



Original Article

Linking genetic merit to sparse behavioral data: behavior and genetic effects on lamb growth in Soay sheep

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Wild quantitative genetic studies have focused on a subset of traits (largely morphological and life history), with others, such as behaviors, receiving much less attention. This is because it is challenging to obtain sufficient data, particularly for behaviors involving interactions between individuals. Here, we explore an indirect approach for pilot investigations of the role of genetic differences in generating variation in parental care. Variation in parental genetic effects for offspring performance is expected to arise from among-parent genetic variation in parental care. Therefore, we used the animal model to predict maternal breeding values for lamb growth and used these predictions to select females for field observation, where maternal and lamb behaviors were recorded. Higher predicted maternal breeding value for lamb growth was associated with greater suckling success, but not with any other measures of suckling behavior. Though our work cannot explicitly estimate the genetic basis of the specific traits involved, it does provide a strategy for hypothesis generation and refinement that we hope could be used to justify data collection costs needed for confirmatory studies. Here, results suggest that behavioral genetic variation is involved in generating maternal genetic effects on lamb growth in Soay sheep. Though important caveats and cautions apply, our approach may extend the ability to initiate more genetic investigations of difficult-to-study behaviors and social interactions in natural populations.

Key words: behavior, breeding value, genomic prediction, parental care, quantitative genetics, Soay sheep.

INTRODUCTION

Understanding the evolutionary trajectory of a trait requires information on the strength of selection on the trait, its genetic basis, and the genetic correlations between it and other traits. By developing methodologies to estimate these parameters, quantitative genetics has enabled empirical tests of evolutionary hypotheses (Falconer and Mackay 1996; Lynch and Walsh 1998). The application of quantitative genetic methods to studies of natural populations has extended our understanding of evolutionary processes in numerous areas, including the importance of genetic correlations between traits for determining evolutionary outcomes (Brommer et al. 2007; Charmantier et al. 2006) and the role of indirect genetic effects (IGEs) in generating phenotypic variation (McAdam et al. 2002; Wilson et al. 2011).

IGEs occur when an individual's phenotype is affected by genetically determined trait(s) in a conspecific (Wolf et al. 1998). Maternal

genetic effects are one specific kind of IGE where maternal genotype affects offspring phenotype over and above the genes that offspring directly inherit. Maternal genetic effects are of considerable interest due to their potential to alter evolutionary responses to selection. For example, theoretical models have shown that depending on the covariance between maternal and offspring traits they can dampen or accelerate evolution (Kirkpatrick and Lande 1989; Wolf et al. 1998; Wolf 2003). This growing appreciation of the role of maternal genetic effects in mediating evolutionary outcomes has meant that maternal genetic effects are now widely incorporated into quantitative genetic studies of wild populations. For example, such studies demonstrate that maternal genetic effects can be substantial (e.g., Wilson et al. 2005a, 2005b; Kruuk and Hadfield 2007; Quéméré et al. 2018) and that failing to account for them can lead to erroneous estimates of heritability (Wilson et al. 2005a; Kruuk and Hadfield 2007) and evolvability (McFarlane et al. 2015).

Despite the fact that it is now commonplace to account for maternal genetic effects in quantitative genetic analyses, we often know very little about the candidate traits mediating these effects.

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Studies on livestock species have revealed significant heritabilities for traits such as milk yield and content (Torres-Vázquez et al. 2009; Aspilcueta-Borquis et al. 2010), but examinations of behavioral traits have produced contrasting results, with some studies reporting substantial heritability for maternal behavior (Hoppe et al. 2008; Brown et al. 2016), while others find little evidence for heritability in behavioral traits (Everett-Hincks et al. 2005; Løvendahl et al. 2005; Gäde et al. 2008). Therefore, the role of genetically derived differences in behavior in mediating maternal genetic effects remains relatively poorly understood.

In general, behavioral traits have received much less attention from quantitative geneticists studying wild systems than morphological and life-history traits (Postma 2014). This is despite the fact that understanding the genetic basis of behavioral traits is vital for understanding how behavioral traits evolve and how they influence the evolution of traits expressed in interacting individuals (Mousseau and Fox 1998; Wolf et al. 1998; Wilson et al. 2011; Bengtson et al. 2018). There are likely many reasons for the relative lack of quantitative genetic studies of behavior. For example, behavior is highly plastic and its repeatability can be hard to demonstrate. Additionally, understanding variation in behaviors involved in interactions between individuals are particularly difficult to investigate because they require the study of more than a single individual. For example, to understand variation in parental care, it is necessary to both quantify parental behaviors and their impact on offspring performance. Furthermore, the lack of behavioral quantitative genetic studies is likely to reflect that quantifying behavior often involves many hours of focal watches on each individual. This limits the number of individuals that can be included, thereby preventing the collection of the high volumes of data necessary to make quantitative genetic approaches tractable. For instance, robust inferences about the role of genetic differences between mothers in generating behavioral variation during maternal care would require the estimation of maternal genetic covariances between offspring performance traits, such as growth, and the measured behavioral traits using a multivariate version of a quantitative genetic approach known as the “animal model.” Such models are notoriously data hungry and are likely to be out of reach even in the most established long-term individual-based studies.

Here, we explore an approach for preliminary investigations of the role of genetic differences between individuals in generating variation in behavioral traits. We advocate this approach cautiously, not as an endpoint in itself, but as a useful way to take data that are often already available to generate hypotheses regarding the genetic basis of behaviors, reducing the set of plausible traits to explore in an exhaustive manner, and thus direct data collection more effectively. For example, although it may not be feasible to measure parental care traits in enough individuals to permit powerful quantitative genetic analysis, we often have large volumes of data on offspring traits, such as growth. These traits are known to determine offspring fitness (e.g., Gaillard et al. 1997; Dantzer et al. 2013), and crucially they are often subject to parental effects (e.g., McAdam and Boutin 2004; Wilson et al. 2007). As mentioned above, using the animal model framework, it is possible to estimate parental effects on offspring phenotypes, and this is now regularly done in studies of wild vertebrates (e.g., Wilson et al. 2005a; McFarlane et al. 2014). Importantly for current purposes, variation in parental genetic effects for offspring performance is expected to arise from among-parent genetic variation in parental care (Cheverud and Moore 1994). We suggest that predicted maternal (or paternal)

genetic merits for offspring performance generated from animal models can be used to select individuals for targeted studies of parental care behaviors to begin to understand the genetic component of behavioral variation. The analysis of predicted genetic (or individual) merits is fraught with statistical issues (Hadfield et al. 2010; Houslay and Wilson 2017) and not all problems are avoided by treating them as predictors rather than responses. Consequently, subsequent inferences about the genetic basis of parental care variation will necessarily be subject to more assumptions and caveats than arise from quantitative genetic analyses of behavioral data (discussed in full later). However, our indirect approach is potentially applicable in situations where large sample sizes are neither available nor readily obtained. Our hope is that it provides a strategy for pilot studies that can ultimately be used to justify allocation of time and funds to the measurement of behavioral traits sufficient for confirmatory studies using more robust quantitative genetic methods.

In this study, we ask whether estimated maternal breeding values for lamb growth predict behavioral variation over the maternal care period in a subset of female Soay sheep selected for targeted observations. This system is well suited as a test case for this approach due to the marked variation in lamb growth (Clutton-Brock et al. 2004), significant maternal genetic effects on early-life traits (Wilson et al. 2005a; Bérénos et al. 2014), availability of high-quality relatedness information, and ability to locate and follow uniquely identifiable individuals in the field. We first fit quantitative genetic models of lamb growth to verify the presence of maternal genetic effects and then used genomic best linear unbiased prediction (gBLUP) to predict maternal breeding values (subsequently MBV_{gBLUP}) from our models. We then used these predicted maternal breeding values to select a subset of females for behavioral phenotyping (in 2014 or 2015). While care is normally viewed as a parental trait (albeit one that is often plastically adjusted in response to offspring phenotype—Royle et al. 2014), we observed both maternal and lamb behaviors here. This is because offspring behaviors can themselves be useful proxies of parental care. For instance, suckling behavior is frequently used as a proxy for care provided by female mammals during lactation (Cameron 1998). We hypothesize that lambs predicted to grow more quickly as a result of maternal (genetic) effects will be provisioned differently by their mothers. Specifically, we predict that mothers with high MBV_{gBLUP} for lamb growth will suckle lambs more frequently or for longer and/or reject fewer suckling attempts. We also hypothesize that any variation in suckling behaviors will influence nonsuckling lamb behaviors, with lambs that suckle less and/or that have their suckling attempts rejected more frequently being predicted to show increased grazing behavior.

MATERIALS AND METHODS

Study population

The Village Bay populations of Soay sheep on the island of Hirta in the St. Kilda archipelago have been studied intensively since 1985. More than 95% of sheep are marked with plastic ear tags making them individually identifiable (Clutton-Brock et al. 2004) and regular mortality checks and censuses enable the monitoring of individual life history on the whole population. Large amounts of phenotypic data, including lamb weights, are obtained through the capture of most lambs shortly after birth and of ~60% of all individuals each August. Samples for genotyping are obtained from all

captured individuals, which enable the additive relatedness matrix among individuals to be determined from high-density single nucleotide polymorphism (SNP) data.

Animal models and female selection

Using ASReml-R (Butler et al. 2007), we built a univariate animal model to partition the variation in lamb growth (treated as a trait of the lamb) into genetic and environmental components. We calculated lamb growth as the change in weight (g/day) between birth and August measurements. We excluded individuals that were more than 5 days old when first weighed to minimize effects due to age at capture. The model contained lamb sex (two-level factor), litter size (two-level factor—singleton or twin), lamb julian birth date (covariate), and maternal age in years (linear and quadratic terms) as fixed effects. We included a series of random effects to partition the variance in lamb growth into a number of environmental and genetic components. As standard, we assumed all random effects were drawn from normal distributions with means of zero and variance to be estimated. We fitted additive genetic merit of the lamb to estimate the additive genetic variance (V_A), and the MBV_{gBLUP} of the mother to estimate the maternal genetic variance (V_{MG}). We also modeled a direct-maternal genetic covariance term (COV_{am}), which was positive but nonsignificant ($COV_{am} = 6.35$, $\chi^2_{(df=1)} = 0.46$, $P = 0.50$). To estimate genetic (co)variance terms, we used SNP-derived maternity identities and relatedness information in the form of a SNP-derived realized genomic relatedness matrix (GRM—see Bérénos et al. 2014 for more details) rather than inferring the (expected) additive relatedness matrix from an explicit pedigree structure. We fitted additional random effects of birth year (V_{yob}), maternal permanent environment (V_{ME}), and maternal identity associated with a matrix containing home range overlap information for all pairs of females. We calculated individual home ranges using lifetime spatial locations for each individual and kernel density estimation methods. We then used Bhattacharyya's affinity to quantify the home range overlap for each pair of females. Individuals have an overlap of one with themselves and zero with an individual whose home range does not overlap at all with their own. We incorporated this information into the model to account for the fact that closely related females often associate spatially as adults and thus to prevent bias in the estimates of genetic components due to spatially derived phenotypic similarity (see Regan et al. 2017).

We initially fitted our animal model in Autumn of 2014 using growth data for 1490 lambs born prior to 2013 and the realized genomic relatedness between these individuals. We found evidence for substantial maternal genetic effects, with the term accounting for 12% (SE = 4%) of the variance in lamb growth (conditional on fixed effects). Dropping the maternal genetic effect resulted in a significantly poorer model fit ($\chi^2_{(df=0,1)} = 22.10$, $P < 0.001$). MBV_{gBLUP} , interpretable as the predicted deviation of a lamb's growth from the fixed-effect mean as a result of maternal genes (over and above additive inheritance) were predicted by $gBLUP$. $gBLUP$ relies on the realized genomic relatedness between individuals rather than their expected relatedness and can therefore provide more accurate predicted breeding values than pedigree-based BLUP (Meuwissen et al. 2016). The use of genomics to predict breeding values, now widespread in animal breeding, has only recently been taken up in quantitative genetics of wild populations (e.g., great tits; Bosse et al. 2017), but holds great promise for the future (Gienapp et al. 2017).

Using these predictions, we compiled a list of 60 females (aged 3–8 years) known to be alive in summer 2014, with the intention of selecting the upper and lower thirds of the MBV_{gBLUP} distribution for behavioral observations in the summer of 2015. In practice, the eventual data structure differed somewhat from this for the following reasons. First, only females surviving over winter and giving birth in the spring were available for selection. Second, because twinning rate was low (12% of litters in 2015), we decided to limit behavioral work to mother–singleton pairs to avoid having to control for litter size effects. Third, we elected to opportunistically increase our sample size by including existing behavioral data collected in 2014 as part of another study (see Regan et al. 2017). For those females observed in 2014, MBV_{gBLUP} were thus predicted after behavioral observation, although we stress the genetic analysis conducted was “blind” with respect to behavioral variation. We observed 64 females and their lambs in either 2014 or 2015, and used data for 33 of these individuals, that were in either the upper or lower quartile of the MBV distribution and therefore represented the extremes (see Supplementary Figure S1), for analysis. These were categorized as either high MBV_{gBLUP} (females with $MBV_{gBLUP} > 2.65$ g day⁻¹) or low MBV_{gBLUP} (females with $MBV_{gBLUP} < 0.29$ g day⁻¹). Analyses using all individuals and treating MBV_{gBLUP} as a covariate produce qualitatively similar results (Supplementary Table S1).

Behavioral observations and trait definition

We conducted 534 observations, each lasting 1 h, on 29 females in 2014 and 35 in 2015, with 286 h of observation on the 33 animals featured in our analysis. Observations were made over three field-work trips per year (April–May, June–July, July–August) to monitor mother and lamb behavior across the maternal care period. Mother–lamb pairs were observed 2–13 times (mean = 8.3). We used binoculars (10x42 - Vortex, USA) and spotting scopes (16-48x - Opticon, UK) to locate individuals and subsequent observations were conducted from a distance of at least 10 m to minimize disturbance. During each observation, we used “Animal behaviour Pro” (Newton-Fisher 2012) to continuously record lamb behavior, noting whether the lamb was suckling, grazing, resting, or playing, while also recording whether the mother was grazing, resting, or moving at 2-min intervals (see Supplementary Table S2 for behavior descriptions). One hour focal observations took place between 08:00 and 19:00 and the observations of each pair were distributed across the day and between observers (two at any one time) to prevent any bias caused by the data collection procedure. Where mothers and lambs separated during an observation, we preferentially kept the lamb in view to accurately record non-suckling behaviors. If either the mother or her lamb entered a cleit (dry-stone structures used for storage by the St. Kildans), we recorded them as “Out of sight,” as in this case, we could be sure that no suckling events were missed. However, we terminated observations when both mother and lamb entered a cleit and excluded the session from further analysis.

From each focal observation, we calculated *suckling frequency* (number of suckling events, whether successful or unsuccessful), *total suckling time* (time in seconds that a lamb spent suckling), *mean suckle duration* (total suckling time divided by suckling frequency), and *suckling success* [proportion of successful suckling events (failed suckles were classified as being shorter than 5 s following Hass 1990; Birgersson and Ekvall 1994; Tollefson et al. 2011)]. Of all failed suckling events, 92% were terminated by the mother and this variable is therefore indicative of the mother rejecting the lamb rather than of lamb satiation.

When calculating these variables, we removed one suckling event that lasted 252 s as it was a clear outlier, having resulted from human disturbance. See Table 1 for details on the correlations between suckling traits. We also characterized nonsuckling behaviors of both the mother and lamb. For the mother, we calculated the frequency of sampling points in which she was grazing, resting and moving (hereafter referred to as *grazing frequency*, *resting frequency*, and *moving frequency*, respectively). For the lamb, we calculated the total time spent grazing, playing and resting during each observation (subsequently referred to as *grazing time*, *playing time*, and *resting time*, respectively).

Statistical analysis

Behavioral data were analyzed using linear and generalized linear mixed models using the packages lme4 (Bates et al. 2015) and glmmADMB (Skaug et al. 2006). We included pair identity (ID of the mother–offspring pair) and observation date as random effects to account for the likely similarity between observations conducted on the same individuals and on the same day. All models included lamb age (covariate), maternal age (covariate), and year (two-level factor) as fixed effects. We compared four models to test whether the behavior of a mother and/or her lamb was predicted by the mother's MBV_{gBLUP} for lamb growth. These models featured the linear or linear and quadratic term for lamb age with and without with the mother's MBV_{gBLUP} group (high or low). All covariates were mean centered and scaled to aid convergence. We used Akaike's Information Criterion corrected for small sample size (AICc) to assess the support for an effect of MBV_{gBLUP} group on each of the behaviors.

We used linear mixed models assuming a Gaussian error distribution for *resting time*, *grazing time* (log transformed), and *total suckling time* (log transformed). All other behaviors were analyzed using generalized linear mixed models. *Suckling frequency*, *grazing frequency*, *resting frequency*, and *moving frequency* were analyzed using negative binomial mixed models (in glmmADMB) because of overdispersion apparent in the residuals when errors were assumed to approximate a Poisson distribution. We assumed a binomial distribution in the case of *suckling success*, with the “bobyqa” optimizer used to aid convergence. Results for *playing time* and the *proportion of suckling events terminated by the female* are not presented because severe zero-inflation resulted in poor model performance. All analyses were conducted in R version 3.1.3 (R Development Core Team 2008).

RESULTS

Lamb behavior

Likelihood ratio tests of models with and without the pair identity effect (and without any MBV_{gBLUP} terms), using a mixture of zero and one degrees of freedom (Self and Liang 1987),

Table 1
Pearson correlation coefficients for suckling traits

	Suckling frequency	Total suckling time	Mean suckling duration	Proportion of successful suckles
Suckling frequency	1.00	0.65	−0.06	−0.25
Total suckling time	0.65	1.00	0.58	0.10
Mean suckling duration	−0.06	0.58	1.00	0.66
Proportion of successful suckles	−0.25	0.10	0.66	1.00

indicated that there was significant between-pair variation in all suckling behaviors (Suckling frequency— $\chi^2(df = 0,1) = 6.7$, $P = 0.003$, Suckling time— $\chi^2(df = 0,1) = 4.69$, $P = 0.01$, Mean suckling duration— $\chi^2(df = 0,1) = 5.88$, $P = 0.005$, Suckling success— $\chi^2(df = 0,1) = 6.19$, $P = 0.004$), but not in nonsuckling behaviors (Resting time— $\chi^2(df = 0,1) = 0$, $P = 1$, Grazing time— $\chi^2(df = 0,1) = 0.04$, $P = 0.59$).

As expected, lamb age was an important predictor in models for all lamb behaviors, with all measures of suckling behavior, as well as resting time, decreasing with age, while grazing time increased as lambs approached weaning (Table 2). The best fit model for suckling success (Supplementary Table S3) included a main effect of MBV_{gBLUP} group, with this term indicating that lambs born to mothers in the low MBV_{gBLUP} group were successful at suckling 13% less often than

Table 2
Parameter estimates (\pm standard error) and AICc values from the full additive models for all lamb behaviors when using predicted maternal breeding values (MBV_{gBLUP}) calculated using the genomic relatedness matrix (covering individuals born between 1985 and 2012). $N_{ind} = 33$, $N_{obs} = 286$

Trait	Term	Est (SEM)	t/z*
Suckling frequency	Intercept	0.77 (0.24)	3.19
	Lamb age	−1.95 (0.26)	−7.49
	Lamb age ²	1.02 (0.28)	3.68
	Maternal age	−0.02 (0.04)	−0.59
	Year (2015)	0.27 (0.14)	1.90
	MBV _{gBLUP} (low)	−0.01 (0.13)	−0.10
AICc = 1169.17 $w_i = 0.26$			
Suckling time	Intercept	2.54 (0.30)	8.53
	Lamb age	−2.88 (0.35)	−8.23
	Lamb age ²	1.72 (0.35)	4.91
	Maternal age	0.05 (0.05)	0.92
	Year (2015)	0.17 (0.18)	0.94
	MBV _{gBLUP} (low)	0.01 (0.16)	0.08
AICc = 948.70 $w_i = 0.26$			
Mean suckle duration	Intercept	2.21 (0.20)	10.94
	Lamb age	−1.09 (0.24)	−4.60
	Lamb age ²	0.96 (0.25)	3.86
	Maternal age	0.06 (0.03)	1.88
	Year (2015)	0.0007 (0.12)	0.006
	MBV _{gBLUP} (low)	−0.08 (0.11)	−0.76
AICc = 483.16 $w_i = 0.32$			
Suckling success	Intercept	0.52 (0.35)	1.46
	Lamb age	−1.36 (0.47)	−2.89
	Lamb age ²	1.77 (0.55)	3.22
	Maternal age	0.17 (0.06)	2.92
	Year (2015)	−0.48 (0.22)	−2.15
	MBV _{gBLUP} (low)	−0.49 (0.19)	−2.56
AICc = 541.74 $w_i = 0.84$			
Resting time	Intercept	1544.42 (230.68)	6.70
	Lamb age	−633.34 (283.64)	−2.23
	Lamb age ²	408.68 (284.18)	1.44
	Maternal age	27.36 (38.55)	0.71
	Year (2015)	−47.70 (145.84)	−0.33
	MBV _{gBLUP} (low)	−100.42 (123.13)	−0.82
AICc = 4739.54 $w_i = 0.17$			
Grazing time	Intercept	5.75 (0.47)	12.28
	Lamb age	6.78 (0.58)	11.65
	Lamb age ²	−5.01 (0.58)	−8.62
	Maternal age	−0.12 (0.08)	−1.49
	Year (2015)	−0.35 (0.29)	−1.19
	MBV _{gBLUP} (low)	0.15 (0.25)	0.61
AICc = 1203.37 $w_i = 0.30$			

*t values for linear mixed effects models and z values for generalized linear mixed effects models.

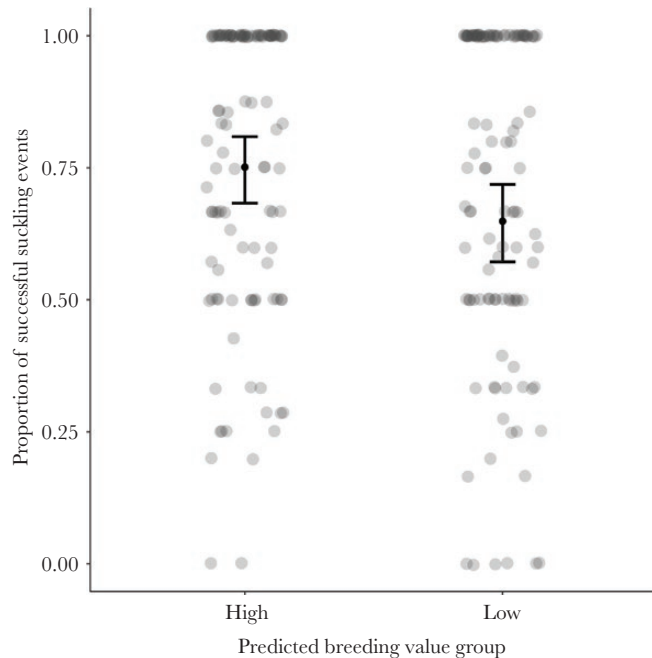


Figure 1

The proportion of successful suckling events was greater for lambs born to females whose predicted breeding value for lamb growth was high. Shown are point estimates and 95% confidence intervals from the best fit generalized linear mixed model using maternal breeding values (MBV_{gBLUP}) predicted with the genomic relatedness matrix. To aid interpretation we plot the relationship for 2015 only.

lambs born to females in the high MBV_{gBLUP} group (Table 2, Figure 1). MBV_{gBLUP} group did not feature in the best fit model for any of the other measures of lamb suckling behavior (Supplementary Tables S3 and S5). Similarly, MBV_{gBLUP} group did not feature in the best fit model for either of the nonsuckling behaviors (Table 2), but when using AICc, it was included in a competitive model for both grazing time ($\Delta AICc = 1.71$) and resting time ($\Delta AICc = 1.38$), indicating that lambs born to females in the high MBV_{gBLUP} group had a tendency to spend more time per hour resting ($MBV_{gBLUP} [low] - Est = -101.38$, $SE = 116.57$) and less time grazing ($MBV_{gBLUP} [low] - Est = 0.1564$, $SE = 0.2412$). See Supplementary Table S3 for AICc values for all lamb behavior models.

Maternal behavior

We found evidence for significant between-pair differences in maternal movement behavior ($\chi^2(1) = 9.79$, $P = 0.002$), but not in maternal grazing or resting frequency (grazing— $\chi^2(1) = 0$, $P = 1$, resting— $\chi^2(1) = 0$, $P = 1$).

As for the lamb behaviors, lamb age was important in explaining variation in the mother's behavior, particularly in the case of *grazing frequency*, which decreased over the maternal care period, and *resting frequency* which increased as lambs approached weaning (Table 3). MBV_{gBLUP} group did not predict grazing frequency, resting frequency, or moving frequency (Table 3). See Supplementary Table S4 for AICc values for all maternal behavior models.

Post hoc analyses

We found that lambs with higher average suckling success tended to have higher growth rates (Supplementary Table S9), and that there was a tendency for females in the high MBV_{gBLUP} group to be

Table 3

Parameter estimates (\pm standard error) and AICc values from the full additive models for all female behaviors when using predicted maternal breeding values (MBV_{gBLUP}) calculated using the genomic relatedness matrix (covering individuals born between 1985 and 2012). $N_{ind} = 33$, $N_{obs} = 286$

Trait	Term	Est (SEM)	t/z*
Grazing frequency	Intercept	3.10 (0.08)	37.89
	Lamb Age	-0.17 (0.10)	-1.73
	Lamb age ²	0.06 (0.10)	0.65
	Maternal age	-0.01 (0.01)	-0.60
	Year (2015)	0.006 (0.05)	0.13
†	$MBV_{gBLUP}(low)$	0.03 (0.05)	0.67
	Intercept	-0.99 (0.44)	-2.23
	Lamb Age	-0.23 (0.38)	-0.60
	Lamb age ²	0.31 (0.38)	0.81
	Maternal age	0.09 (0.07)	1.24
Resting frequency	Year (2015)	0.43 (0.26)	1.62
	$MBV_{gBLUP}(low)$	-0.26 (0.24)	-1.07
	Intercept	1.65 (0.45)	3.65
	Lamb Age	0.93 (0.60)	1.55
	Lamb age ²	-0.49 (0.59)	-0.83
AICc = 1366.20 $w_i = 0.09$	Maternal age	0.02 (0.08)	0.30
	Year (2015)	-0.27 (0.27)	-1.01
	$MBV_{gBLUP}(low)$	-0.15 (0.24)	-0.62

*t values for linear mixed effects models and z values for generalized linear mixed effects models.

†Date was removed due to convergence problems.

heavier, though this relationship was not statistically significant (β (SE) = -2.06 (1.26), $P = 0.12$).

DISCUSSION

Here, we show that MBV_{gBLUP} for lamb growth predicted variation in behavior associated with maternal care in Soay sheep. This suggests that at least some of the maternal genetic variance in lamb growth is likely to be explained by heritable behavioral traits linked to maternal provisioning. We did so using a novel approach that combines information from the quantitative genetic animal model with targeted behavioral observations that could not logistically be conducted on the whole population. Lambs born to mothers with high predicted breeding values for offspring growth had a greater proportion of successful suckling events, and a tendency to spend greater time resting and less time grazing. These results were consistent whether we used breeding values predicted using the genomic relatedness matrix (using phenotype data for lambs born between 1985 and 2012) or an updated pedigree that included individuals born between 2013 and 2016 (see Supplementary Tables S7 and S8). Maternal performance has been linked to body mass in species including sheep (Réale and Festa-Bianchet 2000) and we did find that females in the high MBV_{gBLUP} group tended to be slightly heavier, though not significantly so. This may suggest that differences in body mass were partially driving the behavioral differences we observed, but given that body mass is not necessarily an accurate measure of condition due to size differences between individuals, differences in body mass may not be entirely responsible for the results found.

We found evidence for a relationship between MBV_{gBLUP} and one behavior: suckling success. This finding could, in part, reflect that this behavioral trait is largely under maternal control, with the majority of suckling events being terminated by the mother. However, we cannot rule out differences between females in the quality or quantity of their milk, and resulting differences in

lamb satiation, as a cause of these differences in suckling success. Indeed, the use of suckling behavior as an indicator of milk intake and maternal investment during lactation has been criticized (summarized in Cameron 1998). For example, there might be individual differences in nutritional quality of a female's milk, which may influence the level of satiation of her offspring (Skibieli and Hood 2015), and there may be differences between offspring in the efficiency with which they obtain milk (Cameron 1998). We must also acknowledge that it is possible that our inability to detect a relationship between MBV_{gBLUP} and the other behaviors may be due to a lack of statistical power, particularly given that variation in any one of these behaviors is likely to come from a vast array of sources.

Relatively little work has examined the role of genetic differences in generating variation in the parental care shown by individuals living under natural conditions, with only a small number of studies demonstrating heritability in traits such as yolk and egg mass (Tschirren et al. 2009) and the provisioning rate of passerine parents (MacColl and Hatchwell 2003). Such studies are important for establishing the contribution of genetic effects to variation in parental care that is apparent in natural populations, thereby advancing our understanding of how parental care responds to selection in natural systems. They are also important given the potential environmental dependence of heritability estimates (Charmantier and Garant 2005) and the need to use empirical data on parental and offspring traits to develop the quantitative genetic models that provide the theoretical basis for studying the evolution of parental care (Hadfield 2012). Though sample sizes preclude direct estimation of genetic parameters for behaviors observed, our approach provides some indirect and preliminary evidence that genetic differences between female Soay sheep are associated with variation in behavior over the maternal care period. This in turn suggests that behavioral variation is involved in the pathway that generates maternal genetic effects on lamb growth in this population.

The number of long-term individual-based studies of natural populations has been steadily increasing since the first studies on birds began in the 1940s. Although these studies are largely restricted to mammals and birds, they now cover a range of species that vary substantially in the parental care provided, including passerines (e.g., Verhulst et al. 1997), seabirds (e.g., Grist et al. 2014), marsupials (e.g., Gélín et al. 2013), rodents (e.g., Hayes et al. 2017), and primates (e.g., Alberts et al. 2003). Many of these studies are likely to have the data necessary to calculate a suitable proxy for parental care, thereby making the approach we have used in this paper tractable in these systems. Offspring growth is likely to be one of the most important and widely available measures of offspring performance for two reasons. First, many long-term studies involve the capture of offspring shortly after birth or hatching and before weaning or fledging, thereby providing data on growth for a large number of individuals. Second, offspring growth in both birds and mammals is closely linked with the investment of parents into care given that offspring of many species are entirely reliant on resources from the parent(s) early in life (Clutton-Brock 1991). Our approach provides a promising way to begin to study the genetic basis of parental care in the wild, but it may also prove beneficial to use the approach to direct studies when appropriate data for semicaptive populations are available. This may be particularly useful for taxa other than mammals and birds where it is very difficult to establish long-term individual-based studies. In doing so, it may be possible to begin to understand the role of genetics in

generating variation in a more complete range of parental care patterns. Furthermore, our approach is not necessarily restricted to looking at the mediators of genetic sources of trait variation, given that it is entirely possible to use this method to look more broadly at traits that may be driving overall phenotypic differences between individuals (i.e., consisting of both genetic and environmental determinants).

The use of the animal model to direct field sampling on a subset of individuals could provide a starting point for understanding how genetic differences between individuals result in variation in traits other than those associated with parental care. In our case, we started by estimating the maternal genetic effect (a special case of IGEs) on lamb growth. However, by modeling different IGEs, this approach could be used whenever the interest is in how the genotype of a specific individual influences the phenotype of an interacting individual. Indeed, studies on wild populations are beginning to examine how specific traits in an interacting individual influence a focal individual's phenotype (McGlothlin and Brodie 2009), and incorporate a wider range of IGEs into the animal model, such as IGEs on social dominance (Wilson et al. 2011) and reproductive traits such as laying date (Brommer and Rattiste 2008). Thus, given an appropriate pedigree and a suitable proxy for the trait of interest, our approach provides a tractable means for understanding whether focal individual's predicted breeding value for a trait expressed in an interacting individual can be used to predict how it behaves towards the other individual. This could include understanding how the genes carried by a focal individual influences its reproductive investment or the ways in which individual genetics influences competitive behaviors, such as dominance or aggression, or social behaviors, such as cooperative breeding or antipredator behaviors.

Despite the potential utility of the animal model for directing field sampling schemes, such an approach does come with caveats that must be acknowledged. The power to estimate genetic and environmental components within the animal model relies on the ability to parametrize an appropriate model, which itself is dependent on the pedigree and phenotypic data available (Clément et al. 2001; Kruuk 2004; Kruuk and Hadfield 2007; Wilson et al. 2010). Therefore, we stress that the breeding values we used to select individuals are only predictions (generated by gBLUP), and although genomic estimated breeding values are generally more accurate than breeding values estimated using pedigree data (Clark et al. 2012; Gienapp et al. 2017), they will necessarily be associated with error and bias (Postma 2006; Hadfield et al. 2010). Statistical hypothesis testing can be substantially anticonservative when BLUP are treated as response variables and the uncertainty around them is ignored, a practice that has been shown to be problematic in both evolutionary and behavioral studies (Hadfield et al. 2010; Houslay and Wilson 2017). Here, we use BLUP as a basis for selecting individuals for targeted study, and (analytically) we treat them as predictors rather than response variables. This approach still suffers from the uncertainty around the predicted breeding values and thus violates the assumption of linear models that predictors are measured without error. The result of this failure to propagate error is unclear and is why we strongly advocate this approach not as a general alternative to quantitative genetic modeling of high volume behavioral data where it can be obtained, but very specifically as a necessarily exploratory approach where it cannot.

We reiterate that our results show relationships between predicted, as opposed to true, maternal breeding values and observed behaviors. The accuracy of predicted breeding values is

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