

## ORIGINAL RESEARCH ARTICLE

## Crop Breeding &amp; Genetics

# Estimation of quantitative genetic parameters for dry matter yield and vegetative persistence-related traits in a white clover training population

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## Abstract

White clover (*Trifolium repens* L.), an economically important forage legume in temperate pastures, provides quality herbage and plant-available nitrogen. Enhancing breeding efforts to improve dry matter (DM) yield and vegetative persistence will increase on-farm value of this forage. To increase genetic gain for such traits, breeding tools like genomic selection have proven to be highly valuable in other crops. However, its success relies on a sufficiently large training population and key fundamentals of selective breeding, that is, presence of additive variation. We investigated quantitative genetic parameters for spring DM yield and vegetative persistence in a white clover training population comprising 200 half-sibling (HS) families. This population was established in a replicated cattle-grazed, mixed-sward field trial at two contrasting locations and assessed for spring DM yield and stolon-related vegetative persistence traits over a 3-yr period. The additive variation and genotype  $\times$  environment interactions, comprising the effects from year, season, and location were significant ( $P < .05$ ) for most traits. Narrow-sense heritability for all traits ranged from low (.13; post-summer stolon branches) to high (.73; leaf size) and there was a positive phenotypic correlation (.28) between spring DM yield and stolon number. These results indicate that both spring DM yield and persistence can be concurrently improved through selective breeding in the current population. We also demonstrated that applying a high selection pressure produces the highest predicted genetic gain. There is, however, a trade-off between genetic gain and diversity in the population for the long-term success of a breeding program.

**Abbreviations:** BLUP, best linear unbiased prediction; DM, dry matter;  $G \times E$ , genotype  $\times$  environment Interaction; GS, growth score; HS, half-sibling; LS, leaf size; SB, stolon branches; SBPOS, post-summer stolon branches; SBPRS, pre-summer stolon branches; SN, stolon number; SNPOS, post-summer stolon number; SNPRS, pre-summer stolon number.

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## 1 | INTRODUCTION

White clover (*Trifolium repens* L.) is an important forage legume in temperate regions of the world. It forms a significant legume component in mixed grass/clover swards, providing a high-quality feed for ruminants throughout the grazing season (Caradus et al., 1997). Through fixation of atmospheric nitrogen (N), white clover is an alternative source of N for companion grass species, thereby reducing dependency on artificial N in intense pasture-based grazing systems. However, the potential of white clover is not fully exploited, primarily due to inconsistency in dry matter (DM) yield and poor vegetative persistence (Woodfield & Caradus, 1996). Lack of genetic progress in plant persistence and seasonal DM yield are a consequence of long and complex breeding strategies, on-farm interspecies competition, biotic and abiotic stresses, and poor sward management (Widdup & Barrett, 2011; Woodfield & Caradus, 1996). A decline in DM yield in swards after the first 2 to 3 yr has been reported with an estimated 20 to 35% reduction in DM yield at the end of third year evaluation (Chapman et al., 1996; Piano & Annicchiarico, 1995). Dry matter yield is associated with a number of component traits including leaf size, internode length, and leaf number (Caradus et al., 1993; Mackay, 1991), whereas vegetative persistence is determined primarily by stolon density which plays a major role in the perennation and colonization by white clover in mixed swards (Archer & Robinson, 1989; Caradus et al., 1989; Collins et al., 1997). Many stolon attributes affect stolon density including stolon number and number of branches (Caradus & Williams, 1981; Hay et al., 1987; Jahufer et al., 2012). Vegetative persistence is adversely affected by summer moisture stress and extreme biotic and abiotic stresses (Jahufer et al., 2012) resulting in a rapid loss of stolon number and thus DM yield. The shallow rooting pattern of white clover coupled with the death of the main taproot, which occurs naturally at 6 to 18 mo in the plant life cycle, further exacerbates this issue of vegetative persistence. The ability of the stolon to survive this critical period underpins white clover's longevity in the sward. Evaluation of white clover germplasm under summer drought conditions has been an important breeding strategy for the development of new cultivars with persistence under moisture-stress (Jahufer et al., 2013). Breeding efforts for the simultaneous improvement of vegetative persistence and seasonal DM yield, is hindered by the negative genetic correlation between leaf size and stolon density (Caradus et al., 1989; Jahufer et al., 1994; Williams, 1987). Furthermore, low to moderate heritabilities have been reported for these traits further impeding the genetic advancements of DM yield and vegetative persistence (Caradus & Williams, 1989).

The development of genomic-assisted breeding tools such as genomic selection offers considerable opportunities to drive genetic gains for traits with low to moderate heritabil-

### Core Ideas

- This trial is the largest population of white clover half-sib families evaluated in grazed mixed swards.
- There was significant family variation for dry matter yield and vegetative persistence-related traits.
- A positive correlation between yield and persistence traits is a route for their concurrent improvement.
- Selected families outperformed commercial cultivars, implying genetic potential in the population.
- A trade-off between genetic gain and genetic diversity is needed for long term success of breeding programs.

ities (Calus et al., 2008). The success of genomic selection is heavily reliant upon the fundamentals of selective breeding such as the presence of additive genetic variation for the trait and the choice of breeding strategy. Estimating quantitative genetic parameters such as the magnitude of additive genetic variance to the total phenotypic variance, and contribution of variance associated with genotype  $\times$  environment ( $G \times E$ ) interactions in comparison to the additive genetic variance for the trait, helps to determine the feasibility of genomic selection. Concurrently, quantitative genetic assessments such as genetic gain per cycle, narrow-sense heritability, pattern analysis, and performance of breeding lines in relation to top commercial checks provide insight into the agronomic potential of the training population and choice of breeding strategy.

There are no reports of multiyear, multisite white clover half-sibling (HS) family structured training populations of the size of this current study that assesses quantitative parameters in an animal-grazed mixed sward. Further, this trial approximates standard farm management practices, enabling evaluation of clover performance in a farm-centric context. The objectives of this study were to (a) evaluate the agronomic potential of a training population by performing various quantitative genetic assessments, (b) quantify the magnitude of additive and  $G \times E$  variances associated with DM yield and vegetative persistence-related traits based on multilocation and multiyear cattle-grazed field trial data, and (c) predict the genetic gains for DM yield and vegetative persistence in a white clover HS training population that may be used as a platform for genomic selection.

## 2 | MATERIALS AND METHODS

### 2.1 | Development of breeding population

A training population of 200  $F_3$  HS families was generated from a polycross of 274  $F_2$  generation parental plants derived

from top-performing, broadly adapted breeding lines. The HS family populations were developed in 2012 from 31 clonal cuttings from top performing breeding lines that were polycrossed to form an  $F_1$  synthetic population of 141 HS families. A balanced bulk representation of these 141  $F_1$  HS families was created by selecting an equal number of seeds from each family. From this bulk, a random sample of 141 seeds were grown and this  $F_1$  population was polycrossed to generate 141  $F_2$  HS families, three of which were removed due to insufficient seed. Two individuals were then selected at random from each of the remaining 137 HS families, making a total of 274 individuals that were polycrossed from December 2015 to January 2016 to generate 274  $F_3$  families. The polycrosses were performed in a bee-proof crossing tunnel using bumble bees (*Bombus* sp.) pre-rinsed to remove wild pollen. The plants were re-randomized weekly to ensure a uniform distribution of pollen across all plants and to avoid formation of population substructures. After seed harvest, a subset of 200  $F_3$  HS families was selected based on having sufficient seed for downstream applications. A random sample from each of the selected families was pregerminated and maintained under glasshouse conditions for 2 mo. This was followed by trimming and transferal of the plants outside the glasshouse onto a drained concrete surface for “hardening” to ensure survival and a smooth transition when transplanting into field conditions.

## 2.2 | Field trial establishment and experimental design

The trial was conducted at two locations in New Zealand: AgResearch, Aorangi Research Farm in Palmerston North, Manawatu (40.38 °S, 175.61 °E); and the AgResearch, Ruakura Research Farm in Hamilton, Waikato (37.77 °S, 175.31 °E). The soil types at the Palmerston North and Ruakura sites were Kairanga fine sandy loam and peaty silt loam soil, respectively. Four months prior to transplanting the white clover entries, both locations were sown with a diploid perennial ryegrass (*Lolium perenne* L.) cultivar Ceres One50 containing the *Epichloë* endophyte AR37 at a rate of 18 kg  $ha^{-1}$ . Both locations were sprayed with the herbicide Kamba 500 at a dilution of 800 ml  $ha^{-1}$  to ensure resident white clover plants were eliminated and prevent contamination of the trial.

A row-column experimental design with three replicates was used at each site. Fifteen plants of each of the 200 HS families were hand planted into 0.5- by 0.75-m plots with 1.5 m between plots. Transplanting occurred in August and September 2016 at the Aorangi and Ruakura sites, respectively. In addition to the 200 HS families, each replicate had 24 repeated spatial checks comprised of cultivars Grasslands Kopu II (large-leafed cultivar) and Grasslands Bounty (small-

medium leafed cultivar) resulting in a total of 672 plots at each site. The clover plants were not inoculated with rhizobium.

## 2.3 | Trial management

The trial was managed by applying Cropmaster 15 (Ravensdown) N (15.1%), phosphorus (P; 10%), potassium (K; 10%) and sulfur (S; 7.7%) fertilizer (ravensdown.co.nz) at a rate of 250 kg  $ha^{-1}$  through a direct drill prior to sowing the perennial ryegrass (April 2016), and thereafter applied annually in April. At both locations, standard farming practices were performed by rotationally grazing the trials with cattle when herbage mass on both the plots and inter-plot areas were between 2,500 and 2,800 kg DM  $ha^{-1}$ , as assessed by a rising plate meter. The animals were removed from the trial area immediately after grazing leaving residuals of 1,000 to 1,200 kg DM  $ha^{-1}$ . Inter-row spraying using Kamba 500 was performed as needed to eliminate volunteer clover, weeds, and to prevent spread of HS family stolons into adjacent plots. This herbicide was applied during appropriate weather conditions using targeted nozzles to avoid spray drift. To reduce further likelihood of potential herbicide damage, the trial was sprayed at a sufficient biomass to enable grazing immediately after the withholding period.

## 2.4 | Meteorological conditions

Meteorological data were obtained for both locations and daily observations were aggregated to give monthly total and average values for rainfall and maximum and minimum air temperature respectively (Supplemental Figures S1A and S1B).

## 2.5 | Trait measurements

- (i) Spring DM yield: To assess clover herbage biomass, full-scale DM yield cuts were taken annually from each plot in spring. At each cut, a 0.2-m<sup>2</sup> quadrant was randomly placed in the plot, when herbage mass accumulation was between 2,500 and 2,800 kg DM  $ha^{-1}$ , as assessed by a rising plate meter, and the above-ground biomass cut to a stubble height of approximately 3 cm using mechanical shears. Harvested samples were separated into white clover and ryegrass components and dried in an oven at 80 °C for 48 h and the dry weight for each component recorded. All 672 plots (HS families and checks) were harvested from each trial location annually in mid-late spring (October–November) after the first full year (2017, 2018, and 2019).

- (ii) Seasonal growth: Visual assessment of seasonal clover yield across the trial, termed as growth score (GS), was taken during the spring, summer, autumn and winter, prior to grazing, when herbage mass was between 2,500 and 2,800 kg DM ha<sup>-1</sup>. Scoring was done on a scale of 1 (lowest) to 9 (highest) herbage production per plot, with 0.5-unit increments to allow closer approximation of continuous data.
- (iii) Leaf size (LS): Leaf size was recorded twice each year via visual leaf size score of 1 (smallest) to 5 (largest) with 0.5-units increments.
- (iv) Stolon density: Two stolon traits, stolon numbers (SN) and stolon branches (SB), were measured pre- (PRS) and post-summer (POS) to determine the effect of summer moisture stress on these traits. On each occasion, three cores (5-cm diameter by 4-cm depth) were taken randomly from all plots in the Aorangi site using a soil corer, and from each collected core SN and SB were counted. Data were collected from the second year of growth in late November (pre-summer) and March (post-summer). These traits (SNPRS, SNPOS, SBPRS, and SBPOS) were measured at Aorangi only.

## 2.6 | Statistical analysis

Data were analyzed using a linear mixed-model based on the residual maximum likelihood method (Harville, 1977; Patterson & Thompson, 1971), using DeltaGen (Jahufu & Luo, 2018) that was underpinned by lme4 R package (Bates et al., 2015). These analyses enabled estimation of variance components for genetic and G × E interaction effects, as well as providing best linear unbiased predictors (BLUPs) for Spring DM yield, GS, LS, SN, and SB. The statistical significance of the variance components was assessed using deviance of log-likelihood (Galwey, 2006) and residual plots were assessed for deviations from normality. In the linear mixed-models, years, sites, seasons and repeated check cultivars were considered as fixed effects and HS family, replicates, rows and columns were considered random effects.

To estimate fixed effects, maximum likelihood estimation was used instead of residual maximum likelihood (Zuur et al., 2009). Half-sibling families and check cultivars were considered as fixed effects in order to obtain best linear unbiased estimates (BLUEs) and estimate differences among them. All the linear mixed-models were fitted using the 'lmer' function in the 'lme4' package in R (Bates et al., 2015; R Core Team, 2018), whereas pairwise difference and separation of means was performed by Fisher's least-significant difference (LSD) test in the 'predictmeans' package in R (Luo et al., 2014).

The following linear models were fitted using DeltaGen:

$$Y_{iklm} = \mu + f_i + b_k + r_{lk} + c_{mk} + \varepsilon_{iklm} \quad (1)$$

Where  $Y_{iklm}$  is the value of a trait measured from HS family  $i$  in replicate  $k$  in row  $l$  and column  $m$ , and  $i = 1, \dots, n_f$ ;  $k = 1, \dots, n_b$ ;  $l = 1, \dots, n_r$ ; and  $m = 1, \dots, n_c$ , where,  $f$ ,  $b$ ,  $r$ , and  $c$  are families, replicates, rows, and columns, respectively;  $\mu$  is the overall mean;  $f_i$  is the random effect of the HS family  $i$  and modelled as coming from a normal distribution with the given mean and variance,  $N(0, I\sigma_f^2)$ ;  $b_k$  is the random effect of replicate  $k$ ,  $N(0, I\sigma_b^2)$ ;  $r_{lk}$  is the random effect of row  $l$  in replicate  $k$ ,  $N(0, I\sigma_r^2)$ ;  $c_{mk}$  is the random effect of column  $m$  in replicate  $k$ ,  $N(0, I\sigma_c^2)$  and  $\varepsilon_{iklm}$  is the residual effect of family  $i$  in replicate  $k$  in row  $l$  and column  $m$   $N(0, I\sigma_e^2)$ .

- (i) Across seasons within years and locations,

$$Y_{ijklm} = \mu + f_i + s_j + b_{jk} + (fs)_{ij} + (fb)_{ik} + (bs)_{jk} + r_{jkl} + c_{jklm} + \varepsilon_{ijklm} \quad (2)$$

Where:  $Y_{ijklm}$  is the value of a trait measured from family  $i$  of season  $j$  within replicate  $k$  in row  $l$  and column  $m$  and  $i = 1, \dots, n_f$ ;  $j = 1, \dots, n_s$ ;  $k = 1, \dots, n_b$ ;  $l = 1, \dots, n_r$ , and  $m = 1, \dots, n_c$ , where,  $f$ ,  $s$ ,  $b$ ,  $r$ , and  $c$  are families, seasons, replicates, rows, and columns, respectively;  $\mu$  is the overall mean;  $f_i$  is the random effect of the HS family  $I$  and modelled as coming from a normal distribution with the given mean and variance,  $N(0, I\sigma_f^2)$ ;  $s_j$  is the fixed effect of season  $j$ ,  $b_{jk}$  is the random effect of replicate  $k$  within season  $j$ ,  $N(0, I\sigma_b^2)$ ;  $(fs)_{ij}$  is the random effect of the interaction between family  $i$  and season  $j$ ,  $N(0, I\sigma_{fs}^2)$ ;  $(fb)_{ik}$  is the random effect of interaction between family  $i$  and replicate  $k$ ,  $N(0, I\sigma_{fb}^2)$ ;  $(bs)_{ij}$  is the interaction between replicate  $k$  and season  $j$ ,  $N(0, I\sigma_{bs}^2)$ ;  $r_{jkl}$  is the random effect of row  $l$  in replicate  $k$  within season  $j$ ,  $N(0, I\sigma_r^2)$ ;  $c_{jklm}$  is the random effect of column  $m$  in replicate  $k$ , within season  $j$ ,  $N(0, I\sigma_c^2)$ ; and  $\varepsilon_{ijklm}$  is the residual effect of genotype  $i$  in season  $j$  of replicate  $k$  in row  $l$  and column  $m$ .

- (ii) Across year, seasons and locations,

$$Y_{inojklm} = \mu + f_i + y_o + e_n + (fe)_{in} + s_j + (fs)_{ij} + (fy)_{io} + (sy)_{jo} + (fey)_{ino} + (fsy)_{ioj} + b_{nojk} + r_{nojkl} + c_{nojkm} + \varepsilon_{inojklm} \quad (3)$$

Where  $Y_{inojklm}$  is the value of a trait measured from family  $i$ , at the  $n$ th location of the  $o$ th year in the  $j$ th season, within the  $o$ th year, at the  $n$ th location, within the  $k$ th replicate, in  $l$ th row, and  $m$ th column.  $i = 1, \dots, n_f$ ;  $n = 1, \dots, n_e$ ;  $o = 1, \dots, n_y$ ;  $j = 1, \dots, n_s$ ;  $k = 1, \dots, n_b$ ;  $l = 1, \dots, n_r$ ;  $m = 1, \dots, n_c$ ; where,  $f$ ,  $y$ ,  $e$ ,  $y$ ,  $s$ ,  $b$ ,  $r$ , and  $c$  are families, years, locations, years, seasons, replicates, rows, and columns, respectively.  $\mu$  is the overall mean;  $f_i$  is the random effect of the HS family  $i$  and modelled as coming from a normal distribution with the

given mean and variance  $N(0, I\sigma_f^2)$ ;  $y_o$  is the fixed effect of year  $o$ ;  $e_n$  is the fixed effect of location  $n$ ;  $(fe)_{in}$  is the random effect of the interaction between family  $i$  and location  $n$ ,  $N(0, I\sigma_{fe}^2)$ ;  $s_j$  is the fixed effect of season  $j$ ,  $N(0, I\sigma_s^2)$ ;  $(fs)_{ij}$  is the random effect of the interaction between family  $i$  and season  $j$ ,  $N(0, I\sigma_{fs}^2)$ ;  $(fy)_{io}$  is the random effect of the interaction between family  $i$  and year  $o$ ,  $N(0, I\sigma_{fy}^2)$ ;  $(sy)_{jo}$  is the random effect of the interaction between season  $j$  in year  $o$ ,  $N(0, I\sigma_{sy}^2)$ ;  $(fey)_{ino}$  is the random effect of the interaction between family  $i$  location  $n$  and year  $o$ ,  $N(0, I\sigma_{fey}^2)$ ;  $(fsy)_{ioj}$  is the random effect of the interaction between family  $i$  season  $j$  and year  $o$ ,  $N(0, I\sigma_{fsy}^2)$ ;  $b_{nojk}$  is the random effect of replicate  $k$  within season  $j$  within year  $o$  in location  $n$ ,  $N(0, I\sigma_b^2)$ ;  $r_{nojkl}$  is the random effect of row  $l$  within replicate  $k$  in season  $j$  within year  $o$  in location  $n$ ,  $N(0, I\sigma_r^2)$ ;  $c_{nojkm}$  is the random effect of column  $m$  in replicate  $k$  within season  $j$  within year  $o$  in location  $n$ ,  $N(0, I\sigma_c^2)$ ; and  $\epsilon_{inojklm}$  is the residual effect of genotype  $i$  in location  $n$  within year  $o$  in season  $j$  of replicate  $k$  in row  $l$  and column  $m$  of replicate  $k$  in season  $j$  within year  $o$  in location  $n$ .

As the variance components estimated with HS families explores only one quarter of the additive variance (Falconer, 1989), the heritability derived is narrow-sense and was estimated on a family mean basis according to the equation proposed by Falconer (1989), using the estimates from the linear mixed-models described in Equation 2.

Narrow-sense heritability on a family mean basis estimated across seasons within years and locations:

$$h_n^2 = \frac{\sigma_f^2}{\sigma_f^2 + \frac{\sigma_{fs}^2}{n_s} + \frac{\sigma_{fb}^2}{n_b} + \frac{\sigma_e^2}{n_s n_b}} \quad (4)$$

Where  $h_n^2$  is the narrow-sense heritability;  $\sigma_f^2$  is the family variance component;  $\sigma_{fs}^2$  is the family  $\times$  season interaction variance component;  $\frac{\sigma_{fb}^2}{n_b}$  is the family  $\times$  replicate interaction variance component;  $\sigma_e^2$  is the error variance component; and  $n_s$  and  $n_b$  are the number of seasons and replicates, respectively. The narrow-sense heritability estimates for within years across sites and seasons and across sites seasons and years were calculated using Equation 4 with the denominator expanded to include interaction components as defined in the linear mixed-model Equations 1 and 3.

Pearson's correlation coefficient was determined to evaluate the type (positive or negative) and strength (higher or lower value) of relationship between traits using HS family BLUPs for each trait. Pearson's correlation coefficient and significance testing were performed using the 'cor' package in R. Spearman's correlation coefficient was used to assess the relative ranking performance of the top twenty lines in Year 2 and Year 3.

Principal component analysis (PCA) and cluster analysis were conducted on seasonal GS to generate a graphical summary of the association among measured GS traits and seasons using family BLUPs in DeltaGen (Jahufer & Luo, 2018). Cluster analysis was carried out using a hierarchical agglomerative classification procedure with squared Euclidean distance as a measure of dissimilarity (Burr, 1970; Wishart, 1969), and the Hartigan clustering algorithm (Hartigan, 1975) used as the grouping strategy. The data were standardised to remove scaling effects and have a mean of zero and a variance of one (Fox & Rosielle, 1982; Cooper & DeLacy, 1994).

## 2.7 | Predicted genetic gain

Predicted genetic gain per cycle was estimated using DeltaGen (Jahufer & Luo, 2018) for spring DM yield and second year SNPRS, SNPOS, SBPRS, and SBPOS. The estimations were conducted using the empirical values of family variances, family  $\times$  location and family  $\times$  year variances obtained from the linear mixed-modelling described above. Half-sibling families were selected based on four among-family selection pressures of 20, 10, 5, and 1%. The genetic gain was estimated using the equation described below.

$$\Delta G_{HS} = k_f c \frac{\frac{1}{4}\sigma_A^2}{\sigma_{PF}} \quad (5)$$

Where  $\Delta G_{HS}$  is the genetic gain based on selection and random mating of the top performing HS families;  $k_f$  is the among family selection intensity;  $c$  ( $c = 0.5$  for among HS family selection) is the parental control;  $\sigma_A^2$  is the additive variance; and  $\sigma_{PF}$  is the among-family phenotypic standard deviation.

## 3 | RESULTS

### 3.1 | Variance components and heritability

Family variance ( $\sigma_f^2$ ) for seasonal GS was significant ( $P < .05$ ) across seasons, years and locations, as well as within years and locations (Equations 2 and 1; Table 1). The interactions between family and seasons ( $\sigma_{f,s}^2$ ) were significant ( $P < .05$ ) across years and locations, except at Ruakura in the year 2019. The family  $\times$  location ( $\sigma_{f,l}^2$ ) interactions were significant when the data were combined between the two locations (Table 1). The significant variance components of family  $\times$  location interactions ( $\sigma_{f,l}^2$ ) were larger than the family variance, suggesting a strong change in the ranking of families across the two locations for GS. Family  $\times$  year ( $\sigma_{f,y}^2$ ) interaction was also significant for both sites when analyzed across all years but not significant ( $P > .05$ ) when locations were combined (Table 1). Family

**TABLE 1** Estimated family ( $\sigma_f^2$ ), family-by-season ( $\sigma_{f,s}^2$ ), family-by-year ( $\sigma_{f,y}^2$ ), family-by-location ( $\sigma_{f,l}^2$ ), residual error ( $\sigma_e^2$ ), variance components, their associated interactions, and standard errors ( $\pm$  SE); and family mean narrow-sense heritability ( $h_n^2$ ) for seasonal growth scores, spring dry matter yield, and leaf size for 200 half-sibling white clover families across two locations, Aorangi and Ruakura, and 3 yr

Source of variation	Seasonal growth score			Spring dry matter yield			Leaf size		
	Aorangi	Ruakura	Combined	Aorangi	Ruakura	Combined	Aorangi	Ruakura	Combined
<b>2017</b>									
$\sigma_f^2$	0.12 $\pm$ 0.04*	0.36 $\pm$ 0.11*	0.10 $\pm$ 0.05*	0.19 $\pm$ 0.09*	0.08 $\pm$ 0.08	0.15 $\pm$ 0.04*	0.07 $\pm$ 0.03*	0.06 $\pm$ 0.02*	0.05 $\pm$ 0.01*
$\sigma_{f,s}^2$	0.11 $\pm$ 0.02*	0.21 $\pm$ 0.03*	0.03 $\pm$ 0.01*	n.a. <sup>a</sup>	n.a.	n.a.	n.a.	n.a.	n.a.
$\sigma_{f,l}^2$	n.a.	n.a.	0.46 $\pm$ 0.06*	n.a.	n.a.	0.00	n.a.	n.a.	0.01 $\pm$ 0.01
$\sigma_e^2$	0.76 $\pm$ 0.02	1.52 $\pm$ 0.04	1.83 $\pm$ 0.03	1.60 $\pm$ 0.12	1.16 $\pm$ 0.27	1.40 $\pm$ 0.07	0.55 $\pm$ 0.05	0.29 $\pm$ 0.06	0.47 $\pm$ 0.02
$h_n^2$	0.35 $\pm$ 0.07	0.33 $\pm$ 0.08	0.24 $\pm$ 0.01	0.27 $\pm$ 0.10	n.s. <sup>†</sup>	0.37 $\pm$ 0.08	0.26 $\pm$ 0.10	0.30 $\pm$ 0.09	0.36 $\pm$ 0.09
<b>2018</b>									
$\sigma_f^2$	0.37 $\pm$ 0.08*	0.51 $\pm$ 0.13*	0.20 $\pm$ 0.07*	0.25 $\pm$ 0.06*	0.13 $\pm$ 0.14	0.14 $\pm$ 0.05*	0.08 $\pm$ 0.02*	0.09 $\pm$ 0.02*	0.05 $\pm$ 0.01*
$\sigma_{f,s}^2$	0.14 $\pm$ 0.02*	0.15 $\pm$ 0.03*	0.05 $\pm$ 0.02*	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
$\sigma_{f,l}^2$	n.a.	n.a.	0.66 $\pm$ 0.08*	n.a.	n.a.	0.00	n.a.	n.a.	0.05 $\pm$ 0.01*
$\sigma_e^2$	0.82 $\pm$ 0.02	1.42 $\pm$ 0.05	2.42 $\pm$ 0.04	0.81 $\pm$ 0.06	2.02 $\pm$ 0.16	2.10 $\pm$ 0.07	0.37 $\pm$ 0.02	0.29 $\pm$ 0.02	0.40 $\pm$ 0.01
$h_n^2$	0.50 $\pm$ 0.06	0.42 $\pm$ 0.07	0.24 $\pm$ 0.07	0.48 $\pm$ 0.07	n.s.	0.35 $\pm$ 0.10	0.51 $\pm$ 0.08	0.69 $\pm$ 0.06	0.42 $\pm$ 0.09
<b>2019</b>									
$\sigma_f^2$	0.85 $\pm$ 0.15*	0.53 $\pm$ 0.2*	0.25 $\pm$ 0.12*	0.00	0.19 $\pm$ 0.24	0.01 $\pm$ 0.10	0.20 $\pm$ 0.05*	0.02 $\pm$ 0.03	0.05 $\pm$ 0.01*
$\sigma_{f,s}^2$	0.04 $\pm$ 0.01*	0.00	0.00	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
$\sigma_{f,l}^2$	n.a.	n.a.	1.20 $\pm$ 0.15*	n.a.	n.a.	0.13 $\pm$ 0.06*	n.a.	n.a.	0.03 $\pm$ 0.01*
$\sigma_e^2$	0.49 $\pm$ 0.02	1.25 $\pm$ 0.06	1.60 $\pm$ 0.04	0.68 $\pm$ 0.04	1.12 $\pm$ 0.32	1.20 $\pm$ 0.07	0.70 $\pm$ 0.02	0.01 $\pm$ 0.02	0.56 $\pm$ 0.03
$h_n^2$	0.59 $\pm$ .054	0.29 $\pm$ 0.12	0.27 $\pm$ 0.10	n.s.	n.s.	n.s.	0.46 $\pm$ 0.10	n.s.	0.31 $\pm$ 0.08
<b>Across years</b>									
$\sigma_f^2$	0.26 $\pm$ 0.05*	0.50 $\pm$ 0.07*	0.12 $\pm$ 0.04*	0.11 $\pm$ 0.03*	0.06 $\pm$ 0.05	0.87 $\pm$ 0.02*	0.10 $\pm$ 0.02*	0.05 $\pm$ 0.01*	0.06 $\pm$ 0.01*
$\sigma_{f,l}^2$	n.a.	n.a.	0.35 $\pm$ 0.05*	n.a.	n.a.	0.07 $\pm$ 0.03	n.a.	n.a.	0.04 $\pm$ 0.01*
$\sigma_{f,y}^2$	0.25 $\pm$ 0.03*	0.52 $\pm$ 0.06*	0.04 $\pm$ 0.03	0.03 $\pm$ 0.03	0.05 $\pm$ 0.04	0.00	0.02 $\pm$ 0.01*	0.00	0.00
$\sigma_{f,y,s}^2$	0.01 $\pm$ 0.01	0.02 $\pm$ 0.02	0.04 $\pm$ 0.01*	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
$\sigma_{f,y,l}^2$	n.a.	n.a.	0.34 $\pm$ 0.04*	n.a.	n.a.	0.01 $\pm$ 0.02	n.a.	n.a.	0.01 $\pm$ 0.01*
$\sigma_e^2$	0.90 $\pm$ 0.01	2.40 $\pm$ 0.03	1.73 $\pm$ 0.02	1.05 $\pm$ 0.05	1.46 $\pm$ 0.08	1.40 $\pm$ 0.04	0.40 $\pm$ 0.02	0.30 $\pm$ 0.01	0.42 $\pm$ 0.01
$h_n^2$	0.48 $\pm$ 0.06	0.73 $\pm$ 0.04	0.32 $\pm$ 0.10	0.45 $\pm$ 0.07	n.s.	0.43 $\pm$ 0.09	0.73 $\pm$ 0.06	0.66 $\pm$ 1.8	0.7 $\pm$ 0.06

<sup>a</sup>n.a. not applicable.

\*Significant at the .05 probability level.

<sup>†</sup>n.s. not significant.

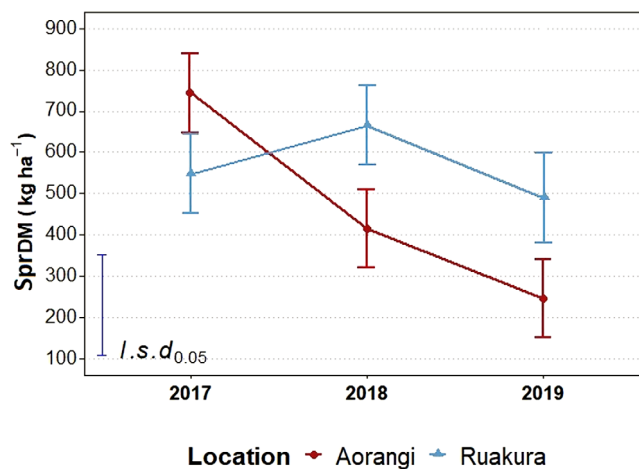
$\times$  year  $\times$  season ( $\sigma_{f,y,s}^2$ ) and family  $\times$  year  $\times$  location ( $\sigma_{f,y,l}^2$ ) were significant ( $P < .05$ ). The residual error variance was larger than family and  $G \times E$  components for most analyses. There was significant ( $P < .05$ ) family variation at individual locations and seasons within years except for year 2017 winter at Aorangi (Supplemental Table S1).

Combined analyses across the years (2017, 2018, and 2019) for each season showed significant family variation among HS families for both locations except spring and summer at Ruakura (Supplemental Table S1). The family  $\times$  year ( $\sigma_{f,y}^2$ ) interaction variance component was usually higher than the family variance component ( $\sigma_f^2$ ; Supplemental Table S1). When compared to Aorangi, Ruakura always had higher residual error variance components ( $\sigma_e^2$ ). Upon combining locations, the family  $\times$  location ( $\sigma_{f,l}^2$ ) interaction was significant ( $P < .05$ ) for individual years and across years and was usually larger than the family variance component ( $\sigma_f^2$ ). Family  $\times$  year ( $\sigma_{f,y}^2$ ) interaction when significant, was lower than the family  $\times$  location ( $\sigma_{f,l}^2$ ) interaction component. Narrow-sense heritability seemed to increase with successive years (Supplemental Table S1).

Significant ( $P < .05$ ) family variation ( $\sigma_f^2$ ) was observed among the HS families for spring DM yield at Aorangi for 2017, 2018, and across all 3 yr, however, no significant ( $P > .05$ ) variation was observed at Ruakura (Table 1). Across-site family variance was significant ( $P < .05$ ) for individual years 2017 and 2018, as well as across all 3 yr combined. Family  $\times$  year ( $\sigma_{f,y}^2$ ) and family  $\times$  year  $\times$  location ( $\sigma_{f,y,l}^2$ ) interactions were not significant ( $P > .05$ ; Table 1).

Significant ( $P < .05$ ) family variation was estimated among the 200 HS families for LS at Aorangi and Ruakura for all years apart from Ruakura 2019 (Table 1). Across-site analyses for individual years, as well as combining all years, also revealed significant ( $P < .05$ ) family variation. Family  $\times$  location ( $\sigma_{f,l}^2$ ) interaction was significant for individual years 2018 and 2019, as well as across all 3 yr. The three-way interaction of family  $\times$  year  $\times$  location ( $\sigma_{f,y,l}^2$ ) was significant ( $P < .05$ ), whereas family  $\times$  year ( $\sigma_{f,y}^2$ ) interaction was only significant at Aorangi when analyses were combined across all years. The residual error variance component was larger than all other components. Family means heritability ranged from .26 to .73 and increased as the trial progressed.

There was significant ( $P < .05$ ) family variation among 200 HS families for SNPRS, SBPRS, SNPOS, and SBPOS in the years 2017–2018 and 2018–2019 (Table 2). The family  $\times$  season ( $\sigma_{f,s}^2$ ) interactions were significant ( $P < .05$ ) for SN, however for SB, significant  $\sigma_{f,s}^2$  was observed only within years. When combining two years of data (2017–2019), there were no significant differences observed among HS families for SNPRS, SBPRS, SNPOS, or SBPOS, and family  $\times$  season ( $\sigma_{f,s}^2$ ) interaction was also nonsignificant. For both SN



**FIGURE 1** The effect of year and location on spring dry matter (SprDM) yield at the two sites (Aorangi and Ruakura), evaluated under dairy cattle grazing systems. The SprDM yield was harvested from all plots in spring of each year. Error bars represent standard errors of the mean.  $l.s.d_{0.05}$ , least significant difference at the .05 probability level

and SB traits, family  $\times$  year ( $\sigma_{f,y}^2$ ) interaction was significant for pre- and post-summer assessments.

### 3.2 | Effect of year and location on spring DM yield

The spring DM yield was harvested from all plots, including check cultivars, in spring of each year from across the two locations. Analysis of variance indicated significant ( $P < .05$ ) year  $\times$  location interaction suggesting a differential performance of HS families across locations and years (Figure 1). There was a significant ( $P < .05$ ) decrease in spring DM yield between the first and third year at Aorangi and Ruakura. The second year at Ruakura and third year at Aorangi were also significantly different and both locations showed a drop in spring DM yield in the third year. All other pairwise comparisons were nonsignificant at the 5% level using Fisher's least significant difference test (Figure 1). Spearman's correlation was used to assess the relative ranking performance of the top 20 HS families in Years 2 and 3 and showed a significant high positive correlation between families  $r_s = .65$ ,  $P < .001$  (Supplemental Figure S2).

### 3.3 | Effects of summer period on stolon traits

The differences between SNPRS and SNPOS were significant ( $P < .01$ ), as well as the effect of year on SN ( $P < .01$ ; Figure 2a). However, for SB only the year effect was significant ( $P < .01$ ; Figure 2b). There was a decrease of 27% in

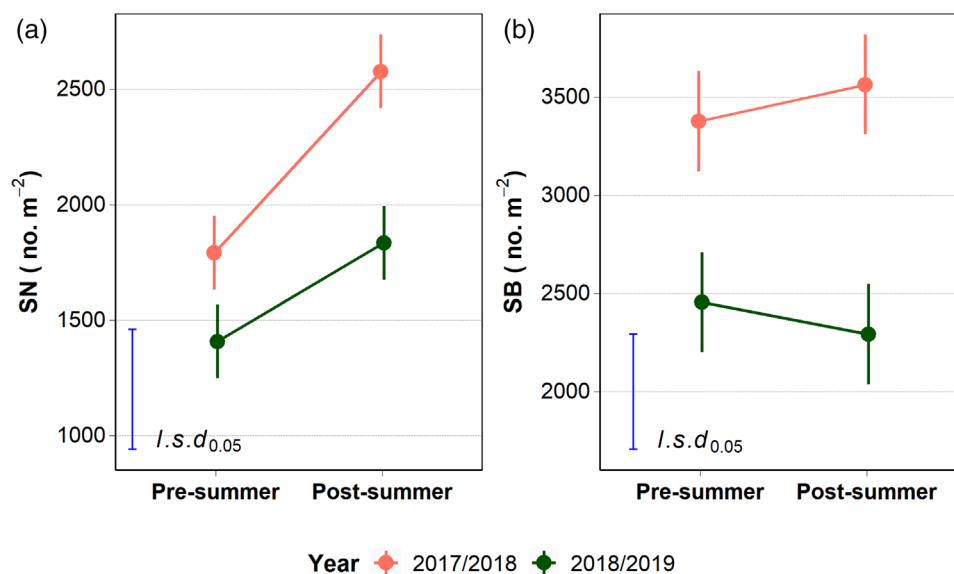
**TABLE 2** Family ( $\sigma_f^2$ ), family-by-season ( $\sigma_{f,s}^2$ ), family-by-year ( $\sigma_{f,y}^2$ ), family-by-year-by-season ( $\sigma_{f,y,s}^2$ ), residual ( $\sigma_\varepsilon^2$ ) variance components and their associated  $\pm$  standard errors, and family mean narrow-sense heritability ( $h_n^2$ ) for white clover morphological traits (stolon number, SN; number of branches, SB) measured from the 200 HS families at Aorangi pre- (PRS) and post-summer (POS) in years 2017–2018 and 2018–2019

Source	Stolon number			Stolon branches		
	SNPRS	SNPOS	Across seasons	SBPRS	SBPOS	Across seasons
<b>2017–2018</b>						
$\sigma_f^2$	0.44 $\pm$ 0.11*	0.31 $\pm$ 0.14*	0.09 $\pm$ 0.09	1.47 $\pm$ 0.45*	1.18 $\pm$ 0.37*	0.75 $\pm$ 0.30*
$\sigma_{f,s}^2$	n.a. <sup>a</sup>	n.a.	0.04 $\pm$ 0.11*	n.a.	n.a.	0.78 $\pm$ 0.34*
$\sigma_\varepsilon^2$	5.16 $\pm$ 0.18	8.33 $\pm$ 0.29	6.80 $\pm$ 0.16	22.87 $\pm$ 0.79	20.39 $\pm$ 0.70	21.88 $\pm$ 0.52
$h_n^2$	0.20 $\pm$ 0.04	0.25 $\pm$ 0.09	0.00	0.37 $\pm$ 0.07	0.15 $\pm$ 0.04	0.10 $\pm$ 0.90
<b>2018–2019</b>						
$\sigma_f^2$	0.21 $\pm$ 0.07*	0.28 $\pm$ 0.1*	0.13 $\pm$ 0.06*	1.32 $\pm$ 0.36*	1.09 $\pm$ 0.29*	0.14 $\pm$ 0.24
$\sigma_{f,s}^2$	n.a.	n.a.	0.13 $\pm$ 0.06*	n.a.	n.a.	1.12 $\pm$ 0.31*
$\sigma_\varepsilon^2$	4.00 $\pm$ 0.14	5.60 $\pm$ 0.19	4.80 $\pm$ 0.12	17.56 $\pm$ 0.60	14.50 $\pm$ 0.49	15.90 $\pm$ 0.38
$h_n^2$	0.14 $\pm$ 0.04	0.13 $\pm$ 0.04	0.28 $\pm$ 0.11	0.18 $\pm$ 0.04	0.19 $\pm$ 0.04	0.09 $\pm$ 0.14
<b>Across years</b>						
$\sigma_f^2$	0.09 $\pm$ 0.07	0.05 $\pm$ 0.09	0.03 $\pm$ 0.05	0.00	0.36 $\pm$ 0.24	0.11 $\pm$ 0.15
$\sigma_{f,y}^2$	0.28 $\pm$ 0.08*	0.30 $\pm$ 0.11*	0.07 $\pm$ 0.07	1.50 $\pm$ 0.29*	0.90 $\pm$ 0.30*	0.27 $\pm$ 0.22
$\sigma_{f,s}^2$	n.a.	n.a.	0.04 $\pm$ 0.07	n.a.	n.a.	0.00
$\sigma_{f,y,s}^2$	n.a.	n.a.	0.22 $\pm$ 0.09*	n.a.	n.a.	0.95 $\pm$ 0.23*
$\sigma_\varepsilon^2$	4.60 $\pm$ 0.11	7.01 $\pm$ 0.17	5.80 $\pm$ 0.09	20.4 $\pm$ 0.40	17.60 $\pm$ 0.40	18.90 $\pm$ 0.32
$h_n^2$	n.s. <sup>†</sup>	n.s.	n.s.	n.s.	n.s.	n.s.

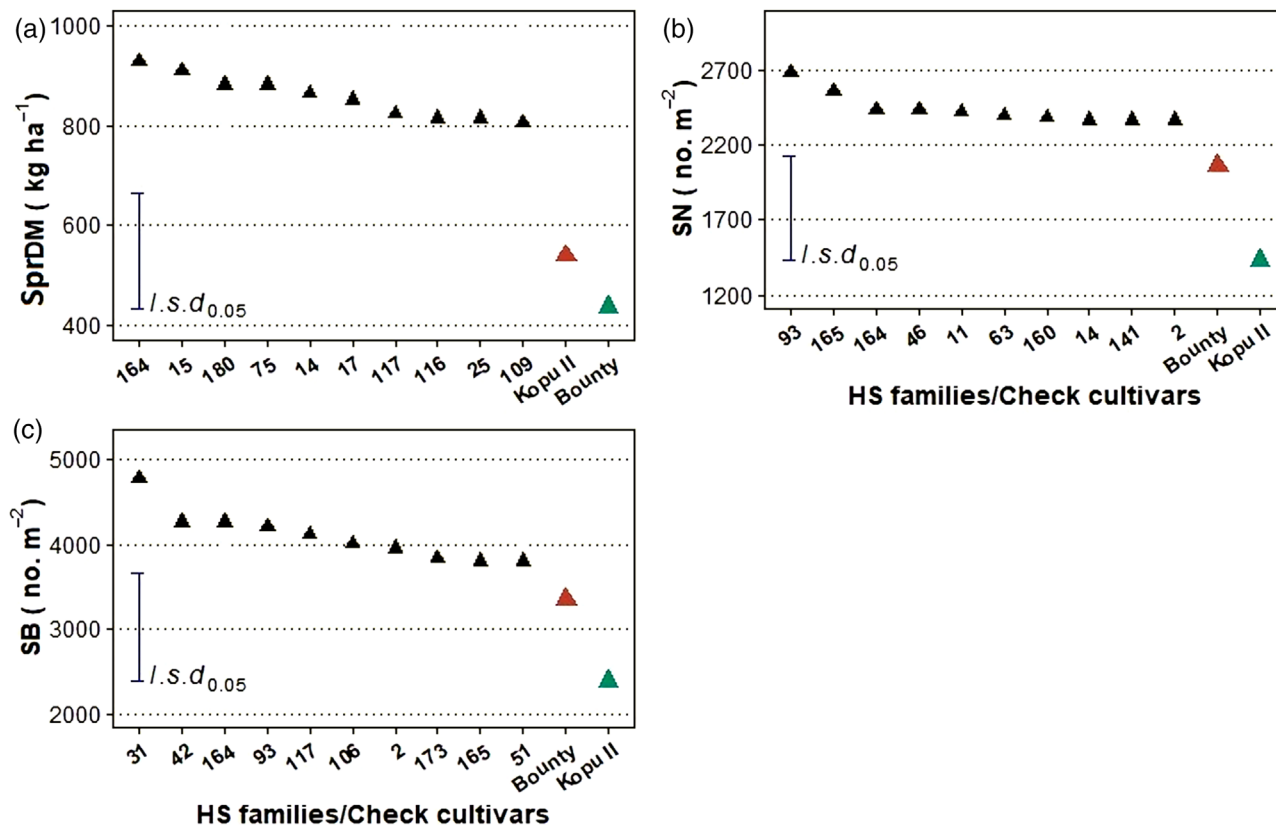
<sup>a</sup>n.a., not applicable

\*Significant at the .05 probability level.

<sup>†</sup>n.s., not significant.



**FIGURE 2** Predicted means and standard errors of the mean of stolon number measured pre- and post-summer (SNPRS, SNPOS, respectively; a) and stolon branches measured pre- and post-summer (SBPRS, SBPOS, respectively; b) for 200 white clover half-sibling (HS) families measured at Aorangi. Error bars represent standard errors of the mean. l.s.d<sub>0.05</sub>, least significant difference at the .05 probability level



**FIGURE 3** Performance of the top 10% of 200 half-sibling (HS) white clover families compared with two commercial check cultivars, Grasslands Kopu II and Grasslands Bounty, for spring dry matter yield (SprDM) which was harvested from all plots in the spring of each year from the trials conducted at Aorangi and Ruakura under dairy cattle grazing across years 2017, 2018, and 2019 (a); mean stolon number (SN, b); and stolon (SB; c). Stolon data were evaluated at Aorangi under dairy cattle grazing across combined summers of years 2017–2019 and 2018–2019.  $l.s.d_{0.05}$ , least significant difference at the .05 probability level

mean value for the SN from Year 2 (2017–2018) to Year 3 (2018–2019), whereas, for SB trait the decrease was 32% from Year 2 to Year 3.

The SN increased significantly ( $P < .05$ ) by an average of 47.5% between pre- and post-summer in Year 2, and in the Year 3 the increase was by 33% but was not found to be significant. For the SB trait, there was an increase in predicted mean value between pre- and post-summer in Year 1 and decrease in the mean value in Year 2, but both differences were nonsignificant.

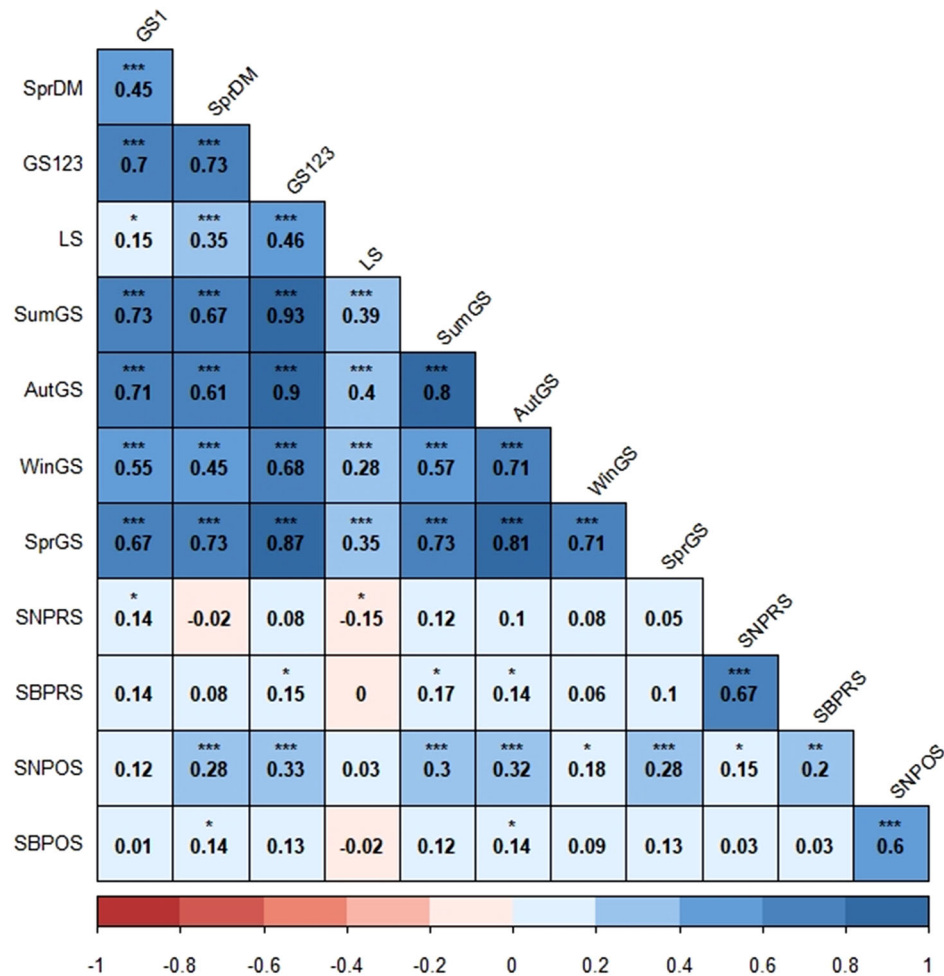
### 3.4 | Evaluating HS families relative to check cultivar performance for spring DM yield and vegetative performance

The performance of the top 10 HS families compared to Grasslands Kopu II and Grasslands Bounty, the two commercial check cultivars included in the trial, is presented in Figure 3. When compared with all 200 HS families, 14 and 42 families had significantly ( $P < .05$ ) higher spring DM yield than Grasslands Kopu II and Grasslands Bounty,

respectively. Compared to the highest performing family, HS164, Grasslands Kopu II and Grasslands Bounty produced 41 and 53% less spring DM yield (Figure 3a). For the traits SN and SB, 49 HS families had significantly ( $P < .05$ ) higher SN than Grasslands Kopu II, whereas Grasslands Bounty performed equivalent to the top HS families (Figure 3b and c). Grasslands Kopu II produced significantly less ( $P > .05$ ) SB than 17 HS families, whereas only one family, HS31, had significantly higher SB (43%) than Grasslands Bounty.

### 3.5 | Trait correlations

Pearson correlation coefficients showed a strong positive correlation (significant at  $P < .001$ ) between full-scale spring DM yield (measured in spring of each year from all plots) and mean seasonal GS across locations, seasons, and years (GS123), as well as seasonal growth scores across locations and years for summer (SumGS), autumn (AutGS), and particularly spring (SprGS) (Figure 4). There was a moderately positive correlation between spring DM yield and



**FIGURE 4** Pearson correlation coefficients among traits estimated from the Best Linear Unbiased Predictor (BLUP) values of the 200 half-sibling (HS) families for Year 1 across location growth score (GS1); 3-yr spring dry matter across locations (SprDM); across all years and location growth scores for all seasons (GS123); across all years and location growth scores for summer (SumGS), Autumn (AutGS), winter (WinGS), spring (SprGS), and leaf size (LS); and Year 3 (2018–2019) pre-summer stolon number (SNPRS), pre-summer stolon branches (SBPRS), post-summer stolon number (SNPOS), and post-summer stolon branches (SBPOS). Locations are Aorangi and Ruakura. Stolon traits were measured at Aorangi only. \*Significant at the .05 probability level. \*\*Significant at the .01 probability level. \*\*\*Significant at the .001 probability level

winter GS, as well as spring DM yield and LS. Furthermore, correlations between GS1 and either spring DM yield or GS123 were moderately or highly positive, respectively, which highlighted associations between establishment in Year 1 and performance of families at later growth stages. There was a low but positive correlation (.28) between Year 3 (2018–2019) SNPOS and spring DM yield (significant at  $P < .001$ ), whereas the other stolon components had low or nonsignificant correlations with yield-type traits. Leaf size was moderately correlated with GS123 and had a significantly negative correlation with Year 3 (2018–2019) SNPRS. There was a significant ( $P < .001$ ) positive correlation between SN and SB measured in both seasons. The pre-summer stolon traits had a low correlation with the post-summer stolon traits (Figure 4).

### 3.6 | Pattern analysis for seasonal growth scores

The biplot generated from the principal component analysis of mean seasonal growth scores across the two locations and years shows that the Principal Components (PC) 1 and PC2 accounted for 46.6 and 29.8% of the variation, respectively (Figure 5). Angles ( $<90^\circ$ ) among the directional vectors indicate a positive but weak relationship between both locations. An interpretation of the biplot indicates that HS Families 18 and 116 (blue circle) could display broad adaptation across both sites, whereas HS Families 14 and 15 (green circle) could perform better at Aorangi compared to Ruakura, and HS Families 4 and 7 (orange circle) could perform better at Ruakura compared to Aorangi.



**FIGURE 5** Biplot generated from pattern analysis using standardized Best Linear Unbiased Predictor (BLUP) values of 200 half-sibling (HS) families assessed in two locations, Aorangi (Ao) and Ruakura (Ru), over 3 yr for seasonal herbage growth scores: summer (SumAo and SumRu), autumn (AutAo and AutRu), winter (WinAo and WinRu), and spring (SprAo and SprRu). Half-sibling families were clustered to Groups 1 (red), 2 (green), 3 (blue), and 4 (purple). PC, principal component

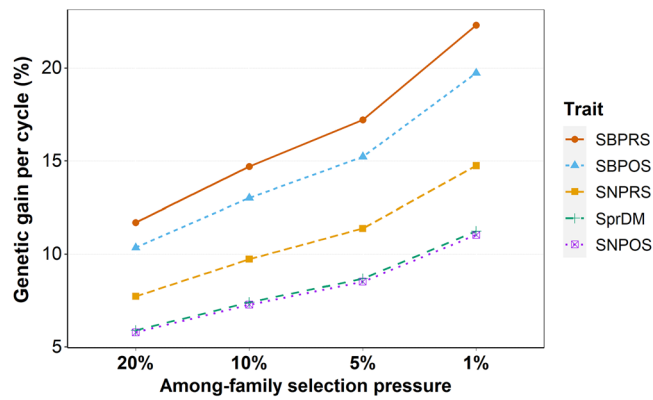
Cluster analysis generated four groups according to the colors of the biplot (Figure 5; Supplemental Table S2). Group 4 (69 families) had the highest mean expression for seasonal growth assessed by growth scores in all four seasons across the two sites. Group 3 had the second highest mean (62 HS families) with the highest yields at Ruakura than at Aorangi. Although Group 1 (45 families) was the third highest with HS families performing better at Aorangi, Group 2 (24 families) consisted of HS families that performed better in some seasons than Groups 1 and 3 but was on average the lowest performing group.

### 3.7 | Predicted genetic gain per cycle

For the spring DM yield estimated across years and locations, using an among-family selection pressure of 20%, the genetic

gain per cycle was predicted to be 5.9%, whereas at a selection pressure of 1%, the gain was 11.24% (Figure 6). When a 20% selection pressure was applied to the 200 HS families, the number of parents available for next selection cycle was 40, but at a 1% selection pressure only two parents would be selected.

For Year 3 (2018–2019) stolon-related traits, as expected, the trend was similar to that observed for spring DM yield, with 1% selection pressure resulting in highest predicted genetic gain per cycle (Figure 6). In relation to each individual trait, the predicted genetic gain per cycle was higher in traits measured pre-summer than post-summer. For instance, at 1% selection pressure, the genetic gain per cycle for SNPRS trait was 14.75%, whereas, for SNPOS trait the increase was only 11.04%. Similar observations were found for SB trait in pre- and post-summer (Figure 6).



**FIGURE 6** Predicted genetic gain per cycle using among family selection strategy with selection pressures of 20, 10, 5, and 1% for spring dry matter (SprDM) yield estimated across all years and locations and third year pre- (PRS) and post (POS)-summer stolon number (SNPRS, SNPOS) and stolon branches (SBPRS, SBPOS)

## 4 | DISCUSSION

Our paper provides a comprehensive study of yield and vegetative persistence related traits in white clover, based on the assessment of 200 HS families across years, seasons, and locations. This is the largest white clover HS family population evaluated as a cattle-grazed mixed sward, multilocation, multi-year trial. Quantitative genetic analysis of the data provided estimates of genetic parameters for the traits measured. Deterministic modelling using the estimated genetic parameters enabled prediction of genetic gain for traits under selection and are indicative of potential genetic advancements that can be achieved using the training population evaluated. These estimates would assist in optimizing breeding strategies for implementing genomic selection.

### 4.1 | Genetic variation and heritability

In this study, significant amounts of family variance were found for spring DM yield, seasonal GS, LS, SN, and SB across years, locations, and seasons. There were significant family  $\times$  year, family  $\times$  location, family  $\times$  season, as well as family  $\times$  year  $\times$  season and family  $\times$  year  $\times$  location interactions for most of the traits. Previous studies by Caradus and Chapman (1991), Caradus et al. (1993), Jahufer et al. (1994), and Jahufer et al. (2009) have also reported significant  $G \times E$  interactions for yield and persistence-related traits in white clover. As expected, for polygenic traits like spring DM yield, GS, SN, and SB, the magnitude of  $G \times E$  interaction was often greater than the family variance, demonstrating a change in relative performance of HS families across spatial and temporal environments (Falconer, 1989; Kang, 2002). By contrast, LS, a trait shown

to be moderately heritable, had higher family variation relative to  $G \times E$ , showing similar performance level of HS family in different environments. This presence of  $G \times E$  implies that spring DM yield at Aorangi would be a poor predictor of spring DM yield at Ruakura as these HS families are highly influenced by the environment type. Although results of the principal component analysis biplot also showed a low correlation between locations, cluster analysis revealed HS families that could be selected for broad adaptation across both locations. For example, HS Families 18 and 116, whereas HS Families 14 and 15 with superior performance in Aorangi, might be selected for specific adaptation to that location. Identifying and harnessing these  $G \times E$  interactions may be a way to ‘future-proof’ forage performance by including trial sites in regions predicted to be under conditions representing a more widespread future climate.

The relative contribution of family  $\times$  year to the total amount of observed variation was lower than family  $\times$  location, indicating that location had a greater effect on seasonal GS than the year of growth. The presence of  $G \times E$  not only complicates comparisons among families but also reduces the efficiency of selection of superior families as the true genetic variation is confounded by environmental effects (Falconer, 1989). Because little success can be expected by phenotypically selecting for these traits in one environment, trials will have to be run across multiple environments and years to assess the true breeding potential of selection candidates, especially if the goal is to breed for broad adaptation (Brown & Caligari, 2008a; Moose & Mumm, 2008; Jahufer et al., 2013).

Heritability estimates for LS was moderate to high (.3 to .73) and were similar to values reported in the previous studies by Caradus and Woodfield (1990), Woodfield and Caradus (1990), and Jahufer et al. (1994). Based on previous findings, traits such as DM yield, GS, SN, and SB, tends to have lower heritability estimates than traits like LS. In this study, heritabilities for spring DM yield were low to moderate (.27–.54) and aligned with those reported by Annicchiarico et al. (1999) (.52), Jahufer et al. (1999) (.38), and Finne et al. (2000) (.44). Heritability for SN (.13–.25) was similar to studies by Caradus and Woodfield (1990), where they reported heritability estimates in the range of .11 to .45. The heritability range for SB (.15–.37) is lower than previously reported value by Rowe and Brink (1993) (.59) and was consistent with Jahufer et al. (1999) (.27) and Caradus and Woodfield (1990) (.37). Low heritabilities can be a result of a trait’s inheritance pattern, insufficient replications in the experimental design, inability to separate spatial variation accurately, and human error in data sampling (Weikai, 2014). Although low heritability value does not signify the lack of genetic differences, it implies that for the observed variation, a substantial proportion is influenced by environmental effects rather than true genetic differences (Visscher et al., 2008).

Genetic improvement through selective breeding for yield and vegetative persistence is possible due to the presence of significant family variation, however, the percentage genetic gain would be low, due to low heritability values. From a breeding perspective, it translates to a difficulty in trait improvement via mass selection or individual phenotypic selection strategies. A more discriminatory breeding strategy such as family-based selection methods, which allows the utilization of both among and within family variation, would significantly drive genetic advancements in the low heritability traits (Casler & Brummer, 2008). The among family phenotypic selection, coupled with genomic selection to accurately select individual plants within the top-performing family, can significantly increase genetic gains for low heritability traits (Faville et al., 2018).

## 4.2 | Year effect on yield

Comparison of three spring DM yield measurements harvested annually across all plots showed that yield differed significantly across years, indicating an influence of year and plant age on yield variability. The decrease in spring DM yield in the third year could be explained by the plants becoming more susceptible to biotic and abiotic stresses, intraspecific competition (Chapman et al., 1996), and reliance on a weak adventitious fibrous root system (Brock & Caradus, 1995). Overall, even though the white clover contribution to yield in a mixed sward is characterized by variability, a major benefit of growing clover with ryegrass is a seasonal complementarity which ensures available feed for livestock all year round (Brougham, 1958; Harris & Thomas, 1973; Caradus et al., 1995).

Despite the high positive correlation between the top 20 HS families identified in Years 2 and 3, the reranking of some families in the third year indicates a switch in HS family performance. Running the trial for 3 yr shows some value in accurately identifying HS families with high vegetative yield and persistence. This is important to inform future selection choices particularly if the goal is not only high biomass yield but also increased longevity of white clover.

## 4.3 | Year and season effect on vegetative persistence

A key problem with white clover is its inability to be sustained in a sufficiently large proportion in mixed swards for its nitrogen fixation and animal production benefits to be realized (Wolledge et al., 1990). Hence, the maintenance of high stolon density is an important trait for white clover vegetative persistence as the stolon is the primary structural unit of the plant (Thomas, 1987). High stolon density allows for bet-

ter capture of light and nutrients to support adequate clover growth (Piano & Annicchiarico, 1995). In our population, the mean stolon number assessed over both years was 1,910 stolons  $m^{-2}$  which was in the range reported by Woodfield and Caradus (1994) (531–2075 stolons  $m^{-2}$ ). Similarly, the mean number of stolons branches assessed in our population over the same period was 2,933 stolon branches  $m^{-2}$  and was in the upper range reported by Jahufer et al. (1995; 1999) (398–3,725 stolon branches  $m^{-2}$ ). It was expected that stolon density, as measured by SN and SB, would be impacted by summer moisture stress, resulting in a decline. However, this was not the case, and two trends were immediately apparent: first, there were more stolons in the HS family trial plot swards after summer than at the start of summer in both years of measurement (2017–2018; 2018–2019) and second, there was a significant decline in the following year's assessment for SN and SB. The increase over summer in the first year was initially assumed to be due to the fragmentation of stolons because of the loss of the taproot system. Despite the relatively dry summers, however, with summer months receiving only 193 and 154 mm of rain over the first two summers, similar results were observed in the following year. Previous research findings on the survival of stolons over summer have been inconsistent, presumably due to the differences in study conditions, climatic patterns, and genetic diversity in HS families used. Whereas Hay et al. (1983), Hay et al. (1987), and Caradus and Williams (1989) found SN to be higher in summer and autumn, Jones (1982) and MacFarlane et al. (1990) reported summer moisture stress caused a decline in stolon numbers. Archer and Robinson (1989) found stolon density to be affected by summer drought only when summer rainfall levels fell below 35 mm and corresponding temperatures were higher than 30 °C. In the current study, mean maximum temperatures were 25 and 24 °C and total rainfall was 193 and 154 mm (Supplemental Figure S1) during the summer periods of both years (2017–2018; 2018–2019). We detected no clear effect on SN and SB at the levels of summer moisture stress and temperature experienced in this trial. This indicates that a combination of higher temperatures and greater moisture deficit may have to be met before stolon density declines.

## 4.4 | Correlation among traits

The high correlation between GS and spring DM yield indicates the suitability to use scores as an indirect measure of spring DM yield. Indirect selections can be made based on visual GS for selecting spring DM yield, if there is a strong correlation between the two traits (Riday, 2009). Because harvesting DM yield requires more time and labor than scores, visual GS allows for more effective resource allocation. Although GS is currently a routine proxy trait for phenotyping DM yield, in the near future, this will change

with advancements in high-throughput phenotyping. However, these tools have limitations to be overcome prior to extensive use in a breeding program. Whereas initial results in white clover look promising in single-spaced plants in bare soil (Inostroza et al., 2016), further development is required to migrate these technologies into mixed sward systems.

The positive correlation between Year 1 growth score (GS1) with growth score Years 1–3 across locations (GS123) and spring DM yield, indicates that good establishment in the first year translated to better performance in later years. Also, the positive significant association between spring DM yield and LS provides an opportunity to select indirectly for increased spring DM yield using LS, when the breeding goal is to develop a large-leaved white clover cultivar, as LS is easier to measure and has a higher heritability (Hallauer & Filho, 1981; Casler, 2012). The trait LS was either not correlated or negatively correlated with SN and SB. This negative correlation is supported by numerous studies (Caradus & Woodfield, 1990; Jahufer et al., 1994; Annicchiarico et al., 1999; Brock & Tilbrook, 2000). The traits SB and SN displayed a significantly high positive correlation, aligning with previous reports by Jahufer et al. (1994). This high correlation can be attributed to pleiotropy or co-inheritance of the favorable alleles for both traits (Lynch & Walsh, 1998).

An interesting finding in our study was the positive phenotypic correlation between SNPOS and spring DM yield (.28) and SNPOS and GS123 (.33). A positive correlation between DM yield and stolon traits has been reported previously by Jahufer et al. (1999), and contrasts with work by Annicchiarico et al. (1999) and Caradus and Williams (1989). This result indicates the population specificity of these correlations, which in this current study, have enabled identification of several promising HS families that are both high yielding and vegetatively persistent. By contrast, the low or nonsignificant correlations between the other stolon components and yield-type traits in this study highlights the challenges faced when attempting to improve stolon-related traits in conjunction with yield.

#### 4.5 | Performance of HS families relative to check cultivars

Assessment of the HS families revealed several that had superior agronomic performance in terms of spring DM yield and vegetative persistence relative to the two commercial checks. However, further investigation into their performance in a wider range of environmental conditions is warranted. Grasslands Bounty, notably, performed significantly better than many of the HS families for SN and SB and was outperformed only by HS Family 31 for SB. These HS families with relatively higher performance relative to the commercial checks give an early insight into the agronomic potential of this train-

ing population and prospects for developing new cultivars that outperform existing cultivars. This could be further enhanced if genomic selection was implemented as additional genetic gains can be made by accessing the three-quarters of additive genetic variation within the family by accurately predicting the breeding values of family selection candidates (Faville et al., 2018; Jahufer et al., 2021).

#### 4.6 | Trade-off between the genetic gain and genetic diversity

From the deterministic modelling conducted, we observed that increasing selection pressure can be used to increase the expected genetic gain. This was expected and aligns clearly with the results reported by Jahufer et al. (2021). These results were due to the selection of families with the highest performance at higher selection pressures. However, application of high selection pressures resulted in only a few parents available for use in the next selection cycle. This could have serious implications in an open-pollinated species like white clover, where breeding schemes aim to increase the frequency of favorable alleles in a population over time while maintaining heterozygosity (Knight, 1979; Brown & Caligari, 2008b). Consequently, there is a trade-off between the percentage of genetic gain that can be achieved per cycle and maintenance of genetic diversity to accumulate favorable alleles over the time in the population to develop cultivars. Furthermore, the predicted genetic gains for spring DM yield and stolon-related traits were based on an among-family phenotypic selection breeding strategy, which exploits only a quarter of total available genetic variance (Casler & Brummer, 2008). Accessing the additional three-quarters of available genetic variance within the family would significantly increase the genetic gain of the target trait. As stated above, by implementing genomic selection the additional three-quarters of genetic variance can be accessed by selecting individual plants accurately within the family based on predicted breeding values (Faville et al., 2018).

### 5 | CONCLUSIONS AND FUTURE DIRECTIONS

A cattle-grazed, multilocation, multiyear field trial of a 200 white clover HS family training population derived from broadly adapted material was established to quantify the magnitude of family and  $G \times E$  variances for key agronomic traits. Family variation and  $G \times E$  interactions were significant for most traits assessed in the two distinct New Zealand environments across multiple years. In particular, significant  $G \times E$  interactions for spring DM yield and stolon traits SN and SB highlight the need for multiyear, multilocation trials. The

presence of family variation and moderate to high narrow-sense heritability suggests that selection in the population can deliver significant genetic gains for yield and persistence. Through deterministic modelling, we have demonstrated that applying high selection pressure produces high genetic gain, however, there needs to be a trade-off between genetic gain and diversity in the population for the long-term success of a breeding program. Furthermore, this modelling was based on an among-HS family breeding strategy and emphasized that methodologies that exploit the within-family variation, such as genomic selection, would expect to show greater genetic gain. In terms of key agronomic traits, the moderate positive correlation observed between spring DM yield and SNPOS has important implications and both traits can be combined to develop selection indices to drive genetic improvement for both yield and vegetative persistence in this population.

The scale of trait phenotyping in a multisite trial of this size was time- and labor-intensive and highlighted the value of developing automated phenotyping methodologies to reduce input. This would release resources that could be diverted into more frequent phenotyping and/or a larger set of families. This trial and population have provided important quantitative genetic assessment of material that can be used to evaluate genomic selection and will be a significant base for developing new white clover cultivars.

#### DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article and Supplementary Material, including best linear unbiased predictors for the traits described. Further inquiries can be directed to the corresponding authors.

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#### AUTHOR CONTRIBUTIONS


O. Grace Ehoche: Data curation; Formal analysis; Investigation; Validation; Visualization; Writing – original draft; Writing – review & editing. Sai Krishna Arojju: Formal analysis; Visualization; Writing – original draft; Writing –

review & editing. Greig Cousins: Investigation; Resources. Jessica R. O'Connor: Investigation. Brian Maw: Investigation. Jennifer A. Tate: Supervision; Writing – original draft; Writing – review & editing. Peter J. Lockhart: Supervision; Writing – original draft; Writing – review & editing. M. Z. Zulfi Jahufer: Conceptualization; Formal analysis; Funding acquisition; Supervision; Writing – original draft; Writing – review & editing. Andrew G. Griffiths: Conceptualization; Funding acquisition; Project administration; Supervision; Writing – original draft; Writing – review & editing.

#### CONFLICT OF INTEREST

Authors declare research was conducted in the absence of any commercial and financial partners involvement in the design and interpretation that could be construed as a potential conflict of interest.

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