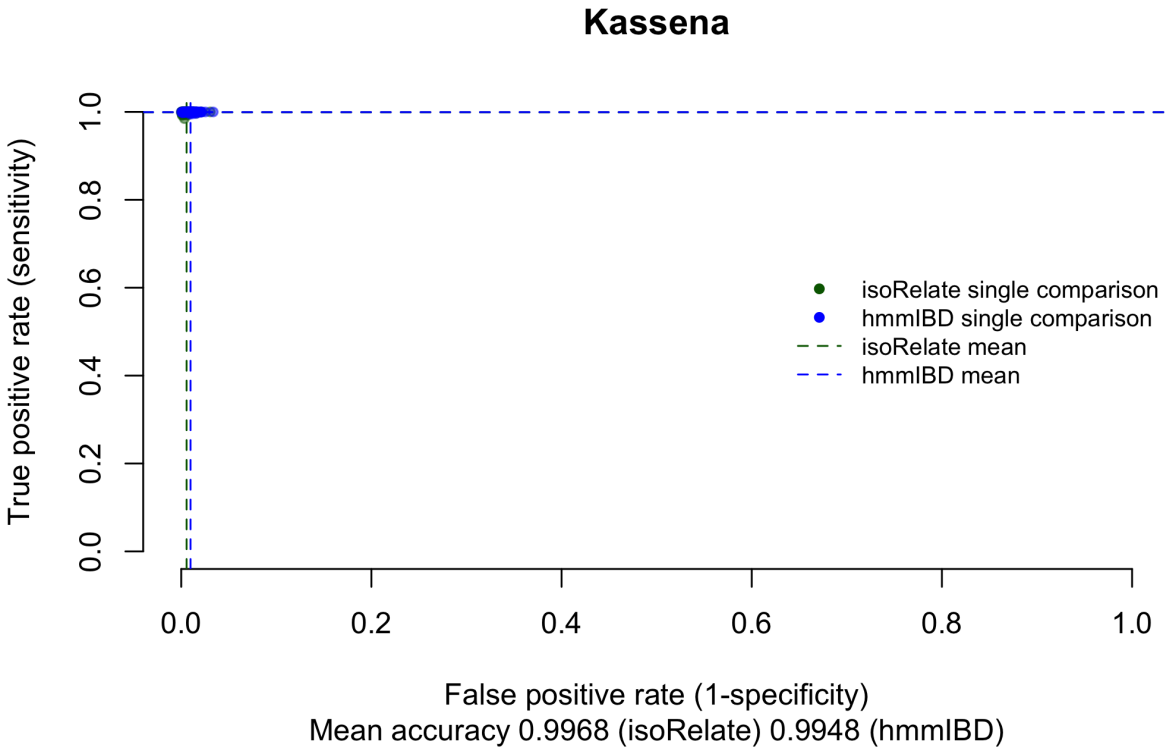
Table 2: Clocktime (sec) per 50 samples

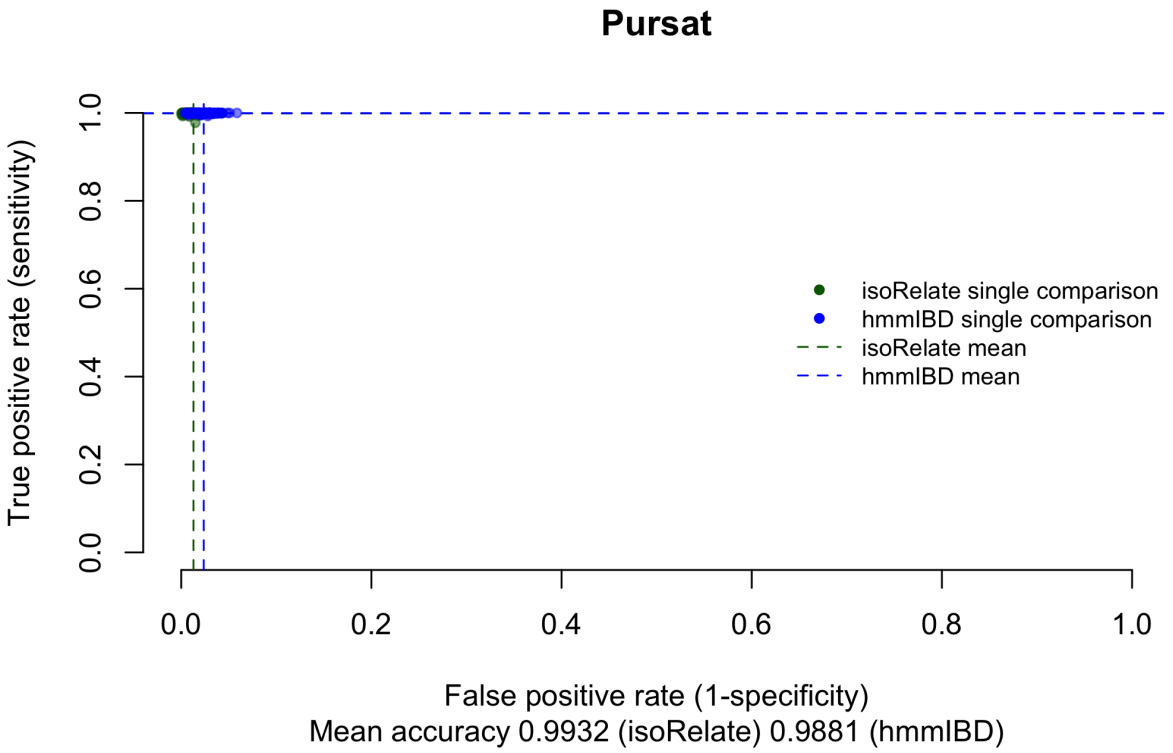
	isoRelate	hmmIBD
Kassena 1	1647.566	68.556
Pursat 1	1242.745	45.657
Thies 1	2035.602	74.431
Kassena 2	1648.765	68.567
Pursat 2	1243.346	45.347
Thies 2	2033.767	74.642
Kassena 3	1645.554	68.498
Pursat 3	1242.854	45.473
Thies 3	2037.765	74.799

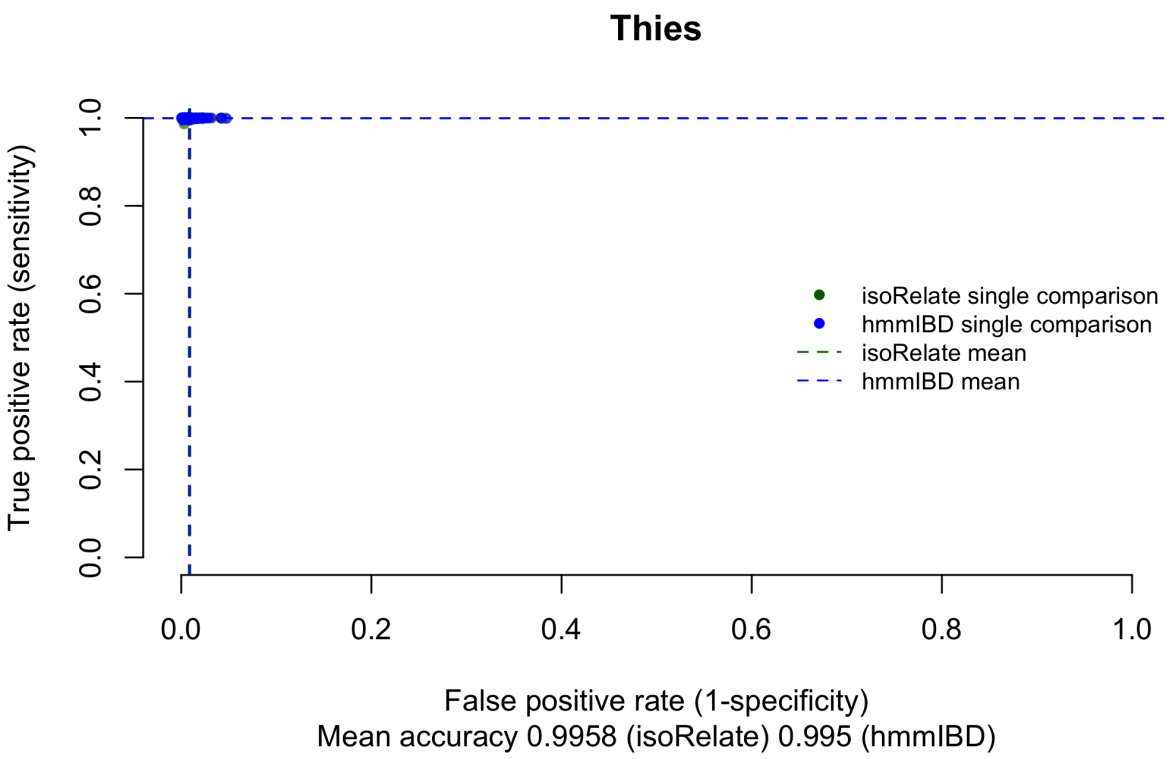
Table 3: CPU time (sec) per 50 samples

	isoRelate	hmmIBD
Kassena 1	1591.945	68.148
Pursat 1	1196.766	45.365
Thies 1	1960.100	73.969
Kassena 2	1592.889	68.024
Pursat 2	1196.816	45.200
Thies 2	1959.130	74.153
Kassena 3	1590.386	68.154
Pursat 3	1197.044	45.321
Thies 3	1963.492	74.262

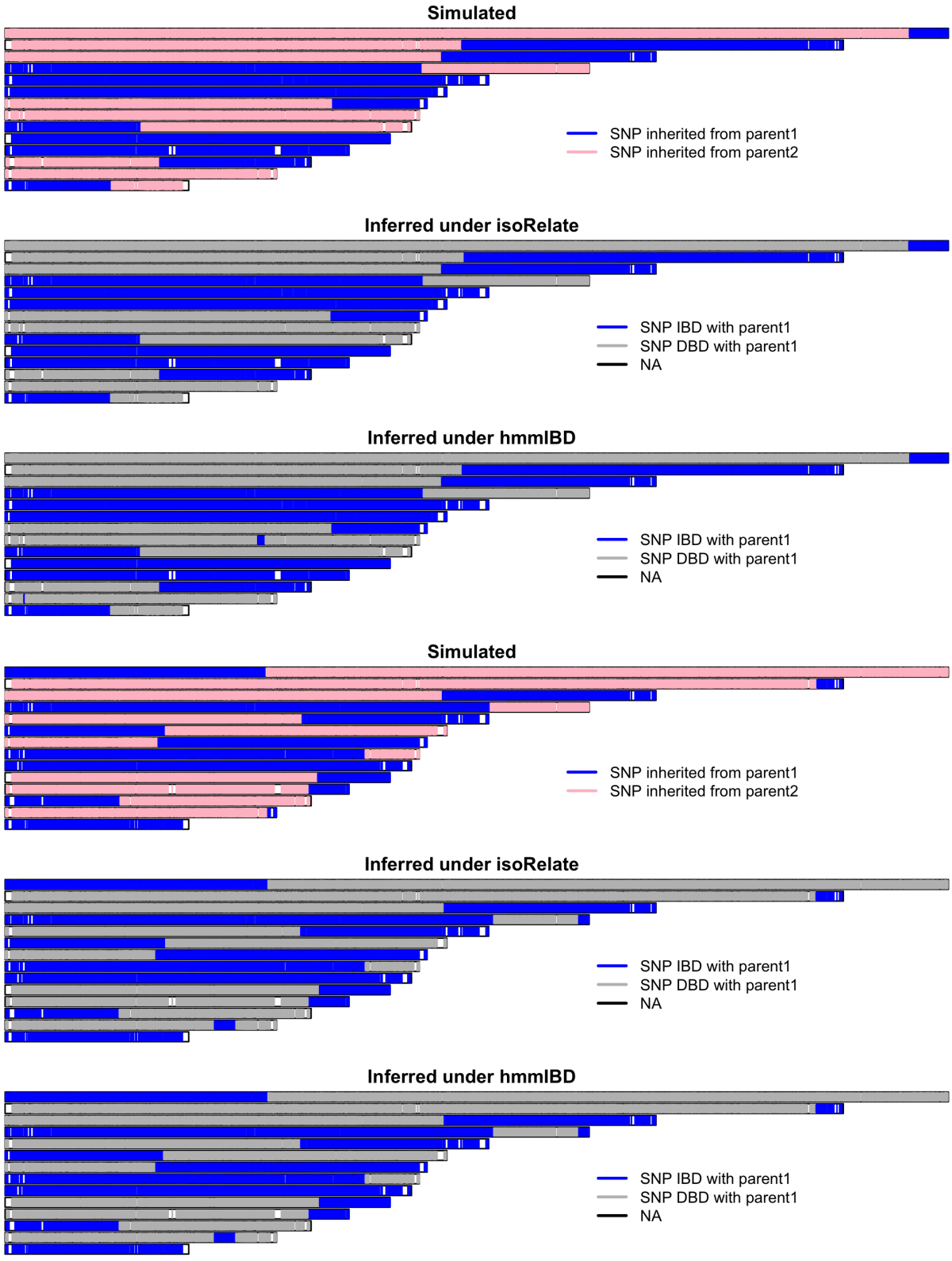
Inference based on non-erroneous chimeric children






Thies
True positive rate (sensitivity)
0.0
0.2
0.4
0.6
0.8
1.0
isoRelate single comparison
hmmIBD single comparison
isoRelate mean
hmmIBD mean
False positive rate (1-specificity)
Mean accuracy 0.9958 (isoRelate) 0.995 (hmmIBD)

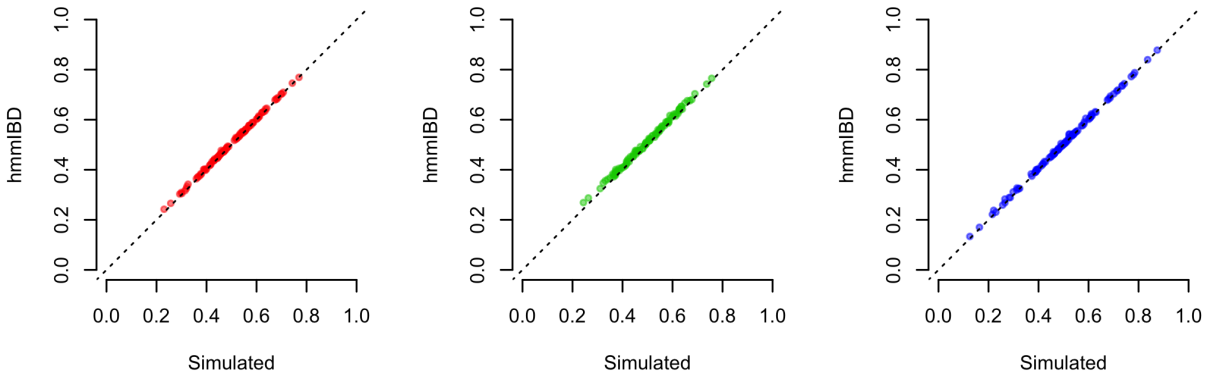
Illustrative assignment plots for two randomly selected pairwise comparisons from Kassena



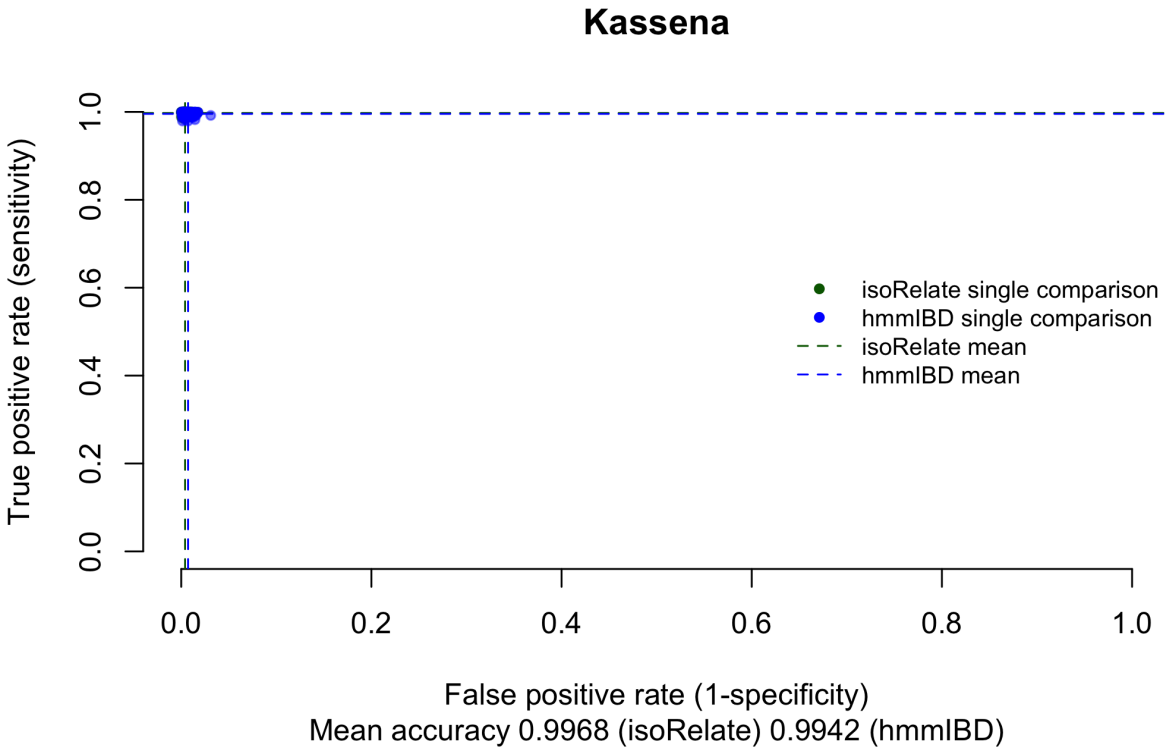
Estimates of numbers of generations

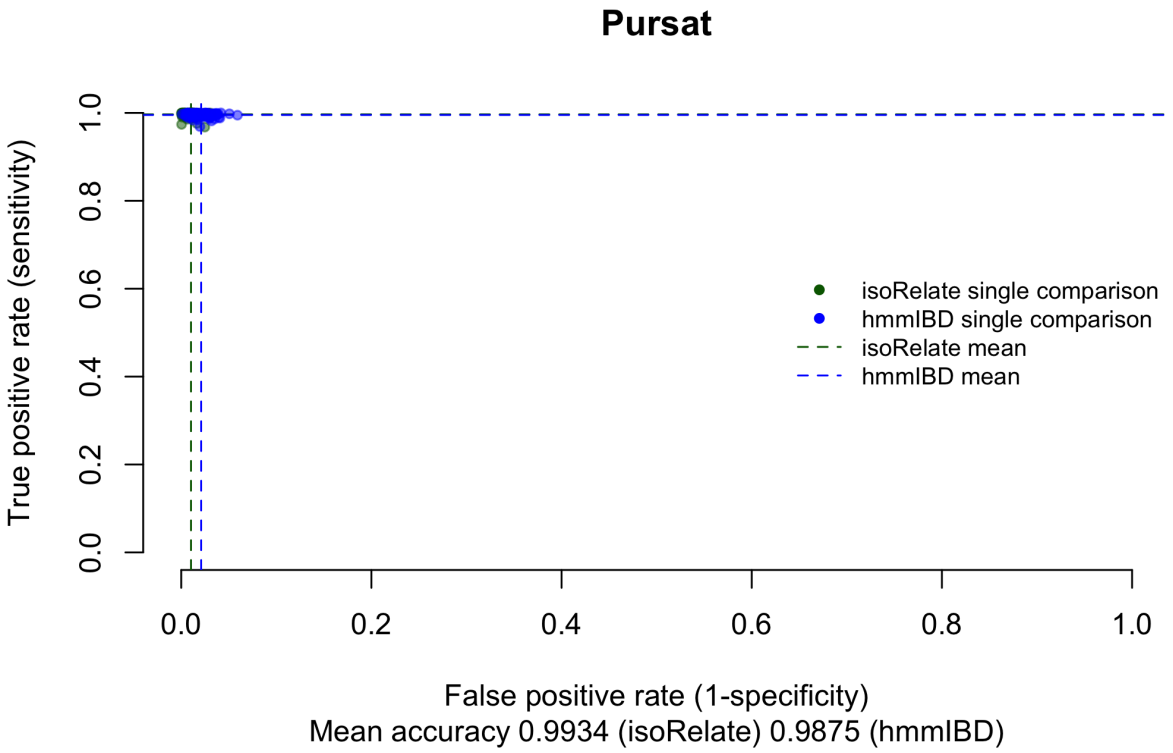
Both methods overestimate the number of generations:

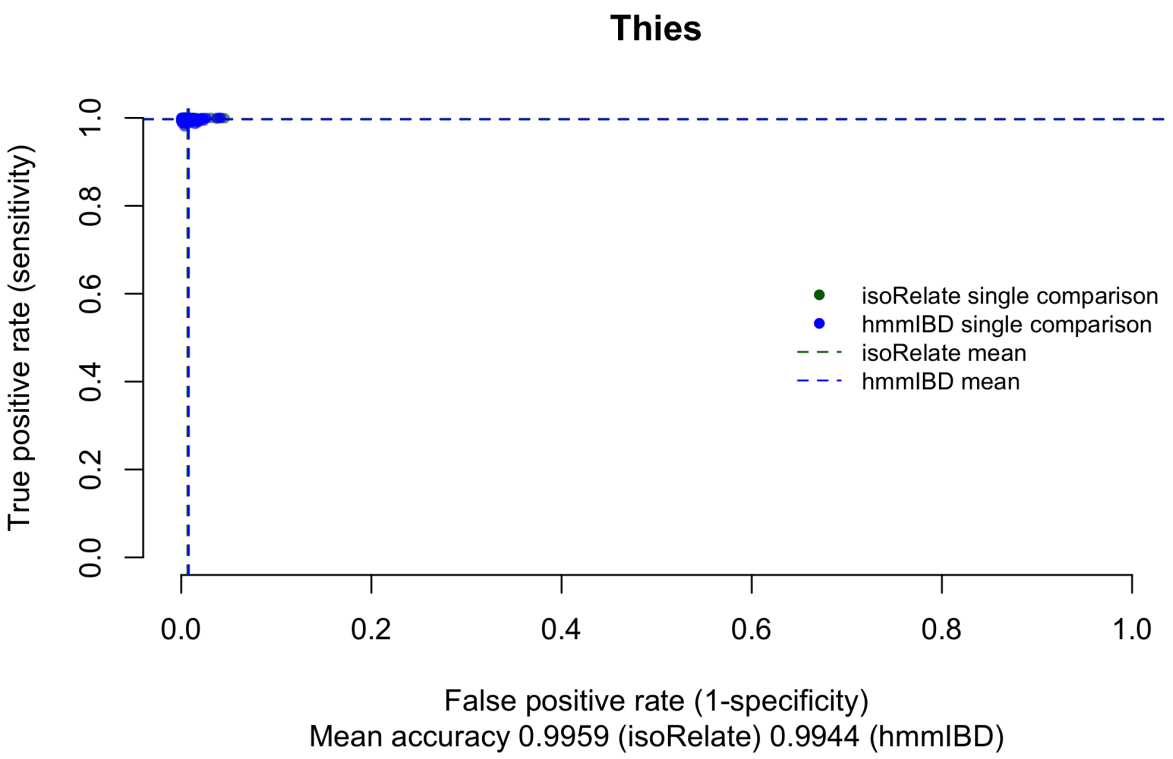
hmmIBD posterior probability of IBD versus proportion simulated IBD



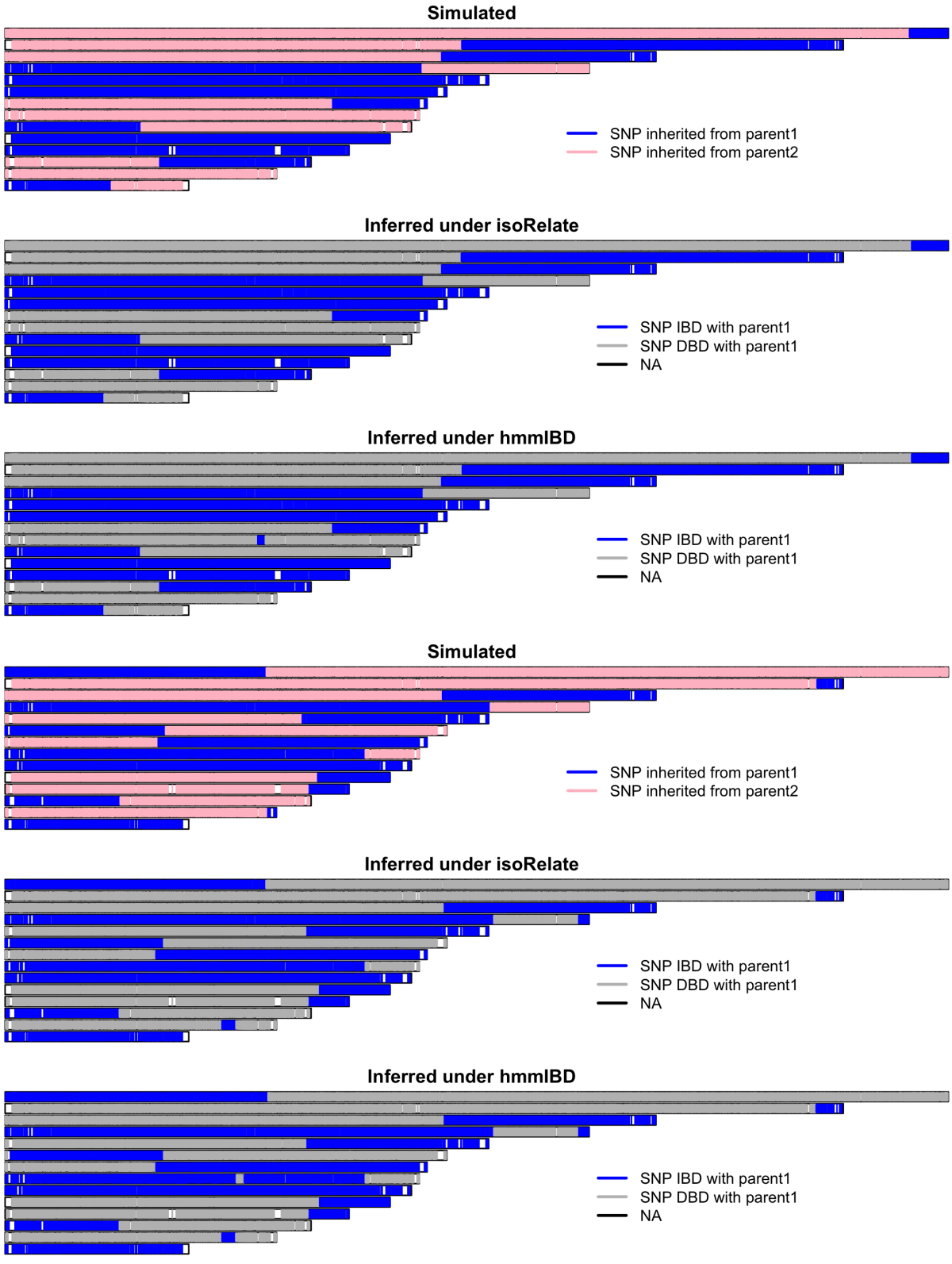
Inference results based on erroneous chimeric children





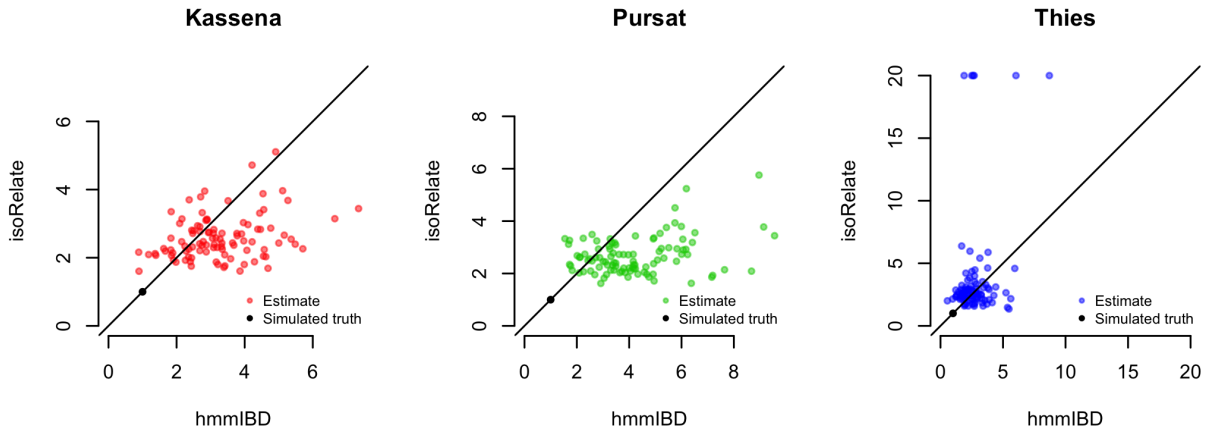

Thies
True positive rate (sensitivity)
0.0
0.2
0.4
0.6
0.8
1.0
isoRelate single comparison
hmmIBD single comparison
isoRelate mean
hmmIBD mean
False positive rate (1-specificity)
Mean accuracy 0.9959 (isoRelate) 0.9944 (hmmIBD)

Illustrative assignment plots for two randomly selected pairwise comparisons from Kassena

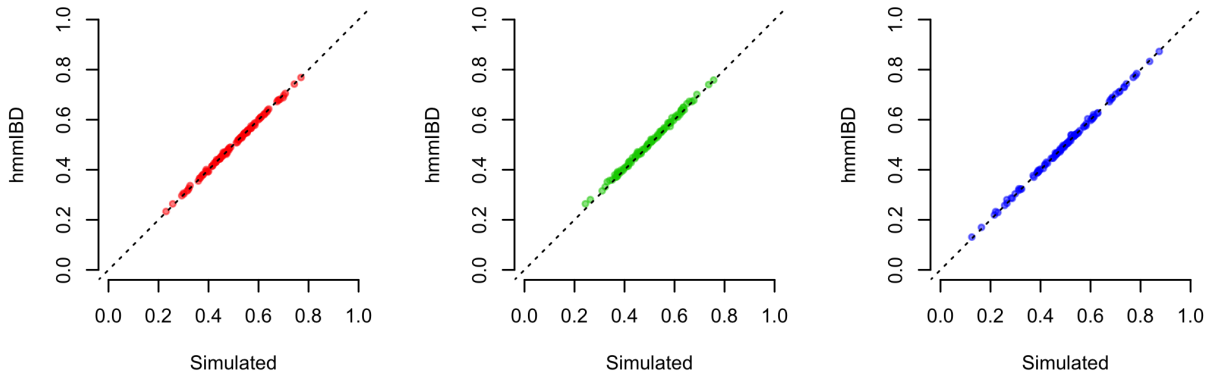


Estimates of numbers of generations

Both methods overestimate the number of generations:



hmmIBD posterior probability of IBD versus proportion simulated IBD



Summary

Both isoRelate and hmmIBD are highly accurate, sensitive and specific, including when genotyping error equal to 0.005 in the data is misspecified under the model at 0.001. In addition to IBD segments, hmmIBD returns the posterior IBD proportion (a measure of relatedness that integrates over all possible IBD segment assignments). Under v0.1.0 of isoRelate, posterior probabilities of the IBD state are not readily accessible, but many auxiliary functions for visualizing model output and assessing significance are provided. On average, hmmIBD was 25 times faster in user CPU time than isoRelate, but both perform adequately in real time.

Table 4: Summary of average run times for 50 samples on a MacBook Air with 1.7 GHz Intel Core i7 processor. Standard deviations in parentheses.

	Clock time (sec)	CPU time (sec)
isoRelate	1641.996 (343.287)	1583.174 (330.901)
hmmIBD	62.886 (13.309)	62.511 (13.172)

Table 5: Summary of average scores based on non-erroneous data with standard deviations in parentheses.

	Accuracy	Sensitivity	Specificity
isoRelate	0.995 (0.004)	0.999 (0.002)	0.991 (0.008)

	Accuracy	Sensitivity	Specificity
hmmIBD	0.993 (0.006)	0.999 (0.001)	0.986 (0.011)

Table 6: Summary of average scores based on erroneous data with standard deviations in parentheses.

	Accuracy	Sensitivity	Specificity
isoRelate	0.995 (0.004)	0.997 (0.004)	0.993 (0.007)
hmmIBD	0.992 (0.006)	0.996 (0.004)	0.988 (0.011)