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DOI:

[10.1111/nph.15284](https://doi.org/10.1111/nph.15284)

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*Document Version*

Peer reviewed version

*Citation for published version (Harvard):*

Luo, Z, Leach, L & Chen, J 2018, 'Orthogonal contrast based models for quantitative genetic analysis in autotetraploid species' *New Phytologist*. <https://doi.org/10.1111/nph.15284>

[Link to publication on Research at Birmingham portal](#)

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Checked for eligibility: 22/05/2018

This is the peer reviewed version of the following article: Chen, J. , Zhang, F. , Wang, L. , Leach, L. and Luo, Z. (2018), Orthogonal contrast based models for quantitative genetic analysis in autotetraploid species. *New Phytol.*, which has been published in final form at: <https://doi.org/10.1111/nph.15284>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

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1 **Orthogonal Contrast Based Models for Quantitative Genetic Analysis in Autotetraploid Species**

2  
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8  
9 Key words: Quantitative Genetic Models, Orthogonal Contrasts, Double Reduction,  
10 Tetrasomic Inheritance, Autotetraploids

11  
12 \* these authors contributed equally to the research

13  
14  
15 Word count total = 6496

16 Introduction (969); Materials and Methods (2076); Results (2065); Discussion (1356);

17 Acknowledgements (30)

18 Number of figures = 1 (figure 1 to be published in colour)

19 Number of tables = 8

20 Supporting information: (2 figures, 5 methods, 1 note and 6 tables)

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### Summary (200 words)

- Dissecting the genetic architecture of quantitative traits is a crucial goal for efficient breeding of polyploid plants, including autotetraploid crop species, such as potato and coffee, and ornamentals such as rose. To meet this goal, a quantitative genetic model is needed to link the genetic effects of genes or genotypes at quantitative trait loci to the phenotype of quantitative traits.
- We present a statistically tractable quantitative genetic model for autotetraploids based on orthogonal contrast comparisons in the general linear model. The new methods are suitable for autotetraploid species with any population genetic structure and take full account of the essential features of autotetrasomic inheritance. The statistical properties of the new methods are explored and compared to an alternative method in the literature by simulation studies.
- We have shown how these methods can be applied for quantitative genetic analysis in autotetraploids by analysing trait phenotype data from an autotetraploid potato segregating population. Using trait segregation analysis, we showed that both highly heritable traits of flowering time and plant height were under the control of major QTL.
- The orthogonal model directly dissects genetic variance into independent components and gives consistent estimates of genetic effects provided that tetrasomic gene segregation is considered.

Key words: autotetraploids, double reduction, orthogonal, polyploid, potato, quantitative genetic model

60 **Introduction**

61 Polyploidy plays an important role in the evolution of eukaryotes, especially for flowering plants,  
62 all of which have undergone at least one round of polyploidization in their evolutionary history  
63 (Otto & Whitton, 2000; Jiao *et al.*, 2011). Between 30-80% of species are currently polyploids,  
64 while the rest exist as paleopolyploids (Wolfe, 2001), having undergone a gradual process of  
65 “diploidization” over evolutionary time. Many of the world’s most important crop species are either  
66 autopolyploid, for example, the autotetraploid potato, coffee, and alfalfa, or allopolyploid, including  
67 wheat, oats and canola. Several economically important aquaculture animals are also autotetraploids,  
68 including Atlantic salmon and trout (Danzmann & Garbi, 2001; Vaughn *et al.*, 2007). Therefore, in  
69 order to address the global food security crisis, rigorous genetic analysis of autopolyploid species  
70 becomes a timely task.

71

72 Most biological characters important in organismal evolution and relevant to plant and animal  
73 breeding, such as reproductive isolation, yield, quality and resistance to biotic and abiotic stresses,  
74 are quantitative traits affected by genes at more than a single locus, as well as by environmental  
75 factors. Understanding the polygenic architecture underlying such quantitative traits is essential to  
76 enable their genetic improvement as part of effective plant or animal breeding programs. However,  
77 progress in quantitative genetic analysis in polyploid species lags far behind compared to that  
78 achieved in diploids for several major reasons.

79

80 Firstly, polyploids display a much more complicated pattern of gene segregation and recombination  
81 than diploids. For example, multiple alleles at individual loci of polyploids cause a substantially  
82 wider spectrum of genotypic segregation. In autopolyploids, multivalent pairing of homologous  
83 chromosomes during meiosis may result in the phenomenon of double reduction, in which identical  
84 alleles carried on sister chromatids enter into the same gamete, resulting in systematic allelic  
85 segregation distortion. Our studies (Luo *et al.*, 2006a) show that recombination frequency between a  
86 pair of loci can be as high as 75% under a tetrasomic model (compared to 50% in diploids) and that  
87 double reduction can occur at a frequency of 25%, showing the remarkable difference in the pattern  
88 of gene segregation and recombination between diploid and autopolyploid species. These factors  
89 have made polysomic genetic analysis one of the most challenging topics in theoretical and applied  
90 genetics since the pioneering works of quantitative geneticists such as Haldane, Mather and Fisher  
91 (Haldane, 1930; Mather, 1936; Fisher, 1947).

92

93 Secondly, the evolution of polyploid genomes is an extremely dynamic process compared to that of  
94 diploids, characterized by extensive genetic and epigenetic changes occurring in the nuclear

95 genome following polyploidization (Soltis & Soltis, 1995; Song *et al.*, 1995; Comai *et al.*, 2000;  
96 Adams & Wendel, 2005). Genome structure and function of polyploids may therefore differ  
97 markedly from that of their diploid relatives. This necessitates that breeding programs targeted at  
98 improving genetic performance of an autopolyploid species should ideally be conducted at the  
99 polyploid level rather than with its diploid counterparts.

100

101 The quantitative genetic model which links genetic effects of genes or genotypes at quantitative trait  
102 loci to the phenotype of quantitative traits is an essential basis for any quantitative genetic analysis.  
103 The theory and methods for modelling and analysing quantitative genetic effects have been well  
104 established and routinely practised in diploid species (Mather & Jinks, 1971; Falconer, 1989; Lynch  
105 & Walsh, 1998). In contrast, there are no methods currently available for modelling quantitative  
106 genetic effects in autotetraploids that take proper account of the complex features of autotetrasomic  
107 inheritance.

108

109 Early models for the quantitative genotypic effects at a single locus in randomly mating  
110 autotetraploid populations (Kempthorne, 1955; Kempthorne, 1957) were intractable for real data  
111 analysis because they involve a large number of genetic parameters. Li (1957) developed a  
112 simplified two-allele version of Kempthorne's model and proposed successive linear regression of  
113 genetic values of genotypes onto the corresponding frequencies in a tetraploid population under  
114 Hardy-Weinberg equilibrium (HWE). This model allowed genetic variance at a single locus to be  
115 represented by only four major components.

116

117 Mather and Jinks (1971) extended their concept of additive and dominance effects for quantitative  
118 genetic analysis in diploids to define these effects in tetraploids (Mather & Jinks, 1971). Analysis  
119 with any quantitative genetic model involves the distribution of genotypes at quantitative trait loci  
120 (QTL) in the population under study. In autotetraploids, this distribution depends on the coefficient  
121 of double reduction (Luo *et al.*, 2004). Killick (1971) therefore explored the influence of double  
122 reduction on Mather and Jink's additive-dominance model for autotetraploids. Nevertheless, all of  
123 the classical additive-dominance models developed either for diploids, autotetraploids, or more  
124 recently autohexaploids (van Geest *et al.*, 2017), share the undesirable property of correlation  
125 between estimates of different types of effects in the model (Li, 1957; Killick, 1971; Wright, 1979;  
126 Li *et al.*, 2010; van Geest *et al.*, 2017). This correlation structure may bias estimation of the model  
127 parameters and variance components of the genetic effects. Addressing this limitation, Cockerham  
128 (1954) pioneered in developing a quantitative genetic model for diploids based on the principle of  
129 orthogonal linear comparison, which enables phenotypic variation of a quantitative trait to be

130 partitioned in a way that ensures independence between different model effects, enabling direct  
131 dissection of genetic variance into independent components (Cockerham, 1954).

132

133 This paper presents a novel and statistically tractable tetrasomic quantitative genetic model based  
134 on orthogonal contrasts that are suitable for use with either natural or artificially created populations  
135 of autotetraploid species. The model represents the first example of quantitative genetic models for  
136 autotetraploids that take account of the essential features of tetrasomic inheritance, including double  
137 reduction, while retaining computational feasibility. The statistical properties of these new models  
138 are explored and compared with another method in the literature by computer simulation analyses.  
139 We have demonstrated their utility in quantitative genetic analyses of autotetraploid species by  
140 analysing trait phenotype data from an outbred segregating population of autotetraploid potato.

141

142

## Materials and Methods

### 143 General one locus model

144 We first consider segregation of two alleles ( $A$  and  $a$ ) at a single locus in an autotetraploid  
145 population. There are a total of 5 possible genotypes at the biallelic locus, namely  $AAAA$   
146 (quadruplex),  $AAAa$  (triplex),  $AAaa$  (duplex),  $Aaaa$  (simplex) and  $aaaa$  (nulliplex). The  $i$ th  
147 genotype  $A_i a_{4-i}$ , is defined with a genotypic value of  $G_i$  and its frequency in the population is  
148 denoted by  $f_i$ , with  $i = 0, 1, \dots, 4$  indicating the number of  $A$  alleles involved in the genotype, as  
149 shown in Table 1. In practice, there may be more than two QTL alleles. However, these may be  
150 grouped into the two classes of either increasing alleles or decreasing alleles, based on their effects  
151 on the trait phenotype. This effectively reduces the maximum number of possible genotypes at a  
152 locus down to 5 (++++, +++-,+---,+----,----) in any population, creating a tractable model.

153

154 We define here the genotypic effect for an individual through a regression model of allelic effects

155

$$156 \quad G = \mu + x_1\theta_1 + x_2\theta_2 + x_3\theta_3 + x_4\theta_4 \quad \text{Eqn 1}$$

157

158 where  $\mu$  is the population mean, and  $\theta_i$  ( $i = 1, \dots, 4$ ) are accordingly the monogenic, digenic, trigenic  
159 and quadrigenic genetic effects of the QTL, and  $x_i$  ( $i = 1, \dots, 4$ ) are the corresponding genetic effect  
160 design variables. The monogenic effect will always be positive and represents the average effect  
161 caused by substituting allele  $A$  for allele  $a$  at the QTL. The digenic effect represents the average  
162 interaction effect between two alleles in a tetraploid genotype, denoted  $I_{Aa}$  in the biallelic model.  
163 The trigenic effect represents the average interaction effects among three alleles. Existence of a  
164 trigenic effect means that the interaction between two alleles differs according to the identity of the

165 third allele. In the model, there are two different three-way interactions, given by  $I_{AAa}$  and  $I_{Aaa}$ .  
 166 The quadrigenic effect represents the average interaction effects among four alleles. Existence of  
 167 quadrigenic effects means that the interaction between two alleles differs depending on the identity  
 168 of the third and fourth alleles. In the biallelic model, there are three different four-way interactions,  
 169 given by  $I_{AAAA}$ ,  $I_{AAaa}$  and  $I_{Aaaa}$ . If there are no two-way, (three-way, four-way) allelic interactions,  
 170 then the corresponding monogenic (trigenic, quadrigenic) genetics effects will be equal to zero. A  
 171 more detailed explanation of the genetic effects is given in Supporting Information Method S1, Fig.  
 172 S1 and Table S1.

173

### 174 **Estimation of genetic effects in the one locus model**

175 In a natural autotetraploid population, genotypic frequencies vary across different loci in the  
 176 genome and are usually not in Hardy-Weinberg equilibrium (Luo *et al.*, 2000). Orthogonal contrasts  
 177 provide a way to partition genetic variance into independent components (Zeng *et al.*, 2005). We  
 178 propose here general orthogonal scales  $w_{ij}$  for the genetic effects of genotype  $i$  for the  $j$ th contrast ( $j$   
 179  $= 1,2,\dots,4$ ), corresponding to monogenic, digenic, trigenic and quadrigenic genetic effects. The  
 180 orthogonal scales are summarized in Table 1 and must satisfy a number of requirements to ensure  
 181 that the comparisons are orthogonal, *i.e.* uncorrelated, as follows.

182 
$$\sum_{i=0}^4 f_i = 1; \text{ and}$$

183 1). For monogenic effects  $w_{i1}$  ( $i = 0,1,\dots,4$ )

184 
$$\begin{cases} \sum_{k=0}^4 w_{k1} f_k = 0 \\ w_{i1} = w_{01} + i \end{cases} \quad (i = 1,2,3,4)$$

185 2). For digenic effects  $w_{i2}$  ( $i = 0,1,\dots,4$ )

186 
$$\begin{cases} w_{(i+1)2} - 2w_{i2} + w_{(i-1)2} = 1 & (i = 1,2,3) \\ \sum_{k=0}^4 w_{k2} f_k = 0 \\ \sum_{k=0}^4 w_{k2} w_{k1} f_k = 0 \end{cases}$$

187 3). For trigenic effects,  $w_{i3}$  ( $i = 0,1,\dots,4$ )

188 
$$\begin{cases} w_{(i+1)3} - 3w_{i3} + 3w_{(i-1)3} - w_{(i-2)3} = 1 & (i = 2,3) \\ \sum_{k=0}^4 w_{k3} f_k = 0 \\ \sum_{k=0}^4 w_{k3} w_{k1} f_k = 0 \\ \sum_{k=0}^4 w_{k3} w_{k2} f_k = 0 \end{cases}$$

189 4). For quadrigenic effects,  $w_{i4}$  ( $i = 0, 1, \dots, 4$ )

$$190 \quad \begin{cases} w_{44} - 4w_{34} + 6w_{24} - 4w_{14} + w_{04} = 1 \\ \sum_{k=0}^4 w_{k4} f_k = 0 \\ \sum_{k=0}^4 w_{k4} w_{k1} f_k = 0 \\ \sum_{k=0}^4 w_{k4} w_{k2} f_k = 0 \\ \sum_{k=0}^4 w_{k4} w_{k3} f_k = 0 \end{cases}$$

191  
 192 The above 1) - 4) ensure the key statistical properties of the orthogonal model as shown by Eqn (1).  
 193 Firstly,  $\sum_{i=0}^4 w_{ij} f_i = 0$  for  $j = 1, \dots, 4$  ensures the statistical definition of  $w_{ij}$  as contrast scales, which  
 194 in turn define the design variables  $x_i$  in Eqn (1). Secondly,  $\sum_{i=0}^4 w_{ij} w_{ik} f_i = 0$  for  $1 \leq j \neq k \leq 4$   
 195 ensures the orthogonality between the contrast scales  $w_{ij}$  and  $w_{ik}$  ( $i = 0, \dots, 4$ ;  $1 \leq j \neq k \leq 4$ ). The  
 196 orthogonal scales calculated as above are then used to derive the genetic effect design variables in  
 197 Eqn (1) as below

$$198 \quad x_j = \begin{cases} w_{4j} & \text{if } G \text{ is } AAAA \\ w_{3j} & \text{if } G \text{ is } AAAa \\ w_{2j} & \text{if } G \text{ is } AAaa \\ w_{1j} & \text{if } G \text{ is } Aaaa \\ w_{0j} & \text{if } G \text{ is } aaaa \end{cases} \quad (j = 1, 2, \dots, 4)$$

200  
 201 We can then express the orthogonal model for the QTL effects at locus A in a matrix form given by

$$202 \quad G_A = \begin{bmatrix} G_4 \\ G_3 \\ G_2 \\ G_1 \\ G_0 \end{bmatrix} = S_A E_A = \begin{bmatrix} 1 & w_{41} & w_{42} & w_{43} & w_{44} \\ 1 & w_{31} & w_{32} & w_{33} & w_{34} \\ 1 & w_{21} & w_{22} & w_{23} & w_{24} \\ 1 & w_{11} & w_{12} & w_{13} & w_{14} \\ 1 & w_{01} & w_{02} & w_{03} & w_{04} \end{bmatrix} \begin{bmatrix} \mu \\ \theta_1 \\ \theta_2 \\ \theta_3 \\ \theta_4 \end{bmatrix} \quad \text{Eqn 2}$$

204  
 205 where  $S_A$  is the genetic effects design matrix and  $E_A$  is the genetic effects of the QTL genotypes,  
 206 which can be calculated from

$$207 \quad E_A = S_A^{-1} G_A \quad \text{Eqn 3}$$

209  
 210 Accordingly, the five QTL genotypic values can be specified under the orthogonal model as

$$211 \quad G_i = \mu + w_{i1} \theta_1 + w_{i2} \theta_2 + w_{i3} \theta_3 + w_{i4} \theta_4 \quad (i = 0, 1, \dots, 4)$$

213



214 The total genetic variance  $V_G$ , contributed by allele segregation at the QTL, can be partitioned into  
 215 four independent components of variance. Each variance component is contributed by its own  
 216 corresponding genetic parameter as

$$217 \sigma_t^2 = \frac{\left(\sum_{i=0}^4 f_i G_i w_{it}\right)^2}{\left(\sum_{i=0}^4 f_i w_{it}^2\right)} \quad \text{Eqn 4}$$

219 where  $t = 1, 2, \dots, 4$  corresponds to the four orthogonal scales defined for the monogenic, digenic,  
 220 trigenic and quadrigenic genetic effects, and  $i (= 0, 1, \dots, 4)$  indicates the number of A alleles in the  
 221 QTL genotype. The significance of the estimated genetic effects can be tested using the one- or  
 222 two-tailed  $t$ -test, with the standard error given by  $\sqrt{\sigma_t^2} / \sqrt{n}$  (degree of freedom equals to  $n-4$ ),

223 where  $\sigma_t^2$  is the estimated variance for the  $t^{\text{th}}$  contrast and can be calculated by  $\hat{\sigma}_e^2 \cdot \sum_{i=0}^4 w_{it}^2 / f_i$ ,

224 where  $\hat{\sigma}_e^2$  is the estimated residual variance and  $n$  is the sample size. In this work, we characterize  
 225 and illustrate the model (1) in two specific populations, an  $S_2$  population (below) and a randomly  
 226 mating population (Supporting Information Method S2), though the model is generic for  
 227 populations with any given genetic structure. It should be noted that the variance components here  
 228 refer to genetic variances contributed by monogenic, digenic, trigenic or quadrigenic effects in the  
 229 model, rather than the variances of the contrasts.

### 230 231 232 **One locus model for an $S_2$ population**

233 In the second generation segregating population, denoted by  $S_2$ , created from crossing two parental  
 234 autotetraploid lines with genotypes AAAA and aaaa, the frequencies of the offspring genotypes can  
 235 be expressed in terms of  $\alpha$ , the coefficient of double reduction at the QTL, as,  $f_0 = (1+2\alpha)^2 / 36$ ,  
 236  $f_1 = 2(1-\alpha)(1+2\alpha) / 9$ ,  $f_2 = [3-4\alpha(1-\alpha)] / 6$ ,  $f_3 = 2(1-\alpha)(1+2\alpha) / 9$  and  $f_4 = (1+2\alpha)^2 / 36$ . The  
 237 corresponding orthogonal contrast scales are summarized in Table 2. The genotypic values  $G_A =$   
 238  $(G_4 \ G_3 \ G_2 \ G_1 \ G_0)^T$  can be presented in a matrix form given by

$$239 \quad G_A = S_A E_A = \begin{bmatrix} 1 & 2 & (5-2\alpha)/3 & 2(1-\alpha)/3 & (1-\alpha)(4\alpha^2-4\alpha+3)/(12(2+\alpha)) \\ 1 & 1 & (1-4\alpha)/6 & -(1+2\alpha)/6 & -(1+2\alpha)(4\alpha^2-4\alpha+3)/(24(2+\alpha)) \\ 1 & 0 & -(1+2\alpha)/3 & 0 & (1-\alpha)(2\alpha+1)^2/(12(2+\alpha)) \\ 1 & -1 & (1-4\alpha)/6 & (1+2\alpha)/6 & -(1+2\alpha)(4\alpha^2-4\alpha+3)/(24(2+\alpha)) \\ 1 & -2 & (5-2\alpha)/3 & -2(1-\alpha)/3 & (1-\alpha)(4\alpha^2-4\alpha+3)/(12(2+\alpha)) \end{bmatrix} \begin{bmatrix} \mu \\ \theta_1 \\ \theta_2 \\ \theta_3 \\ \theta_4 \end{bmatrix}$$

241

242 The genetic effects of the QTL genotypes can be calculated from  $E_A = S_A^{-1}G_A$  where

243

$$S_A^{-1} = \begin{bmatrix} (1+2\alpha)^2/36 & 2(1+2\alpha)(1-\alpha)/9 & (4\alpha^2-4\alpha+3)/6 & 2(1+2\alpha)(1-\alpha)/9 & (1+2\alpha)^2/36 \\ (1+2\alpha)/12 & (1-\alpha)/3 & 0 & -(1-\alpha)/3 & -(1+2\alpha)/12 \\ \frac{(1+2\alpha)(5-2\alpha)}{12(2+\alpha)} & \frac{(\alpha-1)(4\alpha-1)}{3(2+\alpha)} & -\frac{(4\alpha^2-4\alpha+3)}{2(2+\alpha)} & \frac{(\alpha-1)(4\alpha-1)}{3(2+\alpha)} & \frac{(1+2\alpha)(5-2\alpha)}{12(2+\alpha)} \\ 1/2 & -1 & 0 & 1 & -1/2 \\ 1 & -4 & 6 & -4 & 1 \end{bmatrix}$$

245

246 **General two locus model**

247 The one locus method described above is extended to two biallelic loci, A and B, in an  
 248 autotetraploid population with a specified genetic structure. There will be twenty-five possible  
 249 genotypes at the two loci (without accounting for linkage phase). A general form for the two-locus  
 250 tetraploid genotype may be given as  $A_i a_{(4-i)} B_j b_{(4-j)}$  with  $i = 0, 1, \dots, 4$  for the number of A alleles and  
 251  $j = 0, 1, \dots, 4$  for the number of B alleles in the genotype. The genotypic value and genotype  
 252 frequency are denoted by  $G_{ij}$  and  $f_{ij}$ . The marginal frequencies of the genotypes at locus A and  
 253 locus B are denoted by  $f_i$  and  $f_j$  ( $i = 0, 1, \dots, 4$ ). Without loss of generality, locus A is assumed to be  
 254 closer to the centromere than locus B and the coefficients of double reduction at the two loci are  
 255 denoted by  $\alpha$  and  $\beta$ , respectively. A linear model for the genotypic value is comprised of genetic  
 256 effects at each of the two loci and epistatic effects between the genes at the two loci, and is fully  
 257 characterized by a total of twenty-five parameters in the form of a regression model of allelic effects  
 258 analogous to equation (1), as follows:

259

$$G_{ij} = \mu + x_1\theta_1 + x_2\theta_2 + x_3\theta_3 + x_4\theta_4 + y_1\zeta_1 + y_2\zeta_2 + y_3\zeta_3 + y_4\zeta_4 + z_{\theta_1\zeta_1}I_{\theta_1\zeta_1} + z_{\theta_2\zeta_2}I_{\theta_2\zeta_2} +$$

$$z_{\theta_3\zeta_3}I_{\theta_3\zeta_3} + z_{\theta_4\zeta_4}I_{\theta_4\zeta_4} + z_{\theta_2\zeta_1}I_{\theta_2\zeta_1} + z_{\theta_2\zeta_2}I_{\theta_2\zeta_2} + z_{\theta_2\zeta_3}I_{\theta_2\zeta_3} + z_{\theta_2\zeta_4}I_{\theta_2\zeta_4} + z_{\theta_3\zeta_1}I_{\theta_3\zeta_1} +$$

$$z_{\theta_3\zeta_2}I_{\theta_3\zeta_2} + z_{\theta_3\zeta_3}I_{\theta_3\zeta_3} + z_{\theta_3\zeta_4}I_{\theta_3\zeta_4} + z_{\theta_4\zeta_1}I_{\theta_4\zeta_1} + z_{\theta_4\zeta_2}I_{\theta_4\zeta_2} + z_{\theta_4\zeta_3}I_{\theta_4\zeta_3} + z_{\theta_4\zeta_4}I_{\theta_4\zeta_4}$$
Eqn 5

261

262 where  $\mu$  is the population mean,  $\theta_i$  (or  $\zeta_i$ ) ( $i = 1, \dots, 4$ ) are accordingly monogenic, digenic,  
 263 trigenic and quadrigenic genetic effects at locus A (or locus B), and  $x_i$  (or  $y_i$ ) ( $i=1, \dots, 4$ ) are the  
 264 design variables for the corresponding genetic effects at locus A (or locus B), as summarised in  
 265 Table 3.  $I_{\theta_i\zeta_j}$  are the epistasis parameters between the effects  $\theta_i$  and  $\zeta_j$  ( $i=1, \dots, 4; j=1, \dots, 4$ ). For  
 266 example,  $I_{\theta_1\zeta_1}$  is the monogenic x monogenic effect of loci A and B. The corresponding design

267 variables are given by  $z_{\theta, \zeta_j}$ . A full definition of all 25 genetic effect parameters is given in  
 268 Supporting Information Table S2.

269

270 In a similar but algebraically more tedious way to the one locus model, we derived the orthogonal  
 271 contrast scales for the two-locus tetrasomic model under two different settings regarding the mutual  
 272 dependency of genotypes at the two loci: linkage equilibrium (Supporting Information Method S3)  
 273 and linkage disequilibrium (Supporting Information Method S4). We would like make it clear that  
 274 given a feasible sample size, it is impractical to estimate all of the parameters in the above two-  
 275 locus model (Eqn 5). To tackle this practical limitation, we suggest use of a reduced model, in  
 276 which the focus is on the interaction parameters of interest, as shown in Supporting Information  
 277 Method S5.

278

### 279 **Detection of major gene segregation in an outbred autotetraploid population**

280 The segregation analysis models the trait phenotype data distribution as a mixed distribution in  
 281 which each component distribution corresponds to a particular genotype of the major QTL. We  
 282 illustrate a trait phenotype based segregation analysis by modeling the trait phenotype data using the  
 283 following likelihood function of  $m$  mixed normal distributions

284

$$285 \quad L(G, \sigma^2 | Y, G_{P_1}, G_{P_2}, \alpha) = \prod_{i=1}^n \sum_{j=0}^{m-1} f_j(G_{P_1}, G_{P_2}, \alpha) g_j(y_i; G_j, \sigma^2) \quad \text{Eqn 6a}$$

286

287 where  $m$  represents the number of segregating QTL genotypes with the genotypic value vector,  
 288  $G = (G_0 \mathbf{K} G_{m-1})$ ,  $\sigma^2$  is the residual variance,  $G_{P_1}$  and  $G_{P_2}$  denote the two parental QTL genotypes,  
 289  $Y = \{y_1, y_2, \mathbf{K}, y_n\}$  represents the offspring trait data,  $\alpha$  denotes the coefficient of double  
 290 reduction,  $f_j(G_{P_1}, G_{P_2}, \alpha)$  ( $j=0, \dots, m-1$ ) indicates the frequency of the QTL genotype  $Q_j q_{4-j}$  and  
 291  $g_j(y_i; G_j, \sigma^2)$  is the probability density function of a normal distribution with mean  $G_j$  and  
 292 variance  $\sigma^2$ .

293

294 To estimate the genetic effect parameters, we first need to calculate the mean for each QTL  
 295 genotype from the offspring population, which is equivalent to estimating the means for a finite  
 296 mixture of component distributions. For any given parental QTL genotypes and the coefficient of  
 297 double reduction at the putative major QTL, the parameters can be estimated from standard normal  
 298 mixture model analysis using the EM algorithm (Dempster, 1977). In the present context, offspring  
 299 QTL genotypes were unknown but can be inferred either from the individual's genotype

300 information at marker loci nearby to the QTL, as we developed previously (Luo *et al.*, 2000; Luo *et al.*, 2004), or from their parental genotypes at the QTL. A modified version of equation (6a)  
 301 incorporating parental marker information, given by  $M_{P_1}$ ,  $M_{P_2}$ , and offspring marker information  
 302 given by  $O_i$ , is given as follows  
 303

$$304 \quad L(G, \sigma^2 | Y, G_{P_1}, G_{P_2}, \alpha) = \prod_{i=1}^n \sum_{j=0}^{m-1} f_{ij}(G_{P_1}, G_{P_2}, \alpha, r, M_{P_1}, M_{P_2}, O_i) g_j(y_i; G_j, \sigma^2) \quad \text{Eqn 6b}$$

306 where  $f_{ij}$  is the QTL genotype frequency for the  $i$ th individual and the  $j$ th QTL genotype,  
 307 calculated according to equations (3) and (4) in the multi-locus linkage analysis we developed  
 308 previously (Leach *et al.*, 2010).  
 309

310 Assuming biallelic segregation at a putative QTL, there are a total of twelve possible autotetraploid  
 311 parental genotype configurations, listed as (1)  $aaaa \times Aaaa$ , (2)  $aaaa \times AAaa$ , (3)  $aaaa \times AAAa$ , (4)  
 312  $Aaaa \times AAaa$ , (5)  $Aaaa \times AAAa$ , (6)  $Aaaa \times AAAA$ , (7)  $AAaa \times AAAa$ , (8)  $AAaa \times AAAA$ , (9)  
 313  $AAAa \times AAAA$ , (10)  $Aaaa \times Aaaa$ , (11)  $AAaa \times AAaa$ , and (12)  $AAAa \times AAAa$ . We conducted a  
 314 scan of the likelihood function (Eqn 6a) over all twelve parental genotype configurations and over  
 315 all different levels of double reduction from its minimum value of 0.00 to the maximum of 0.25, at  
 316 every increment of 0.005. Given a parental genotype configuration  $(G_{P_1}, G_{P_2})$ , the frequency of  
 317 QTL genotype  $Q_j q_{4-j}$  denoted  $f_j(G_{P_1}, G_{P_2}, \alpha)$  ( $j=0, \dots, m-1$ ), can be calculated as a function of  
 318 the coefficient of double reduction  $\alpha$ .  
 319

320 The EM algorithm is initialised with starting values for the QTL genotypic values by using k-means  
 321 cluster analysis. The sample variance is used to initialise  $\sigma^2$ . It then involves iterating the E-step  
 322 that calculates the conditional probability of the  $i^{\text{th}}$  individual having the QTL genotype  $Q_j q_{4-j}$ , i.e.  
 323

$$324 \quad \omega_{ij} = \frac{f_j(G_{P_1}, G_{P_2}, \alpha) g_j(y_i; G_j, \sigma^2)}{\sum_{k=0}^{m-1} f_k(G_{P_1}, G_{P_2}, \alpha) g_k(y_i; G_k, \sigma^2)} \quad \text{Eqn 7}$$

325 and the M-step that calculates the maximum likelihood estimates (MLEs) of the model parameters  
 326 given the conditional probabilities from the above E step from the following formula

$$327 \quad \hat{G}_j = \frac{\sum_{i=1}^n \omega_{ij} y_i}{\sum_{i=1}^n \omega_{ij}} \quad \text{Eqn 8}$$

328 
$$\hat{\sigma}^2 = \sum_{i=1}^n \sum_{j=0}^{m-1} \omega_{ij} (y_i - \hat{G}_j)^2 / n$$
 Eqn 9

329 The E-step and M-step are repeated iteratively until convergence.

330

331 We calculated the log-likelihood ratio statistic (LRT)

332

333 
$$LRT = 2 \left[ L(\hat{G}^*, \hat{\sigma}^{*2} | Y, G_{P_1}, G_{P_2}, \alpha) - L(\bar{G}, \bar{\sigma}^2 | Y, G_{P_1}, G_{P_2}, \alpha) \right]$$
 Eqn 10

334

335 as a statistical test for significance of major QTL segregation in the population under study. In Eqn  
 336 10,  $\hat{G}^*$  and  $\hat{\sigma}^{*2}$  are the MLEs of the genotypic means and residual variance, while  $\bar{G}$  and  $\bar{\sigma}^2$  are  
 337 the mean and variance of the trait calculated from all individuals. Each model was compared with  
 338 the null model assuming no major gene to be segregating in the population, by applying the  
 339 likelihood-ratio test (LRT). The LRT statistic in the present context asymptotically follows a chi-  
 340 square distribution with  $m-1$  degrees of freedom, with  $m$  equal to the number of QTL genotypes  
 341 segregating as defined above.

342

### 343 **Estimation of overlap between normal densities**

344 We proposed an average overlapping coefficient (*aOVL*) to define a disparity index for quantifying  
 345 the average difference between any two component normal distributions. The overlap coefficient  
 346 between two normal distributions has been defined (Inman & Bradley, 1989) as

347

348 
$$OVL = 2\Phi\left(-\frac{|\mu_1 - \mu_2|}{\sigma}\right)$$
 Eqn 11

349 where  $\Phi$  denotes the cumulative distribution function of the standard normal distribution,  $\mu_1$  and  
 350  $\mu_2$  are the means of the two component normal distributions, and  $\sigma^2$  is the variance for the  
 351 component normal distribution. In the tetraploid case, there are  $k$  ( $k = 2, \dots, 5$ ) components in the  
 352 mixture normal distribution and the corresponding *aOVL* could be calculated by

353

354 
$$aOVL = \sum_{i=1}^k \sum_{j=i+1}^k 2\Phi\left(-\frac{|G_i - G_j|}{\sigma}\right) / \binom{2}{k}$$
 Eqn 12

355

356 *aOVL* takes a value between 0 and 1, with larger values indicating that the component normal  
 357 distributions are less well separated.

358

### 359 **Simulated autotetraploid populations**

360 Simulated populations were created by developing programs to mimic the gametogenesis of an  
361 autotetraploid individual with a given genotype and random union of gametes to generate a zygote.  
362 Segregation and recombination of alleles at the loci of interest were simulated under tetrasomic  
363 inheritance, as explained in detail elsewhere (Luo *et al.*, 2000). Given a simulated genotype for any  
364 offspring individual, the phenotype of the individual was determined as the sum of the genotypic  
365 value calculated from the corresponding simulated model, developed here as shown in equations (1)  
366 or (5), or developed by Killick (1971) (see Supporting Information Note S1), and a variable  
367 randomly sampled from a normal distribution,  $N(0, \sigma^2)$ . The residual variance was calculated  
368 according to the prior phenotypic variance of the trait in question and the heritability of the  
369 simulated QTL.

370

### 371 **Segregating autotetraploid potato population**

372 A first generation segregating population ( $S_1$ ) of autotetraploid potato (*Solanum tuberosum*) was  
373 created by crossing two parental cultivars, with the American cultivar Atlantic as the maternal  
374 parent and the Chinese cultivar Longshu-3 as the paternal parent. A second generation ( $S_2$ )  
375 segregating population consisting of 304 full-sib individuals ( $S_2$ ) was derived by crossing two  
376 individuals (5-12 and 1-20) from the  $S_1$  population. The  $S_2$  population was planted together with  
377 their parental lines in three different field trials in 2015, each with five replicates per individual, by  
378 propagating the individuals asexually using tubers. A series of morphological and agronomic traits  
379 were scored, including plant height and flowering time.

380

### 381 **Data availability**

382 Programs and data for statistical analyses presented in the paper are freely available from  
383 <https://github.com/LJLeach/QuantModelTetra> and the link is included on our group website at  
384 [www.statisticalgenetics.info](http://www.statisticalgenetics.info).

385

386

## Results

### 387 **Detecting major genes in outbred autotetraploid populations**

388 To illustrate the use of our new method, we considered the detection of major effect QTL in a  
389 segregating population, one of the most popular quantitative genetic analyses using trait phenotype  
390 data. If a major gene makes a sufficiently large contribution to the phenotypic variation of a  
391 quantitative trait relative to the background genetic and environmental variation, then the  
392 phenotypic distribution will be multimodal (Falconer, 1989). In an outbred autotetraploid  $S_2$   
393 population, the distribution may be bimodal, trimodal, quadrimodal or quinquemodal under a  
394 biallelic model, depending on the parental genotype configuration and the occurrence of double  
395 reduction. Simulated examples of each are shown in Fig. 1a-d.

396

397 We simulated a quantitative trait for 300, 500 or 1,000  $S_2$  individuals generated by crossing parental  
398 genotypes  $AAaa \times AAaa$  and with a range of heritability values for the major gene, from 10% to  
399 35%. The monogenic, digenic, trigenic and quadrigenic effects for this gene and the population  
400 mean were all set equal to 1. The coefficient of double reduction at this QTL was equal to 0.1.  
401 Accordingly, the genetic variance of the major gene,  $V_G$ , was calculated as 1.132, according to  
402 equation (4). The residual variances of the trait were chosen based on the simulated heritability and  
403 genetic variance of the major gene.

404

405 In practice, the parental genotype configuration is usually unknown in outbred autotetraploid  
406 populations, therefore trait segregation analysis was carried out across all twelve possible parental  
407 genotype configurations, (for example  $AAAA \times AAAa$ ), across the range of possible values (0-0.25)  
408 for the rate of double reduction (Luo *et al.*, 2006a). The value corresponding to the highest  
409 likelihood value was taken as the MLE of the double reduction parameter. For a given parental  
410 genotype configuration and coefficient of double reduction, the expected offspring genotype  
411 frequency distribution could be calculated directly in terms of  $\alpha$ , and then used to calculate the  
412 general orthogonal scales  $w_{ij}$  for the genetic effects of genotype  $i$  for the  $j$ th contrast ( $j = 1, 2, \dots, 4$ ).

413 We implemented the EM algorithm as described in Equations (7)-(9) to calculate the MLEs of the  
414 genotypic values for each QTL genotype,  $\hat{G}_j$ . The genetic effect parameters for the major QTL,  $E_A$ ,  
415 could then be calculated according to equation (3), and the significance of major QTL segregation  
416 was tested as shown in equation (10).

417

418 Segregation analysis of phenotypic data in autotetraploids has a very poor statistical power for  
419 major gene detection when heritability is low (10%) (Table 4), which reflects the high degree of

420 overlap between the component normal distributions, as indicated by a high average overlapping  
421 coefficient ( $aOVL > 0.5$ ). When heritability is doubled to 20%, then the statistical power reaches an  
422 acceptable level of 79%, though only when there is large population size of at least 1,000 here. Only  
423 when there is a large degree of separation between component distributions, for example  $h^2 = 0.30$   
424 and  $aOVL = 0.3575$ , does major gene detection have adequate power with more realistic population  
425 sizes ( $n \geq 300$ ). When trait heritability is low, we caution that the genetic variance of major QTL is  
426 significantly overestimated by using trait segregation analysis in autotetraploids. Segregation  
427 analysis was able to correctly infer the parental QTL genotypes only in less than 10% of simulations.  
428

### 429 **Comparison with Killick's model**

430 We used a numerical example to explore the statistical properties of our model compared with an  
431 additive-dominance model, such as Killick's model. We simulated two biallelic loci,  $Q_A$  and  $Q_B$ ,  
432 with alleles segregating in linkage equilibrium at the two loci in an  $S_2$  population created from  
433 crossing two homozygous autotetraploid parents. The population mean, all genetic effects of the  
434 two loci and epistatic effects were simulated to be equal to 1. It should be noted that the simulation  
435 was designed without incorporating an environmental variable, with a purpose to minimize the  
436 influence of random sampling variation in the comparison of the methods. Shown in Supporting  
437 Information Table S3 are the genotypic values calculated either from a two locus orthogonal  
438 contrast based model defined according to equation (5) or from Killick's model with two loci. In  
439 Scenario 1, double reduction was absent at both loci, and genotypic values were generated either  
440 under our model (Scenario 1<sup>O</sup>, Supporting Information Table S3a) or under Killick's model  
441 (Scenario 1<sup>K</sup>, Supporting Information Table S3b); in Scenario 2, the coefficient of double reduction  
442 was equal to 0.05 for locus  $Q_A$  and 0.10 for  $Q_B$ , and the data was simulated under our model  
443 (Scenario 2<sup>O</sup>).

444  
445 As expected from the orthogonal property of our model, estimates of monogenic, digenic, trigenic  
446 and quadrigenic effects under both scenarios are independent of the estimation of epistatic effects  
447 (Table 5). All genetic effects can be consistently estimated under both single-locus (reduced)  
448 models and two-locus (full) models, when data are simulated under either our model or Killick's  
449 model. For example, under Scenario 1<sup>O</sup>, all estimated genetic effects take the same value of 1  
450 regardless of whether the model is fitted for one locus or for both loci, including epistatic  
451 parameters. In contrast, estimates of additive and dominance effects are markedly biased from their  
452 true values, particularly when epistatic effects are fitted in Killick's model, suggesting the model is  
453 vulnerable to the inclusion of various effects in the genetic models. For example, in Scenario 1<sup>K</sup>,  
454 additive and dominance effects are estimated to be equal to 1.94 in either reduced (single locus)



455 model, but all effects across both loci are correctly estimated to be equal to 1.00 when the full  
456 model is used. A further limitation of Killick's model arises because the genetic effect parameters  
457 are only defined on the basis of genotypic values and not using the genotypic frequencies (though  
458 genotypic frequencies are considered when estimating the population mean). This explains why the  
459 estimates of genetic effects from Killick's model (and other additive-dominance models in the  
460 current literature) involving both loci remained the same under Scenarios 1° and 2°. In contrast, our  
461 model confers a statistically appropriate and feasible way to estimate the various genetic effects in  
462 populations with various genotypic frequency distributions.

463

#### 464 **Parameter estimation under the orthogonal model**

465 We carried out a simulation study to test for reliability of the theoretical models presented above  
466 and to explore the statistical properties of the methods developed for estimating the model  
467 parameters under the orthogonal model, including the impact of the double reduction parameter on  
468 the estimation of genetic effect parameters. We considered a biallelic quantitative trait locus,  $Q_A$ ,  
469 segregating in an  $S_1$  population created from parental genotypes  $AAaa$  and  $AAaa$ . Segregation at the  
470 simulated QTL contributed 20% of the phenotypic variance of the trait. All genetic effects at the  
471 QTL,  $E_A = (\mu \theta_1 \theta_2 \theta_3 \theta_4)^T$ , were set equal to 1, and the residual variance was determined  
472 accordingly. Given the parental genotypes, offspring genotypes were generated under two levels of  
473 double reduction,  $\alpha = 0$  or 0.15, the former corresponding to bivalent pairing of homologous  
474 chromosomes and the latter to quadrivalent pairing in the autotetraploid meiosis.

475

476 Our previous simulation studies showed that trait segregation analysis based on phenotype data  
477 alone had limited power to detect segregation of major genes for traits with low heritability (see  
478 Table 4). It is well established that use of genetic markers is effective in recovering genotype  
479 information at QTL, leading to an increase in statistical power for detecting the QTL. We therefore  
480 simulated a chromosome with a single QTL closest to the centromere and an additional 10 genetic  
481 marker loci equally spaced at 10 cM intervals downstream from the QTL (Table 6). We  
482 implemented a modified version of the trait segregation analysis (equation 6b), which incorporates  
483 the parental and offspring marker information. This is expected to make the distribution of offspring  
484 QTL genotypes more informative, with an expected gain in statistical efficiency of parameter  
485 estimation from the mixture model.

486

487 Based on the simulated parental QTL genotype configuration ( $AAaa$  and  $AAaa$ ), the EM algorithm  
488 was used as described in equations (7) to (9) to give the MLEs for the QTL genotypic values. The  
489 genetic effect parameters were then estimated using our orthogonal model based on a range of

490 assumed values for the coefficient of double reduction. Table 7 shows the means and standard  
491 errors of the estimated genetic effects based on 500 repeated simulations. The genetic effect  
492 parameters and their variance components were predicted adequately as long as double reduction  
493 was properly taken into account, while the corresponding estimates were comparatively poor when  
494 double reduction was ignored. It is clear from the heritability estimates ( $h^2$ ) that an overestimated  
495 double reduction parameter may lead to overestimation of the genetic variance, and thus  
496 overestimation of trait heritability.

497

#### 498 **Case study of flowering time and plant height in autotetraploid potato**

499 To demonstrate the application of the methods we developed for modeling and analyzing real  
500 experimental data collected from autotetraploid species, we analyzed the phenotype data of two  
501 quantitative traits, flowering time and plant height, scored on 304 offspring from a cross between  
502 two varieties of cultivated autotetraploid potato (*Solanum tuberosum*). We found significant  
503 variation in the trait phenotype scores across three separate field trials, showing the major effect of  
504 the environment on both traits (ANOVA,  $p < 0.001$ , Supporting Information Tables S4, S5). We  
505 also observed a significant genotype by environment (G x E) interaction for plant height (ANOVA,  
506  $p < 0.001$ , Supporting Information Table S5).

507

508 We observed highly significant statistical evidence for segregation of major QTL genes for both  
509 flowering time and plant height. Figure S2 shows the LOD score profiles for different  
510 configurations of parental genotypes of QTL for both traits, which enable identification of the most  
511 likely parental QTL genotype(s). For flowering time, there was significant evidence for a major  
512 QTL ( $p < 0.0001$ ) under three alternative configurations, namely [5] ( $Aaaa \times AAAa$ ), [10]  
513 ( $Aaaa \times Aaaa$ ) and [11] ( $AAaa \times AAaa$ ), each with similar LOD score profiles and maximum LOD  
514 scores (Fig. S2a). Under each alternative model, the estimated mixture normal distribution is  
515 composed of five component normal distributions. In the absence of any marker information, model  
516 [11] was chosen as the most likely parental genotype configuration (Table 8, Fig. 1e), because the  
517 average parental trait scores (illustrated by arrows in Fig. 1e) were most likely to have come from  
518 the third component normal distribution corresponding to genotype  $AAaa$ . Meanwhile, model [5]  
519 was deemed unlikely because the parental phenotype scores were unlikely to have come from  
520 component genotypes  $Aaaa$  and  $AAAa$ , as shown in Fig. S3a. However, model [10] was only  
521 slightly less likely than model [11], as shown in Fig. S3b, illustrating the difficulty in clearly  
522 distinguishing parental genotype configuration through trait segregation analysis in autotetraploids.

523

524 For plant height, there was significant evidence for a major QTL ( $p = 0.003$ ) under genotype  
525 configurations [3]  $aaaa \times AAAa$ , [9] ( $AAAa \times AAAA$ ) and [11] ( $AAaa \times AAaa$ ) (Fig. S2b). Model  
526 [9] was chosen as the most likely parental genotype configuration (Table 8, Fig. 1f), because the  
527 average parental trait scores were more likely to have come from the component normal  
528 distributions for genotypes  $AAAa$  and  $AAAA$  (Fig. 1f) than from the component distributions for  
529 genotypes  $aaaa$  and  $AAAa$  (Fig. S3c) or  $AAaa$  and  $AAaa$  (Fig. S3d). Preliminary prediction of  
530 parental genotype configuration in this way can be valuable for various downstream analyses,  
531 including linkage and linkage disequilibrium based QTL analyses.

532

533 We estimated the narrow heritability of flowering time and plant height using the linear mixed  
534 model, as described in Supporting Information Method S6, based on the most likely value of the  
535 coefficient of double reduction inferred from the trait segregation analysis (Table 8). High values of  
536 narrow heritability were estimated for both flowering time (79%) and plant height (73%), and the  
537 assumed values for the double reduction rate had very little effect (Supporting Information Table  
538 S6). For both traits, the major QTL effect explained a significant proportion of the total phenotypic  
539 variance (29-39%), and up to one half of the total genetic variance (40-50%, Table 8).

540

541 All of the estimated genetic effects at the major QTL for both traits were highly significant ( $p <$   
542  $0.0001$ ). These estimates reveal valuable information on the genetic architecture of the trait, which  
543 may be useful for identifying useful QTL for selection purposes. For flowering time, a monogenic  
544 effect of 5.92 indicates that the average effect of replacing allele  $a$  with allele  $A$  at the major QTL in  
545 any autotetraploid genotype will be to delay flowering by around 6 days. Significance of the  
546 trigenic and quadrigenic genetic effects means that the interaction between the increasing alleles ( $A$ )  
547 and decreasing alleles ( $a$ ) depends on the identity of the third and fourth alleles in an autotetraploid  
548 genotype. However, the corresponding genetic variance components at trigenic and quadrigenic  
549 levels contributed very little to the total genetic variance of the major QTL (0.07% and 0.32%),  
550 suggesting that higher level allelic interactions exert limited effects on the genotypic values for this  
551 trait. For plant height, we observed a monogenic effect of 12.46, meaning that on average, replacing  
552 allele  $a$  with allele  $A$  at the major effect QTL will increase height by around 12cm, while a  
553 significant digenic effect of -6.84 suggests the importance of two-way allelic interactions at the  
554 major QTL for plant height, though such interactions make a relatively small contribution (2.48%)  
555 to the total genetic variance.

556

557

## Discussion

558 In recent decades, considerable progress has been made in the genetic analysis of quantitative traits  
559 in diploid plant, animal and human species. For example, new technologies and statistical methods  
560 have enabled genome-wide mapping of genetic variation and led to the detection of individual  
561 genomic regions (Quantitative Trait Loci), or more rarely individual quantitative trait nucleotides,  
562 that directly or indirectly influence trait phenotypic variation. However, progress has been limited  
563 by many factors (Hill, 2012), including difficulties in disentangling pleiotropic and epistatic effects  
564 of genes, and the complicated inheritance systems in polyploid species.

565

566 A crucial foundation for all quantitative genetic analyses in species of any ploidy level, including  
567 QTL analysis and evaluation of quantitative genetic parameters, is a quantitative genetic model that  
568 links together the genetic effects of genes with the quantitative trait phenotype. Historically, the  
569 field of quantitative genetics has focused on diploid species, and as such, the quantitative genetic  
570 model, theory and methods for various quantitative genetic analyses have been well established and  
571 routinely practiced (Mather & Jinks, 1971; Falconer, 1989; Lynch & Walsh, 1998). Meanwhile,  
572 progress in the genetic analysis of polyploid species, particularly autotetraploids, has lagged far  
573 behind. Built upon Kempthorne's model, Hackett *et al.*, (2001) proposed a quantitative genetic  
574 model for use in interval mapping of QTL in autotetraploids, though this model was not based on a  
575 strict tetrasomic inheritance model, and does not possess the orthogonal property between different  
576 model parameters and their estimates.

577

578 In this article, we have contributed a quantitative genetic model for autotetraploid species based on  
579 the orthogonal contrast scales model developed for diploids (Cockerham, 1954). The model relates  
580 the phenotype score of an autotetraploid individual for a quantitative trait to the alleles at the loci  
581 that contribute to trait variation, in terms of monogenic, digenic, trigenic, and quadrigenic effects at  
582 individual loci, and epistatic effects between loci. The orthogonal property of the model ensures that  
583 the genetic effects can be independently estimated for one or two loci, assuming only pair-wise  
584 epistatic effects. This property is very useful for obtaining reliable estimates of genetic effects and  
585 genetic variance components, even when the number of QTL involved is unknown, which is usually  
586 the case (Zeng *et al.*, 2005). The model could be extended to three or more loci, assuming epistasis  
587 only occurs between pairs of loci. It has been recognised that use of an orthogonal model for QTL  
588 mapping would be advantageous in various ways (Kao & Zeng, 2002; Zeng *et al.*, 2005). For  
589 example, different QTL genetic effects may be tested and estimated separately. Parameter estimates  
590 are also independent of which, if any, epistatic effects are fitted in the model, which will simplify  
591 genetic interpretation of the underlying genetic architecture.

592

593 Our model decreases the number of parameters used to describe the genetic effects of QTL from  
594 255 (Kempthorne, 1955; Kempthorne, 1957) down to 24 for a two-locus analysis, making it  
595 statistically tractable for real data analysis. We have shown its suitability for use in populations with  
596 various genetic structures, including segregating populations (e.g.  $S_2$ ) and natural populations of  
597 unrelated individuals, either in linkage equilibrium or linkage disequilibrium. While there is  
598 evidence for multiple allele segregation at individual QTL genes in diploid populations (Barton &  
599 Keightley, 2002), each allele may only increase or decrease the trait phenotype, therefore the  
600 biallelic setting is appropriate for quantitative genetic analysis in autotetraploids.

601

602 We have shown that our model can accurately estimate the genetic effects in a segregating  
603 population under either one locus (reduced model) or two locus (full model) settings. Our model  
604 takes proper account of the complex features of autotetrasomic inheritance, including double  
605 reduction, unlike other quantitative genetic models developed for autotetraploids (Killick, 1971).  
606 We showed that the double reduction parameter has a significant impact on the genetic parameter  
607 estimation, and thus advise that this parameter should be taken into account in any quantitative  
608 genetic analysis in autotetraploid species to avoid bias in the estimation of genetic effects. We have  
609 provided statistical tests for the significance of double reduction and methods for its accurate  
610 estimation using molecular marker data in our previous work (Luo *et al.*, 2004; Luo *et al.*, 2006a).  
611 Since double reduction is a location dependent parameter, the marker data can provide an  
612 approximation of the double reduction parameter near to the QTL.

613

614 We carried out trait segregation analysis to illustrate the practical application of our quantitative  
615 genetic model for the analysis of trait phenotype data in autotetraploids. Trait segregation analysis  
616 has been an important topic in the history of quantitative genetics in diploids (Falconer, 1989). It  
617 serves as an important intermediate step prior to collection of genomic marker data, allowing major  
618 genes affecting quantitative traits to be detected prior to designing further genomic analyses, such  
619 as QTL analysis, and also enabling more efficient selection in breeding programs of agronomic  
620 traits in autotetraploid crops or animals (Falconer, 1989).

621

622 Segregation analysis involves the estimation of normal mixtures, which is well known to be an ill-  
623 posed problem, particularly when the disparity between the component distributions is small (Xiao  
624 *et al.*, 2007; Lourens *et al.*, 2013). Methods for segregation analysis may therefore suffer from low  
625 statistical power in diploids, and even more so in autotetraploids (Xiao *et al.*, 2007). We extended  
626 the concept of the overlapping coefficient (Inman & Bradley, 1989) to quantify the disparity

627 between multiple component normal distributions for segregation analysis in autotetraploids. Our  
628 results echo previous work showing that maximum likelihood estimation may perform poorly when  
629 component distributions are poorly separated, and substantial bias may be observed when OVL  
630 exceeds 0.45 (Lourens *et al.*, 2013). Additionally, when the disparity is low, within population  
631 variance will be underestimated; in the present context, the proportion of the trait phenotypic  
632 variation explained by the QTL, *i.e.* its heritability, may therefore be markedly overestimated. This  
633 could be an inherent weakness of the statistical method implemented for segregation analysis and  
634 care must thus be taken when interpreting the results of phenotype-based analysis in autotetraploids.  
635 We observed the statistical power to detect QTL segregation is low when heritability is low ( $\leq 20\%$ )  
636 and the OVL is greater than 0.4, while the power became adequate for detecting QTL segregation  
637 when heritability was at least 30%, in populations with a modest size of at least 300.

638

639 We have demonstrated the utility of our quantitative genetic model for autotetraploids by analyzing  
640 real data on flowering time and plant height in a segregating population of potato. We estimated  
641 high values of narrow heritability for both flowering time (79%) and plant height (73%), consistent  
642 with various other potato populations (Khan *et al.*, 2013; Ozturk & Yildirim, 2014), and also crops  
643 such as barley, which have shown high heritability ( $>90\%$ ) for flowering time (Maurer *et al.*, 2015).  
644 Trait segregation analysis showed evidence for segregation of a major gene affecting flowering time  
645 in this population. Work in *Arabidopsis*, rice and tomato, led to the identification of FLOWERING  
646 LOCUS T (FT) as the mobile signal “florigen” that plays a central role in the floral transition,  
647 travelling from the leaves to the shoot apical meristem to promote flowering (Turk *et al.*, 2008).  
648 More recently, several functional homologues of the key *Arabidopsis* flowering time genes have  
649 been identified in potato, including *StSP3D* as the mobile signal “florigen”, and *StSP6A* as the  
650 mobile signal “tuberigen” responsible for the stolon to tuber transition (Navarro *et al.*, 2011). A  
651 CONSTANS (CO) homologue, *StCO*, has also been discovered with a role in repression of  
652 tuberisation (Gonzalez-Schain *et al.*, 2012), as well as homologues of other key flowering time  
653 genes, including *StCDF1* (Kloosterman *et al.*, 2013). The major regulators of flowering time in  
654 potato are therefore conserved with *Arabidopsis*, but have also been recruited to control the  
655 developmental switch involved in storage organ formation.

656

657 The quantitative genetic model we presented here lays the foundation for quantitative genetic  
658 analysis in autotetraploid species. In addition to the classical phenotype based analysis of  
659 quantitative traits, such as segregation analysis, estimation of breeding values, and genetic variance  
660 components, the model fulfills an essential requirement for DNA molecular marker assisted QTL  
661 analysis under both linkage and linkage disequilibrium based settings. The model will therefore

662 facilitate future studies of the genetic architecture and evolution of quantitative traits in important  
663 crop species such as potato (D’Hoop *et al.*, 2008; Massa *et al.*, 2015), and coffee (del Pilar  
664 Moncada *et al.*, 2016), forest legumes such as alfalfa (Yu *et al.*, 2016), and ornamental species such  
665 as rose (Gitonga *et al.*, 2016).

666

667 **Acknowledgements** The study was supported by research grants from BBSRC (BB/N008952/1) in  
668 the United Kingdom, the National Nature Science Foundation of China (grant numbers 81172006  
669 and 91231114) and the Leverhulme Trust.

670

671 **Author contribution** Z.L. conceived of and designed the study. Z.L. designed the theoretical  
672 model and statistical methods. J.C. implemented the statistical methods. Z.L., F.Z. and L.W. created  
673 the potato segregating population, implemented field trials and collected the phenotypic data. J.C.  
674 analysed the data with inputs from Z.L. L.L., Z.L., J.C. and L.L. wrote the paper.

675

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834 **Supporting Information**

835 Additional Supporting Information may be found online in the Supporting Information tab for this  
836 article:

837

838 **Fig S1** Impact of genetic effect parameters on genotypic values in the orthogonal model.

839

840 **Fig S2** LOD score profiles for flowering time (a) and plant height (b) under different configurations  
841 of parental genotypes at a putative QTL.

842

843 **Fig S3** Mixture normal distribution and inferred component normal distributions for flowering time  
844 and plant height under alternative parental QTL genotype configurations.

845

846 **Methods S1** Notations and definition of the orthogonal model in autotetraploids.

847

848 **Methods S2** One locus model for a randomly mating population.

849

850 **Methods S3** Two locus model under linkage equilibrium.

851

852 **Methods S4** Two locus model under linkage disequilibrium.

853

854 **Methods S5** Reduced two-locus model and analysis.

855

856 **Methods S6** Estimation of narrow-sense heritability in autotetraploids.

857

858 **Note S1** Definition of the Killick quantitative genetic model.

859

860 **Table S1** The number of digenic, trigenic and quadrigenic allelic interactions in autotetraploid  
861 genotypes.

862

863 **Table S2** Definition of the 25 genetic parameters for the two locus quantitative genetic model in  
864 autotetraploids.

865

866 **Table S3** Numerical examples with genotypic values generated under our orthogonal model (a) or  
867 under Killick's model (b).

868

869 **Table S4** One-way ANOVA for flowering time in autotetraploid potato.

870

871 **Table S5** Type II ANOVA for plant height in autotetraploid potato.

872

873 **Table S6** Narrow sense heritability of two autotetraploid potato quantitative traits (flowering time  
874 and plant height) using the linear mixed model.

875

## 876 **Figure Legends**

877

878 **Figure 1. Segregation analysis for quantitative traits in simulated and real outbred**  
879 **autotetraploid segregating populations.**

880 The quantitative trait phenotype may show a bimodal (a), trimodal (b), quadrimodal (c) or  
881 quinquemodal (d) distribution in a segregating population derived from a cross between parental  
882 genotypes as indicated above each panel and with a given value of the coefficient of double  
883 reduction,  $\alpha$ . Flowering time (e) in the potato segregating population showed a quinquemodal  
884 distribution with the most likely parental genotype configuration being *AAaa* x *AAaa*. Plant height  
885 (f) showed a trimodal distribution with the most likely parental genotype configuration being *AAAA*  
886 x *AAAA*. Average parental phenotype scores for  $P_1$  and  $P_2$  parental varieties are indicated using  
887 orange and green arrows respectively. Red lines indicate the mixture normal distribution and dotted  
888 blue lines indicate the inferred component normal distributions, numbered to indicate genotypes 1)  
889 *aaaa*; 2) *Aaaa*; 3) *AAaa*; 4) *AAAA*; and 5) *AAAA*.

890

891 **Tables**

892 **Table 1. The general orthogonal contrast scales model for one locus.**

	$i$	4	3	2	1	0
	Genotype	AAAA	AAAa	AAaa	Aaaa	aaaa
	Frequency	$f_4$	$f_3$	$f_2$	$f_1$	$f_0$
	Genotypic value	$G_4$	$G_3$	$G_2$	$G_1$	$G_0$
$\theta_1$	$W_1$	$w_{41}$	$w_{31}$	$w_{21}$	$w_{11}$	$w_{01}$
$\theta_2$	$W_2$	$w_{42}$	$w_{32}$	$w_{22}$	$w_{12}$	$w_{02}$
$\theta_3$	$W_3$	$w_{43}$	$w_{33}$	$w_{23}$	$w_{13}$	$w_{03}$
$\theta_4$	$W_4$	$w_{44}$	$w_{34}$	$w_{24}$	$w_{14}$	$w_{04}$

893  $G_i$  and  $f_i$  denote the genotypic values and genotypic frequencies for the five genotypes  
894 with  $i$  copies of the A allele.  $\theta_i$  ( $i=1,2,\dots,4$ ) are the monogenic, digenic, trigenic and  
895 quadrigenic genetic effects respectively.  $w_{ij}$  ( $i=0,1,\dots,4; j=1,\dots,4$ ) is the scale component  
896 of genotype  $i$  for the  $j^{th}$  contrast.

897

898 **Table 2. Orthogonal contrast scales for one locus in a biallelic autotetraploid  $S_2$  population.**

Genotype		AAAA	AAAa	AAaa	Aaaa	aaaa
Frequency		$f_4$	$f_3$	$f_2$	$f_1$	$f_0$
		$G_4$	$G_3$	$G_2$	$G_1$	$G_0$
$\theta_1$	$W_1$	2	1	0	-1	-2
$\theta_2$	$W_2$	$(5-2\alpha)/3$	$(1-4\alpha)/6$	$-(1+2\alpha)/3$	$(1-4\alpha)/6$	$(5-2\alpha)/3$
$\theta_3$	$W_3$	$2(1-\alpha)/3$	$-(1+2\alpha)/6$	0	$(1+2\alpha)/6$	$-2(1-\alpha)/3$
$\theta_4$	$W_4$	$\frac{(1-\alpha)(4\alpha^2-4\alpha+3)}{12(2+\alpha)}$	$-\frac{(1+2\alpha)(4\alpha^2-4\alpha+3)}{24(2+\alpha)}$	$\frac{(1-\alpha)(2\alpha+1)^2}{12(2+\alpha)}$	$-\frac{(1+2\alpha)(4\alpha^2-4\alpha+3)}{24(2+\alpha)}$	$\frac{(1-\alpha)(4\alpha^2-4\alpha+3)}{12(2+\alpha)}$

899  $\theta_i$  ( $i=1, \dots, 4$ ) are the monogenic, digenic, trigenic, and quadrigenic genetic effects of locus A.  $f_i$  and  $G_i$  ( $i=0, 1, \dots, 4$ ) are the frequency and genotypic  
900 values for the  $i^{\text{th}}$  genotype  $A_i a_{4-i}$ , respectively.  $\alpha$  is the coefficient of double reduction at locus A.

901



902 **Table 3. The general orthogonal contrast scales model for two loci (A and B).**

	Genotype	AAAA	AAAa	AAaa	Aaaa	aaaa		Genotype	BBBB	BBBb	BBbb	Bbbb	bbbb
	Frequency	$f_4$	$f_3$	$f_2$	$f_1$	$f_0$		Frequency	$f_4$	$f_3$	$f_2$	$f_1$	$f_0$
	G	$G_4$	$G_3$	$G_2$	$G_1$	$G_0$		G	$G_4$	$G_3$	$G_2$	$G_1$	$G_0$
$\theta_1$	$W_{A1}$	$w_{41}$	$w_{31}$	$w_{21}$	$w_{11}$	$w_{01}$	$\zeta_1$	$V_{B1}$	$v_{41}$	$v_{31}$	$v_{21}$	$v_{11}$	$v_{01}$
$\theta_2$	$W_{A2}$	$w_{42}$	$w_{32}$	$w_{22}$	$w_{12}$	$w_{02}$	$\zeta_2$	$V_{B2}$	$v_{42}$	$v_{32}$	$v_{22}$	$v_{12}$	$v_{02}$
$\theta_3$	$W_{A3}$	$w_{43}$	$w_{33}$	$w_{23}$	$w_{13}$	$w_{03}$	$\zeta_3$	$V_{B3}$	$v_{43}$	$v_{33}$	$v_{23}$	$v_{13}$	$v_{03}$
$\theta_4$	$W_{A4}$	$w_{44}$	$w_{34}$	$w_{24}$	$w_{14}$	$w_{04}$	$\zeta_4$	$V_{B4}$	$v_{44}$	$v_{34}$	$v_{24}$	$v_{14}$	$v_{04}$

903

904  $G_i$  ( $G_i$ ) and  $f_i$  ( $f_i$ ) ( $i = 0, 1, \dots, 4$ ) denote the genotypic values and genotypic frequencies for the five genotypes of locus A (locus B).  $\theta_i$  ( $\zeta_i$ ) ( $i=1,$   
905  $2, \dots, 4$ ) are the monogenic, digenic, trigenic and quadrigenic effects for locus A and locus B, respectively. Here  $w_{ij}$  and  $v_{ij}$  ( $i=0,1,\dots,4; j=1,2,\dots,4$ ), are  
906 the orthogonal contrast scales of genotype  $i$  for the  $j^{th}$  contrast, calculated separately for each locus using the general biallelic one locus model.

907

908 **Table 4. Statistical power of major gene detection in outbred autotetraploid populations.**

Heritability ( $h^2$ )	Sample Size ( $n$ )	$aOVL$	power (%)	Genetic Variance ( $\hat{V}_{QTL}$ )	
				mean	s.e.
0.10	300	0.5275	17	4.8786	0.2343
	500		18	4.1234	0.2345
	1000		23	3.4567	0.2325
0.15	300	0.4634	29	2.8886	0.1575
	500		35	2.5018	0.1477
	1000		53	2.4363	0.1306
0.20	300	0.4193	46	2.3815	0.1156
	500		54	2.1323	0.1004
	1000		79	1.6360	0.0746
0.25	300	0.3856	59	1.8227	0.0825
	500		75	1.5201	0.0703
	1000		99	1.4111	0.0504
0.30	300	0.3575	71	1.6470	0.0705
	500		93	1.4608	0.0611
	1000		99	1.2765	0.044
0.35	300	0.3329	77	1.5475	0.0626
	500		100	1.3251	0.0465
	1000		100	1.2272	0.0352

909  $aOVL$  is the average overlapping coefficient between normal distributions. The empirical statistical  
 910 power for major gene detection is given at significance level 5% based on 100 replicates. The  
 911 simulated value of  $V_G$  was equal to 1.132.

912 **Table 5. Estimates of genetic effects using Killick’s model and the orthogonal contrast scales based model, under Scenario 1 (without double**  
 913 **reduction,  $\alpha_A = \alpha_B = 0.00$ ) or Scenario 2 (with double reduction,  $\alpha_A = 0.05, \alpha_B = 0.10$ ).**

Killick’s model (ref. 23)																									
Scenario	$\mu$	$a$	$d_1$	$d_2$	$d_3$	$b$	$h_1$	$h_2$	$h_3$	$I_{ab}$	$I_{ah_1}$	$I_{ah_2}$	$I_{ah_3}$	$I_{d_1b}$	$I_{d_1h_1}$	$I_{d_1h_2}$	$I_{d_1h_3}$	$I_{d_2b}$	$I_{d_2h_1}$	$I_{d_2h_2}$	$I_{d_2h_3}$	$I_{d_3b}$	$I_{d_3h_1}$	$I_{d_3h_2}$	$I_{d_3h_3}$
1 <sup>O</sup>	1.00	2.67	-2.52	-2.08	-0.85	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1.00	-	-	-	-	2.67	-2.52	-2.08	-0.85	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1.00	7.44	-7.03	-5.82	-2.38	7.44	-7.03	-5.82	-2.38	7.11	-6.72	-5.56	-2.28	-6.72	6.35	5.25	2.15	-5.56	5.25	4.34	1.78	-2.27	2.15	1.78	0.73
1 <sup>K</sup>	3.78	1.94	1.94	1.94	1.94																				
	3.78					1.94	1.94	1.94	1.94																
	3.78	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2 <sup>O</sup>	1.10	2.84	-2.62	-2.22	-0.91	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1.10	-	-	-	-	2.75	-2.60	-2.15	-0.88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1.10	7.44	-7.03	-5.82	-2.38	7.44	-7.04	-5.82	-2.38	7.11	-6.72	-5.56	-2.28	-6.72	6.35	5.25	2.15	-5.56	5.25	4.34	1.78	-2.27	2.15	1.78	0.73
orthogonal contrast scales based model																									
Scenario	$\mu$	$\theta_1$	$\theta_2$	$\theta_3$	$\theta_4$	$\zeta_1$	$\zeta_2$	$\zeta_3$	$\zeta_4$	$I_{\theta_1\zeta_1}$	$I_{\theta_1\zeta_2}$	$I_{\theta_1\zeta_3}$	$I_{\theta_1\zeta_4}$	$I_{\theta_2\zeta_1}$	$I_{\theta_2\zeta_2}$	$I_{\theta_2\zeta_3}$	$I_{\theta_2\zeta_4}$	$I_{\theta_3\zeta_1}$	$I_{\theta_3\zeta_2}$	$I_{\theta_3\zeta_3}$	$I_{\theta_3\zeta_4}$	$I_{\theta_4\zeta_1}$	$I_{\theta_4\zeta_2}$	$I_{\theta_4\zeta_3}$	$I_{\theta_4\zeta_4}$
1 <sup>O</sup>	1.00	1.00	1.00	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1.00	-	-	-	-	1.00	1.00	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1 <sup>K</sup>	3.78	0.32	-0.81	1.94	-3.89																				
	3.78					0.32	-0.81	1.94	-3.89																
	3.78	0.32	-0.81	1.94	-3.89	0.32	-0.81	1.94	-3.89	0.03	-0.07	1.67	-0.33	-0.07	0.17	-0.42	0.83	0.17	-0.42	1.00	-2.00	-0.33	0.83	-2.00	4.00
2 <sup>O</sup>	1.10	1.08	1.08	1.07	1.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1.10	-	-	-	-	1.07	1.05	1.03	1.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1.10	1.08	1.08	1.07	1.07	1.07	1.05	1.03	1.03	1.05	1.04	1.02	1.02	1.04	1.03	1.01	1.01	1.03	1.02	1.00	1.00	1.03	1.02	1.00	1.00

914  $\mu$  is the population mean.  $a(b)$  indicate the additive effect for locus  $Q_A(Q_B)$ .  $d_1, d_2$  and  $d_3(h_1, h_2$  and  $h_3)$  indicate three unique dominance effects  
915 for the simplex, duplex and triplex heterozygote genotypes for locus  $Q_A(Q_B)$ .  $\theta_i$  (or  $\zeta_i$ ) ( $i = 1, \dots, 4$ ) are the monogenic, digenic, trigenic and  
916 quadrigenic genetic effects at locus  $Q_A$  (or  $Q_B$ ).  $I_{\theta_i \zeta_j}$  denote epistasis between the effects  $\theta_i$  and  $\zeta_j$  ( $i=1, \dots, 4; j=1, \dots, 4$ ). '–' indicates the parameter  
917 was not estimated. The population mean, all genetic effects of the two loci and epistatic effects were simulated to be 1.0. In scenario 1, the simulation  
918 data was generated under either Killick's model ( $1^K$ ) or our orthogonal contrast scales based model ( $1^O$ ).

919 **Table 6. Simulation settings based on a single QTL with 10 linked marker loci.**

Locus	$r$	Parental genotypes		Genetic parameters		
		P <sub>1</sub>	P <sub>2</sub>	$\mu, \theta_1, \theta_2, \theta_3, \theta_4, = 1$	$\alpha$	$\alpha$
QTL	0.00	<i>AAaa</i>	<i>AAaa</i>		$\alpha = 0$	$\alpha = 0$
$L_1$	0.02	$M_1M_2M_3M_3$	$M_5M_6M_7M_8$			
$L_2$	0.11	$M_1M_2M_2M_4$	$M_5M_2M_2M_6$	$G_4$	5.458	5.215
$L_3$	0.19	$M_1M_1M_3M_4$	$M_3M_5M_6M_7$	$G_3$	1.938	1.787
$L_4$	0.26	$M_4M_2M_3M_4$	$M_5M_6M_6M_8$	$G_2$	0.708	0.622
$L_5$	0.32	$M_2M_1M_4M_2$	$M_4M_5M_7M_6$	$G_1$	0.271	0.229
$L_6$	0.38	$M_3M_2M_1M_1$	$M_5M_5M_6M_7$	$G_0$	0.125	0.089
$L_7$	0.42	$M_1M_3M_3M_4$	$M_4M_3M_5M_6$	$\sigma$	1.928	2.223
$L_8$	0.46	$M_1M_1M_3M_4$	$M_5M_2M_6M_7$			
$L_9$	0.50	$M_2M_2M_1M_3$	$M_1M_5M_6M_7$			
$L_{10}$	0.53	$M_2M_3M_4M_4$	$M_2M_5M_5M_6$			

932 Markers were located on the same side of the QTL, which is closest to the centromere.  $r$  denotes the  
 933 recombination frequency between the QTL and marker loci. The offspring population of size  $n =$   
 934 300 was generated under a tetrasomic inheritance model with double reduction rate set equal to 0.00  
 935 or 0.15. Heritability was assumed to be 0.2. Alleles listed in the same column had the same linkage  
 936 phase.

937 **Table 7. Means and standard errors of the parameter estimates based on 500 repeated simulations of the single QTL model.**

Offspring data generated with double reduction rate $\alpha = 0$												
Simulated values				Estimated values								
				$\alpha = 0$			$\alpha = 0.05$		$\alpha = 0.10$		$\alpha = 0.15$	
$\mu$	1.000			0.992 (0.005)			1.016 (0.005)		1.043 (0.005)		1.072 (0.005)	
$\theta_1$	1.000	$V_1$	0.667	0.947 (0.006)	0.632 (0.008)	0.959 (0.006)	0.688 (0.009)	0.973 (0.006)	0.773 (0.010)	0.988 (0.006)	0.864 (0.011)	
$\theta_2$	1.000	$V_2$	0.222	0.953 (0.012)	0.217 (0.005)	0.965 (0.012)	0.251 (0.006)	0.976 (0.012)	0.287 (0.007)	0.986 (0.012)	0.325 (0.007)	
$\theta_3$	1.000	$V_3$	0.037	1.039 (0.029)	0.055 (0.002)	1.057 (0.028)	0.065 (0.003)	1.068 (0.028)	0.074 (0.003)	1.077 (0.028)	0.083 (0.003)	
$\theta_4$	1.000	$V_4$	0.003	1.053 (0.092)	0.019 (0.001)	1.095 (0.091)	0.020 (0.003)	1.123 (0.091)	0.020 (0.001)	1.147 (0.091)	0.021 (0.001)	
$\sigma$	1.928			1.915 (0.003)			1.915 (0.003)		1.916 (0.003)		1.916 (0.003)	
$h^2$	0.200			0.200 (0.002)			0.217 (0.002)		0.238 (0.002)		0.258 (0.002)	

  

Offspring data generated with double reduction rate $\alpha = 0.15$												
Simulated values				Estimated values								
				$\alpha = 0.15$			$\alpha = 0.00$		$\alpha = 0.05$		$\alpha = 0.20$	
$\mu$	1.000			0.999 (0.005)			0.938 (0.006)		0.950 (0.005)		1.027 (0.005)	
$\theta_1$	1.000	$V_1$	0.867	0.960 (0.006)	0.814 (0.010)	0.906 (0.006)	0.561 (0.008)	0.925 (0.006)	0.641 (0.008)	0.977 (0.006)	0.908 (0.011)	
$\theta_2$	1.000	$V_2$	0.311	0.969 (0.011)	0.310 (0.007)	0.903 (0.011)	0.195 (0.004)	0.936 (0.011)	0.234 (0.005)	0.981 (0.011)	0.350 (0.007)	
$\theta_3$	1.000	$V_3$	0.053	1.118 (0.027)	0.086 (0.004)	1.108 (0.027)	0.059 (0.002)	1.111 (0.027)	0.068 (0.003)	1.122 (0.027)	0.095 (0.004)	
$\theta_4$	1.000	$V_4$	0.004	1.312 (0.101)	0.026 (0.002)	1.435 (0.100)	0.025 (0.002)	1.343 (0.101)	0.025 (0.002)	1.313 (0.101)	0.027 (0.002)	
$\sigma$	2.222			2.212 (0.004)			2.216 (0.004)		2.213 (0.002)		2.212 (0.002)	
$h^2$	0.200			0.201 (0.002)			0.146 (0.001)		0.164 (0.002)		0.219 (0.002)	

938  $\mu$  is the population mean and  $\theta_i$  ( $i=1,\dots,4$ ) are accordingly monogenic, digenic, trigenic and quadrigenic genetic effects of the QTL.  $\sigma$  is the  
939 environmental error and  $h^2$  is the heritability.  $V_1$ ,  $V_2$ ,  $V_3$  and  $V_4$  represent monogenic, digenic, trigenic and quadrigenic genetic variance components,  
940 respectively. The estimation procedure was carried out assuming a range of values for the coefficient of double reduction  $\alpha$ . The simulated values of  $\alpha$   
941 are highlighted in bold.

942 **Table 8. Inference of major QTL genes affecting flowering time and plant height in a**  
 943 **segregating population of autotetraploid potato.**

	Flowering time (days)	Plant height (cm)
$G_{P_1} / G_{P_2}$	<i>QQqq / QQqq</i>	<i>QQQq / QQQQ</i>
$\hat{\alpha}$	0.055	0.165
$-\ln(L)$	939.47	1099.89
LOD score	13.83	5.70
P value	0.0000	0.003
$\hat{h}^2$	79.38	72.67
Mean	33.62	45.97
Monogenic	5.92 (25.97) ***	12.46 (51.61) ***
Digenic	2.83 (2.03) ***	-6.84 (1.31) ***
Trigenic	0.59 (0.02) ***	-
Quadrigenic	-5.09 (0.09) ***	-
$V_{QTL}/V_{Total}$ (%)	39.7	29.7
$V_{QTL}/V_{genetic}$ (%)	50.1	40.8

944 Estimated parameters of the quantitative genetic model are given based on the most likely  
 945 parental genotype configuration. Monogenic, digenic, trigenic and quadrigenic genetic effects  
 946 estimated from the orthogonal contrast scales model are shown, with the genetic variance  
 947 component in brackets. \*\*\*  $p$ -value < 0.0001 from the two-tailed  $t$ -test.