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Genomic Variation of Inbreeding and Ancestry in the Remaining Two Isle Royale Wolves

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Abstract

Inbreeding, relatedness, and ancestry have traditionally been estimated with pedigree information, however, molecular genomic data can provide more detailed examination of these properties. For example, pedigree information provides estimation of the expected value of these measures but molecular genomic data can estimate the realized values of these measures in individuals. Here, we generate the theoretical distribution of inbreeding, relatedness, and ancestry for the individuals in the pedigree of the Isle Royale wolves, the first examination of such variation in a wild population with a known pedigree. We use the 38 autosomes of the dog genome and their estimated map lengths in our genomic analysis. Although it is known that the remaining wolves are highly inbred, closely related, and descend from only 3 ancestors, our analyses suggest that there is significant variation in the realized inbreeding and relatedness around pedigree expectations. For example, the expected inbreeding in a hypothetical offspring from the 2 remaining wolves is 0.438 but the realized 95% genomic confidence interval is from 0.311 to 0.565. For individual chromosomes, a substantial proportion of the whole chromosomes are completely identical by descent. This examination provides a background to use when analyzing molecular genomic data for individual levels of inbreeding, relatedness, and ancestry. The level of variation in these measures is a function of the time to the common ancestor(s), the number of chromosomes, and the rate of recombination. In the Isle Royale wolf population, the few generations to a common ancestor results in the high variance in genomic inbreeding.

Subject area: Conservation genetics and biodiversity; Bioinformatics and computational genetics
Key words: genome, identity by descent (IBD), pedigree, relatedness, runs of homozygosity

Until very recently, it was thought that inbreeding and inbreeding depression were best analyzed using a good pedigree (Pemberton 2008). However, with the advent of genomic markers in many species, determining the inbreeding level for different individuals using many molecular markers is providing more precision (Keller et al. 2011; Kardos et al. 2015). In fact, many genomic markers can provide the realized inbreeding levels in individuals even without a known pedigree.

Using pedigree data, the expectation of the level of individual inbreeding (F_P), relatedness between individuals (r), and ancestry (the fraction of an individual's genome deriving from a particular ancestor) can be determined. However, for specific individuals with

the same known pedigree, the realized levels of these measures are expected to show substantial variation (Franklin 1977; Stam 1980; Weir et al. 1980; Hill and Weir 2011; Kardos et al. 2015, 2016; Wang 2016). For example, the proportion of the genome that is expected to be inbred or identical by descent (IBD) from a first-cousin mating is 0.0625 but the standard deviation of this expectation in humans is substantial at 0.024 (Hill and Weir 2011). Of particular interest here is that molecular genomic measures of inbreeding, and these other measures, should reflect this expected variation among individuals having the same known pedigree.

The amount of genomic variation in these measures among individuals with the same pedigree depends upon the number of generations to the common ancestors, the number of chromosomes, and rate of recombination. The highest variation around the expected inbreeding level occurs when there are fewer generations to the common ancestors, there are fewer chromosomes, and the rate of recombination is lower (Franklin 1977; Stam 1980; Hill and Weir 2011; Kardos et al. 2015). This occurs because the extent of the genome that occurs in linked blocks declines as the number of generations to the common ancestors increases, the number of chromosomes increases, and the rate of recombination (per physical chromosome length) increases. For example, the lengths of chromosomal regions that are IBD are larger when there are fewer generations to the common ancestor. The expected length of a region in cM (centiMorgans) IBD for a common ancestor t generations in the past is $100/2t$ (Thompson 2013). However, the variance around this expectation is large (Thompson 2013; Kardos et al. 2016) as is the variance around the expectation for IBD length when there are few chromosomes or low recombination.

In the Isle Royale wolf population, there are only 2 remaining wolves (see details about this population below). Although the expectation of relatedness for the 2 remaining wolves is high and the expectation of inbreeding in a hypothetical offspring from them is high, the realized relatedness, inbreeding, and ancestry from the 3 ancestors could be much higher or lower than these expectations. To quantify this, we evaluated the distribution of relatedness, ancestry, and inbreeding for the 2 remaining wolves and/or a potential offspring from them to determine how high and low these measures are expected to be. These distributions were determined by gene-drop simulation for single genes and by simulating the whole genome using the domestic dog genome as a model. This study provides the background for understanding the variance observed in measures based on molecular data for small populations with high inbreeding and pedigrees of only a few generations.

Materials and Methods

Isle Royale Wolf Population

The Isle Royale wolf population has provided important lessons and insights about genetics and evolution in a small population. In particular, immigration of a single large male, known as M93 (M indicates male), or Old Grey Guy, in 1997 resulted in genetic rescue and a "genomic sweep" where the ancestry from this migrant individual, the proportion of all genes in the population that can be traced back to this individual, increased quickly to an expected value of 59.4% of the population in 2008 (Hedrick et al. 2014, Table 1). From 2005 on, all the ancestry in the Isle Royale population has been descended from only 3 individuals; the male immigrant M93, F99 (F indicates female) his first mate, and F67, another female population resident. In other words, what appeared to be initially positive genetic aspects of the immigration of M93 had the subsequent apparent negative effect of reducing the gene pool by eliminating ancestry from other individuals except his mate and one other female. In addition, the level of inbreeding substantially increased, mainly due to IBD from M93, and the pedigree-based estimates of relatedness between the remaining individuals in the population became very high (Hedrick et al. 2014). These high rates of inbreeding were also associated with elevated levels of bone malformation (Räikkönen et al. 2009) and demographic collapse (Hedrick et al. 2014).

In the last few years, the population numbers of Isle Royale wolves have declined dramatically and there were likely only 2 wolves remaining, a male and a female, in early 2016. Figure 1 is a photo of the 3 wolves observed in 2015, the adult female F193 to the right, the adult male M183 in the middle, and a third animal thought to be their pup, which was not seen in 2016. These 2 adults are very closely related, and in fact they are the most closely related pair of the 4 males and 4 females that were present in the

Table 1. The annual number of wolves and number of packs in the Isle Royale population since M93 immigrated in 1997, the proportion of ancestry from M93, and the mean inbreeding coefficient (F_P)

Year	Number of individuals	Number of packs	M93 ancestry	F_P
1997	24	4	0.042	0
1998	14	3	0.071	0
1999	25	3	0.180	0
2000	29	3	0.250	0
2001	19	3	0.333	0
2002	17	3	0.347	0
2003	19	3	0.473	0.057
2004	29	3	0.465	0.087
2005	30	3	0.521	0.143
2006	30	3	0.531	0.150
2007	21	3	0.558	0.189
2008	23	4	0.594	0.224
2009	24	4	0.567	0.230
2010	19	2	0.522	0.221
2011	16	2	0.453	0.183
2012	9	2	0.391	0.133
2013	8	2	0.357	0.140
2014	9	2	0.372	0.159
2015	3	1	0.344	0.292
2016	2	1	0.344	0.218

Figure 1. Photo of the 3 wolves in the Isle Royale populations observed in 2015 with the female F193 to the right, the male M183 in the middle, and a pup to the left that was not seen in 2016.

population in 2013 (Hedrick et al. 2014). Also, as we will discuss below, the expected inbreeding coefficient of an offspring from them was the highest of any pair at 0.438 (Hedrick et al. 2014). Notice that the putative pup in the photo appears to have an unusual tail and posture and is relatively small, indicators of potential inbreeding depression effects.

A pedigree of the Isle Royale wolf population for the years 1998–2016 was constructed, based on 18 microsatellite loci that were derived from samples of feces and blood of wolves (for methodological details, see Adams et al. 2011). Here, we trimmed the pedigree (Figure 2) to examine only the ancestors of the 2 wolves likely remaining in 2016, M183 and F193. Notice that these 2 individuals are both father and daughter and half siblings because they have the same mother, F160. Based on this pedigree, we carried out both single-gene and whole genome simulations (see below) to determine the distribution of inbreeding F, relatedness r, and ancestry expected in these 2 wolves and a potential offspring. Note that the single-gene relatedness distributions examined were between the 2 remaining individuals and the single-gene ancestry distributions were the same for the 2 remaining individuals. On the other hand, the genomic distributions of inbreeding and ancestry were for a potential offspring of the 2 remaining wolves.

Single-Gene Simulations

First, we carried out single-gene, gene-drop simulations (after MacCluer et al. 1986) using the pedigree in Figure 2 and assigning 2 unique alleles to each ancestor to estimate the distribution and variance of relatedness r (Hedrick and Lacy 2014) between the 2 remaining wolves, M183 and F193. For relatedness between 2 individuals, there are 4 different levels of relatedness for a given locus, 0.0, 0.5, 0.75, and 1.0 (Hedrick and Lacy 2015; see below). Because there has been past inbreeding, note that $r < 2F$ (Hedrick and Lacy 2015). Next, we carried out single-gene, gene-drop simulations to estimate the ancestry in the 2 remaining wolves from each ancestor (wolves F67, M93, and F99). For ancestry of the 2 remaining individuals, there were 5 different categories, that is, 0.0, 0.25, 0.5, 0.75, and 1.0 for each locus from each of the 3 ancestors. For both measures, we ran 10^6 independent simulations, each for a single gene, and calculated the distribution and variance over these replicates.

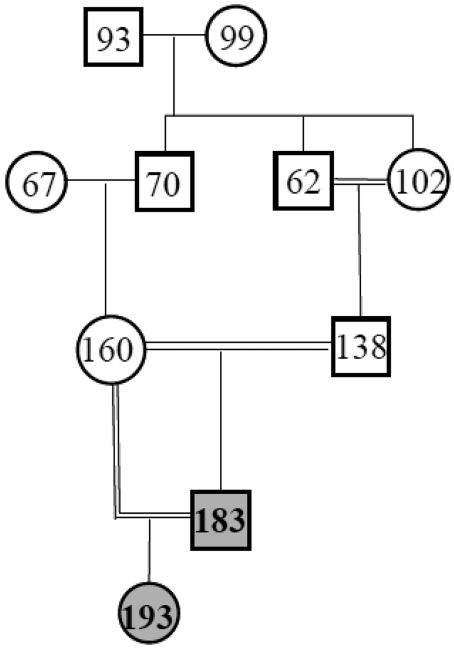

Figure 2. Pedigree showing the remaining 2 wolves, M183 and F193, shaded and their known ancestors, M93, F99, and F67, in the Isle Royale population. Double lines indicate matings between relatives, squares indicate males, and circles indicate females.

Genomic Simulations

Next, we carried out pedigree-based simulations of the wolf genome assuming Mendelian segregation and recombination to evaluate the expected distribution and variance in inbreeding F and ancestry for a hypothetical offspring of wolves M183 and F193. To do this, we modified the simulations of Kardos et al. (2015) to incorporate the karyotype and linkage map of the domestic dog (Wong et al. 2010) using the pedigree of the 2 remaining Isle Royale wolves (Figure 2). We simulated the 38 autosomes with genetic map lengths ranging from 42.4 to 82.5 cM (total for the genome = 2085.1 cM) (Wong et al. 2010). Each ancestor (wolves F67, M93, and F99) was assigned 2 unique copies of each autosome and mating was then simulated according to the Isle Royale pedigree. We assumed no crossover interference and the number of crossovers during meiosis was drawn randomly from a Poisson distribution (using the *rpois* function in R) with mean and variance equal to the length of the chromosome in cM divided by 100. We ran the simulations in R version 3.2.0 (R Core Team 2015). Our simulation script is available from M. Kardos (martykardos@gmail.com).

We ran 5000 simulation repetitions for each individual of interest (wolves M183, F193, and an offspring from them). Recombination and Mendelian segregation were simulated through the entire pedigree for each repetition. This was done to account for recombination and segregation events throughout the entire pedigree as sources of variance in the realized ancestry and F among simulation repetitions for each individual. The F of each simulated individual was calculated as the proportion of the genome (in cM units) that was IBD. IBD chromosome segments occurred where an individual carried 2 copies of a segment that originated from a single copy in an ancestor and was uninterrupted by recombination in the pedigree. We calculated the ancestry for each simulated individual as the proportion of the autosomes that arose from each particular ancestor. We also determined the IBD (inbreeding level) from each of the 3 common ancestors separately.

Results

Single-Gene Simulations

The 2 remaining wolves were known to be the most closely related pair possible in 2013 in the remaining 8 wolves at that time (Hedrick et al. 2014). Hedrick et al. (2014) calculated that, given the known pedigree, the expected relatedness r (Hedrick and Lacy 2015) for this pair was very high at 0.734, and the expected inbreeding for an offspring from this pair was also very high at 0.438. The expected proportions of ancestry from M93, F99, and F67 for this pair (and an offspring from this pair) were 0.344, 0.344, and 0.312, respectively.

From single-gene simulation, there was large variation around these expectations. First, the standard deviation for the ancestry from M93 or F99 in the 2 remaining wolves was 0.252, over 73% that of the mean 0.344. The distribution in Table 2 shows the basis for this high variation for M93 and F99, with ancestry in the 2 remaining wolves varying from no ancestry from M93 or F99 to all ancestry from M93 or F99. The mean and variation from F67 is somewhat lower, partly because it is not possible for all the ancestry

in the remaining 2 wolves to be from F67 (M138, the male parent of M183, has no ancestry from F67).

Similarly, there is large expected variation around the expected relatedness of 0.734 between the 2 remaining wolves (Table 2). The distribution shows that the 2 remaining wolves have nearly equal chances of relatedness at the levels of 0.5, 0.75, and 1.0 for individual loci. For insight into these values, Table 3 gives the 5 identity states, denoted as Δ_i, of the 2 individuals that give nonzero relatedness using the measure developed by Hedrick and Lacy (2015), an approach which includes individuals with past inbreeding.

For example, the relatedness values of 0.75 for identity states Δ_3 and Δ_5 are based on the following logic. Assume that M183 has the genotype A_1A_1 (IBD) and F193 has the genotype A_1A_2. M183 shares all of its alleles with F193, that is, M183 only has A_1 alleles, which F193 also has. On the other hand, F193 shares half of its alleles with M138, that is, A_1 is shared and A_2 is not. Weighting these 2 levels of sharing of alleles equally, then the contribution to relatedness for this combination of genotypes is 0.75.

Notice that the pair of genotypes with the highest probability is Δ_8 or A_1A_2–A_1A_3 (0.377) where the 2 individuals share one allele and neither individual is IBD at this locus. The probability for the other 2 relatedness levels (0.75 and 1.0) are equal. However, it is noteworthy that the probability of Δ_5 or A_1A_2–A_1A_1 (0.250) is much higher than the complement Δ_3 or A_1A_1–A_1A_2 (0.062) because the inbreeding (IBD) level is higher for F193 (second individual in the pair) than M183 (the first individual in the pair). The probability of the r contribution with 1.0 is highest when the 2 individuals share both alleles and are heterozygous Δ_7 or A_1A_2–A_1A_2 (0.249) than when all 4 alleles are shared Δ_1 or A_1A_1–A_1A_1 (0.062) and there is identity by descent for the same alleles in both individuals.

Genomic Simulations

Because there are only a few generations (and meioses) separating the ancestors and a hypothetical offspring of the 2 remaining wolves, very large chromosome blocks are expected to be uninterrupted by recombination within the pedigree. Thus, IBD segments are expected be extremely long (Fisher 1954, 1965; Thompson 2013; Kardos et al. 2016). One way to illustrate this is given in Figure 3 where chromosomes 1–10 are generated from simulation (columns) for 10 hypothetical offspring (rows) from M183 and F193. Here, blue indicates regions that are IBD and nonblue indicates regions non-IBD (chromosomes are alternately gray and white). For example, offspring 4 in the second row is IBD for all of chromosomes 1, 6, and 10 but is not identical by descent for any of chromosome 3, 7, and 9. Other chromosomes are identical by descent for part of a chromosome in a given individual depending upon random Mendelian segregation and where recombination has taken place.

The patterns depicted in Figure 3 can be summarized over independently generated offspring to give the observed distribution of identity by descent (F) for a given chromosome. As an example, the top panel of Figure 4 gives the observed distribution of the proportion of chromosome 1 that was IBD for 5000 simulated individuals. Notice that there are 2 peaks, about 20% of these randomly generated chromosomes have no IBD and about 12% have IBD for the whole chromosome. The other chromosomes are nearly uniformly distributed over the values between 0 and 1, based upon where recombination has taken place. The mean inbreeding is 0.438, as expected from the pedigree, but the standard deviation is quite extreme (0.355) over different independent chromosomes. If there was no recombination on this chromosome, then 0.562 and 0.438 of the chromosomes would be not IBD or IBD, respectively, the same results that occur for a single gene.

When IBD is calculated for all 38 pairs of autosomes for 5000 different hypothetical offspring, then the distribution of F is centered around the expected mean with a large variance and with the 95% confidence interval ranging from 0.311 to 0.565 (bottom panel of Figure 4). In other words, even averaging over 38 chromosomes that individually can have complete IBD, no IBD, or values in between, there is still a very high variation in genomic inbreeding over independently simulated individuals.

Table 2. (a) The probability of different levels of ancestry in the 2 remaining wolves in the Isle Royale population, M183 and F193, from their 3 ancestors, M93 or F99 and F67 and (b) The probability of different levels of relatedness between the 2 remaining wolves in the Isle Royale population, M183 and F193

	0	0.25	0.5	0.75	1	Mean (SD)
(a) Ancestry						
M93 or F99	0.316	0.244	0.252	0.128	0.060	0.344 (0.252)
F67	0.246	0.380	0.254	0.120	0	0.312 (0.120)
(b) Relatedness	0	0	0.377	0.316	0.311	0.734 (0.207)

Table 3. Example genotypes, where A_1, A_2, and A_3 indicate alleles different by descent, for the 5 identity state combinations of nonzero contributions to relatedness for the 2 remaining wolves in the Isle Royale population, M183 and F193, and the probability determined by simulation

Identity state	M183	F193	r contribution	Probability
Δ_1	A_1A_1	A_1A_1	1.0	0.062
Δ_3	A_1A_1	A_1A_2	0.75	0.062
Δ_5	A_1A_2	A_1A_1	0.75	0.250
Δ_7	A_1A_2	A_1A_2	1.0	0.249
Δ_8	A_1A_2	A_1A_3	0.5	0.377

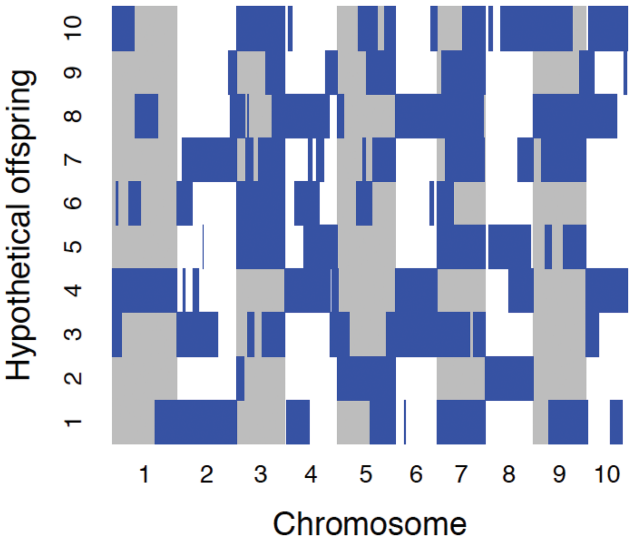

Figure 3. The extent of identity by descent (blue) for chromosomes 1–10 (horizontal axis alternating gray and white) in 10 independently simulated offspring (vertical axis) from wolves M183 and F193 in the Isle Royale population.

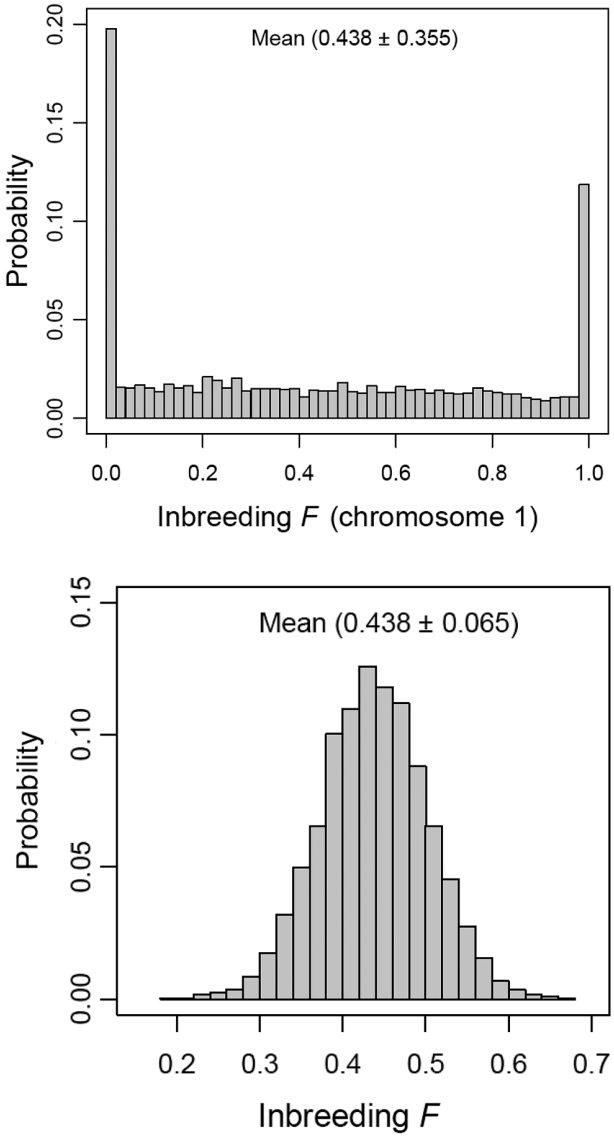

Figure 4. Estimated distribution of IBD (inbreeding) on chromosome 1 for 5000 independently simulated offspring from the 2 remaining wolves, M183 and F193, in the Isle Royale population. In the top panel is given the distribution for chromosome 1 and in the bottom panel is given the distribution for the whole genome (38 autosomes). The mean and standard deviation of the level of inbreeding (*F*) are given in parentheses.

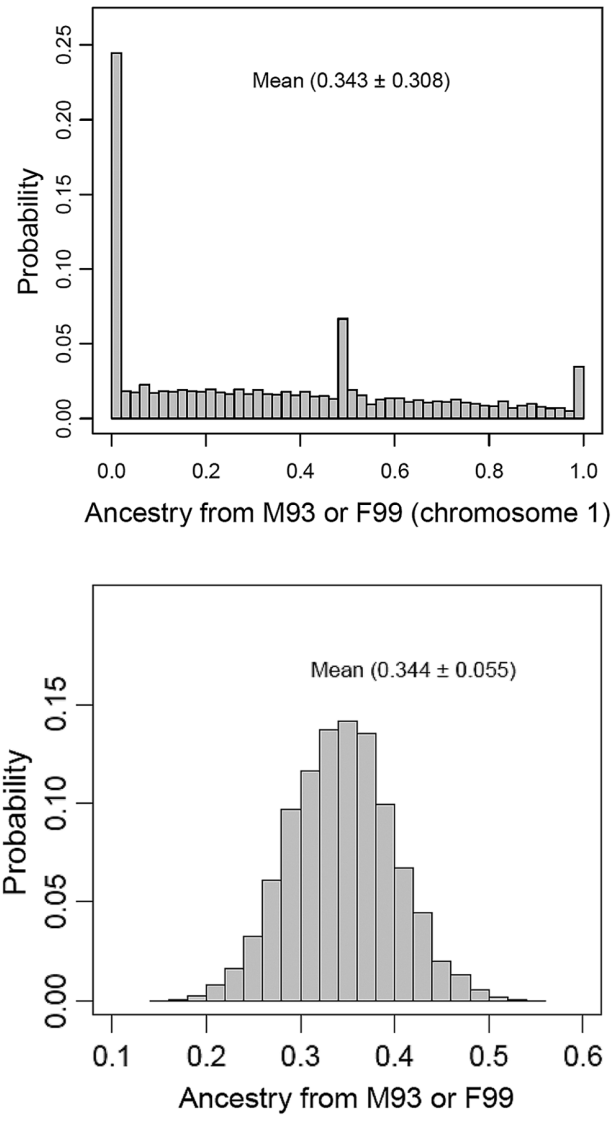

Figure 5. The distribution of genomic ancestry from M93 or F99 in 5000 independently simulated offspring from the 2 remaining wolves in the Isle Royale population. The top panel gives the distribution for chromosome 1 and the bottom panel gives the distribution for the whole genome (38 autosomes) and in parentheses are the mean and standard deviation of the level of ancestry.

In addition, the level of IBD arising from each ancestor can be calculated for independently generated offspring. For example, the distribution of IBD from M93 has a mean of 0.156 and 95% confidence interval of 0.062 to 0.250. In other words, the extent of inbreeding from the one ancestor M93 could be quite substantial.

The ancestry from the 3 ancestors in a hypothetical offspring from 2 remaining wolves can also be calculated. The top panel of Figure 5 gives the observed distribution of the proportion of chromosome 1 that was descended from M93 or F99 or (the pattern of the distribution for F67 is similar, not shown). Notice that there are 3 peaks, the highest with no ancestry but that both for 50% or 100% ancestry there are also peaks. The other chromosomes are nearly uniformly distributed over the values between 0 and 1 (but declining as ancestry increases), based upon where recombination has taken place.

The distribution of ancestry for 5000 different hypothetical offspring is given for M93 and M99 (bottom panel of Figure 5). Again the mean values are as calculated from the pedigree previously, 0.344 from M93 and F99 (and 0.312 from F67), but again there is substantial variation with the 95% confidence interval for M93 and F99 from 0.236 to 0.452 and for F67 from 0.218 to 0.406. In other words, even though the expected ancestry from the 3 ancestors has equalized in recent years (Hedrick et al. 2014), it is possible that individual animals might have very substantial ancestry from particular ancestors, and ancestry from only one ancestor for particular chromosomes.

Discussion

The concepts of inbreeding, relatedness, and ancestry have been central to the fields of evolutionary genetics and conservation biology for more than a century (Darwin 1876). Indeed, estimating individual inbreeding and relatedness between individuals are crucial to numerous lines of research in evolutionary biology. Previous research

has shown that pedigrees provide imprecise estimates of inbreeding and relatedness under different demographic scenarios (Keller et al. 2011; Kardos et al. 2015; Wang 2016). In this study, we evaluated the variance in inbreeding and relatedness around the expected values of these measures derived from a pedigree of wild wolves on Isle Royale. Our simulations demonstrate that realized inbreeding and relatedness likely deviate substantially from pedigree-based expectations of these measures in Isle Royale. Thus, our results show that pedigree-based measures of inbreeding and relatedness provide basic guideposts, but realized values can vary greatly in the Isle Royale study system. Similar high levels of variation are to be expected for other small pedigreed populations of conservation concern with high levels of inbreeding.

The remaining wolves in the Isle Royal population are closely related because they are both father and daughter and half-siblings. As a result, the expected relatedness between them is 0.734 and the expected inbreeding from an offspring from them is 0.438. Further, based on our analysis here, at 31.2% of their genes they share both copies IBD, and the 95% confidence interval for the *F* from an offspring is from 0.311 to 0.565. In other words, as high as the expectations for these measures are, it is very likely that individuals have genomic relatedness or inbreeding values that deviate substantially from the pedigree-based expectations.

Ideally one would want to examine the genomic variation in inbreeding and relatedness of all of the individuals in the Isle Royale population, past and present, and compare their genomes to the distributions of relatedness, inbreeding, and ancestry we have generated (or could generate). However, the policy at Isle Royale National Park is that handling of individuals is minimized. As a result, there are not blood or muscle samples from many of the wolves, which are needed for complete genome analysis. For example, there are not samples from either of the 2 remaining wolves M183 and F193, or the pup that was observed in 2015 but is no longer present. Also, there are not samples for ancestors either M93 or M99. However, there is a sample for ancestor F67 and there are samples from 2 offspring of M93 and M99 (F58 and M62) and 3 siblings of M183. Overall, genomic analysis of these animals and comparison to the expected distributions from our analysis could provide an important understanding of the genetics of the Isle Royale population. We are endeavoring to carry out such genomic analysis.

Genomic estimates of *F* based on analysis of runs of homozygosity identified with many thousands of SNPs are expected to be higher than predicted from our pedigree because of common ancestry of F99 and F67 in resident ancestors that are not included in our pedigree. Including the unknown resident ancestors of F99 and F67 might have result in reduced variance in *F* compared to the distributions we generated. Unfortunately, such an approach was not possible because the complete pedigree of F99 and F67 is unknown. It is not obvious how the observed variance in distribution of *F* would be influenced when there are multiple common ancestors from different time periods.

The Isle Royale wolf population has shown the impact of inbreeding with a high rate of bone malformation (58% overall, including 100% of animals born after 1994) (Räikkönen et al. 2009). As mentioned above, the putative pup of M183 and F913 seen in 2015, had a predicted inbreeding coefficient of 0.438, but as we have discussed its actual inbreeding coefficient could have been considerably higher (or lower) because of the large 95% confidence limits around this estimate. This pup had an abnormal phenotypic appearance with a quite unusual, short tightly curled tail, appeared to have an unusual posture, and was relatively small. Further, field observations suggest that this offspring was short lived and died as a pup (Peterson and Vucetich 2016). These malformations and the pup's short life suggest the negative impact of inbreeding depression on its phenotype and survival.

The mean level of *F* in the Isle Royale population reached a high in 2009 of 0.230 (Table 1) and 76.1% of this inbreeding was from immigrant M93 because he mated with his daughter F58 and had 21 offspring. Note that neither the immigrant M93 nor his daughter F58 were inbred (according to the known pedigree) but all of their offspring had F_P = 0.25. All 21 of these inbred offspring subsequently died and did not leave any descendants so the mean inbreeding F_P declined to 0.133 in 2012. In other words, the immigrant and his daughter, both noninbred, produced a very large number of inbred progeny that did not subsequently contribute and might have resulted in longer term negative effects on the population. The inbreeding level then increased in 2015 to 0.292 when only M183, F193, and their pup were in the population with F_P of 0.125, 0.312, and 0.438, respectively. When the pup died, the mean inbreeding level declined slightly to 0.219 in 2016. The decrease in inbreeding level from the high in 2009 to that in 2012 suggests that there was selection against the more inbred wolves in the population.

Detailed analysis of the presence and frequency of the ancestral chromosomes might provide some understanding of the type of selection operating in the population. For example, if given ancestral chromosomes (or regions of chromosomes) were never found as homozygotes, then this could indicate the presence of a recessive lethal (or composite lethal) on that chromosome or chromosomal region. This could result in a realized distribution of inbreeding that is lower than expected and indicate selection against inbred individuals. Missing homozygosity regions were used to suggest the presence of lethals on certain regions of the *Eucalyptus grandis* genome in selfed progeny (Hedrick et al. 2016). In this case, the expected inbreeding coefficient was 0.5 but that observed was only 0.345, indicating strong selection against inbred individuals. Similarly, comparisons of the frequency of the 6 ancestral chromosomes (or regions from them) from each of the 38 chromosomes in their descendants to their expectation might indicate selection either against or favoring particular chromosomal regions present in the ancestral individuals.

In this regard, it is possible that some recessive detrimental variants with large effects were introduced by the immigration of M93. Because he came from a presumably very large population in Canada, there might not have been past purging of detrimental variation as could possibly have occurred in the much smaller Isle Royale population. The initial progeny from M93 and his mate (F99) might have had higher fitness than other wolves because some detrimental alleles accumulated in the Isle Royale population were heterozygous in these initial offspring and the success of these offspring could have increased the frequency of detrimental variants brought in by M93. With inbreeding, these detrimental alleles were subsequently expressed as homozygotes and resulted in lowered fitness. As we have shown, the genomic level of inbreeding and ancestry from M93 had a large range and individuals with values in the high end of this range could have had low fitness. Both the ancestry and inbreeding contribution from M93 has declined in recent years (the proportion of inbreeding from M93 declined from 76.1% in 2009 to 35.6% in a hypothetical offspring from M183 and F193), suggesting that the combination of detrimental variation and inbreeding from M93 has reduced these values.

The major factor generating the high expected variation in the single-gene and genomic measures of inbreeding, ancestry, and relatedness in the Isle Royale wolves is the few generations to the

ancestors. The number of generations ranged from only 2 generations between ancestor F67 and remaining male wolf M183 to 3 or 4 between ancestors M93 and F99 and remaining female wolf F193. On the other hand, the 2 other factors that can impact the variation for genomic measures, the number of chromosomes and the rate of recombination, were either high (38 pairs of autosomes) or not unusual (2085.1 total cM in the genome). For example, if the number of chromosomes were much smaller, as essentially 2 autosomes as in *Drosophila melanogaster*, then the variance observed in the bottom panels of Figures 4 and 5 would have been much larger.

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