Supplementary Information for Haasl & Payseur.



SUPPLEMENTARY FIGURE 1. Using a version of equation (7) that is specific to the actual sample size can lead to large overestimates of  $\theta$ . In this case, 10 data sets of n = 25 were simulated with true  $\theta = 10$ . This particular set of 10 data sets was chosen for its extremity. The  $n_a$  were: 5, 5, 5, 6, 6, 7, 7, 8, 10, 10. These values of  $n_a$  are mapped to estimates  $\hat{\theta}_{NA}$ using the n = 25 regression (left panel) and n = 1000 regression (right panel). Despite the fact that the  $n_a$  vs  $\theta$  curve in the left panel accurately corresponds to the average  $n_a$  for each  $\theta$  when n = 25, its flat slope can lead to wild overestimates of  $\theta$  when an unusually large value of  $n_a$  is obtained. Using the n = 1000 regression buffers against such overestimates, leading to an MSE that is 1/10th of that using the n = 25 regression.



SUPPLEMENTARY FIGURE 2. When estimating  $\theta$  based on the vector of observed allele frequencies, the exponential approximation to the microsatellite frequency spectrum leads to more accurate estimates of  $\theta$  than the gamma approximation. This is despite the fact that the gamma approximation describes empirical frequencies much better than the gamma approximation. Here, spectra for  $\theta = 5, 10, 25, 50$ , and 100 are plotted on the interval (0, 0.3], with the gamma approximation on the left and the exponential approximation on the right. As  $\theta$  changes, changes to the empirical approximation are most extreme along the very allele frequency intervals that change the most with  $\theta$ . For example, exponential approximations for  $\theta = 5$  and  $\theta = 25$  are quite divergent for allele frequencies on the interval (0, 0.05], while the corresponding gamma approximations show much less divergence. To a lesser extent, divergence between the expoential approximations on the allele frequency interval (0.075, 0.3) are greater than those between the corresponding gamma approximations. As  $\theta$ increases, allele frequencies < 0.05 (especially < 0.01) become much more common and allele frequencies > 0.1 become much more rare. The form of the exponential approximation is better suited to diagnose these differences.

SUPPLEMENTARY TABLE 1. Intercepts and regression coefficients for equations (7) and (8).

	equation $(7)$			equation $(8)$		
n	$c_0$	$c_1$	$c_2$	$a_0$		
25	1.5384	-0.05558	-0.01983	0.57991		
50	1.6831	-0.03792	-0.01655	0.53729		
100	1.8157	-0.04686	-0.01017	0.49990		
150	1.8816	-0.05574	-0.00673	0.46722		
200	1.9210	-0.05735	-0.00557	0.45206		
250	1.9486	-0.06295	-0.00387	0.43252		
500	2.0357	-0.07910	-0.00007	0.39704		
1000	2.0845	-0.08290	0.00110	0.35311		

SUPPLEMENTARY TABLE 2. Sample size and  $\theta$  estimation. MSE and bias (in parentheses) are shown. All statistics based on 150 independent data sets. n and  $\theta$  in the two leftmost columns refer to the simulation parameters. The first row of the header refers to the version of equation (7) used to perform estimation. The results imply a complicated relationship between  $\theta$ , n, and our methods of estimation. Generally, MSE and bias are reduced by using a version of equation (7) specific to a value of n that is greater than actual sample size.

		n = 25		$\underline{n=50}$		n = 100		n = 150		n = 250		n = 1000	
n	θ	$\hat{\theta}_{EFS}$	$\hat{\theta}_{NA}$										
25	10	165 (5.6)	148 (5.2)	118(3.6)	96(3.3)	25.8 (-2.2)	25.2 (-2.4)	25.2 (-3.2)	25.3 (-3.3)	26.2 (-3.6)	26.2 (-3.7)	31.9 (-4.7)	32.0 (-4.8)
	100	19920 (89)	15843 (73)	18664 (82)	14799~(66)	3753 (-59)	4004 (-61)	4280 (-6)	4530 (-66)	4710 (-67)	4946 (-70)	5536 (-74)	5338 (-75)
50	10	-	-	75.4(2.5)	75.2(2.7)	36.2(0)	35.7(0.12)	28.2 (-1.1)	27.8 (-1)	24.9 (-1.9)	24.2 (-1.8)	25.4 (-3.1)	24.6(-3)
	100	-	-	14196(55)	8334 (35)	2342 (-12)	1822 (-21)	2003 (-27)	1967 (-33)	2266 (-36)	2352 (-41)	2977 (-49)	3224 (-53)
100	10	-	-	-	-	38.5(0.7)	40.3(1.0)	37.4(0.5)	38.9(0.8)	32.7 (-0.1)	33.6 (0.21)	24.3 (-2)	24.3 (-1.8)
	100	-	-	-	-	3102 (5.8)	2343 (-0.8)	3252 (8.8)	2470(2.1)	2282 (-6.2)	1831 (-12)	2032 (-25.6)	1928 (-30)

SUPPLEMENTARY TABLE 3. Effect of subsampling on  $\theta$  estimation. All subsampling results are based on 10,000 independent estimates of  $\theta$ . For ease of comparison, the results from non-subsampling estimates are repeated from Table 1. Boldface statistics indicate combinations of  $\theta$  and n for which subsampling improved estimation in terms of MSE.

θ	n	$\hat{ heta}_{ar{x}}$	$\hat{ heta}_{NA}$	$\hat{ heta}_{EFS}$
5	150 subsampling	5.75 (-1.00)	6.77 (-0.85)	6.88 (-1.02)
	150	6.66 (-0.36)	8.22 (-0.12)	7.89 (-0.35)
	250 subsampling	5.64 (-0.93)	6.68 (-0.78)	6.79 (-0.96)
	250	7.30 (-0.11)	9.29(0.17)	8.65 (-0.15)
	500 subsampling	5.52 (-0.78)	6.68 (-0.61)	6.72 (-0.80)
	500	7.60(0.06)	11.78(0.71)	9.95(0.27)
	1000 subsampling	5.41 (-0.85)	6.45 (-0.68)	6.53 (-0.88)
	1000	8.34 (0.30)	$13.33\ (0.99)$	10.79(0.48)
10	150 subsampling	20.03 (-2.67)	22.14 (-2.02)	22.80 (-2.23)
	150	20.84 (-1.46)	27.45 (-0.61)	26.22 (-0.98)
	250 subsampling	19.67 (-1.94)	22.42 (-1.72)	$23.01 \ (-1.94)$
	250	22.58 (-0.82)	23.43(0.15)	28.68 (-0.27)
	500 subsampling	19.10 (-2.26)	22.16 (-1.53)	22.66 (-1.78)
	500	22.92 (-0.66)	37.99(1.04)	33.26(0.38)
	1000 subsampling	18.75 (-2.14)	22.00(-1.39)	22.47 (-1.64)
	1000	24.72 (-0.14)	43.79(1.70)	36.86(0.90)
25	150 subsampling	130.81 (-6.48)	119.96 (-6.48)	$125.61 \ (-6.58)$
	150	114.45 (-6.08)	138.76(-2.57)	138.17 (-3.07)
	250 subsampling	126.28 (-8.65)	$117.47 \ (-7.01)$	$123.37 \ (-6.15)$
	250	113.93 (-4.53)	$161.29\ (0.55)$	152.06 (-1.34)
	500 subsampling	119.64 (-8.12)	$113.95 \ (-5.35)$	$120.03 \ (-5.49)$
	500	118.14 (-3.67)	200.40(1.92)	$188.00\ (0.88)$
	1000 subsampling	$118.21 \ (-7.92)$	$114.35 \ (-5.10)$	120.74 (-5.24)
	1000	122.70 (-2.57)	231.52(3.42)	210.54 (2.12)
75	150 subsampling	1587.9 (-37.05)	1166.4 (-27.81)	1159.8 (-26.12)
	150	1144 (-26.32)	963.6 (-13.19)	1044 (-13.37)
	250 subsampling	1474.6 (-35.15)	1076.5 (-25.24)	1084.9 (-23.39)
	250	1010 (-20.38)	1061 (-5.35)	1178 (-6.08)
	500 subsampling	1386.6 (-33.42)	1024.2 (-22.88)	1054.4 (-20.79)
	500	1001 (-17.60)	1560(3.69)	1730(4.08)
	1000 subsampling	1350.4 (-32.77)	999.1 (-22.00)	$1037.5 \ (-19.85)$
	1000	987 (-13.56)	1917 (9.76)	2044 (9.32)