Genetic and environmental variation in methane emissions of sheep at pasture[1]

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ABSTRACT: A total of 2,600 methane (CH_4) and 1,847 CO_2 measurements of sheep housed for 1 h in portable accumulation chambers (PAC) were recorded at 5 sites from the Australian Sheep CRC Information Nucleus, which was set up to test leading young industry sires for an extensive range of current and novel production traits. The final validated dataset had 2,455 methane records from 2,279 animals, which were the progeny of 187 sires and 1,653 dams with 7,690 animals in the pedigree file. The protocol involved rounding up animals from pasture into a holding paddock before the first measurement on each day and then measuring in groups of up to 16 sheep over the course of the day. Methane emissions declined linearly (with different slopes for each site) with time since the sheep were drafted into the holding area. After log transformation, estimated repeatability (rpt) and heritability (h^2) of liveweight-adjusted CH_4 emissions averaged 25% and 11.7%, respectively, for a single 1-h measurement. Sire × site interactions were small and nonsignificant. Correlations between EBV for methane emissions and Sheep Genetics Australia EBV for production traits were used as approximations to genetic correlations. Apart from small positive correlations with weaning and yearling weights (r = 0.21–0.25, $P < 0.05$), there were no significant relationships between production trait and methane EBV (calculated from a model adjusting for liveweight by fitting separate slopes for each site). To improve accuracy, future protocols should use the mean of 2 (rpt = 39%, h^2 = 18.6%) or 3 (rpt = 48%, h^2 = 23.2%) PAC measurements. Repeat tests under different pasture conditions and time of year should also be considered, as well as protocols measuring animals directly off pasture instead of rounding them up in the morning. Reducing the time in the PAC from 1 h to 40 min would have a relatively small effect on overall accuracy and partly offset the additional time needed for more tests per animal. Field testing in PAC has the potential to provide accurate comparisons of animal and site methane emissions, with potentially lower cost/increased accuracy compared to alternatives such as SF_6 tracers or open path lasers. If similar results are obtained from tests with different protocols/seasonal conditions, use of PAC measurements in a multitrait selection index with production traits could potentially reduce methane emissions from Australian sheep for the same production level.

Key words: enteric methane, genetic parameters, grazing ruminants, heritability, sheep

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INTRODUCTION

Enteric methane (**CH_4**) from livestock represents a large proportion of anthropogenic greenhouse gas emissions—in Australia, 68% of agricultural (and 9.3% of all) emissions (DCCEE, 2012). With worldwide demand for meat and milk predicted to double by

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2050 (Gerber et al., 2010), it will be increasingly important to meet this anticipated increase in demand and also reduce greenhouse gas emissions.

For heritable traits, genetic improvement offers many advantages. Selection can continue for many generations and achieve large, permanent changes. Ruminant CH_4 production from pasture results in the loss of 6 to 10% of gross (8–14% of digestible) energy intake (Cottle et al., 2011). Reducing methane could help improve production efficiency as well as reduce global warming.

There have been relatively few investigations into the heritability (h^2) of CH_4 emissions, often involving a single environment, for example, 13% for h^2 of 1-h CH_4 emissions (adjusted for liveweight) of 708 genetically diverse mature ewes (Robinson et al., 2010) and 13% for 1-d respiration chamber (**RC**) measurements of methane yield (**MY**; CH_4 emissions divided by feed intake) of 1,225 sheep (Pinares-Patiño et al., 2013). Some studies suggest the presence of genotype × environment (**G×E**) interactions (Jones et al., 2011; Pinares-Patiño et al., 2011b).

Here, we report and discuss 1-h CH_4 measurements of 2,404 sheep in 5 CRC Information Nucleus (**IN**; Fogarty et al., 2007) flocks distributed across Australia, grazing pasture that varied in quality and availability. The IN sheep are representative of, and have genetic links with, the genotypes currently used in the Australian sheep industry. Our aim was to evaluate the use of portable chambers under field conditions, estimate genetic variation and h^2 for protocols involving 1 or repeated measurements, and assess G×E interactions across the 5 sites and the feasibility of using portable accumulation chamber (**PAC**) field measurements to select for reduced CH_4 emissions (adjusted for liveweight, as a proxy for production) of sheep at pasture.

MATERIALS AND METHODS

Animals and Measurements

Use of animals and the procedures performed in this study were approved by Animal Care and Ethics Committees of the University of New England (approval AEC 09/098), New South Wales Department of Primary Industries (approval ORA 10/007), Victorian Department of Primary Industries 5 (approval 2010-25) and Department of Agriculture and Food WA (approval 04-08-23).

Portable accumulation chambers (Goopy et al., 2011) were used to measure 1-h methane emissions at 5 sites from the Australian Sheep CRC IN flocks. The IN is described in detail by Fogarty et al. (2007) and van der Werf et al. (2010). The design and operation of the PAC is described by Goopy et al. (2011). Briefly, the PAC is a polycarbonate chamber 1,228 (length) × 1,237 (height) × 534 mm (width), with internal volume of 0.795 m^3 and open at the bottom. The procedure involved drafting the sheep into a race over which 14 to 16 PAC were suspended and then, from the front to the back of the race, lowering a PAC over each animal. Elasticized straps were used to apply downward pressure, sealing the lower edges of the PAC (which are covered with medium-density foam rubber tape) against 5 mm industrial rubber belting on the floor of the race. After 60 min, methane concentration (**[CH_4]** μL/L) was measured using a flame ionization detector (MX100053; ENVCO, Wellington, New Zealand) via a tube introduced through a 3-mm sampling port, which was sealed with tape when not in use. At 4 of the 5 sites, [**CO_2**] μL/L was measured using a Foxbox gas analysis system (Sable Instruments, Las Vegas, NV).

Table 1. Dates of methane testing at each site, numbers of test days, test sessions, animals tested, and total number of records

Site[1]	Test period	No. of test days	Test sessions	No. of animals	No. of records
COW	29 Nov.–3 Dec., 2010	5	30 (1.0)[2]	394	412
WA	22–24 and 29–30 Nov., 2010	5	51 (1.6)	708	753
Kir	15–18 and 21–24 Feb., 2011	8	48 (2.0)	571	619
Rut	31 Jan.–5 Feb. and 7 Feb., 2011	7	30 (4.9)	357	447
Tra	13–16 Dec., 2010	4	24 (1.0)	374	369
Total		29	183 (2.1)	2,404	2,600

[1]COW = Cowra; WA = Western Australia; Kir = Kirby; Rut = Rutherglen; Tra = Trangie.

[2]Number of test sessions (in parentheses: average number of animals per session with a repeat measurement in another test session).

A total of 5 IN sites were used: Cowra (**COW**), Kirby (**Kir**), and Trangie (**Tra**) in New South Wales; Rutherglen (**Rut**) in Victoria; and Katanning in WA. At the sites in New South Wales and Victoria, there were 6 measurement sessions per day, commencing at approximately 0810, 0940, 1110, 1240, 1410, and 1540 h. In WA, there were 11 sessions per day on Day 1, 2, 4, and 5, with tests conducted from 0600 to 1830 h, and 7 sessions on Day 3 from 0840 to 1630 h. Table 1 shows the number of measurement days and sessions at each site.

To provide statistical links between the measurement sessions and provide an indication of repeatability, all sessions had at least 1 sheep that was tested in another session. The average number of animals per session with a repeat measurement in another session varied from 1.0 in COW and Tra to 4.9 at Rut.

Before the start of the first test of the day at each site, the sheep to be measured were rounded up and kept in a holding paddock until the time of their test session. Carbon dioxide was not measured at 1 site (WA); instead, the buildup of methane over time was monitored by measuring [CH_4] at 20 and 40 min as well as the standard

60 min measurement. All sheep were weighed at the end of their measurement session. Measured 60-min [CH_4] µL/L was converted into liters by multiplying by the volume of air in the PAC (assuming the volume occupied by the sheep was 1.0 L/kg liveweight) and then converted to milligrams using available temperature and air pressure measurements. If the time in the PAC was not exactly 60 min, measurements were adjusted to the desired 1-h time period by multiplying by 60/(min in the PAC).

Data Structure and Checks

A total of 2,600 1-h methane measurements were collected (Table 1). All records were carefully scrutinized and a small number (34) deleted from animals noted to be sick, lame, or agitated (which might potentially compromise the seal of the PAC). As an additional safeguard that the PAC seal remained intact throughout the recording period, 46 records with CO_2 measurements less than 1.2% at the end of the 60-min test session were also removed. Data for WA (where CO_2 was not measured) were validated by examining the buildup of CH_4 over time, which was noted to be generally stable, resulting in a correlation, $r = 0.94$, between the 40- and 60-min measurements (Fig. 1). A total of 5 records corresponding to outliers from this generally consistent relationship were deleted.

An additional 60 records corresponding to animals with unknown sires or dams were also deleted, leaving a total of 2,455 records on 2,279 animals from 187 sires, representing an average of 12.1 offspring per sire. The pedigree file contained information on 7,690 animals, with 43 genetic groups to adjust for differences between foundation animals with unknown sires and dams. Numbers of sires by numbers of offspring and numbers of sires tested at 1, 2, 3, 4, or all 5 sites are shown in Table 2. Numbers of animals, dams, and animals by birth year are shown in Table 3.

Statistical Analysis

Exploratory models were fitted to the data from each individual site (COW, WA, Kir, Rut, and Tra) to identify the most important factors affecting measurements at each individual site. Factors considered included time of measurement (hours since 0730 h) as a linear and quadratic effect, test day and session within day, breed type (Merino, maternal × Merino, and terminal × Merino), sire breed, sire, dam breed (Merino or polled Merino), dam, animal, year of birth, and other relevant information; for example, at 1 site (COW), some sheep were shorn and others were woolly. Exploratory analyses were also performed of the relationship between CH_4 and CO_2 emissions at the 4 sites where CO_2 was measured.

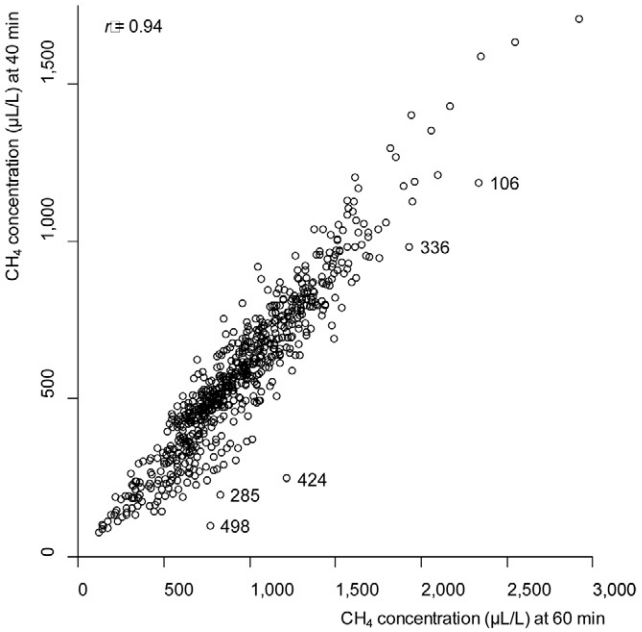

Figure 1. Relationship between CH_4 concentrations (µL/L) at 40 min and 1 h ($C_{40} = 0.61$ [±0.003] C_{60}; the intercept was not significantly different from 0, so it was omitted). Numbered values were identified as outliers and excluded from the final analysis.

All sites were then combined into a single dataset and analyses conducted of both the untransformed data (CH_4; mg/h) and after a logarithmic transformations (LTCH4 and LTCO2, defined in Eq. [1]) to overcome the skewness and tendency for increasing variances with increasing means:

$$LTCH_4 = 10 \times \ln[CH_4 \text{ (mg/h)}] \text{ and}$$

$$LTCO_2 = 10 \times \ln[CO_2 \text{ (g/h)}]. \quad [1]$$

Wald tests were used to assess the significance of terms in the fixed effects model, and likelihood ratio

Table 2. Number of sires (after data validation) by number of offspring, and numbers of sires with offspring at 1, 2, 3, 4, or all 5 Information Nucleus sites

	Number of offspring									
	1–4	5–8	9-12	13-16	17-20	21-24	25-28	29-32	35	Total
No. of sires with above no. of offspring	27	37	30	46	21	13	9	3	1	187
			Number of sites							
			1	2	3	4	5			Total
No. of sires used at the above number of sites			32	24	75	49	7			187

Table 3. Numbers of animals (after data validation) by site and birth year and numbers of dams by numbers of offspring

	Total no. of		Animals by birth year			Dams with 1, 2, or 3+ offspring		
Site[1]	Animals	Dams	2007	2008	2009	1	2	3+
COW	352	237	83	90	179	151	63	23
WA	667	533	155	267	245	425	87	21
Kir	548	431	124	168	256	337	73	21
Rut	365	227	63	115	187	132	63	32
Tra	347	225	0	158	189	129	71	25
All	2,279	1,653	425	798	1,056	1,174	357	122

[1]COW = Cowra; WA = Western Australia; Kir = Kirby; Rut = Rutherglen; Tra = Trangie.

tests used to assess the significance of random terms, including the need for different residual variances at each site. The models considered were

$$\text{emissions} = \text{environmental effects} + \text{birth\_year} + \text{breed\_type} + \text{AG} + D + \text{AP} + \text{error and} \quad [2a]$$

$$\text{emissions} = \text{environmental effects} + \text{birth\_year} + \text{breed\_type} + S + D + \text{AP} + \text{error}, \quad [2b]$$

in which the final model for environmental effects is discussed in the Results section; **AG** represents the animal genetic effect, based on all available pedigree information—parents, grandparents, great-grandparents, etc.—with Var(AG) = $A\sigma^2_a$ for additive genetic variance σ^2_a and A = the numerator relationship matrix (**NRM**); D is the effect of the animal's dam (an independent randomly distributed effect, ignoring genetic relationships) with variance σ^2_d; and **AP** is a permanent animal effect (an independent randomly distributed permanent environmental effect of the animal [ignoring genetic relationships] with variance σ^2_{ap}).

Equation [2b] is the same as Eq. [2a] but with AG replaced by S, the effect of the animal's sire (an independent randomly distributed effect, ignoring genetic relationships) with variance σ^2_s. Fitting terms with and without genetic relationships allows additional insights into the data structure and assists with model validation, for example, checking whether variances are consistent with the genetic relationships from the NRM. The significance of sire, dam, animal genetic, and other random effects can also be assessed using likelihood ratio tests.

The P-value for different residual variances at each site was highly significant ($P < 10^{-50}$). Heritability (h^2) at site i was therefore estimated as

$$\text{est-}h^2_i = \sigma^2_a/(\sigma^2_a + \sigma^2_d + \sigma^2_{ap} + \text{RV}_i) \text{ and} \quad [3a]$$

$$\text{est-}h^2_i = 4 \times \sigma^2_s/(\sigma^2_s + \sigma^2_d + \sigma^2_{ap} + \text{RV}_i), \quad [3b]$$

in which σ^2_a, σ^2_s, σ^2_d, and σ^2_{ap} are as described above and RV_i is the residual variance (**RV**) of the trait at site i (from Eq. [2a] or [2b]). Equation [3a] applies to models fitting AG effects and Eq. [3b] applies to models fitting sire effects.

These same equations estimate heritability for a trait consisting of the mean of n independent measurements on each animal. The only difference is that the residual variance for the mean of n independent observations, $\text{RV}_{i,n} = \text{RV}_i/n$, is used instead of RV_i.

Repeatability (correlation between repeat measurements on the same animal) was estimated for each site as

$$\text{Rpt}_{i,n} = \text{TA}/(\text{TA} + \text{RV}_{i,n}), \quad [4]$$

in which $\text{RV}_{i,n}$ is the residual variance for the site and the trait (which, as described above, can be a single measurement or the mean of several measurements per animal) and **TA** is the total variance of animal effects, including breed type, birth year, animal genetic, and permanent environmental effects of the dam and animal, that is, TA = Var(breed_type) + Var(birth_year) + $\sigma^2_a + \sigma^2_d + \sigma^2_{ap}$.

Genetic relationships with production traits were assessed using EBV for methane (**EBVCH$_4$**) and CO_2 emissions (**EBVCO$_2$**) of the sires, calculated from univariate analyses fitting random S, D, and AP effects as well as other terms in the final model (Eq. [5], below). Correlations were then calculated between EBVCH_4 and EBVCO_2 and Australian sheep breeding values (**ASBV**; D. J. Brown, AGBU, Armidale, Australia, personal communication) from the Merino analysis for 104 sires with accuracies for yearling weight of at least 67%.

RESULTS

Means, Variances, and Relationships with CO_2 and Liveweight

Means, minima, maxima, and variances for CH_4 and CO_2 emissions and log-transformed values are shown in Table 4. Mean liveweight at the 5 sites ranged from 46 to 71 kg, average CH_4 emissions ranged from 383 to 665 mg/h, and CO_2 emissions ranged from 26 to 38 g/h. In general, within-site variances of the log-transformed variables were more homogeneous, but there were still significant differences between the residual variances at each site ($P < 0.00001$).

There was a strong positive correlation between CH_4 and CO_2 emission rates (Fig. 2, left; $r = 0.65$) and evidence of a declining CH_4:CO_2 ratio with time of day (Fig. 2, right). The declining relationship with time T (in

Table 4. Liveweights, means, minima, maxima and observed variances of 1-h CH_4 and CO_2 emissions, with and without logarithmic transformation, for sheep at the 5 Information Nucleus sites

Site[1]	Mean live weight, kg	Untransformed CH_4 emissions, mg/h				Log-transformed CH_4 (Eq. [1])			
		Mean	Min[2]	Max[2]	Var[2]	Mean	Min	Max	Var
COW	71.4	383	14	1,292	47,969	57.9	26.5	71.6	34.1
WA	53.6	448	58	1,359	32,124	60.1	40.6	72.1	20.6
Kir	46.1	485	68	1,116	33,291	61.1	42.2	70.2	16.1
Rut	53.2	665	11	1,543	104,557	63.6	23.5	73.4	36.7
Tra	66.9	551	81	1,467	61,596	62.0	43.9	72.9	24.3
Mean	58.2	506	46	1,355	55,907	60.9	35.3	72.1	26.3
		Untransformed CO_2 emissions, g/h				Log-transformed CO_2 (Eq. [1])			
Site		Mean	Min	Max	Var	Mean	Min	Max	Var
COW		29.5	15.8	54.3	59.5	33.5	27.6	39.9	6.9
Kir		26.3	13.5	50.0	42.9	32.4	26.0	39.1	6.5
Rut		38.5	18.6	80.2	104.4	36.2	29.2	43.9	6.5
Tra		33.5	16.4	52.6	29.7	35.0	27.9	39.6	2.7
Mean		31.9	16.0	59.3	59.1	34.3	27.7	40.6	5.6

[1]COW = Cowra; WA = Western Australia; Kir = Kirby; Rut = Rutherglen; Tra = Trangie.

[2]Min = minimum; Max = maximum; Var = observed variance.

hours since 0730 h) was explored by fitting the model $CH_4 = (\beta - \tau T) \times CO_2 + \alpha$. Estimates from the model (adjusted $R^2 = 0.66$) were $\alpha = -53.4$ (±13.3), $\beta = 25.03$ (±0.44), and $\tau = 1.52$ (±0.04). Further analysis showed significantly different relationships between sites ($P < 0.001$), which produced a marginal increase in the adjusted R^2 to 0.73.

Mean CH_4 and CO_2 emissions (±SD) for each test site by time of day are shown in Fig. 3. The sheep had limited opportunity to graze after being rounded up into the holding paddock, so CH_4 emissions (which at the start of the day differed significantly between sites, $P < 0.001$) declined over the course of the day, as might be expected from the increasing amounts of time off feed (Fig. 3, left). There were small but significant ($P < 0.001$) differences in slopes for CH_4 emissions at the different sites. The reduction in CO_2 emissions over time (Fig. 3, right) was significant ($P < 0.001$) but much smaller, with different intercepts for each site but no significant differences in slopes between sites ($P = 0.25$).

Figure 4 illustrates the relationships of emissions with liveweight. There was no consistent overall relationship with liveweight across all sites ($R^2 = 0.02$). However, there were significant (but different) slopes within sites (adjusted $R^2 = 0.197$), with a marginally better fit according to the Akaike information criterion for the model with different slopes and intercepts, although other statistics (adjusted $R^2 = 0.198$ and P-value for adding separate intercepts = 0.09) demonstrate that the difference was marginal. Carbon dioxide emissions were more strongly related to liveweight-adjusted $R^2 = 0.35$ fitting different slopes, again with significantly different slopes ($P < 0.001$) for each flock but not intercepts ($P = 0.16$; Fig. 4, right).

Final Model

The final model for the genetic analysis of emissions was

$$\text{emissions} = \text{site} + \text{site.time} + \text{site.lwt} + \text{birth\_year} + \text{site.birth\_year} + \text{site.PAC} + \text{site.day} + \text{test\_session} + \text{breed\_type} + \text{animal\_effects}, \quad [5]$$

in which, as described in the Statistical Analysis section, likelihood ratios were used to evaluate the significance of animal effects such as AG, *D*, AP, and *S*. The first 3 terms in Eq. [5] were fitted as fixed, with the covariates for time of measurement (**time**) and liveweight (**lwt**) standardized to have mean 0 and, for analyses of untransfomed data, different slopes for each of the 5 sites. Different slopes were not required for liveweight in the analysis of $LTCH_4$ or for time in the analysis of $LTCO_2$. All slopes for liveweight were positive, implying that, within each site, heavier animals tended to have greater emissions. However, the site with the heaviest animals (COW) had the lowest average methane emissions (Table 4), indicating that the relationships with liveweight observed within sites do not hold across sites. This suggests that other factors (e.g., quantity and availability of feed) can have a major influence on the overall level of emissions at each site.

There was no effect on emissions of birth or rearing type (single, twin, or multiple), sex, age of dam, or contemporary group within site, so these effects were not fitted. Significant random effects included sire breed type (Merino, maternal, or terminal sire), year of birth (birth_year = 2007, 2008, or 2009), birth_year.site, day of measurement (within site), session (within day and site), and PAC (within site). Specific sire or dam breed

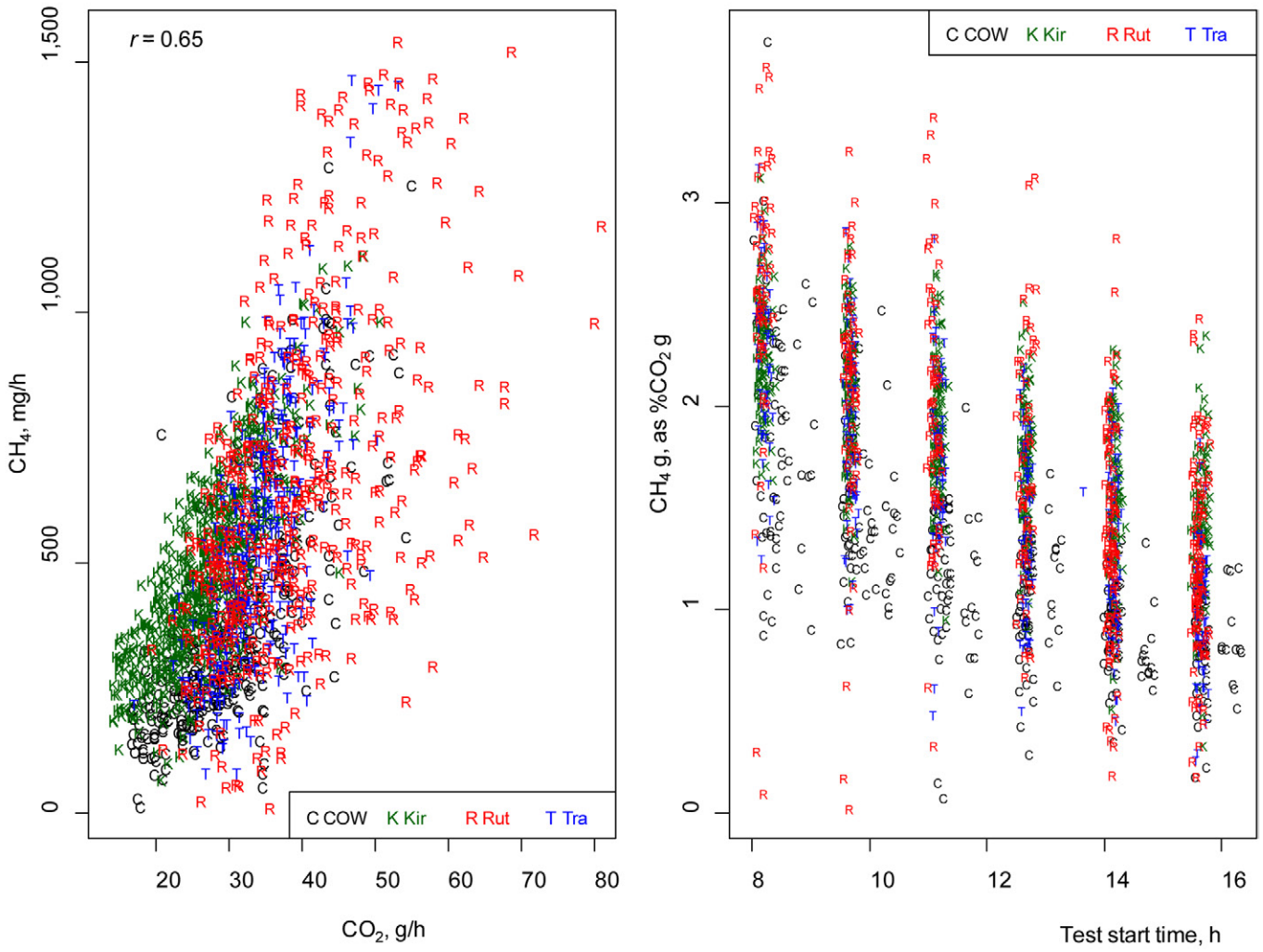

Figure 2. Relationships between unadjusted CH_4 and CO_2 emission rates (left) and CH_4 emissions (g) as percent of CO_2 emissions by time of day (h) for each flock. COW = Cowra; Kir = Kirby; Rut = Rutherglen; Tra = Trangie. See online version for figures in color.

codes within breed type explained no variation for either $LTCH_4$ or $LTCO_2$ and so were not included in the final model.

Methane

Table 5 shows *P*-values from likelihood ratio tests for adding or dropping terms for animal effects for $LTCH_4$. The most significant *P*-value for adding a single animal term to Eq. [5] was for the AG. The next most significant (which was also significant when added to Eq. [5] plus the AG) was AP.

The more complex models of Eq. [5] plus either $S + D$ + AP or AG + D + AP had almost identical log likelihoods (difference 0.15), which were not significantly more likely than Eq. [5] + AG + AP. Therefore, the observed sire and dam effects were not inconsistent with information from the genetic relationships in their pedigrees. The overall consistency of sire effects across sites was examined by adding a sire × site interaction to model $S + D$ + AP; its estimated variance was small compared to the estimated sire variation and nonsignificant, implying there was very little evidence of inconsistencies across sites.

Table 6 presents the results for the models of $LTCH_4$ as well as untransformed data. The estimates from the 3 models for $LTCH_4$ are generally similar but with a slightly higher estimated heritability (11.7%) from model AG + AP, which did not fit a permanent environmental effect of dam.

Carbon Dioxide

Table 7 shows *P*-values for adding and dropping animal terms for $LTCO_2$. Similar to the analysis of CH_4, the AG was the most significant animal term. After fitting AG, the *P*-value for the next most significant term, the AP, was 0.091, with no significant improvement in likelihood from adding either *S* or *D* individually to Eq. [5] + AG + AP. However, exploratory analyses fitting other combinations of animal terms revealed that model S + D + AP was a better fit than AG + AP ($P = 0.007$).

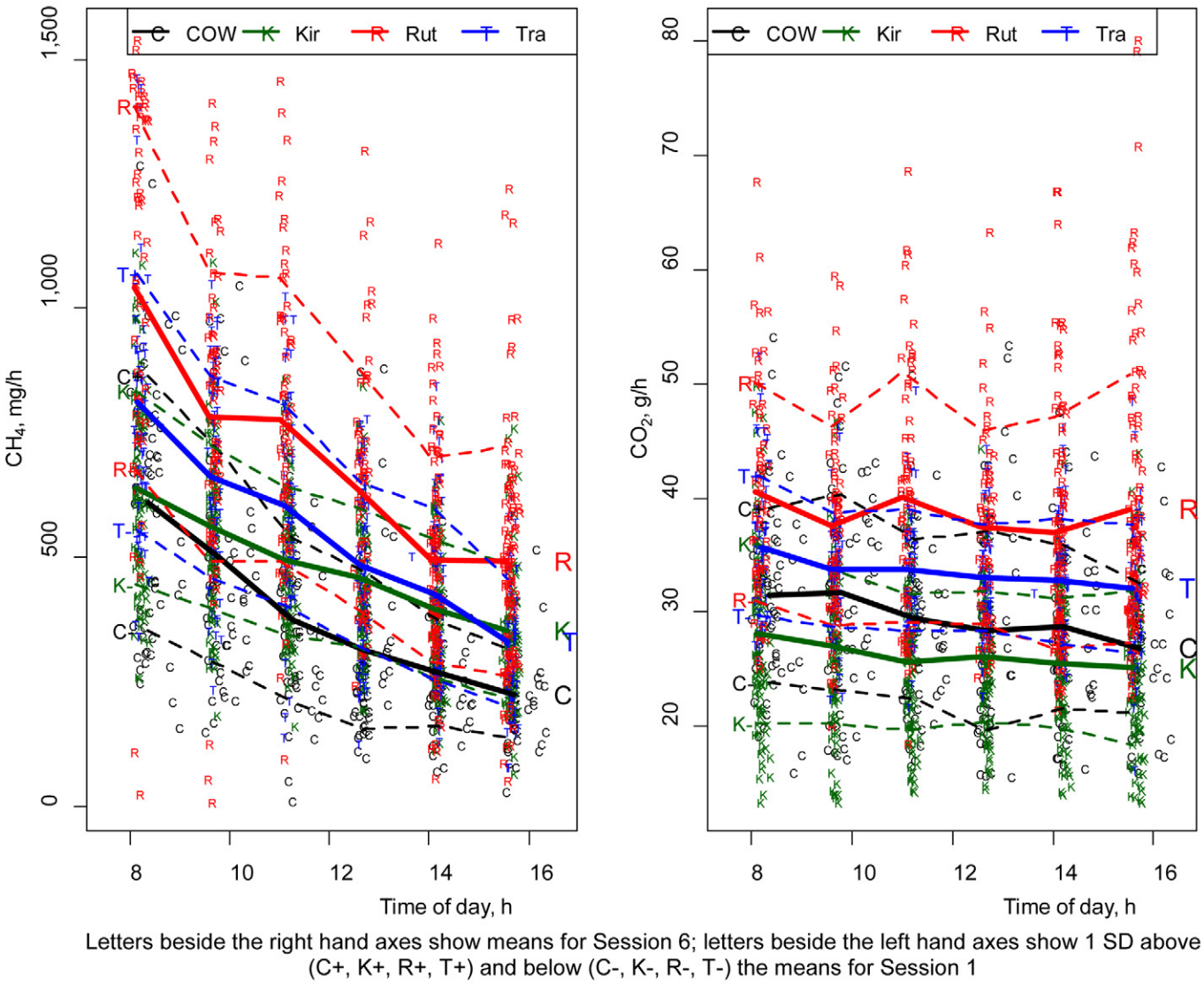

Figure 3. Methane (left) and CO_2 (right) emissions by site and time of day. Means are shown by solid lines; dotted lines indicate 1 SD above and below the means. COW = Cowra; Kir = Kirby; Rut = Rutherglen; Tra = Trangie. See online version for figures in color.

Because of the high correlations between $LTCH_4$ and $LTCO_2$, bivariate analyses of the 2 traits were performed for the 4 sites where CO_2 was measured, which had 1,747 records from 1,612 animals and 163 sires, an average of 9.9 offspring per sire (72 sires used at 2 sites and 67 at 3 or more sites). Results are shown for models $S + D$ + AP and AG + AP (Table 8). Both models had similar residual variances for each site, with broadly similar estimates of h^2 (9.7 and 11.8%) for $LTCH_4$, similar to the estimates of 10.0 and 11.7% from the univariate analysis fitting the same models to the data from all 5 sites (Table 6). As might be expected, given the difference in likelihoods, there were larger discrepancies in heritability estimates for CO_2 (13.8% for $S + D$ + AP and 20.6% for AP + AG; Table 8).

Although the data structure provides substantial information to estimate sire effects, the power to discriminate between other animal effects might be more limited. For $LTCO_2$, the significantly better fit for model $S + D$ + AP (ignoring genetic relationships) compared to AG + AP in the univariate analysis and similar result for the bivariate $S + D$ + AP (9-parameter) model compared to the bivariate AG + AP model (6 parameters; $\chi^2 = 8.2$, $P = 0.04$) is therefore a matter for speculation. Possibilities include a chance effect, a tendency for the offspring of a few unrelated sires to become agitated and have outlying CO_2 emissions, or other factors possibly relating to activity or energy use. Although, as noted earlier, the estimated variation due to sire breeds was 0, this would not necessarily rule out unusual situations such as an absence of variation for most breeds but an outlying breed with only a small number of animals. Further work will be required to shed light on this issue.

Despite the uncertainty over why $S + D$ + AP was a better fit for $LTCO_2$ (but not $LTCH_4$), the overall results of both the univariate and bivariate analyses suggest there is genetic variation in methane and CO_2 measured

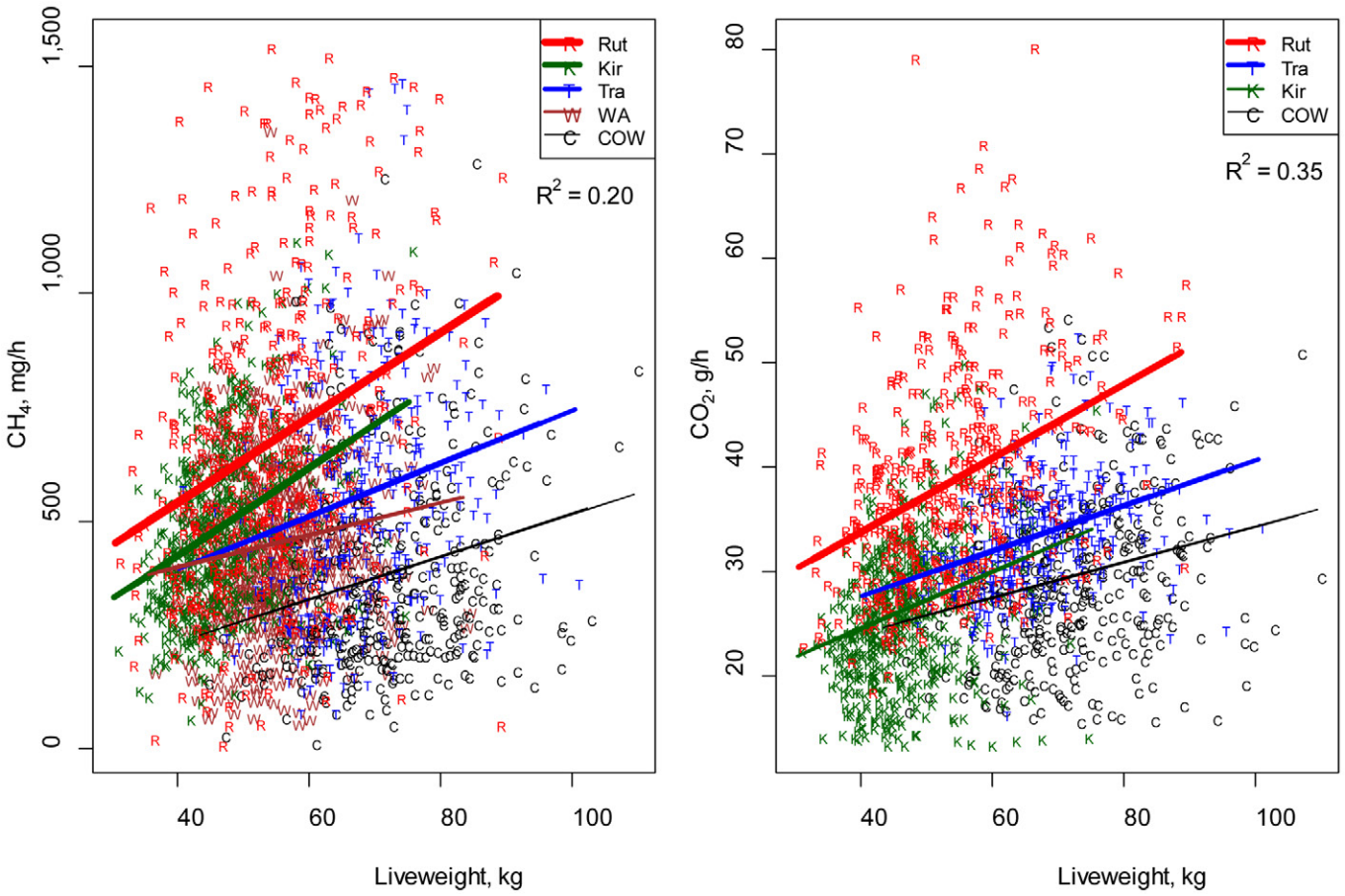

Figure 4. Relationships between liveweight and CH_4 emissions (left) and CO_2 emissions (right) for each site. The R^2 are the percentages of variation explained by the models (the lines on the graphs show the fitted values) with different intercepts and slopes for each site. Rut = Rutherglen; Kir = Kirby; Tra = Trangie; WA = Western Australia; COW = Cowra. See online version for figures in color.

using our test protocol, with quite high genetic and phenotypic correlations between CO_2 and CH_4 emissions.

Effect of Increasing the Number of Tests per Animal

Estimates of heritability and repeatability, calculated from Eq. [3] and [4], using estimates of variances from model AG + AP for individual sites and either 1, 2, or 3 methane measurements per animal are shown in Table 9, together with estimated correlations between animal means for protocols using a single measurement or the mean of 2 or 3 measurements per animal.

Table 5. Probability values for adding/dropping individual animal terms for log-transformed CH_4 ($LTCH_4$[1]).

Model terms[2]	AG	AP	D	S
Equation [5][3] +	0.0000	0.0005	0.0008	0.0007
Equation [5] + AG +		0.0436	0.0973	1.0000
Equation [5] + AG + AP +			0.3662	0.3136
Drop from full model[4]	0.0024	0.0533	0.1853	0.0028

[1]$LTCH_4$ = 10 x ln[CH_4 (mg/h)], see Eq. [1].

[2]AG = animal genetic effect (fitted using the relationship matrix calculated from the pedigree file); AP = permanent animal effect; S = effects of the animals' sires (ignoring pedigree); D = effects of the animals' dams (ignoring pedigree).

[3]Equation [5] is: emissions = site + lwt + site.time + birth_year + site.birth_year + site.PAC + site.day + test_session + breed_type + animal_effects, with animal effects = various combinations of AG, AP, S and D.

[4]Effect of dropping S, D, or AP from the S + D + AP model and dropping AG from the AG + D + AP model.

Approximate Genetic Relationships with Production Traits

Table 10 shows the correlations of sire EBV (calculated using Eq. [5] + S + D + AP) for $LTCH_4$ and $LTCO_2$ with ASBV for 104 sires with accuracies of at least 67% for yearling weight. Although Eq. [5] includes covariates to adjust for liveweight, some residual correlations of both $LTCH_4$ and $LTCO_2$ EBV with weight ASBV remained, but sire EBV for $LTCH_4$ were uncorrelated with ASBV for fleece weight, fiber diameter, fat, or muscle. For $LTCO_2$, sire EBV were also positively correlated with ASBV for fleece weight and fiber diameter.

Table 6. Estimated variances (EVar) of log-transformed data ($LTCH_4$[1]; fitting models AG + AP[2], $S + D$ + AP[2], and AG + D + AP[2]) and untransformed data (CH_4; model AG + AP[2])

	Trait[1] and model[2]							
	$LTCH_4$: AG + AP		$LTCH_4$:$S + D$ + AP		$LTCH_4$:AG + D + AP		CH_4, mg/hAG + AP	
Model term[3]	EVar	*SE*	EVar	*SE*	EVar	*SE*	EVar	*SE*
Sire/AG	1.47	0.65	0.30	0.12	1.24	0.63	2,448	1,189
Dam (D)			0.59	0.43	0.42	0.44		
Permanent animal (AP)	1.41	0.73	1.42	0.78	1.11	0.80	2,377	1,437
Breed type	0.29	0.47	0.36	0.46	0.30	0.47	4,228	5,946
Birth_year (2007, 08 or 09)	0.44	0.52	0.28	0.35	0.43	0.52	1,020	1,186
Site.birth_year	0.22	0.18	0.19	0.16	0.22	0.18	475	361
PAC (within site)	1.17	0.28	1.17	0.28	1.17	0.28	2,167	515
Test day (within site)	1.99	0.63	1.98	0.63	1.99	0.63	3,482	1,133
Test session (within site and day)	0.11	0.11	0.13	0.11	0.11	0.11	535	238
Residual-WA (Rw)	7.92	0.85	7.89	0.85	7.93	0.86	11,564	1,519
Residual-COW (Rc)	15.01	1.50	14.99	1.50	15.03	1.50	20,346	2,270
Residual-Kir (Rk)	4.93	0.71	4.88	0.71	4.95	0.72	10,462	1,466
Residual-Tra (Rt)	8.76	1.06	8.62	1.05	8.71	1.06	23,840	2,526
Residual-Rut (Rr)	25.13	1.98	25.17	1.99	25.11	1.98	56,823	4,412
Mean residual variance	12.3		12.3		12.3		24,607	
Mean estimated heritability	11.7%	4.8%	10.0%	4.8%	10.2%		10.6	

[1]Trait = $LTCH_4$ = 10 x ln[CH_4 (mg/h)], see Eq. [1], or CH_4 (mg/h).

[2]Models are: emissions = site + lwt + site.time + birth_year + site.birth_year + site.PAC + site.day + test_session + breed_type + animal_effects, with animal effects = various combinations of AG, AP, S and D.

[3]AG = animal genetic effect, AP = permanent animal effect, S = effects of the animals' sires (ignoring pedigree); D =effects of the animals' dams (ignoring pedigree), see Eq. [2a] and [2b] for detailed description. PAC = portable accumulation chamber; WA = Western Australia; COW = Cowra; Kir = Kirby, Tra = Trangie, Rut = Rutherglen. Estimated heritability (est-h^2) for each site was calculated as: est-h^2 = Var(AG)/(Var(AG) + Var(D) + Var(AP) + RV) for models AG + AP, AG + D + AP, and est-h2 = 4*Var(S)/(Var(S) + Var(D) + Var(AP) + RV) for model S + D + AP and RV = residual variance (Rw, Rc, Rk, Rt, Rr) at each site. Mean residual variance = mean (Rw, Rc, Rk, Rt, Rr); Mean estimated heritability = mean(est-h2) over all 5 sites.

DISCUSSION

The results from measuring 2,404 animals from 5 industry-representative sites provide evidence of genetic differences in methane emissions measured by our test protocol. For untransformed values of CH_4 emissions adjusted for liveweight, estimates from model AG + AP correspond to a genetic SD of about 9.8% of the mean. Breeding values of the top 50% of sires (a selection intensity, S, of 0.80) are therefore expected to average 0.8 × 9.8% = 7.8% of the mean (see, for example, Robinson, 2009). The relatively low correlations in this study between sire EBV and production traits suggest that it should be possible to select the top 50% of sires without greatly affecting the selection intensity for other traits, generating a reduction in CH_4 emissions of offspring by about 3.9% × A, in which A is the accuracy of the EBV used for selection, with potentially greater improvement if selection pressure can also be applied to dams. The costs and benefits of such a strategy merit further investigation, as do the best protocols for measuring animals.

Improving the Test Protocol

The protocol in this study was based on a single 1-h measurement, with batches of up to 16 animals tested over the course of a day, after rounding the sheep off pasture before the first test in the morning. Due to the modest repeatability of single 1-h measurements, it seems advisable for future tests to average out error variation by using at least 2 measurements per animal. The correlation between measured CH_4 at 40 min and

Table 7. Probability-values for adding/dropping individual animal terms for $LTCO_2$[1]

Model terms[2]	AG	AP	*D*	*S*
Equation [5][3] +	0.0001	0.0003	0.0005	0.0003
Equation [5] + AG +		0.0911	0.3113	1.0000
Equation [5] + AG + AP +			0.2983	0.0511
Drop from full model[4]	0.3660	0.0257	0.0665	0.0086

[1]$LTCO_2$ = 10 × ln[CO_2 (g/h)], see Eq. [1].

[2]AG = animal genetic effect (fitted using the relationship matrix calculated from the pedigree file); AP = permanent animal effect; S = effects of the animals' sires (ignoring pedigree); D = effects of the animals' dams (ignoring pedigree).

[3]Equation [5] emissions = site + time + site.lwt + birth_year + site.birth_year + site.PAC + site.day + test_session + breed_type + animal_effects, with animal effects = various combinations of AG, AP, S and D.

[4]Effect of dropping S, D or AP from the S + D + AP model and dropping AG from the AG +D + AP model. The difference in log likelihoods of S + D + AP vs. AG + AP was 3.59, implying that the former was the preferred model.

1 h (r = 0.94) was higher than the average correlation of 0.79 between protocols with 1 measurement and protocols with 2 measurements per animal, suggesting that the additional information gained from a second measurement per animal could be partially offset by some reduction in the time spent in the PAC per test.

Repeat tests should ideally be performed after an interval of at least 3 d to avoid any residual correlations with the amount of feed ingested before testing. This recommendation is based on results from RC measurements, which are influenced by feed intake in the previous 2 d as well as feed eaten in the RC (Robinson et al., 2014). If practical, longer time intervals of at least 2 wk might be worthwhile, given the much lower repeatability of 0.26 (compared to 0.89 on consecutive days) for RC measurements of daily MY (methane emissions/feed intake) after 2 wk (Pinares-Patiño et al., 2013).

Methane emissions of grazing animals are strongly related to feed intake, which is likely to vary with seasonal pasture conditions. Careful statistical modeling was therefore required to account for all relevant factors in our data, for example, the different residual variances at each site, different slopes for effects of liveweight and time off feed, and the need for log transformations.

Even though the estimated variance of the sire × site interaction was small (implying that sire effects in our data were consistent across sites with varying pasture quality and quantity and, by implication, perhaps also consistent over seasonal conditions), other researchers have found significant G×E interactions. For example, significant sire variation (equivalent to a heritability of 13%) was observed in 708 ewes grazing dryland lucerne pasture, using a protocol involving an overnight fast and then access to baled wheaten hay for 60 min commencing 2 h before CH_4 emissions were measured for 1 h in PAC (Robinson et al., 2010). However, when a subset of 207 animals (the highest and lowest 15%) were tested under more abundant pasture conditions at a different location, no consistent sire variation was evident (J. P. Goopy, unpublished data). A subset of 160 of the same ewes was also tested in RC, where they ate an average of 94.1% of the feed provided. Significant sire variation was noted in the percentage of feed that was eaten but not MY, calculated as methane emissions divided by a weighted index of feed intake in chamber and 2 previous days, using weights proportional to the effect on measured methane emissions.

Another example of G×E interactions is that when grazing high-quality but not low-quality pasture, low–residual feed intake (**RFI**) cows had lower CH_4 emissions (per kilogram liveweight of cows and their calves, if present; Jones et al., 2011). Diet significantly altered rumen microbial communities; high- and low-RFI cows had significantly different archaeal and methanogenic communities only when fed high-quality pasture (Torok et al., 2011). Although we found significant sire effects (with only a small, nonsignificant sire × site interaction), further research is desirable to determine the most appropriate pasture conditions and time of year to identify low-methane animals and whether tests should be repeated at different times of year.

Further research is also desirable to investigate the effect of diet selection and genetic differences in diurnal grazing patterns. If present, the effect of the latter could be reduced by measuring all animals at least twice at different times of day to average out any genetic differences in diurnal grazing patterns. Rounding up animals immediately before testing (perhaps in conjunction with shorter tests) could also potentially improve accuracy by reducing the impact of time off feed. In our test protocol, all animals were rounded up

Table 8. Estimated variances (EVar) and correlations (r) from the bivariate analysis of log-transformed CH_4 and CO_2 ($LTCH_4$[1] and $LTCO_{21}$) for the 4 sites where CO_2 was measured

	$LTCH_4$		$LTCO_2$			
Model term	Evar	*SE*	Evar	*SE*	*r*	*SE*
Model S + D + AP[2]						
Sire	0.30	0.14	0.09	0.04	0.64	0.19
Dam	0.25	0.45	0.19	0.11	0.73	0.43
AP	1.72	0.80	0.65	0.24	0.96	0.15
Residual-COW(Rc)	14.66	1.46	2.64	0.35	0.61	0.05
Residual-Kir (Rk)	4.90	0.71	2.51	0.28	0.72	0.05
Residual-Tra (Rt)	8.81	1.06	1.02	0.26	0.55	0.08
Residual-Rut (Rr)	25.70	2.01	1.62	0.24	0.44	0.06
Mean RV	13.52		1.95			
Mean h^2	9.7%	4.6%	13.8%	6.3%		
Model AG + AP[2]						
AG	1.56	0.80	0.62	0.26	0.70	0.17
AP	1.36	0.76	0.59	0.25	0.98	0.17
Residual-COW (Rc)	14.65	1.46	2.64	0.35	0.61	0.05
Residual-Kir (Rk)	4.97	0.72	2.52	0.28	0.72	0.05
Residual-Tra (Rt)	8.88	1.06	1.01	0.26	0.56	0.08
Residual-Rut (Rr)	25.59	2.00	1.60	0.23	0.44	0.06
Mean RV	13.52		1.94			
Mean h^2	11.8%	5.6%	20.6%	7.6%		

[1]$LTCH_4$ = 10 x ln[CH_4 (mg/h)]; $LTCO_2$ = 10 × ln[CO_2 (g/h)], see Eq. [1].

[2]Models are: emissions = site + site.lwt + site.time + birth_year + site.birth_year + site.PAC + site.day + test_session + breed_type + animal_effects, with animal effects = S + D + AP or AG + AP and AG = animal genetic effect, AP = permanent animal effect, S = effects of the animals' sires (ignoring pedigree); D = effects of the animals' dams (ignoring pedigree); see Eq. [2a] and [2b] for detailed description; r = correlation between estimated effects for $LTCH_4$ and $LTCO_2$ for each model term, e.g. for AG, r is the estimated genetic correlation; for sires, r is also an estimate of the genetic correlation derived from information on sires. For AP, r represents the correlation between permanent environmental effects of the animal; for residual terms, r represents the correlation between residual error variances for measurements on the same animal at that site. Mean RV = mean residual variance = mean (Rc, Rk, Rt, Rr); Mean h^2 = mean of estimated heritabilities for the 4 sites, where h2 at each site was calculated using Eq. [3a] and [3b].

Table 9. Estimates of repeatability (rpt) and heritability of log-transformed CH_4 ($LTCH_4$[1]), plus estimated correlations between animal means for protocols with 1, 2 or 3 measurements per animal

	One measurement			Mean of 2			Mean of 3		
Site[2]	rpt	h^2	SE (h^2)	rpt	h^2	r, 1-meas.[3]	rpt	h^2	r, 2-meas.[4]
WA	29%	13.6%	5.7%	45%	21.5%	80%	55%	26.6%	90%
COW	17%	8.2%	3.6%	30%	14.2%	77%	39%	18.7%	88%
Kir	39%	18.8%	7.6%	56%	27.5%	83%	66%	32.5%	92%
Tra	27%	12.6%	5.3%	42%	20.3%	80%	52%	25.4%	90%
Rut	11%	5.3%	2.3%	20%	9.5%	75%	27%	13.1%	86%
Mean	25%	11.7%	4.9%	39%	18.6%	79%	48%	23.2%	89%

[1]$LTCH_4 = 10 \times \ln[CH_4\ (mg/h)]$; $LTCO_2 = 10 \times \ln[CO_2\ (g/h)]$, see Eq. [1].

[2]Estimated repeatabilities and heritabilities calculated using Eq. [3] and [4] and variances for each site; WA = Western Australia; COW = Cowra; Kir = Kirby; Tra = Trangie; Rut = Rutherglen; results derived from the model fitting animal genetic and permanent animal effects and separate residual variances for each site.

[3]Correlation of the mean of 2 measurements with the first measurement.

[4]Correlation of the mean of 3 measurements with the mean of the first 2 measurements.

first thing in the morning, so the significant sire differences in CH_4 emissions could possibly relate to differences in diurnal grazing patterns, as well as increased efficiency or lower MY.

Alternative Measurement Techniques for Individual Animals

Until PAC were developed, RC measurements over 22 or 23 h were considered the most accurate way to measure methane emissions. In an experiment where the same animals were measured in the RC and immediately afterward in PAC, Bickell et al. (2011) estimated that three 1-h PAC measurements would be at least as repeatable as a 23-h RC measurement.

Animals enclosed in a chamber have restricted movement and lack the opportunity to select their diet or interact with their peers or the natural environment. O'Kelly and Spiers (1992) questioned attempts to extrapolate measurements under highly standardized, controlled conditions to the free ranging situations of most livestock farming systems. There is evidence that confinement depresses feed intake (Robinson et al., 2011), with the offspring of some sires more likely to be affected than others (Robinson et al., 2014). Despite using an accepted and published acclimatization procedure to habituate the animals to the RC, for sheep with ad libitum access to feed, Bickell et al. (2014) reported a 15 to 25% reduction in feed intake of sheep in the RC compared to their intake in the animal house the previous week. Interpretation of RC measurements therefore requires considerable care to ensure that all relevant factors relating to the artificial environment, including feed intake depression and the amount of feed eaten on previous days, have been considered as potential sources of bias.

Sulfur hexafluoride tracers (Grainger et al., 2007) are an alternative to PAC for measuring individual animals at pasture. For sheep fed at 1.2 × maintenance, SF_6 measurements had higher between- and within-animal variation than RC measurements of the same animals (Pinares-Patiño et al., 2011a). Simultaneous SF_6 and RC estimates of CH_4 were more highly correlated ($r = 0.57$) than when RC measurements took place a few days after SF_6 measurements ($r = 0.14$). This suggests the presence of animal × time interactions and that higher overall accuracy is likely to be achieved by several cheaper but less accurate measurements (such as PAC measurements of grazing animals) to average out both measurement

Table 10. Correlations (%) of Australian Sheep Breeding Values[1] with sire EBV (estimated in this study by fitting Eq. [5] + S + D + AP)[2] for log-transformed CH_4 and CO_2 ($LTCH_4$ and $LTCO_2$)[3]

Emissions	Australian Sheep Breeding Value[3]									
trait	Wwt	Ywt	Yemd	Yfc	Ygfw	Yfd	Yfdcv	Ysl	Yss	Ebwr
$LTCH_4$	21%	25%	–3%	–5%	6%	10%	3%	11%	–4%	–7%
$LTCO_2$	24%	30%	–2%	–5%	24%	25%	10%	16%	4%	–4%
Mean acc.[4]	94%	95%	88%	85%	93%	96%	94%	93%	90%	91%

[1]Australian Sheep Breeding Values (ASBV) provided by D. J. Brown, AGBU, Armidale, Australia, personal communication

[2]Model fitted = site + lwt + site.time + birth_year + site.birth_year + site.PAC + site.day + test_session + breed_type + S + D + AP, with S = effect of the animals' sires (ignoring pedigree); D = effect of the animals' dams (ignoring pedigree), AP = permanent animal effect, see Eq. [2b] for detailed description.

[3]Wwt, Ywt = weaning, yearling weight; Yemd = yearling rib eye muscle depth; Yfc = yearling fat (ultrasound scanned, C-site) Ygfw = yearling greasy fleece weight; Yfd, Yfdcv = Yearling fibre diameter and CV of fibre diameter; Ysl, Yss = yearling staple length and strength; Ebwr = early breech wrinkle.

[4]Mean acc = mean accuracy of the ASBV.

Values > 19.2% ($LTCH_4$) and 20.1% ($LTCO_2$) differ from 0 ($P < 0.05$).

errors and any animal × time of day or measurement period interactions (see Robinson, 2009).

Measuring either the CH_4:CO_2 or the amount of CH_4 eructed during feeding sessions is another possible way to estimate individual animal emissions. The technology has been evaluated in dairy cows (Madsen et al., 2010; Lassen et al., 2012) and has potential for measuring animals at pasture (Garnett, 2012; Storm et al., 2012). Such systems are capable of estimating CH_4 emissions from livestock, but deployment and replication must be carefully considered to ensure adequate numbers of measurements are obtained (Hammond et al., 2013).

Methane Intensity and Residual Feed Intake

The preferred metric for New Zealand's emissions trading scheme is methane emissions intensity (methane intensity [**MI**]; CH_4 g/kg product; Hegarty and McEwan, 2010) with the Animal Selection, Genetics and Genomics Network meeting in 2012 also recognizing that, in the short term, a MI target, rather than total animal emissions, would better fit consumer expectations (ASGGN, 2012). Methane intensity is a compound trait that could be improved either by reducing emissions per unit of feed intake (**MY**) or by reducing feed intake for the same level of production (a broadly similar trait to **RFI**) or a combination of the 2.

Selection for improved RFI has been suggested as 1 way to reduce the cost of livestock production (Arthur et al., 2012) and there is some evidence that low-RFI animals have lower methane emissions for the same liveweight or level of production (Nkrumah et al., 2006; Fitzsimons et al., 2013). However, when emissions are adjusted for feed intake, the relationship appears to change with the environment. Low-RFI steers on a concentrate diet had less energy lost as methane (Nkrumah et al., 2006), but for heifers fed 60% corn silage, 30% alfalfa hay, and 10% grains, in a model that also included DMI, methane production (g/d) increased with increasing BW gain:DMI (Freetly and Brown-Brandl, 2013). Jones et al. (2011) reported that low-RFI cows grazing high-quality pasture had lower CH_4 emissions per kilogram BW of cows and calves (if present), but there was no apparent different on low-quality pasture. For beef heifers fed grass silage, MY varied with test period (42 vs. 31 g/kg DMI; $P < 0.001$) with a tendency for MY in the second measurement period to be negatively associated with RFI ($P = 0.07$; Fitzsimons et al., 2013).

Feed efficiency has also been noted to vary with diet, feeding method, and stage of development. Even small limitations, for example, automatic feeder pens where animals may have to wait to use the feeder, can result in moderate improvements in efficiency (e.g., 7.6%; Robinson et al., 2013). The review of Basarab et al. (2013) noted that estimated genetic correlations (r_g) of RFI on different diets ranged from 0.55 to 0.67, with a slightly lower estimate ($r_g = 0.50$) for the same group of steers measured in growing vs. finishing periods (Durunna et al., 2011). Similar correlations are likely for grazing vs. lot-fed animals.

This suggests that a low-cost test, repeated at different stages of life, could be more effective than a single, more expensive test. In addition, if, as suggested above, liveweight (**LW**)-adjusted methane emissions of mature grazing ruminants (or LW- and growth-adjusted CH_4 emissions for growing animals) are genetically correlated with RFI under the same grazing conditions, a secondary benefit of using PAC measurements to select for improvements in this trait might be a correlated improvement in the difficult-to-measure trait of RFI in grazing animals.

Methane Intensity and Methane Yield

The trait of methane adjusted for liveweight (**CH_4adjLW**), analyzed in this study, is a measure of MI (with liveweight representing the "product") that avoids problems relating to the analysis of ratios (Allison et al., 1995). Our estimated heritability (18.6% for the mean of 2 measurements) and genetic SD (9.8% of the mean) compare favorably to the heritability of 13% and genetic SD (3.7% the mean) for 1-d RC measurements of MY in New Zealand (Pinares-Patiño et al., 2013). Our mean repeatability of 25% (measurements on nonconsecutive days) was similar to other estimates of the repeatability of PAC measurements of CH_4adjLW in the same week (0.32 [Robinson et al., 2010] and 0.33–0.43 [J. P. Goopy, unpublished data), as well as PAC measurements of CH_4 adjusted for feed intake and LW after a 4-wk interval (0.24; Bickell et al., 2011) and RC measurements of MY measurements after 2 wk (0.26; Pinares-Patiño et al., 2013), but less than the repeatability of daily methane production in the RC (adjusted for feed intake and LW) after 4 wk (0.49; Bickell et al., 2011).

Interactions of MY with diet appear to be complex and not simply related to the quality or energy density of the diet. When high- and low-MY sheep from the New Zealand study were retested, the initial 7.9% difference observed when the sheep were fed a restricted diet of molassed perennial ryegrass silage increased to 13% when the sheep were fed grass in the RC and 36% when fed pellets (Pinares-Patiño et al., 2011b). An Australian study found that sheep phenotypically selected for low MY tend to have smaller rumens and higher passage rates, potentially affecting digestibility and perhaps leading to higher feed intake on unrestricted diets (Goopy et al., 2014a).

For RC measurements of 532 Angus bulls fed at 1.2 times maintenance, Herd et al. (2013) reported a correlation of –0.59 ($P < 0.001$) for MY with feed intake; some care may therefore be needed to ensure that animals with low MY do not have higher feed intake. Preliminary heritability estimates for MY ($h^2 = 0.19$) and CH_4adjLW ($h^2 = 0.21$) were similar, with a high r_g (0.96; Donoghue et al., 2013), suggesting that selection for either trait could be equally effective if included in an index of all relevant traits. Therefore, even though it is not practical to measure feed intake in PAC, the high r_g of 0.96 noted above suggests that, especially with some refinement to the measurement protocol, selection based on PAC measurements of CH_4 to reduce emissions adjusted for liveweight could also improve MY.

Relationship of CH_4 and CO_2 Emissions

After excluding sick and agitated animals, our results show strong relationships between CH_4 and CO_2 emissions of sheep in PAC plus significant sire or genetic variation in CO_2 emissions. Carbon dioxide is produced from oxidation of substrates in the body and feed fermentation in the rumen. Its rate of emission is related to energy expenditure (e.g., Whitelaw et al., 1972) and heat production (Storm et al., 2012). When CH_4 production cannot be measured continuously (e.g., if sniffers are used), some researchers (Madsen et al., 2010; Lassen et al., 2012) have suggested that the CH_4:CO_2 could be used to estimate the proportion of carbon not metabolized to CO_2 and therefore total methane emissions. This ratio varies with feed intake, weight gain, fat deposition/utilization, and milk production (Madsen et al., 2010; Lassen et al., 2012), so adjustments for these factors might be required. The strong relationships between CO_2 and energy expenditure, and hence intake, suggests that CO_2 might be a possible proxy for feed intake. However, potentially elevated CO_2 emissions of agitated animals might confound the results; estimates of regression relationships can be subject to considerable bias when covariates are subject to measurement or other errors (Robinson, 2005).

Additional Use of Portable Accumulation Chambers

A useful feature of PAC, especially if animals are rounded up immediately before testing, is the potential to provide a relatively low cost unbiased estimate of total greenhouse gas emissions for an entire site or compare effects of different treatments or different types of pasture or the effects of dietary supplements that might possibly reduce emissions. Even if the interest is not individual animal data but estimating treatment or other effects, PAC offer potential advantages of cost and convenience over alternatives such as open path lasers (Jones et al., 2011; Tomkins et al., 2011) or SF_6 tracers (Pinares-Patiño et al., 2011a).

Conclusions

Portable accumulation chambers for up to 60 min are a practical way to measure methane emissions of grazing sheep. Estimates of repeatability (25%) and heritability (11.7%) of a single 1-h measurement were relatively low, so future tests should ideally be based on 2 or more measurements per animal. The additional time taken per animal could be offset by reducing the time in the PAC to, for example, 40 min. Although still modest, h^2 estimates (18.6% for the mean of 2 measurements and 23.2% for the mean of 3 measurements) are sufficient for genetic improvement of CH_4 emissions adjusted for liveweight or production. Further research will be required to validate the results and explore possible alternative test protocols, for example, allowing animals to continue grazing until the start of the test. Research to explore G×E interactions and breed and breed type effects and provide more accurate estimates of genetic parameters including correlations with production traits is also required. However, based on the 2,600 records in this study, the observed genetic variation and low correlations of EBV for methane and production traits would appear to be sufficient to enable methane emissions to be included in a multitrait analysis of production traits, leading to strategies to reduce methane emissions from Australian agriculture while still improving production.

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