ORIGINAL ARTICLE

Measuring individual inbreeding in the age of genomics: marker-based measures are better than pedigrees

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Inbreeding (mating between relatives) can dramatically reduce the fitness of offspring by causing parts of the genome to be identical by descent. Thus, measuring individual inbreeding is crucial for ecology, evolution and conservation biology. We used computer simulations to test whether the realized proportion of the genome that is identical by descent (IBD_G) is predicted better by the pedigree inbreeding coefficient (F_P) or by genomic (marker-based) measures of inbreeding. Genomic estimators of IBD_G included the increase in individual homozygosity relative to mean Hardy–Weinberg expected homozygosity (F_H), and two measures (F_{ROH} and F_E) that use mapped genetic markers to estimate IBD_G . IBD_G was more strongly correlated with F_H , F_E and F_{ROH} than with F_P across a broad range of simulated scenarios when thousands of SNPs were used. For example, IBD_G was more strongly correlated with F_{ROH} , F_H and F_E (estimated with $\geq 10\,000$ SNPs) than with F_P (estimated with 20 generations of complete pedigree) in populations with a recent reduction in the effective populations size (from $N_e = 500$ to $N_e = 75$). F_{ROH} , F_H and F_E generally explained $>90\%$ of the variance in IBD_G (among individuals) when 35 K or more SNPs were used. F_P explained $<80\%$ of the variation in IBD_G on average in all simulated scenarios, even when pedigrees included 20 generations. Our results demonstrate that IBD_G can be more precisely estimated with large numbers of genetic markers than with pedigrees. We encourage researchers to adopt genomic marker-based measures of IBD_G as thousands of loci can now be genotyped in any species.

Heredity (2015) 115, 63–72; doi:10.1038/hdy.2015.17; published online 18 March 2015

INTRODUCTION

Biologists have long recognized that inbred individuals (those whose parents are closely related) often have lower fitness than the offspring of unrelated parents (Darwin, 1868; Ives and Whitlock, 2002). The cumulative effects of inbreeding on individual fitness can reduce the population growth rate and the probability of persistence (O'Grady *et al.*, 2006; Kenney *et al.*, 2014). Effects of inbreeding on individual fitness and population dynamics make measuring individual inbreeding a crucial part of many studies in ecology, evolution and conservation biology.

Inbred individuals have lower genome-wide heterozygosity because a fraction of loci are 'identical by descent' (IBD). A locus is identical by descent if it carries two gene copies that both originated from a single copy in a common ancestor of the parents. All measures of individual inbreeding seek to predict the proportion of the genome that is identical by descent (IBD_G). The classical measure of individual inbreeding is the pedigree inbreeding coefficient F_P (Wright, 1922; Keller and Waller, 2002). F_P predicts the IBD_G due to the known common ancestors of parents and assumes that the pedigree founders are unrelated and non-inbred. F_P has often been considered as the best measure of individual inbreeding (Pemberton, 2004, 2008). However, F_P may be an imprecise measure of IBD_G because IBD_G can vary substantially among individuals with the same pedigree (for example, siblings, Figure 1 and Supplementary Figure S1) (Franklin, 1977; Hill and Weir, 2011; Forstmeier *et al.*, 2012). The standard deviation of IBD_G among individuals with the same pedigree is highest in species

with few chromosomes and short genetic maps (Franklin, 1977; Stam, 1980; Hill and Weir, 2011; Figure 1 and Supplementary Figure S1). Furthermore, F_P cannot account for inbreeding caused by distant ancestors not included in a pedigree.

The increasing availability of large-scale molecular genetic data might make it possible to more precisely estimate IBD_G with genetic markers than with pedigrees (Keller *et al.*, 2011; Hoffman *et al.*, 2014). For example, IBD_G can be estimated as the increase in individual homozygosity relative to Hardy–Weinberg expected homozygosity (F_H) (Purcell *et al.*, 2007). Other measures of IBD_G use mapped genetic markers to identify IBD chromosome segments (referred to here as IBD tracts), which are characterized by long contiguous runs of homozygosity (ROH) at mapped genetic markers (Chapman and Thompson, 2003; McQuillan *et al.*, 2008). The F_{ROH} statistic estimates IBD_G as the proportion of the genome within inferred ROH that satisfy user-defined criteria (for example, number of SNPs, physical length and SNP density) (Purcell *et al.*, 2007; McQuillan *et al.*, 2012). Leutenegger *et al.* (2003) introduced a maximum likelihood estimator (F_E) that uses mapped genetic markers and a hidden Markov model to identify putative IBD tracts and to estimate IBD_G .

A thorough evaluation of the relative performance of pedigree- versus marker-based measures of IBD_G in small populations would advance our understanding of how individual inbreeding should be measured in natural populations of conservation concern as we enter an era when thousands of loci can be genotyped for any organism.

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Received 22 July 2014; revised 3 February 2015; accepted 13 February 2015; published online 18 March 2015

Figure 1 The distribution of IBD_G among 5000 simulated offspring of full siblings with 20 chromosomes of equal length, and 800 cM (upper panel) or 3600 cM (lower panel) genomes.

Using simulations, Keller *et al.* (2011) found that the number of homozygous rare alleles within an individual was more strongly correlated with marker-based measures of inbreeding than with F_P . However, they simulated large populations with 220 cM genomes and only 2 chromosomes. Thus, their results likely underestimate the precision of F_P as a measure of IBD_G in humans and other species with much longer genetic maps and more chromosomes (Figure 1 and Supplementary Figure S1; Franklin, 1977).

Our objective was to test whether IBD_G is predicted better by pedigrees or by genetic markers in small populations. Specifically, we evaluated the precision and bias of F_P , F_{ROH} , F_E and F_H in small populations that are of the greatest concern for conservation with genomic characteristics typical of mammals. Our simulations accounted for the depth of the pedigree used to estimate F_P , the number of markers used to estimate F_{ROH} , F_E and F_H , the genetic map length of the genome and demographic history. We emphasize comparisons of the performance of F_P versus the marker-based measures of IBD_G .

MATERIALS AND METHODS

Computer simulation model

We used a stochastic, individual-based simulation program (Kardos *et al.*, 2014) for R version 3.0.1 (R Core Team, 2013). We simulated a sexually reproducing, random mating, non-selfing species with non-overlapping generations. We simulated hermaphrodites to avoid temporal fluctuations in the effective populations size (N_e) due to stochastic variation in the sex ratio. We simulated recombination and kept track of the ancestral origin of each chromosome segment, which was known to the base pair. We simulated genomic characteristics typical of mammals, including 3 Gb genomes with 20 chromosomes of equal size, and mutation with a base change probability of 1.2×10^{-8} per base pair per generation. We simulated only 20 chromosomes because chromosome number does not strongly affect the standard

deviation of IBD_G among individuals with the same pedigree ($\sigma(IBD_G)$) when genetic map lengths are in the range we simulated (Supplementary Figure S1). We used two different recombination rates to evaluate the effect of genetic map length on the precision of F_P , F_{ROH} , F_E and F_H . We simulated genomes with 1.2 cM/Mb (that is, an average of approximately 0.012 recombinations per Mb per meiosis) which is similar to humans (Dumont and Payseur, 2008) and resulted in a genetic map length of 3600 cM. We also simulated genomes with 0.27 cM/Mb which is typical of the lower end of the distribution of recombination rates among mammals (Dumont and Payseur, 2008) and resulted in a genetic map length of 800 cM.

Our model of recombination follows the simulations of Chapman and Thompson (2003) and is based on Fisher's theory of junctions (Fisher, 1965). We assume no interference, that the number of crossovers along a chromosome is Poisson distributed, and that the recombination probability is constant across the genome and among individuals.

Population founders were unrelated and non-inbred. Thus, IBD_G for an individual is relative to the population founders. Founders for each simulated population were drawn from a hypothetical source population in Hardy-Weinberg equilibrium. Allele frequencies at 400 K ($K=1000$) SNPs in the source populations were assigned so that the mean expected heterozygosity (H_e) was approximately 0.3 at the end of the simulations before SNP filtering steps (see below). To account for the effects of SNP heterozygosity (Miller *et al.*, 2014) on our results, we repeated simulations of populations with 3600 cM genomes with founder allele frequencies assigned so that H_e was only approximately 0.2, and analyzed these data without filtering based on minor allele frequency (MAF) (beyond removing fixed loci). SNP positions were determined using a random number generator so that each nucleotide position in the genome was equally likely to be polymorphic in the source population. Individual founder genotypes were assigned by randomly choosing two alleles at each locus based on their frequencies in the source population (see Kardos *et al.*, 2014 for further details on the assignment of founder genotypes). Simulation output included IBD_G and genotypes at 300–350 K diallelic SNPs, which were left after removing loci with $MAF < 0.05$ for each individual in the final generation. SNPs with $MAF < 0.05$ were removed to maximize the information content of the genotypes. Statistical analyses were performed only on individuals from the last generation to hold the number of pedigree generations used to estimate F_P constant among individuals within each analysis, and to hold the sample sizes for statistical tests constant across all simulations. An R script for the simulations is available in Dryad (see Data Accessibility).

Defining IBD_G

We defined IBD_G as the realized proportion of the physical genome in IBD tracts. IBD_G was measured without error because the exact boundaries of IBD tracts were known to the base pair. Thus, IBD_G is a parameter representing the true individual genomic inbreeding. The IBD_G was calculated from the simulated genomic data once for each individual. The mean and variance of IBD_G for each simulated scenario are shown in Supplementary Table S2.

Simulated demographic scenarios

We simulated two demographic scenarios to account for differences in the variance of IBD_G , and to mimic populations where researchers are often interested in inbreeding and its effects on fitness. First, we simulated partially isolated populations with a constant local N_e of 75 for 150 generations, receiving occasional immigrants ($m=0.013$, one

immigrant per generation on average). This scenario mimics small populations on habitat islands with occasional immigration from a large source population. Immigrants were unrelated to each other and to residents. Immigrants were drawn from the same hypothetical source population as the founders and thus had genotypes drawn from the same allele frequency distribution as the founders. Second, we simulated closed populations that were large (constant $N_e = 500$) for 90 generations, and experienced a reduction in size to $N_e = 75$ for 10 additional generations. We ran 20 replicate simulations for each scenario.

We wanted to test whether our results were substantially different when all inbreeding was due to recent ancestors. Therefore, we reran the simulations as described above, except now running the simulations for only 20 generations (not 100–150 generations as above). Here, all inbreeding is due to recent (within 20 generations) ancestors, and F_P estimated with a 20 generation pedigree accounts for all common ancestors of parents. For these simulations, partially isolated populations had a constant local N_e of 75 for 20 generations, and received one immigrant per generation on average ($m = 0.013$). Populations with recently reduced N_e were again closed ($m = 0$) and had $N_e = 500$ for the first 10 generations, then $N_e = 75$ for the last 10 generations.

Linkage disequilibrium pruning

Before estimating F_{ROH} , F_E and F_H , we used the—indep-pairwise function in PLINK version 1.07 (Purcell *et al.*, 2007) to reduce linkage disequilibrium among the 300–350 K SNPs remaining after MAF-based filtering. We conducted ‘LD pruning’ because F_E assumes all markers are in linkage equilibrium (Polašek *et al.*, 2010). Additionally, F_{ROH} is typically estimated after LD pruning to avoid detecting fixed or nearly fixed ROH arising due to ancient population processes (for example, selective sweeps) (for example, McQuillan *et al.*, 2012). For LD pruning, we used a sliding window of 25 SNPs, a step size of 10 SNPs and we removed one SNP from each pair in a window above the LD threshold of $r^2 = 0.5$. Our results were not substantively affected by increasing the size of the sliding window for LD pruning to 50 SNPs, or by using a more stringent LD pruning threshold ($r^2 = 0.25$) (data not shown).

We conducted statistical analyses on 500 SNPs to 100 K SNPs randomly sampled from those remaining after MAF- and LD-based filtering. We used only up to 100 K SNPs because preliminary simulations showed that using more loci did not substantively affect our results (data not shown). Mean H_e after filtering based on MAF and LD was 0.31 in partially isolated populations with 3600 cm genomes, and 0.33 on average in partially isolated populations with 800 cm genomes. H_e was 0.35 on average in populations with recently reduced N_e . These levels of SNP heterozygosity are similar to large SNP chip data sets from mammals and birds (for example, Deatwyler *et al.*, 2010; Kawakami *et al.*, 2014). In our simulations of populations with low heterozygosity SNPs ($H_e \approx 0.2$), the mean realized H_e after removing fixed loci and LD pruning was 0.21 in populations with recently reduced N_e and 0.19 in partially isolated populations.

Estimators of IBD_G

We used three marker-based measures of IBD_G . We used the—homozyg function in PLINK to estimate F_{ROH} using 5–100 K SNPs. PLINK uses a sliding window approach and user defined criteria to identify ROH, which are inferred to be putative IBD tracts. F_{ROH} is estimated as the proportion of the genome in ROH, and ranges from zero to one. Thus, F_{ROH} is directly proportional to IBD_G . We used only ≥ 5 K SNPs to estimate F_{ROH} because we believed that SNP density would be too low to reliably detect ROH when fewer loci are

used, regardless of the parameter settings used in PLINK. For example, fewer than two SNPs are expected to occur in an 8-Mb IBD tract on average when only 500 randomly distributed SNPs are used in a 3-Gb genome. We adjusted the required number of SNPs and SNP density (maximum Kb per SNP on average within an ROH) per inferred ROH based on the number of loci used (Supplementary Table S1). We allowed a single heterozygous position in any inferred ROH to account for the occasional mutation occurring within otherwise IBD chromosome segments. Initial testing showed that adjusting PLINK parameters according to the number of SNPs and LD pruning substantially improved the performance of F_{ROH} when ≤ 25 K SNPs were used. Consistent with our definition of IBD_G above, we estimated F_{ROH} as the sum of the lengths of all detected ROH detected by PLINK divided by the physical genome size (3 Gb).

The second measure of IBD_G we used (F_H) measures the excess in the observed number of homozygous genotypes within an individual relative to the mean number of homozygous genotypes expected under random mating:

$$F_{H_i} = \frac{O(Hom_i) - E(Hom)}{m - E(Hom)},$$

where $O(Hom_i)$ is the observed number of homozygous loci for the i th individual, and $E(Hom)$ is the Hardy–Weinberg expected mean number of homozygous genotypes across m loci (Keller *et al.*, 2011). The average $O(Hom)$ is expected to equal $E(Hom)$ on average in random mating populations. F_H can thus take negative values (like some other marker-based measures of inbreeding or relatedness; Wang, 2014), and will be centered near zero in random mating populations when allele frequencies are taken from the current sample of individuals. F_H should thus be considered as a proxy rather than a direct estimator of IBD_G , which is a proportion with range 0–1. We estimated F_H using the—het function in PLINK.

The third marker-based estimator (F_E) is implemented in the program FEstim and uses maximum likelihood and a hidden Markov model to determine the values of IBD_G that maximize the likelihood of the observed genotypes at mapped loci (Leutenegger *et al.*, 2003). F_E has a theoretical range of 0–1 and is thus directly proportional to IBD_G . Starting values for both F_E and the IBD rate of change parameter were 0.1. Default FEstim settings were used for other parameters. FEstim did not consistently run to completion on data from partially isolated populations when more than 10 K SNPs were used, even when using more stringent LD pruning settings in PLINK (LD threshold of $r^2 = 0.25$, and a sliding window size of 50 SNPs). This is likely due to higher LD remaining among closely linked SNPs in the partially isolated populations than in populations with recently reduced N_e after LD pruning (Supplementary Figure S2). Using more stringent LD pruning thresholds (for example, $r^2 = 0.1$) left too few SNPs to usefully compare F_E and the other IBD_G estimators. Thus, we present results from F_E in partially isolated populations for 10 K and fewer SNPs only. Allele frequencies used to estimate F_H and F_E were from the 75 individuals in the last generation of each simulation. We used the kinship2 package in R to estimate F_P for each individual using 3–20 generations of pedigree.

Comparing the performance of F_P , F_H , F_E and F_{ROH}

We used the proportion of variance in IBD_G explained by F_{ROH} , F_E , F_H and F_P (r^2) from linear regression models to evaluate the precision of F_{ROH} , F_E , F_H and F_P ($N =$ all 75 individuals from the last simulated generation). We conducted separate regressions of F_{ROH} , F_H , F_E and F_P versus IBD_G for individuals in the final generation of each simulated population. We used two-tailed t -tests to determine whether

the mean r^2 from regressions with IBD_G (among 20 replicate simulations) was statistically significantly different for F_{ROH} , F_H , F_E and F_P . We compared all possible combinations of pedigree depth (for F_P) and number of SNP used (for F_H , F_E , and F_{ROH}) when testing if the mean r^2 with IBD_G was statistically significantly different for F_P , F_H , F_E and F_{ROH} . We also used t -tests to determine whether the mean r^2 from regressions with IBD_G was statistically significantly different for F_{ROH} , F_E , F_H and F_P when the genetic map length was 800 cM instead of 3600 cM, and when simulations were run for 20 generations instead of 100–150 generations.

We measured bias of the IBD_G estimators as the mean amount by which F_P , F_H , F_E and F_{ROH} over- or under-estimated IBD_G (that is, the mean of the values of F_P , F_H , F_E or F_{ROH} minus IBD_G). Note that F_H is expected to underestimate IBD_G when allele frequencies are taken from the current population and mating occurs at random. Thus, we expect F_H to underestimate IBD_G in our simulated random mating populations. We also wanted to measure bias of the estimated differences in IBD_G among individuals. For this we estimated the ratio of the standard deviation of each of the estimators (F_P , F_H , F_E and F_{ROH}) to the standard deviation of IBD_G to measure bias of the estimated differences in IBD_G among individuals. A ratio of the standard deviation of an estimator to the standard deviation of IBD_G near 1 indicates that the estimated differences in IBD_G among individuals are unbiased. Ratios of below or above 1 indicate that estimated differences in IBD_G among individuals are substantially under- or overestimated.

RESULTS

We first present results on the precision and bias of F_P , F_H , F_E and F_{ROH} in simulations of 3600 cM genomes where inbreeding was due to both recent and distant ancestors (that is, simulations ran for 100–150 generations). We then summarize how the results change when inbreeding is due only to recent ancestors (that is, simulations ran for only 20 generations), when genetic map length is shortened to 800 cM, and finally when SNP $H_e \approx 0.2$ (instead of $H_e \approx 0.3$).

Precision of IBD_G estimators

F_P had low precision in both demographic scenarios (Figure 2). The mean r^2 from regressions of F_P versus IBD_G across 20 simulation repetitions ($\bar{r}_{F_P-IBD_G}^2$) was 0.72 in partially isolated populations and 0.71 in populations with recently reduced N_e when pedigrees included 20 generations. Using more than five pedigree generations had little effect on $\bar{r}_{F_P-IBD_G}^2$ in populations with recently reduced N_e . However, $\bar{r}_{F_P-IBD_G}^2$ increased when deeper pedigrees were used in partially isolated populations.

F_{ROH} and F_H had high precision in both demographic scenarios when large number of SNPs were used. The mean r^2 from regressions with IBD_G was statistically significantly higher for F_H ($\bar{r}_{F_H-IBD_G}^2$) than for F_E ($\bar{r}_{F_E-IBD_G}^2$) or F_{ROH} ($\bar{r}_{F_{ROH}-IBD_G}^2$) in partially isolated populations for all numbers of loci used ($P < 0.05$, Figure 2). $\bar{r}_{F_H-IBD_G}^2$ was > 0.9 when 10 K or more SNPs were used in both demographic scenarios. $\bar{r}_{F_{ROH}-IBD_G}^2$ was > 0.9 when 100 K SNPs were used in partially isolated populations, and when ≥ 15 K SNPs were used in populations with recently reduced N_e . $\bar{r}_{F_E-IBD_G}^2$ was always statistically significantly

Figure 2 Barplots of the mean r^2 (± 1 s.d. among 20 simulated populations) from regressions of F_P , F_E , F_H and F_{ROH} versus IBD_G . The data shown are from 20 partially isolated populations (top row) and also 20 populations with recently reduced N_e (bottom row) and 3600 cM genomes. Dashed lines at $r^2 = 0.9$ are to aid comparison of r^2 for F_P , F_H , F_E and F_{ROH} .

higher than $\bar{r}_{F_H-IBD_G}^2$ and $\bar{r}_{F_{ROH}-IBD_G}^2$ in populations with recently reduced N_e ($P < 0.001$).

F_{ROH} , F_E and F_H were always more precise than F_P when large numbers of SNPs were used. For example, $\bar{r}_{F_H-IBD_G}^2$ measured with ≥ 5 K SNPs and $\bar{r}_{F_{ROH}-IBD_G}^2$ measured with ≥ 35 K SNPs were both statistically significantly higher than $\bar{r}_{F_P-IBD_G}^2$ based on 20 generation pedigrees in partially isolated populations ($P < 0.001$; Figure 2). The mean r^2 from regressions with IBD_G was statistically significantly higher for F_{ROH} , F_E and F_H estimated with ≥ 10 K SNPs than for F_P estimated with 20 generations in populations with recently reduced N_e ($P < 0.001$).

Bias of IBD_G estimators

F_P consistently underestimated IBD_G in both simulated demographic scenarios (Figure 3). For example, F_P underestimated IBD_G by 0.19 with 3 generation pedigrees, and by 0.1 with 20 generation pedigrees on average in partially isolated populations. F_P was less downwardly biased in populations with recently reduced N_e where F_P underestimated IBD_G by 0.13 and 0.07 on average when pedigrees included 3 and 20 generations, respectively.

Marker-based estimators were all substantially downwardly biased when few SNPs were used, and this downward bias was largest in partially isolated populations (Figure 3). F_E and F_{ROH} became less downwardly biased when larger numbers of SNPs were used. For example, F_E underestimated IBD_G by 0.11 when 500 SNPs were used, and by only 0.02 when 100 K SNPs were used in populations with

recently reduced N_e . F_{ROH} underestimated IBD_G by 0.14 when 5 K SNPs were used, and by 0.05 on average when 100 K SNPs were used in populations with recently reduced N_e . F_H was the most downwardly biased of all ($P < 0.0001$) and the number of SNPs had no effect on bias. F_H underestimated IBD_G by 0.21 on average in partially isolated populations and by 0.16 in populations with recently reduced N_e .

Both F_E and F_{ROH} were less downwardly biased than F_P in both demographic scenarios when large numbers of SNPs were used (Figure 3). F_{ROH} estimated with 10 K and 50 K SNPs was less downwardly biased than F_P estimated with 3 and 20 generations, respectively, in populations with recently reduced N_e ($P < 0.0001$). F_E estimated with 500 and 10 K SNPs was less downwardly biased than F_P estimated with 3 and 20 generations, respectively, in populations with recently reduced N_e ($P < 0.0001$). F_H had larger downward bias than F_P in all cases (Figure 3). The precision and bias of F_{ROH} , F_E , F_H and F_P relative to IBD_G in a representative population is shown in Figure 4.

Bias in estimated differences in IBD_G among individuals

The mean ratio of the standard deviation of F_P to the standard deviation of IBD_G ($\sigma_{F_P}/\sigma_{IBD_G}$) across 20 simulation repetitions was < 1 in all scenarios (Figure 5). $\sigma_{F_P}/\sigma_{IBD_G}$ was 0.61 in partially isolated populations and 0.86 in populations with recently reduced N_e when pedigrees included 20 generations. Using pedigrees of more than five generations did not substantively affect $\sigma_{F_P}/\sigma_{IBD_G}$ in populations with recently reduced N_e .

Figure 3 Barplots of the mean bias of F_P , F_H , F_E and F_{ROH} (± 1 s.d. among 20 simulated populations). Bias was measured as the mean error (F_P , F_H , F_E or F_{ROH} minus IBD_G) across all individuals in each simulated population. The data shown are from 20 partially isolated populations (top row) and 20 populations with recently reduced N_e (bottom row) and 3600 cM genomes.

Figure 4 F_P (a), F_H (b), F_{ROH} (c) and F_E (d) versus IBD_G in a population with recently reduced N_e and 3600 cM genomes. F_P was estimated with five generations. F_H , F_E and F_{ROH} were estimated with 25 K SNPs. The dashed lines have intercept of zero and slope of one. Points below the lines represent underestimates of IBD_G .

Figure 5 Ratios of the standard deviation of F_P , F_E , F_H and F_{ROH} to the standard deviation of IBD_G . The data shown are from partially isolated populations (top row) and populations with recently reduced N_e (bottom row) and 3600 cM genomes. The dashed lines represent a ratio of 1. Bars extending above and below the line represent over- and underestimates, respectively, of the difference in IBD_G among individuals.

F_H always overestimated the differences in IBD_G among individuals, particularly when relatively few SNPs were used (Figure 5). For example, the mean ratio of the standard deviation of F_H to the standard deviation of IBD_G ($\sigma_{F_H}/\sigma_{IBD_G}$) across 20 simulation repetitions was approximately 1.7 when 5 K SNPs were used, and 1.25 when 15 K SNPs were used in partially isolated populations.

The mean ratio of the standard deviation of F_{ROH} to the standard deviation of IBD_G ($\sigma_{F_{ROH}}/\sigma_{IBD_G}$) was consistently below 1 in partially isolated populations (Figure 5). $\sigma_{F_{ROH}}/\sigma_{IBD_G}$ was 0.70 when 100 K SNPs were used in partially isolated populations. However, in populations with recently reduced N_e , $\sigma_{F_{ROH}}/\sigma_{IBD_G}$ was approximately 1.05 on average when ≥ 25 K SNPs were used (Figure 5). The mean ratio of the standard deviation of F_E to the standard deviation of IBD_G ($\sigma_{F_E}/\sigma_{IBD_G}$) was 1.1 when 500 SNPs were used, and 1.0 when 100 K SNPs were used in populations with recently reduced N_e (Figure 5). However, $\sigma_{F_E}/\sigma_{IBD_G}$ was approximately 0.72 with 10 K SNPs in partially isolated populations.

$\sigma_{F_E}/\sigma_{IBD_G}$ and $\sigma_{F_{ROH}}/\sigma_{IBD_G}$ were always $> \sigma_{F_P}/\sigma_{IBD_G}$ when large numbers of SNPs were used (Figure 5). For example, $\sigma_{F_E}/\sigma_{IBD_G}$ was statistically significantly higher than $\sigma_{F_P}/\sigma_{IBD_G}$ estimated with 20 generations when 10 K SNPs were used in partially isolated populations ($P=0.009$) and when ≥ 500 SNPs were used in populations with recently reduced N_e ($P<0.0001$). $\sigma_{F_{ROH}}/\sigma_{IBD_G}$ was statistically significantly higher than $\sigma_{F_P}/\sigma_{IBD_G}$ estimated with 20 generations when 100 K SNPs were used in partially isolated populations ($P=0.003$), and when ≥ 15 K SNPs were used in populations with recently reduced N_e ($P\leq 0.025$).

In the following sections, we summarize how altering simulation parameters (genetic map length, number of simulated generations and SNP heterozygosity) caused results to differ substantially from those presented above. Detailed results from these scenarios are provided in Supplementary Figures S3–S14.

Effects of the genetic map length on precision and bias

$\bar{r}_{F_P-IBD_G}^2$ was always statistically significantly lower in populations with 800 cM genomes than in populations with 3600 cM genomes ($P\leq 0.005$), except when pedigrees included only 3–5 generations in partially isolated populations (Figure 2 and Supplementary Figure S3). For example, $\bar{r}_{F_P-IBD_G}^2$ based on three pedigree generations was 0.54 when the genetic map was 3600 cM, and 0.36 when the genetic map was 800 cM in populations with recently reduced N_e .

F_E and F_{ROH} always had statistically significantly higher precision in partially isolated populations with 800 cM genomes than with 3600 cM genomes ($P<0.002$), with the exception of when F_{ROH} was estimated with 100 K SNPs (Figure 2 and Supplementary Figure S3). For example $\bar{r}_{F_E-IBD_G}^2$ and $\bar{r}_{F_{ROH}-IBD_G}^2$ were 0.83 and 0.88, respectively, when 10 K SNPs were used in partially isolated populations with 800 cM genomes. However, $\bar{r}_{F_E-IBD_G}^2$ and $\bar{r}_{F_{ROH}-IBD_G}^2$ were 0.65 and 0.5, respectively, when 10 K SNPs in partially isolated populations with 3600 cM genomes. F_{ROH} and F_E were often statistically significantly less downwardly biased when genomes were 800 cM instead of 3600 cM, but these differences in bias were always small (≤ 0.06) (Figure 3 and Supplementary Figure S4).

$\sigma_{F_P}/\sigma_{IBD_G}$ based on pedigrees including five or more generations was always statistically significantly lower when genomes were 800 cM than when genomes were 3600 cM in populations with recently reduced N_e ($P\leq 0.001$; Figure 5 and Supplementary Figure S5). For example, $\sigma_{F_P}/\sigma_{IBD_G}$ based on pedigrees including five generations was 0.90 with 3600 cM genomes, and 0.75 with 800 cM genomes in populations with recently reduced N_e . $\sigma_{F_{ROH}}/\sigma_{IBD_G}$ was 0.2 units higher (closer to 1) on average in partially isolated populations with

800 cM genomes than in partially isolated populations with 3600 cM genomes ($P<0.0001$, Figure 5 and Supplementary Figure S5). $\sigma_{F_E}/\sigma_{IBD_G}$ was 0.16 units higher (closer to 1) on average with 800 cM genomes than with 3600 cM genomes in partially isolated populations ($P<0.002$).

Performance of IBD_G estimators when inbreeding is due only to recent ancestors

F_P was imprecise when all inbreeding was due to recent ancestors (that is, in simulations run for only 20 generations). For example, $\bar{r}_{F_P-IBD_G}^2$ estimated with 20 generation pedigrees was 0.78 in partially isolated populations with 3600 cM genomes (Supplementary Figure S6) and 0.53 in partially isolated populations with 800 cM genomes (Supplementary Figure S9).

The precision of the marker-based measures of IBD_G in 20 generation simulations was generally very similar to the results described above for simulations that ran for 100–150 generations (Supplementary Figures S6 and S9). However, $\bar{r}_{F_E-IBD_G}^2$ was considerably higher in partially isolated populations when the simulations ran for only 20 generations instead of 150 generations (Figure 2 and Supplementary Figure S6).

F_{ROH} , F_E , F_H and F_P had lower bias in simulations that ran for only 20 generations (Supplementary Figures S7 and S10) than in simulations that ran for 100–150 generations (Figure 3 and Supplementary Figure S4). In particular, F_P estimated with pedigrees including all 20 generations was unbiased, as expected when pedigree founders are unrelated and non-inbred.

The standard deviations of F_{ROH} , F_E and F_P more closely matched the standard deviation of IBD_G in simulations of partially isolated populations that ran for only 20 generations instead of 150 generations (Figure 5 and Supplementary Figure S8). For example, $\sigma_{F_P}/\sigma_{IBD_G}$ was always < 0.65 in partially isolated populations with 3600 cM genomes simulated for 150 generations. However, $\sigma_{F_P}/\sigma_{IBD_G}$ was > 0.8 when F_P was estimated with ≥ 5 generations of pedigree in partially isolated populations simulated for only 20 generations. $\sigma_{F_E}/\sigma_{IBD_G}$ and $\sigma_{F_{ROH}}/\sigma_{IBD_G}$ were always < 0.75 in partially isolated populations run for 150 generations. However, in partially isolated populations simulated for only 20 generations, $\sigma_{F_E}/\sigma_{IBD_G}$ was > 0.9 for all numbers of loci used, and $\sigma_{F_{ROH}}/\sigma_{IBD_G}$ was > 0.9 when F_{ROH} was estimated with 100 K SNPs.

Effects of SNP heterozygosity

F_H and F_{ROH} were less precise in simulations with lower heterozygosity ($H_e\approx 0.2$) when relatively few loci were used. For example, $\bar{r}_{F_{ROH}-IBD_G}^2$ was 0.35 with $H_e\approx 0.2$ and 0.74 with $H_e\approx 0.3$ when 5 K SNPs were used in populations with 3600 cM genomes and recently reduced N_e (Figure 2 and Supplementary Figure S12). However, the number of SNPs necessary for high precision ($r^2\geq 0.9$) was unaffected by SNP heterozygosity for any of the marker-based measures of IBD_G .

DISCUSSION

Pedigrees have long been the preferred tool to measure individual inbreeding (Pemberton, 2004,2008). However, we found that the realized IBD proportion of the genome (IBD_G) is better predicted by large numbers of genetic markers than by pedigrees. Our results are consistent with two recent studies that used different approaches to evaluate the precision of marker- versus pedigree-based measures of individual inbreeding when using thousands of SNPs. Hoffman *et al.* (2014) found a moderately high correlation between heterozygosity estimated at $> 13\,000$ SNPs and F_P ($r^2=0.74$) in oldfield mice (*Peromyscus polionotus*). However, the predicted r^2 between heterozygosity and IBD_G (based on estimated identity disequilibrium in their

oldfield mice) was ≈ 1 ; thus, IBD_G was likely more precisely predicted by heterozygosity than by F_P in their study population. Keller *et al.* (2011) found that the number of homozygous rare alleles within an individual was more strongly correlated with marker-based estimates of inbreeding than with F_P in large simulated populations with short genetic maps (220 cM) and two chromosomes.

We expanded on previous work (Keller *et al.*, 2011; Hoffman *et al.*, 2014) by evaluating the performance of marker- and pedigree-based measures of IBD_G using simulations where IBD_G was known for each individual without error. Additionally, we accounted for variation in the genetic map length, population demography, heterozygosity of the genetic markers and pedigree depth. Finally, we specifically simulated relatively small populations that are of the greatest concern for conservation. Our results suggest that marker-based measures of IBD_G computed from thousands of loci should be preferred over measures based on F_P in future studies of inbreeding depression in natural populations.

Precision and bias

F_P had low precision and underestimated the differences in IBD_G among individuals in all simulated scenarios. Additionally, F_P was always downwardly biased except when pedigrees included 20 generations in simulations that ran for only 20 generations and pedigree founders were thus unrelated and non-inbred (Supplementary Figures S7 and S10). Errors in F_P have two major sources. As discussed above, $\sigma(IBD_G)$ can be high among individuals with the same pedigree (Figure 1; Franklin, 1977). High $\sigma(IBD_G)$ partially explains why multiple locus heterozygosity is usually only weakly related to F_P , and may also explain why heterozygosity-fitness correlations are sometimes observed when F_P -fitness correlations are absent (Forstmeier *et al.*, 2012). Linkage and a finite genetic map length are expected to weaken the correlation between F_P and IBD_G , but not to cause F_P to be downwardly biased.

The downward bias in F_P can easily be explained by relatedness and inbreeding of pedigree founders. When founders are related, inbreeding due to common ancestors deeper in an individual's ancestry is not accounted for, thus causing F_P to underestimate IBD_G . F_P will also underestimate IBD_G for individuals whose parents have a common ancestor that is an inbred founder. Moving the base population further back in time (that is, including more generations in a pedigree) accounts for a larger proportion of the common ancestors of parents and the inbreeding of those common ancestors, thus decreasing the downward bias of F_P (Figure 3 and Supplementary Figure S15). High variance in founder relatedness may reduce the precision of F_P by causing variation among individuals in the magnitude of the underestimation of IBD_G . A focus of future research should be to determine the relative influence of related or inbred pedigree founders versus linkage and recombination on the imprecision of F_P .

All of the marker-based measures of IBD_G were highly precise when large number of SNPs were used. Additionally, genetic markers showed lower bias than F_P in estimated differences in IBD_G among individuals. Thousands of SNPs can currently be typed for any organism, for example, with SNP chips (for example, Kawakami *et al.*, 2014), restriction site-associated DNA sequencing (RAD-seq) (Davey *et al.*, 2011), or by whole genome sequencing (Ellegren, 2014). These technologies along with the increasing number of whole genome reference assemblies make it possible to precisely measure IBD_G with molecular markers in any species. Genotyping large numbers of SNPs is still expensive, but this cost will almost always pale in comparison with the expense and difficulty of obtaining deep pedigrees in free-ranging natural populations.

F_H was always very strongly correlated with IBD_G (r^2 near or above 0.9) when 10 K or more SNPs were used (Figure 2 and Supplementary Figure S3). However, as expected, F_H underestimated IBD_G (Figure 3 and Supplementary Figure S4) and the magnitude of this bias was unaffected by the number of SNPs. This bias can be explained by a discrepancy between the assumed and actual base populations used to estimate F_H (Wang, 2014). As mentioned above, F_H can take negative values, and is expected to be centered near zero when allele frequencies are estimated from the current population and mating is random (for example, Figure 4). For F_H to provide unbiased estimates of IBD_G , allele frequencies must be derived from a base population where all individuals would be unrelated if mating occurred randomly (Supplementary Figure S16). Allele frequencies usually can only be estimated using the individuals for which inbreeding measurements are desired. Mean pairwise relatedness can be high in populations with small N_e (Wang, 2014), which are often the focus of studies of inbreeding and inbreeding depression. Thus, researchers should expect F_H to substantially underestimate IBD_G in most small natural populations.

F_P consistently underestimated the differences in IBD_G among individuals (Figure 5 and Supplementary Figure S5). This is likely due to pedigrees failing to account for the variation in IBD_G due to distant ancestors, and linkage and recombination. We found that F_{ROH} and F_E had lower bias than F_P in estimated differences in IBD_G among individuals when large numbers of SNPs were used (for example, Figure 5). Downward bias in estimates of the differences in IBD_G (among individuals) might lead to overestimation of the strength of inbreeding depression (Keller, 1998) because of a greater decrease in fitness per unit increase in F_P than per unit increase in IBD_G . This suggests that the magnitude of inbreeding depression (that is, the number of lethal equivalents, Keller and Waller, 2002) might be overestimated in pedigree-based tests for inbreeding depression. However, estimates of the number of lethal equivalents might have lower bias when using F_{ROH} and F_E with large numbers of SNPs. The statistical performance of marker- versus pedigree-based estimates of the strength of inbreeding depression should be formally evaluated in future research.

How many SNPs are needed?

The number of markers necessary for precise and low-bias estimates of IBD_G in a particular study system will depend on the genome size, whether inbreeding due to distant ancestors is of interest, marker heterozygosity and proportion of missing genotypes, and the variance of IBD_G . The ability to reliably detect IBD tracts depends on marker density (SNPs/cm); thus, the necessary number of markers increases with genome map length. Additionally, higher density markers are necessary to detect short IBD tracts caused by distant ancestors or a very high recombination rate (Supplementary Figure S17). The expected correlation between heterozygosity-based measures of individual inbreeding and IBD_G increases with heterozygosity and the variance of IBD_G (Miller *et al.*, 2014). This was evident in our results where F_{ROH} and F_H often had higher precision when H_e was high. Thus, more loci may be necessary for marker-based measures of IBD_G to have higher precision than F_P in studies where H_e is low.

The discussion above suggests that there is not a single number of loci that will always achieve high precision and low bias estimates of IBD_G . Simulations with genomic and demographic parameters set to match a particular study system can be used to evaluate the number of markers necessary to precisely estimate IBD_G . However, we believe the number of SNPs typically available with SNP chips or RAD-seq ($\geq 10^4$ SNPs, Allendorf *et al.*, 2010) should in most cases provide highly

precise estimates of IBD_G when used with one or more of the marker-based measures of IBD_G included in the present study.

Effects of genetic map length on the performance of IBD_G estimators

We found that the advantages of marker-based measures of IBD_G over F_P are greatest in organisms with short genetic map lengths. The reduced precision of F_P among organisms with shorter map lengths is easily attributed to the higher $\sigma(IBD_G)$ because of fewer crossovers per meiosis on average (Franklin, 1977). Unlike F_P , F_E and F_{ROH} were often more strongly correlated with IBD_G in populations with shorter genetic maps (Figure 2 and Supplementary Figure S3). The increased precision of F_E and F_{ROH} in populations with shorter genetic maps likely occurs because the longer IBD tracts resulting from a lower recombination rate (Supplementary Figure S17) are more easily detected by the hidden Markov model and sliding window algorithms used to estimate F_{ROH} and F_E . These results demonstrate that the marker-based measures of IBD_G are sensitive to the higher variance in IBD_G in organisms with shorter genetic maps. Therefore, marker-based measures of IBD_G should be even more strongly preferred over F_P in organisms with short genetic maps.

We simulated genomes typical of mammals in terms of the physical size (3 Gb), number of chromosomes and genetic map length (800–3600 cM; Dumont and Payseur, 2008). F_P will be less precise in species with shorter genetic maps or fewer chromosomes due to higher variance in IBD_G among individuals with the same pedigree (Franklin, 1977; Supplementary Figure S1). Thus, the advantage of marker-based measures of IBD_G over F_P is expected to be greater in species with fewer chromosomes or shorter genetic maps. However, the precision of F_P will increase in species with longer genetic maps or more chromosomes, and more loci will be required to have equivalent or higher precision than F_P in such cases.

Relative value of F_{ROH} , F_E and F_H

The major advantages of F_H are that it does not require mapped genetic markers and it was highly precise in all simulated scenarios when using as few as 10 K SNPs (Figure 2 and Supplementary Figure S3). Physical genome maps are currently available for few taxa (Ellegren, 2014). Therefore, our results are encouraging for studies on organisms lacking genome maps and have no choice but to measure individual inbreeding with unmapped loci (for example, Hoffman *et al.*, 2014).

Though IBD_G can be measured precisely with unmapped genetic markers, there are advantages to using mapped markers when they are available. In addition to showing high precision and low bias when large numbers of markers are used, F_{ROH} can distinguish inbreeding due to recent versus distant ancestors by including only specific ROH length categories in statistical analyses (for example, Kirin *et al.*, 2010). Inbreeding depression could be caused mainly by inbreeding due to recent ancestors when a large fraction of deleterious recessive alleles are purged over many generations. For example, Szpeich *et al.* (2013) found that very long ROH (caused mainly by recent ancestors) was more highly enriched for deleterious recessive alleles than short ROH (caused mainly by distant ancestors) in humans. Therefore, tests for inbreeding depression may often be more powerful when F_{ROH} is estimated only with very long ROH. However, it could be important to account for inbreeding due to distant ancestors (for example, by including short ROH in F_{ROH} , or by using F_E or F_H) when purging is inefficient at removing the genetic load (for example, when inbreeding depression is caused by many deleterious recessive alleles with small effects).

F_E had higher precision and often lower bias than F_H and F_{ROH} when relatively few markers were used (for example, ≤ 10 K SNPs) (Figures 2,3 and 5). Therefore, F_E may often be the most powerful measure of IBD_G in studies employing relatively few mapped SNPs. However, the current implementation of F_E (FEstimator) almost always failed to run to completion when > 10 K SNPs were used in partially isolated populations, presumably due to high LD remaining among linked SNPs (Supplementary Figure S2). Lowering the N_e of the simulated populations from $N_e = 75$ to $N_e = 20$, which increases the magnitude of LD (Hill, 1981), resulted in FEstimator failing to run to completion in the great majority of simulation repetitions in both demographic scenarios (data not shown). This suggests that studies of populations with very small N_e (where strong LD extends across the entire genome) will often be limited to using small numbers of SNPs with FEstimator.

Assumptions and limitations

Our simulations assumed no crossover interference. However, interference can be strong in some vertebrates (Broman and Weber, 2000). We are not aware that the effects of interference on the spatial and length distributions of IBD tracts have been investigated. Therefore, it is unclear how interference affects the performance of pedigree- and marker-based measures of IBD_G .

We also assumed pedigrees and genotypes were error free. However, genotyping (Pompanon *et al.*, 2005) and pedigree errors (Pemberton, 2008) are common. Incorrect heterozygous versus homozygous calls will increase errors in marker-based statistics. Likewise, incorrect or missing parentage assignments will increase errors in F_P (Pemberton, 2008). The effects of genotyping and pedigree errors were beyond the scope of this study. However, plausible rates of genotyping or pedigree errors should be considered in empirical inbreeding studies. Further, we did not simulate inbreeding depression. It is not clear how inbreeding depression, where the most highly inbred individuals are less likely to survive and be sampled in natural populations, might affect the performance of marker- and pedigree-based measures of IBD_G . Finally, we simulated genetic markers with H_e in the approximate range of 0.2–0.3. Using loci with lower heterozygosity is expected to decrease the precision of marker-based estimates of IBD_G .

CONCLUSIONS

Our results show that marker-based measures of IBD_G are substantially more precise and often less biased than F_P . This and other recent findings (for example, Hoffman *et al.*, 2014; Keller *et al.*, 2011) are in stark contrast to previous studies that used few genetic markers and did not consider the effects of linkage and recombination on the relative performance of pedigree- versus marker-based measures of inbreeding (for example, Balloux *et al.*, 2004; Slate *et al.*, 2004; Santure *et al.*, 2010). The current explosion of DNA sequence data from non-model organisms is providing the tools necessary to precisely measure IBD_G with genetic markers in any organism. We encourage researchers to adopt marker-based measures of IBD_G as large numbers of markers and genome maps quickly become available for non-model organisms.

DATA ARCHIVING

Our simulation program is available in Dryad: <http://dx.doi.org/10.5061/dryad.f8p63>.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

MK was supported by the National Science Foundation (NSF) through the Montana Institute on Ecosystems (EPS-1101342) and the Montana Ecology of Infectious Diseases IGERT program (DGE-0504628). GL and FWA were partially supported by NSF grants DEB-0742181 and 1258203. GL was also partially supported by DEB-0723928. We greatly appreciate helpful comments from the reviewers.

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Supplementary Information accompanies this paper on Heredity website (<http://www.nature.com/hdy>)