



Inbreeding depression causes reduced fecundity in Golden Retrievers

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Abstract

Inbreeding depression has been demonstrated to impact vital rates, productivity, and performance in human populations, wild and endangered species, and in recent years, the domestic species. In all cases, standardized, high-quality phenotype data on all individuals are invaluable for longitudinal analyses such as those required to evaluate vital rates of a study cohort. Further, many investigators agree upon the preference for and utility of genomic measures of inbreeding in lieu of pedigree-based estimates of inbreeding. We evaluated the association of measures of reproductive fitness in 93 Golden Retrievers enrolled in the Golden Retriever Lifetime Study with a genomic measurement of inbreeding, F_{ROH} . We demonstrate a statistically significant negative correlation between fecundity and F_{ROH} . This work sets the stage for larger scale analyses to investigate genomic regions associated with fecundity and other measures of fitness.

Introduction

The term “inbreeding depression” encompasses a reduction of a trait, often associated with lifetime fitness, as a sequela to a sustained rate of breeding of closely related individuals (reviewed in Charlesworth and Willis 2009; Hedrick and Garcia-Dorado 2016). While inbreeding depression has been extensively explored in plants (Lande and Schemske 1985), geographically isolated wild animal populations

(Furlan et al. 2012; Hagenblad et al. 2009), and endangered and zoo populations (Roelke et al. 1993), much research of late has addressed the same phenomenon in domestic species, many of which have been selectively bred for performance, production, and companionship. The correlation between inbreeding and impaired production in the dairy, wool, and meat industry has been well described (Ercanbrack and Knight 1991; Norén et al. 2016; Mokhtari et al. 2014; Pereira et al. 2016; Perez et al. 2017). More recently, inbreeding has been correlated with reduced performance in Australian Thoroughbred horses (Todd et al. 2018).

In the past, the estimation of inbreeding has relied on in-depth pedigrees, whereby a coefficient of inbreeding (COI), estimated from pedigree-based relationships between ancestors (F_{PED}), is used in lieu of measurement of true autozygosity (Wright 1922). Genomic measures of the COI based on runs of homozygosity (F_{ROH}) preclude the need for pedigree-based COIs, which depend heavily on pedigree depth and accuracy (Zhang et al. 2015); even with detailed pedigrees, estimated COIs can deviate substantially from true autozygosity due to recombination and segregation (Hill and Weir 2011; Keller et al. 2011). Rather, F_{ROH} is a direct measurement of the fraction of the genome actually contained in long homozygous stretches and therefore more likely to be identical by descent; making F_{ROH} a more accurate assessment of an individual dog’s inbreeding level. With the availability of high-density SNP arrays and affordable DNA sequencing, F_{ROH} has proven more effective than pedigrees (Huisman et al. 2016) or limited microsatellite panels (Hoffman

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et al. 2014) in assessing inbreeding and fitness in animal and human populations (Brüniche-Olsen et al. 2018).

As accurate as genome-wide assessments of inbreeding have proven, equally high-quality phenotype data are necessary to detect inbreeding depression. In humans and wild populations, inbreeding depression can be assessed by tracking vital rates—birth rate, mortality rate—in a population over time (Robert et al. 2005, 2009; Johnson et al. 2011). In domestic species, additional measures of inbreeding depression include litter size, reproductive success, body size, and performance traits are used (as discussed earlier). Naturally, these analyses can be clouded by external factors including environment, demographics, record completeness and accessibility, and genetic heterogeneity (Fox and Reed 2011). In that specific regard, the domestic dog, *Canis familiaris*, is an ideal candidate species in which to assess inbreeding depression. In effect, purebred dogs represent naturally occurring populations with limited genetic variation, the result of closed breed registries and strict breed standards for appearance and behavior. Further, dogs have an average gestational period of 2 months and are polytocous, providing rapid collection of fecundity data, and have an average lifespan of roughly 10% of the average human lifespan, permitting timely collection of multigenerational mortality data.

Initiatives for banking of biological samples in combination with standardized, detailed phenotype data are gaining greater traction in the canine community as a means to identify genetic, epigenetic, and environmental variants that impact canine health and longevity. One such initiative, the Morris Animal Foundation's (MAF) Golden Retriever Lifetime Study (GRLS), seeks to identify genetic and environmental variables that impact longevity in the Golden Retriever (Guy et al. 2015). Known for its sunny coat and disposition, the Golden Retriever is widely recognized as one of America's favorite dog breeds and is consistently ranked in the top-five highest breeds in AKC registrations annually (American Kennel Club 2019a). Unfortunately, Golden Retrievers are also overrepresented in neoplasia cases, with more documented mortalities due to cancer than nearly any other breed (Kent et al. 2018; Dobson 2013). And while some genetic variants have been associated with increased risk for certain cancers (Arendt et al. 2015), other major genetic contributors to Golden Retriever lifespan and fitness remain unidentified.

In 4 years, the GRLS has amassed a sample set of over 3000 Golden Retrievers, complete with annual biological samples and standardized phenotype data collection from owners and veterinarians (Simpson et al. 2017), and represents a one-of-a-kind dataset for genomic analysis. Here, we combine detailed reproductive data gathered on 93 GRLS participants with high-density SNP genotyping. We evaluate the correlation of the genomic coefficient of inbreeding, F_{ROH} , with various indicators of female reproductive

success, and we identify a negative correlation between F_{ROH} and live litter size.

Results

Study participants were drawn from the GRLS cohort of 3044 dogs. 1504 were female; 239 of these had been bred at least once. A random stratified sample of 100 dogs, termed the Embark-GRLS cohort, was selected based on number of attempted breedings to enrich for dogs who had been bred several times and had the potential of producing several litters (summarizing statistics available in Table S1). 93 dogs were successfully genotyped, ranging from 1 to 7 years of age. A total of 407 heats were recorded; heat frequency ranged from 0 to 4 heats per dog per year. Recorded heats for dogs over the age of 5 years decreased dramatically, likely reflecting the relative youth of the GRLS cohort as well as increased likelihood for elective spay in older bitches. 66 dogs had produced at least one litter, with a total of 99 litters observed. F_{ROH} ranged from 0.187 to 0.479, with mean F_{ROH} of 0.316 (Fig. 1).

Many have demonstrated a negative impact of F_{ROH} on body size (Fredrickson and Hedrick 2002; Lacy and Alaks 2013; Fareed and Afzal 2014; Cecchi et al. 2018). We regressed the median shoulder measurement for each dog against F_{ROH} and found that in this dataset, F_{ROH} was not appreciably correlated with median reported height at the shoulder (Fig. S1a, $P=0.71$).

Body size has been observed to impact both age at first estrus, ovulation frequency, and parity across dog breeds

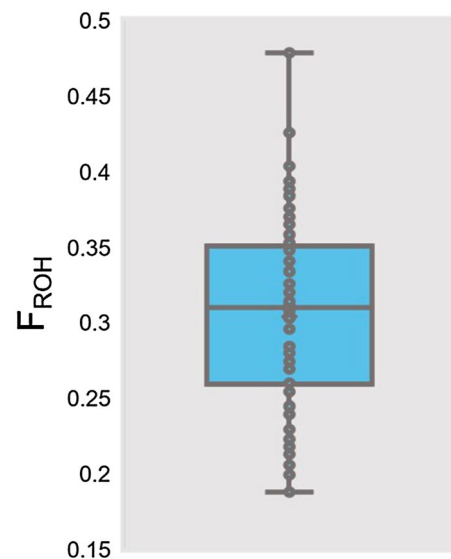


Fig. 1 Box and whisker plot of F_{ROH} for 93 genotyped dogs in the Embark-GRLS cohort. F_{ROH} ranged from 0.187 to 0.479, with mean F_{ROH} of 0.316

(Borge et al. 2011). To ascertain whether body size was impacting litter size in this cohort, we regressed litter size against median shoulder height. We found a statistically insignificant positive association between median height at the shoulder and litter size (Fig. S1b, $P=0.19$).

Finally, age at time of parturition has been shown to impact litter size (Borge et al. 2011; Mandigers et al. 1994). We regressed litter size against the dog's age at the time of litter recording and did not observe an appreciable correlation between these two factors (Fig. S1c, $P=0.65$).

The canine interestrus cycle is roughly 7 months with high variation across breeds; bitches can also vary individually in their interestrus cycle depending on age and season (Sokolowski et al. 1977; Concannon 1986; Davidson 2006). Shorter interestrus periods, ergo, more frequent estrous cycles (heats), provide greater opportunities for conception and could therefore contribute to high conception rates. We plotted recorded annual heat frequency versus F_{ROH} , separating samples by calendar age. We saw no significant correlation between estrous cycle frequency and F_{ROH} at any age. (Fig. S2); however, we did note that dogs who had more than 1 heat per year were likely to maintain this higher than average heat frequency over all years recorded.

We next measured the association of successful conception rate (SCR) versus F_{ROH} . SCR is a derived value calculated from total number of litters produced over total number of attempted breedings. Dogs who had been bred one or less times were excluded from this analysis under the assumption that a single breeding (which would result in an SCR of either 0% or 100%) may not be reflective of a dog's potential for SCR. We found that, while dogs with lower F_{ROH} had subjectively higher SCR, this result was not statistically significant (Fig. S3).

We next regressed F_{ROH} against the number of live puppies born per litter using a mixed-effects linear model,

considering F_{ROH} , median height, and age at time of litter log as fixed effect variables and dam ID as a random effect variable. We found a statistically significant negative correlation between F_{ROH} and number of live puppies (Fig. S4, $R^2=0.102$, $P=0.02$); binning dams by F_{ROH} into lower, middle, and upper thirds demonstrates appreciably lower recorded litter sizes in the uppermost or most inbred third (Fig. 2).

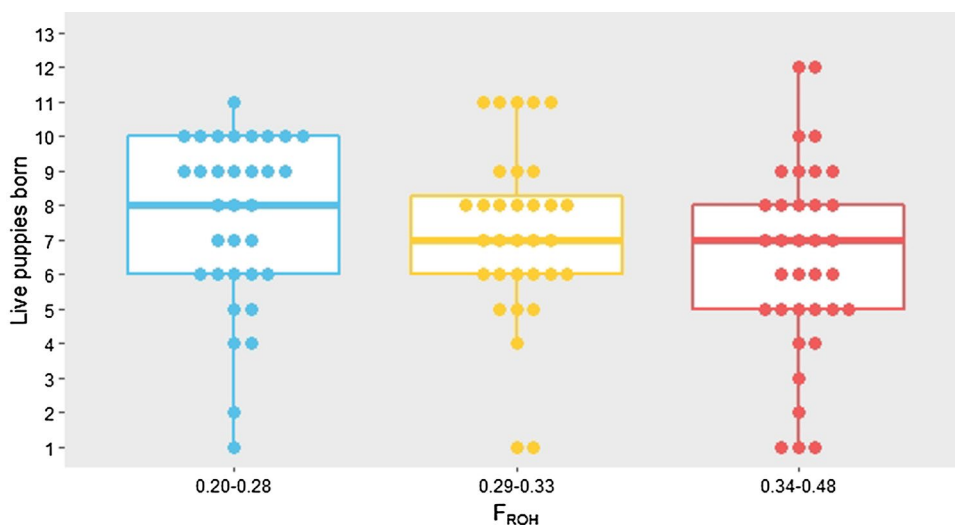
An alternative mixed-effects linear model was performed using F_{ROH} , median height, and age at time of litter log as fixed effect variables and dam ID as a random variable, defining a standardized kinship matrix generated from GEMMA as the variance family to be used for the dam ID. This model also yielded a statistically significant negative correlation between F_{ROH} and number of live puppies ($P=0.02$).

While other measures of reproductive success could include variables for parturition and post-natal care, our dataset included just five reported cases of dystocia and one case of mastitis; data on puppy survival and progress post-partum were not available in all cases. However, post-natal measurements for reproductive success are likely to be much more complex in nature, and will likely require a much larger dataset to inform them.

Discussion

We and others have already demonstrated the potential of direct-to-consumer genomics to discover novel genetic variants affecting coloration (Deane-Coe et al. 2018; Eriksson et al. 2010), behavior (Hyde et al. 2016), and disease risk (Chang et al. 2017). Our present findings also emphasize the power of multi-institutional collaboration to expedite and improve the process of data-driven discovery. The

Fig. 2 Higher F_{ROH} is associated with lower litter size. Dams are binned into lower (blue), middle (yellow), and upper thirds (red) by F_{ROH} . Each point represents the average number of puppies born in a single litter. Median litter size is similar between middle and upper third F_{ROH} bins, but the uppermost or most inbred third also has appreciably more litters with below-average litter size (Color figure online)



longitudinal, all-encompassing nature of the GRLS represents a wealth of phenotypic data. Combined with high-quality, high-density SNP genotyping, the potential for rapid identification of genetic contributions to lifespan and healthspan in the Golden Retriever is unprecedented. The work described here is clear evidence: even with a relatively small sample size of purebred Golden Retrievers, we describe a statistically significant negative correlation between F_{ROH} and litter size.

The effects of inbreeding on reproductive success can be obscured by genotypic and phenotypic variation in the sample population. By using a subset of GRLS participants, we find ourselves in the lucky position of assessing this complex relationship in a natural population with, by definition, minimal variation. We do not observe a significant correlation between litter size and maternal body weight, though this has been described by others (Borge et al. 2011). However, litter size trends have historically been documented across, but not within breeds, and it could be possible that body size variation within a breed with an already narrow range of acceptable body size could be insufficient to impact litter size. This hypothesis could be more definitively assessed in a larger sample set. Similarly, the negative effect of inbreeding on body weight has been explored in many species (reviewed in Leroy 2014). While we observe a subtle negative relationship between F_{ROH} and median shoulder height, in this cohort, this correlation was not significant, suggesting that a larger sample set could prove more informative.

Strikingly, the only variable that significantly impacts litter size in this cohort is F_{ROH} . A negative correlation between pedigree-based estimates of inbreeding and litter size has been reported (LeRoy et al. 2015). To our knowledge, our work is the first to identify a significant correlation between a genomic estimate of inbreeding, F_{ROH} , and fecundity, predicting a roughly one puppy reduction in litter size with every 10% increase in F_{ROH} .

We also identify a suggestive negative correlation between successful conception rate, a measure derived from number of attempted breeding versus number of litters born. Given the many variables upon which successful conception depends upon, for example, appropriate timing of breeding relative to estrus, semen viability, and method of breeding, it is perhaps unsurprising that in this small cohort, this correlation was statistically insignificant. As such, we intend to examine SCR and other measures of fecundity in a larger cohort of Golden Retrievers. In addition, pending availability of phenotype, we would be eager to examine the effects of inbreeding on other indices of fertility including early fetal resorption, incidence of dystocia or perinatal complications, or, from the male point of view, sperm count or motility.

Purebred animal registries are no stranger to popular sire effect. Animals with significant titles and accomplishments

are more likely to contribute to the next generation with the hopes that progeny will exhibit the same excellent performance, conformation, or work ethic of the parent. Perhaps the most dramatic example of popular sire effect exists within the Thoroughbred racehorse industry (Catton and Wezerek 2018). However, selective use of just a few highly accomplished individuals essentially pushes the population into an artificial bottleneck, leading to reduced genetic diversity in the next generation. In the purebred dog world, certain measures do exist to control popular sire effect (Federation Cynologique Internationale 2019; American Kennel Club 2019b); further, most purebred dog breeders keep meticulous records in order to monitor and control the relatedness of their breeding animals. However, pedigree analysis of large populations of dogs still demonstrates a reduction in effective breeding population over the past 50 years (Calboli et al. 2008). Though our analyses remain preliminary, it is possible that the consequences of popular sire usage and the contribution of just a select number of individuals to the next generation have come to roost for many well-known dog breeds. We believe that this work sets the stage for a much larger population analyses by which regions of the genome associated with aspects of inbreeding depression—higher mortality, reduced reproductive success—could be pinpointed and breeding recommendations could be made to increase heterozygosity in these regions. In this regard, high-density, high resolution genotyping could be invaluable for the maintenance and perpetuation of popular dog breeds.

Materials and methods

Genomic DNA and phenotype information relative to reproductive status and success was requested from 100 female intact Golden Retriever dogs enrolled in the GRLS study had been bred at least once (Table S1).

Phenotype information was compiled and provided by the MAF; information was gathered via veterinary- and owner-submitted questionnaire annually and at each veterinary visit per MAF guidelines. Participants' date of birth, physical exam findings, most recent estrous (heat) cycle and duration, date and method of last breeding and litter, litter size (puppies born, puppies weaned), and reproductive complications (dystocia, pyometra) were included.

Peripheral blood mononuclear cell (PBMC)-derived gDNA for each dog was provided by the MAF. gDNA was diluted to roughly 200 ng/ μ L; 50 μ L of each sample as submitted for genotyping using on the Embark 220K SNP array platform as previously described (Deane-Coe et al. 2018). F_{ROH} was calculated using runs of homozygosity \geq 500 kb as described in Sams and Boyko (2018). Successful conception rate (SCR) was calculated as the ratio of attempted breedings to number of litters born for each dog;

dogs with zero attempted breedings were excluded from analysis. Violin plots of SCR relative to COI quartiles and regression plots for litter size relative to COI were generated with `ggplot2` (Wickham 2016).

Litter size was calculated as the variable `livepup`, number of live puppies born, compiled from MAF records. A linear mixed model (`coi.with.barcode`) was generated with the `lmer` function (`lme4`, Bates et al. 2015) in R, considering F_{ROH} (`coi_with_public`), median withers height (`median_height`), and age in years at the time of litter recording (`age_at_visit_year`) as fixed effect variables and unique dam ID (`barcode`) as a random effect variable (as described in Cnaan et al. 1997 and implemented in Lüpold et al. 2010, Koch et al. 2018):

```
coi.with.barcode <- lmer(livepup ~ coi_with_public + age_at_visit_year + median_height + (1|barcode), data = all_data_for_kinship)
```

A second linear mixed-effects model (`coi.with.kinship`) was performed using `lme4` function in `coxme` (Therneau 2018):

```
coi.with.kinship <- lme4(livepup ~ coi_with_public + median_height + age_at_visit_year + (1|barcode), data = all_data_for_kinship, varlist = kinship.matrix.pdv)
```

Using a standardized kinship matrix (`kinship.matrix.pdv`) generated with GEMMA (version 0.97) as a random effective variable (Zhou and Stephens 2012).

For all regressions, significance of Pearson's correlation coefficient is reported as *P*.

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Author contributions ETC analyzed the data and wrote the paper. AJS analyzed data. ARB and AJS jointly directed the research and writing. ARB wrote the proposal requesting samples to the MAF. MJ provided phenotype data and genomic DNA samples from MAF participants. KD and RP take management and leadership roles in the MAF and provided comments on the paper.

Data availability Additional data and complete summary statistics from the analyses in this paper will be made available to researchers through Embark Veterinary Inc., under an agreement with Embark that protects the privacy of Embark customers and their dogs. Please contact the corresponding author for more information and to apply for access to the data.

Compliance with ethical standards

Conflict of interest ETC, ARB, and AJS are employees of Embark Veterinary, a canine DNA testing company. ARB is co-founder and part owner of Embark. Correspondence and requests for materials should be addressed to ETC (chue@embarkvet.com), ARB (adam@embarkvet.com), or AJS (asams@embarkvet.com).

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