The use of multi-response models to improve inferences about natural selection

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1. Abstract

Natural selection, the relationship between trait and fitness, is a key determinant of evolutionary change and population adaptation. Therefore, accurate estimation of natural selection is important. In 1983, Lande and Arnold proposed a simple regression-based approach which allows the measurement of selection on a range of traits whilst accounting for confounding variables. However, issues remain with its application to observational data from wild populations which can bias estimates, including assumptions around causality and whether selection on a trait is hard or soft. We highlight how, when fitness and traits are measured repeatedly across individuals and/or common environments, we can identify these issues in observational studies by comparing directional selection gradients decomposed across different hierarchical levels. We outline the theory behind this and show how multi-response models provide a readily available statistical tool to implement this approach. We then use an empirical example to illustrate our method and how to interpret the results. Our approach builds upon previous work to allow greater inference to be drawn from existing

observational datasets, particularly when no genetic information is available. This should facilitate improved interpretation of estimates of selection in wild populations, and ultimately, our understanding of the selection process.

Keywords: directional selection, bias, repeated measures, soft selection, hard selection, quantitative genetics

2. Introduction

Natural selection is the difference in survival and/or reproduction of individuals as a result of differences in their trait phenotypes, which is broadly measured as the relationship between fitness and a trait (Darwin, 1859; Falconer & Mackay, 1996). The strength of natural selection is an important determinant of the rate of adaptation of a population (Charmantier et al., 2014; Falconer & Mackay, 1996; Hoekstra et al., 2001). Therefore, understanding variation in the strength and direction of natural selection can provide crucial insights into potential causes of differences in fitness between individuals and how populations might evolve in response to changing environmental pressures (Caruso et al., 2017; Siepielski et al., 2009). As a result, accurately estimating natural selection is a key goal in evolutionary biology that has implications across a range of disciplines. Under the assumption of low temporal and spatial variation in selection (Morrissey & Hadfield, 2012; Siepielski et al., 2013), there is often interest in estimating an over-all selection gradient i.e. the average selection gradient across time and/or space. Lande and Arnold (1983) proposed a simple linear regression-based approach to estimating the strength of natural selection (hereafter the 'Lande-Arnold' method) that can account for confounding variables. This has allowed evolutionary biologists and ecologists to measure selection on a range of traits, using the regression of relative fitness *w* on a trait *z*:

$$w_i = \mu_0 + \beta_z z_i + e_i. \quad [1]$$

Where w_i is the fitness of an individual relative to the population mean at observation *i*, μ_0 is the global intercept, *z* is the trait value and *e* is the residual. The linear effect of the trait on fitness, β_z, is the directional selection gradient. This Lande-Arnold selection gradient is straightforward to measure and can be expanded to measure quadratic and correlational selection (Lande & Arnold, 1983; Stinchcombe et al., 2008; Svensson, 2023). This has led to a rapid expansion in the number of published estimates of selection, to the extent that, most

meta-analyses on the strength of selection have more estimates of Lande-Arnold derived selection gradients than estimates from other methods (Caruso et al., 2017; Charmantier et al., 2024; Hoekstra et al., 2001; Kingsolver et al., 2001; Morrissey & Hadfield, 2012; Siepielski et al., 2009; Svensson, 2023).

Despite the benefits of the Lande-Arnold approach to estimating natural selection, and its widespread use in studies of observational data from wild populations, issues remain with its application and interpretation; here we focus on two. The first, well-documented, issue is that the Lande-Arnold approach inherently assumes a causal relationship, i.e., that fitness changes as a direct result of a change in the trait being analysed (Hadfield, 2008; Mitchell-Olds & Shaw, 1987; Morrissey et al., 2010; Rausher, 1992; Svensson, 2023). This can be particularly problematic in wild populations where many confounding environmental factors may result in a trait-fitness relationship and thus a non-zero estimate of selection, when in reality there is no causal relationship between the trait and fitness (Kingsolver et al., 2012; Rausher, 1992). Confounding factors can be dealt with by including them as covariates, which allows measures of selection to be corrected when confounding variables are both known and measured (Morrissey & Henshaw, 2022). However, in wild observational studies there are theoretically an unlimited number of unknown confounding variables, which poses a huge challenge when relying on Lande-Arnold based approaches to estimate selection and infer causality (Svensson, 2023).

The second, less well-known issue is the impact of hard and soft selection on the accuracy of the Lande-Arnold approach. Here, we define hard selection as cases where the fitness of individuals depends solely on an individual's absolute trait value and is independent of the trait values of other conspecifics in the local environment (i.e. selection is density and

84 frequency independent). We define soft selection as cases where the fitness of individuals
85 depends on their trait value relative to the trait value of other conspecifics in the local
86 environment with which they interact (i.e. selection is density and frequency dependent) (Bell
87 et al., 2021; Wallace, 1975; Wallace & Lewontin, 1968). For more detailed examples and a
88 discussion of soft and hard selection see Bell et al. (2021). If selection is soft, a single/global
89 average selection gradient (i.e. estimated across environments) may be biased towards zero
90 depending on how fitness has been relativised (see section 3.2 for details). Together, these
91 issues may contribute to the mismatch between the observed and predicted response to
92 selection that is common across wild populations (Brookfield, 2016; Hansen & Houle, 2004;
93 Merilä et al., 2001; Pemberton, 2010; Pujol et al., 2018).
94
95 In wild populations that are monitored repeatedly across time and/or space, trait(s) of interest
96 and fitness (measured as an individual's contribution to the population at the next time point
97 (Sæther & Engen, 2015)) or fitness components (e.g. survival and/or reproduction in the next
98 time point) are often also measured repeatedly on the same individuals across time and/or
99 space. As a result, fitness and trait(s) will be measured repeatedly across environments that
100 individuals share with each other. This means that any environmental conditions, such as
101 weather, food availability or population density effects that occur in a specific location or
102 timepoint and affect an individual's trait and/or fitness measurements are shared by
103 individuals within that environment.
104
105 Here, we will introduce how we can use repeated measures of traits and fitness to decompose
106 the association between them (i.e. the estimates of selection) across hierarchical levels, such
107 as time and space. This decomposition of selection into hierarchical levels can be used to: (i)
108 assess whether selection is consistent with causality; and (ii) test consistency with, and

correct selection estimates for, soft and hard selection in observational data. We then show how, without additional data, multi-response models (often referred to as multivariate models) provide a readily available statistical tool to implement this approach. Whilst the statistical models we promote have been previously described and widely used, their application to trait-fitness relationships is mostly limited to separating associations into genetic and non-genetic hierarchical levels and thus directly estimating the predicted evolutionary change in a trait without any explicit model of selection (Morrissey et al., 2012; Rausher, 1992). This article highlights how, even without genetic information, multi-response models can be applied more broadly to improve our understanding of trait-fitness relationships and thus the process of natural selection.

3. Theory

3.1 Decomposing selection gradients to assess consistency with causality

To understand how we can infer if our estimates of selection from observational data are consistent with causality, we reconsider the Lande-Arnold equation in its simplest form (equation [1]), but where a trait and fitness have been measured repeatedly across individuals and common environments:

$$w_{ijk} = \mu_0 + \beta_z z_{ijk} + o_{ijk}. \quad [2]$$

Where w_{ijk} is relative fitness that has been relativised by the global mean fitness. For simplicity, *o* is everything that is not explained by *z*: $o_{ijk} = u_j + c_k + e_{ijk}$ where u_j and c_k are the random effects of individual identity *j* and common environment *k*. As both the trait and fitness are measured repeatedly across individuals and common environments, we can also decompose the phenotypic value of the trait:

$$z_{ijk} = u_{z,j} + c_{z,k} + e_{z,i}. \quad [3]$$

Where u, c and e are individual, common environment and residual effects on the trait z, respectively. We combine equations [2] and [3], to give:

$$w_{ijk} = \mu_0 + \beta_z(u_{z,j} + c_{z,k} + e_{z,i}) + o_{ijk}, \quad [4]$$

which, when expanded, gives us:

$$w_{ijk} = \mu_0 + \beta_z u_{z,j} + \beta_z c_{z,k} + \beta_z e_{z,i} + o_{ijk}. \quad [5]$$

Equation [5] shows that if there is a causal effect of *z* on *w* (i.e. an increase in any component of the trait phenotype leads to the same increase in fitness), the gradients estimated for each hierarchical level should be the same. This is because the Lande-Arnold method is estimating a single selection gradient β_z, which inherently assumes that all decomposed gradients are of equivalent magnitude and direction. If this is not true, this suggests the relationship between a trait and fitness is not causal and β_z would give a biased estimate of selection. These decomposed gradients represent associations between trait and fitness at different hierarchical levels as follows:

$$w_{ijk} = \mu_0 + \beta_u u_{z,j} + \beta_c c_{z,k} + \beta_e e_{z,i} + o_{ijk}. \quad [6]$$

Where the β's represent the linear relationship between different hierarchical levels of the trait and relative fitness: β_u among individuals, β_c among common environments, and β_e within individuals, respectively. The selection gradient estimated in a Lande-Arnold regression is composed of these decomposed gradients as shown below:

$$\beta_z = \beta_u \frac{\sigma^2_{u_z}}{\sigma^2_z} + \beta_c \frac{\sigma^2_{c_z}}{\sigma^2_z} + \beta_e \frac{\sigma^2_{e_z}}{\sigma^2_z}. \quad [7]$$

Where σ^2's represents the total (σ^2_z), among individuals ($\sigma^2_{u_z}$), among common environments ($\sigma^2_{c_z}$), and within individual ($\sigma^2_{e_z}$) variances of the trait. The Lande-Arnold derived selection gradient (β_z) is therefore composed of all these gradients, weighted by the proportion of the total phenotypic variance of the trait explained by variation at these different hierarchical

levels. The derivation of equation [7] from equation [6] can be found in supplementary material S1.

As shown above, under a simple model of causality, where the trait directly affects fitness, we expect the selection gradients at each hierarchical level to be equal in both direction and magnitude to each other and the overall β_z (Equation [6]; Figure 1A; (Bonnet & Postma, 2018; Gauzere et al., 2021; Hadfield et al., 2013; Pick et al., 2016). Thus, comparing the magnitude and direction of these decomposed selection gradients, can be used to infer whether selection on a trait is consistent with causality. An exception to this would be when selection is measured on a trait with considerable measurement error, which may result in β_e estimates that are biased downwards (Dingemanse et al., 2021). Inconsistency with causality would be indicated if one or more of the decomposed selection gradients differed from each other. For example, if there is a non-causal relationship between relative fitness and a trait driven by an environmental variable that changes among common environments, this would result in an increase in β_c which would upwardly bias β_z (Eq, 8; Figure 1b), and thus impact our interpretation of selection estimated using the Lande-Arnold method (Rausher, 1992; Stinchcombe et al., 2002).

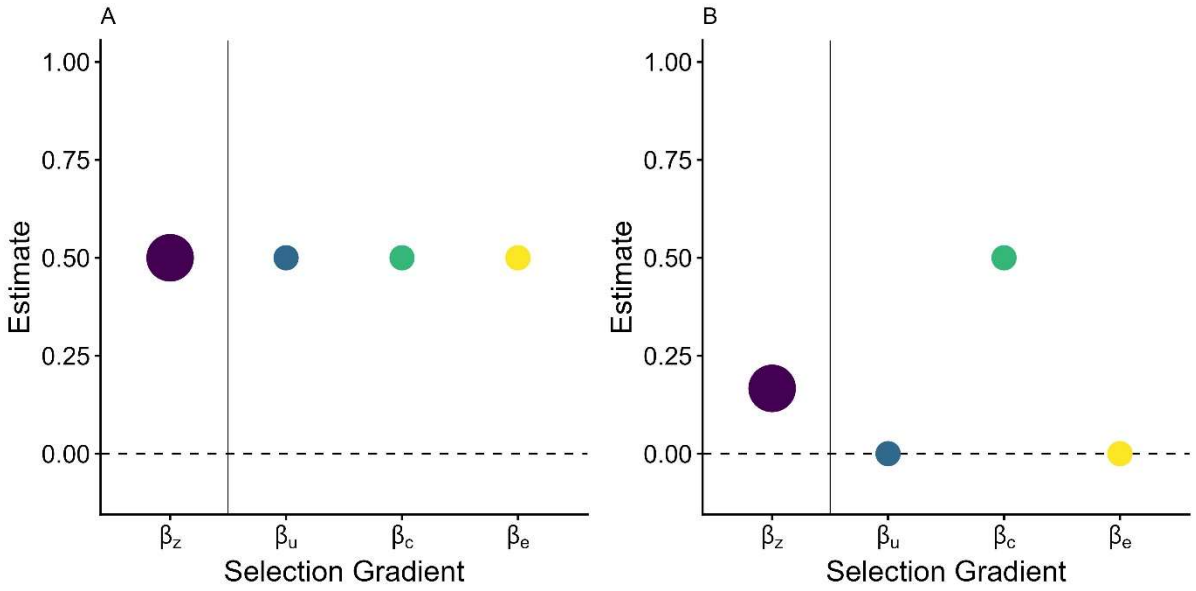

Figure 1: Examples of Lande-Arnold derived selection gradients and their decomposition to selection gradients at different hierarchical levels when selection is: (A) consistent with causality; versus (B) inconsistent with causality and instead confounded by an environmental variable that changes across common environments. β_z (purple) is the Lande-Arnold derived selection gradient, β_u (blue) is the among individual selection gradient, β_c (green) is the common environment selection gradient and β_e (yellow) is the within individual selection gradient.

3.2 Decomposing selection gradients to assess consistency with, and correct for, soft and hard selection

When estimating a single, global selection gradient across environments, the Lande-Arnold method can bias estimates of selection toward zero depending on both how selection operates and how fitness is relativised. Although selection may actually be on a continuum between hard and soft selection, we discuss these as discrete modes of selection for clarity. To illustrate this, Figures 2A – D show a theoretical example where the selection gradients for

192 trait *z* were estimated across two years of measurements under scenarios of hard selection
193 (first column; A and C) and soft selection (second column; B and D), when fitness is
194 relativised either across years (top row; A and B) or within years (bottom row; C and D).
195 Here, all individuals live within the same local environment, and the mean value of the trait
196 changes between years, being larger in year 2. The true strength of selection is the same
197 across years in both scenarios (solid black lines). When selection is hard the fitness of an
198 individual is dependent on its trait value independent of the population trait mean. Therefore,
199 as the population trait mean changes across years, so too does mean absolute fitness. Under
200 this scenario, we would obtain the correct selection gradient using the Lande-Arnold method
201 when selection is measured across common environments and fitness is relativised across
202 years (Figure 2A). However, when fitness is relativised within years and hard selection is
203 occurring, relative fitness is not reflective of the change in absolute fitness that is occurring
204 between years, causing the selection gradient to be biased downwards (Figure 2C).
205
206 When selection is soft, the absolute fitness of individuals in year 2 falls into the same range
207 as year 1 because mean fitness is the same in both years. In this scenario of soft selection, the
208 selection gradient is always underestimated when measured from data pooled across both
209 years. This occurs even if the selection gradient is the same in both years and regardless of
210 how fitness is relativised (Figures 2B & 2D).

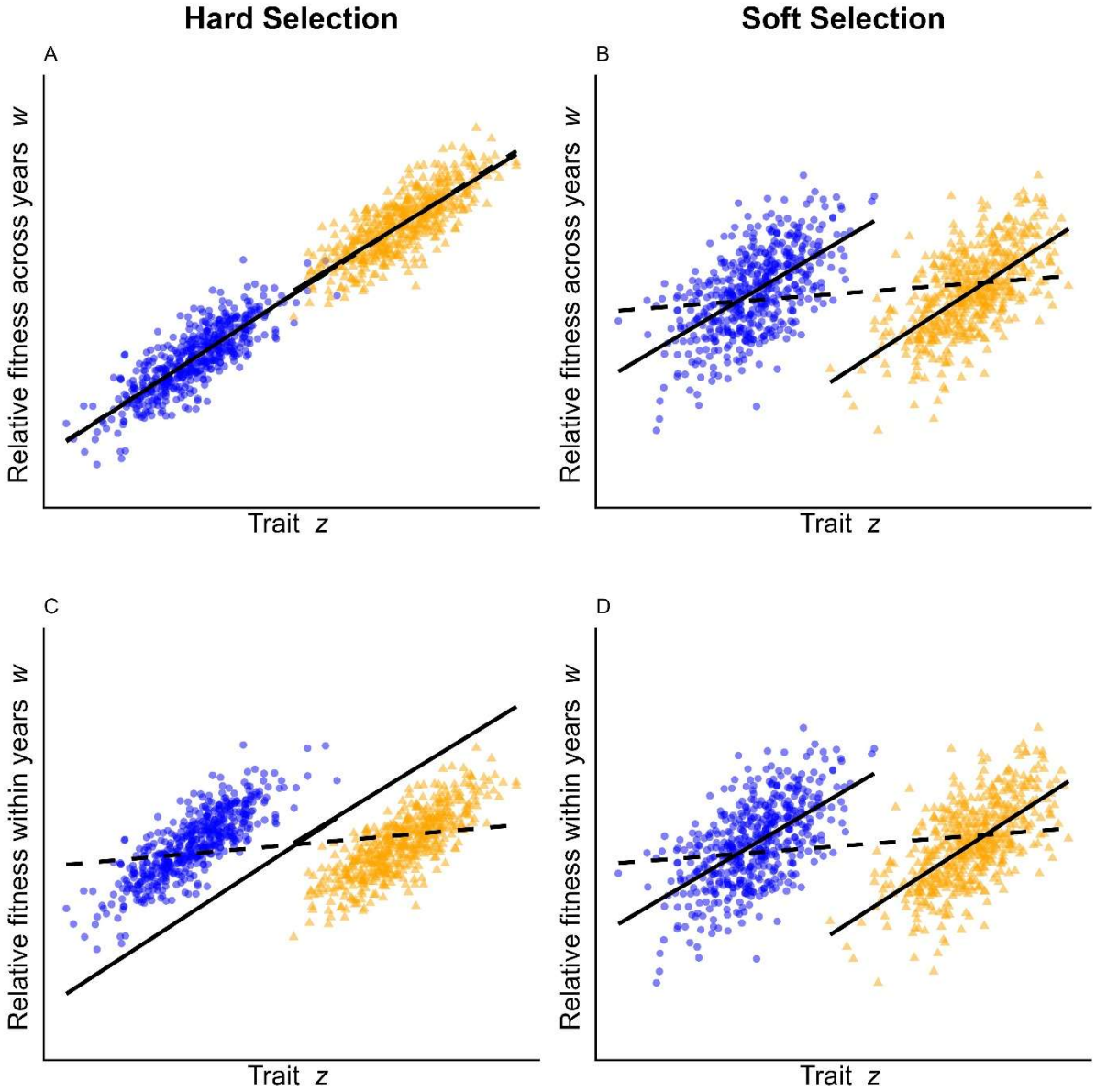

Figure 2: The linear relationship between relative fitness *w* and trait *z* (i.e. the selection gradient) where fitness has been relativised by the global mean (A & B) and the within year mean (C & D), when selection is hard (A & C) and soft (B & D). Coloured points denote *w* (treated as described above) and *z* values measured in year 1 (blue) and year 2 (orange). Solid lines show the true selection gradients would be recovered by analysing each year separately, while dashed lines show the selection gradients measured when data from both years are

pooled together and fitness is relativised by the global mean. In (A) the dashed line cannot be seen as it is identical to the solid line. Note that the slopes of the true selection gradients do not change between years, which would occur if the strength of selection was varying across years.

Before we describe how selection gradient estimates can be corrected when selection is consistent with soft selection, we must first discuss how the pattern of decomposed selection gradients that is consistent with causality changes depending on whether selection is hard or soft. When hard selection occurs the fitness of an individual is independent of the trait mean of the population. If the trait mean of the population changes across common environments, the associated relative fitness also changes from year to year (Figure 2A). Therefore, we expect the among common environment selection gradient (β_c) to be different from zero and equivalent in both direction and magnitude to all other selection gradients when selection is both causal and hard (Figure 3A). However, if selection is soft, there will be no difference in fitness, and thus relative fitness of individuals among common environments (Figure 2B). Therefore, we do not expect there to be a relationship between mean trait value and mean relative fitness between common environments if the trait mean varies across those common environments. Consistency with soft selection, is therefore provided by a common environment selection gradient (β_c) of zero, but other selection gradients (e.g., β_u and β_e) different from zero and equivalent to each other. If selection is consistent with soft selection and causality, we should still see the relationship between trait and relative fitness present both within and among individuals (Figure 3B). Soft selection will result in a downward bias in β_z if the contribution of the negligible among common environment selection gradient is not corrected (Figures 2B & 3B), as estimates of β_z will include β_c as per equation [7].

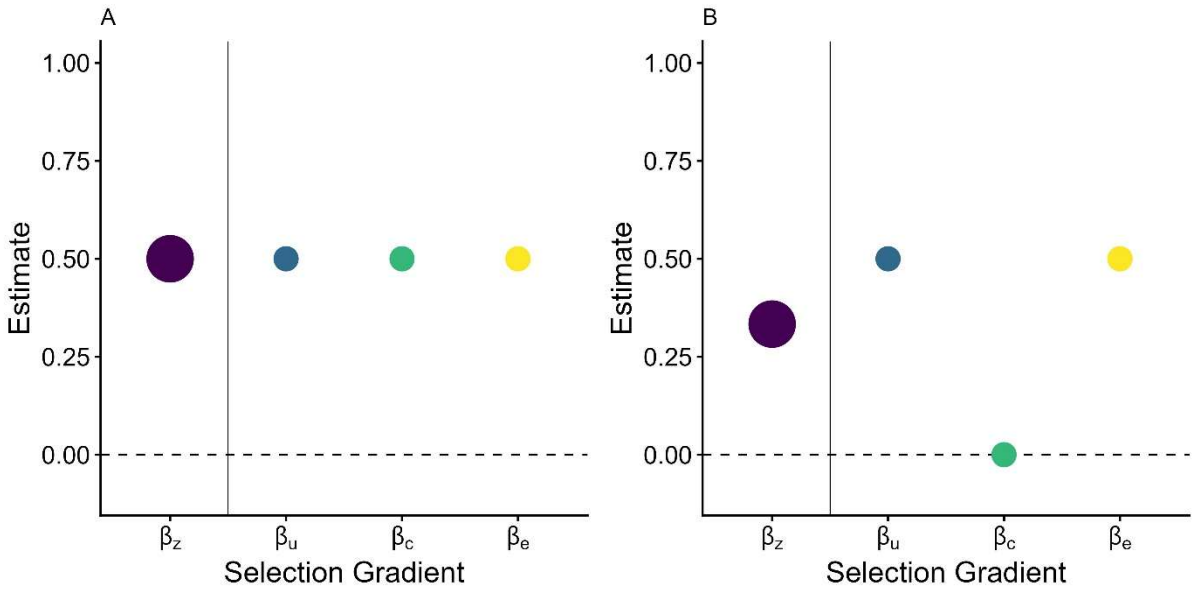

Figure 3: Examples of Lande-Arnold derived selection gradients and their decomposition at different hierarchical levels when selection is: (A) consistent with hard selection; versus (B) consistent with soft selection. β_z (purple) is the Lande-Arnold derived selection gradient, β_u (blue) is the among individual selection gradient, β_c (green) is the common environment selection gradient and β_e (yellow) is the within individual selection gradient.

Corrected estimates of the selection gradient (β'_z) can be obtained by removing any variation in relative fitness or the trait across common environments as well as their relationship to each other from equation 7. This includes removal from the total variance σ_z^2. Thus, in Figure 3B the corrected estimate of the selection gradient (β'_z) is:

$$\beta'_z = \beta_u \frac{\sigma_{u_z}^2}{\sigma_{u_z}^2 + \sigma_{e_z}^2} + \beta_e \frac{\sigma_{e_z}^2}{\sigma_{u_z}^2 + \sigma_{e_z}^2}. \quad [8]$$

It is also important to note that other methods have been developed to correct and test for consistency with soft selection. More in-depth discussion of how these different approaches affect selection gradient estimates and test for consistency with hard vs soft selection is in the supplementary material S2.

3.3 Using genetic information to decompose selection gradients

Previous studies have decomposed the covariance between fitness and trait into genetic versus non-genetic hierarchical levels (Morrissey et al., 2012; Rausher, 1992; Stinchcombe et al., 2002; Stinchcombe et al., 2014). This can be achieved when data on the relatedness among individuals is available (Kruuk, 2004). However, to date these approaches have not discussed what can be learnt from partitioning the non-genetic association further into any of its components, instead focusing on the genetic association. However, in isolation this genetic association does not provide an explicit model of selection because a genetic association between a trait and fitness could occur due to genetic correlations with other traits that are causally related to fitness (i.e. under selection; Morrissey et al. (2012); Rausher (1992); Thomson et al. (2017)). Below we highlight how the model we present above can be extended to obtain further information on whether selection on a trait is likely to be causal by partitioning β_u into additive genetic and permanent environmental hierarchical levels as shown below:

$$\beta_u u_{z,j} = \beta_a a_{z,j} + \beta_{pe} pe_{z,j}. \quad [9]$$

Where the additive genetic selection gradient (β_a) is the linear relationship between individual breeding values for a trait and individual breeding values for fitness. The permanent environment selection gradient (β_{pe}) is the linear relationship between mean trait value across individuals' permanent environments and mean relative fitness across individuals' permanent environments. Thus, β_u is weighted by both β_a and β_{pe} as follows:

$$\beta_u = \beta_a \frac{\sigma^2_{a_z}}{\sigma^2_{a_z} + \sigma^2_{pe_z}} + \beta_{pe} \frac{\sigma^2_{pe_z}}{\sigma^2_{a_z} + \sigma^2_{pe_z}}. \quad [10]$$

Therefore, equation [6] becomes:

$$w_{ijk} = \mu_0 + \beta_a a_{z,j} + \beta_{pe} pe_{z,j} + \beta_c c_{z,k} + \beta_e e_{z,i} + e_{w,i}. \quad [11]$$

Comparison of the genetic selection gradient with other selection gradients measured in the same model provides more information about whether a causal relationship between the trait and relative fitness is likely (Figure 4). When a trait has a causal effect on fitness, we expect the selection gradient between trait and relative fitness between individuals' breeding values and permanent environments to be equivalent to each other. Therefore, assuming hard selection, selection is consistent with causality when all selection gradients are equivalent (Figure 4A). Decomposing β_u into permanent environment and genetic selection gradients can also facilitate the interpretation of the relationship between a trait and fitness in more complex scenarios. For example, if β_z is non-zero, but both within individual (β_e) and among common environment (β_c) selection gradients are zero (suggesting the association is not causal), decomposition of β_u into β_a and β_{pe} can help us separate cases where β_z is confounded by an environmental variable that is consistent among individuals (Figure 4B) from cases where β_z is being confounded by genetic correlations with another trait that is under selection (Figure 4C). Additionally, decomposing β_u into permanent environment and genetic selection gradients can help us identify cases where selection on the trait is consistent with causality, but the trait is genetically correlated with another trait which is being selected in the opposite direction to the genetic correlation. In this scenario we expect all selection gradients to be equal in magnitude and direction to each other except the genetic selection gradient β_a, which may be biased downwards by any magnitude depending on the strength of the genetic correlation with the other trait and the strength of selection acting on the other trait (Figure 4D).

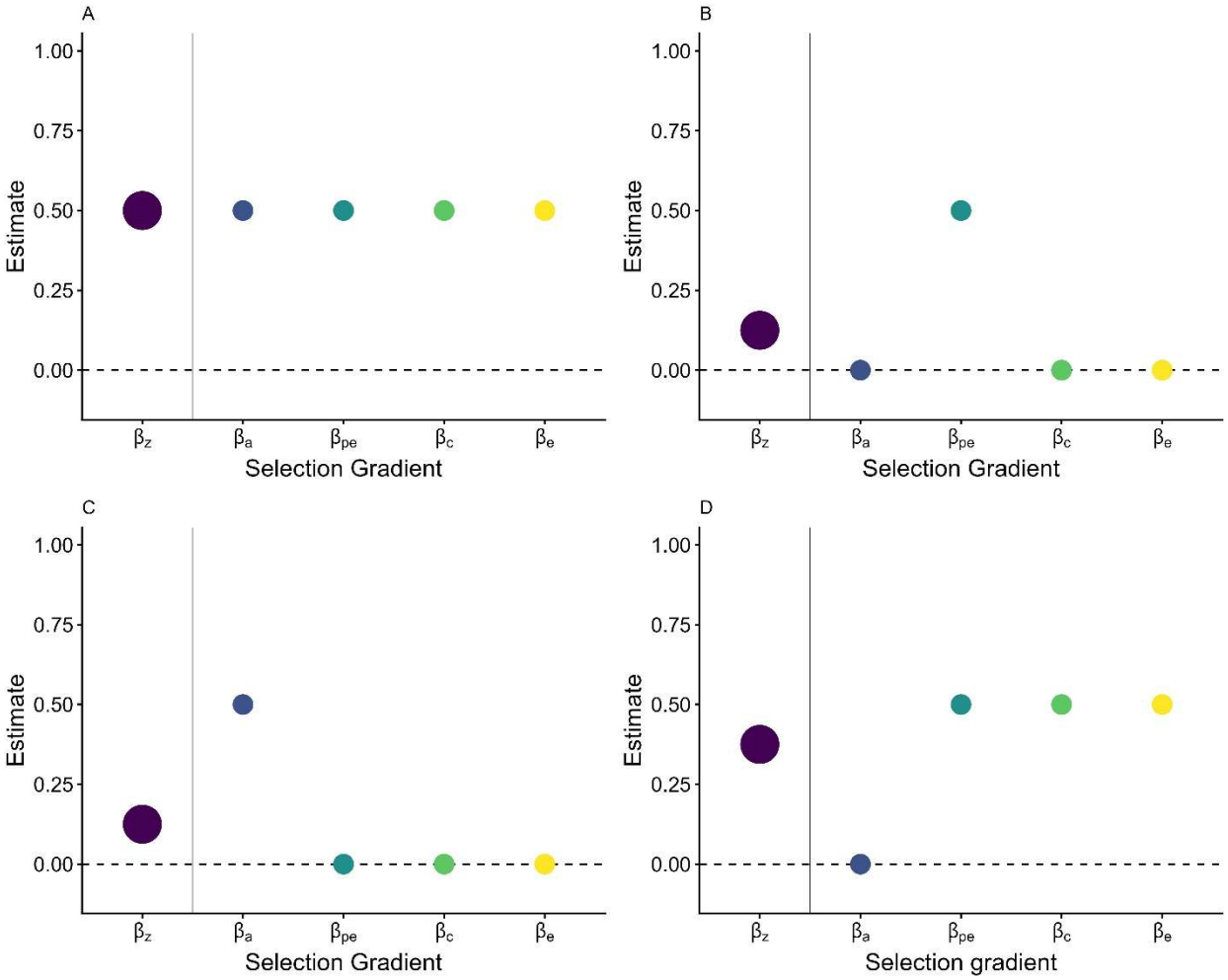

Figure 4. Examples of Lande-Arnold derived selection gradients and decomposed selection gradients where genetic information is available. (A) Pattern is consistent with causality, (B) Pattern is selection is inconsistent with causality and instead suggests a confounding variable that is consistent among individuals, (C) Pattern is inconsistent with causality and instead suggests confounding by a genetic correlation with a trait that is selected to increase and (D) Pattern is consistent with causality but the trait is genetically correlated with a trait that is is selected to decrease. Where β_z (purple) is the Lande-Arnold derived selection gradient, β_a (blue) is the genetic selection gradient, β_{pe} (teal) is the permanent environment selection gradient, β_c (green) is the among common environment selection gradient and β_e (yellow) is the within individual selection gradient. Hard selection is assumed to be occurring in all scenarios. In D, the genetic selection gradient depicted is zero; but it can take any magnitude

depending on the strength of the genetic correlation with the other trait and the strength of selection acting on the other trait.

4. Estimating decomposed selection gradients

4.1 Multi-response models

The decomposition of directional selection gradients can be readily achieved with multi-response models. Where multiple response variables are simultaneously modelled, the variances for each response variable and covariances between them at different hierarchical levels can be estimated. Multi-response models can be run in Bayesian or Frequentist frameworks. However, a Bayesian approach allows uncertainty in parameters to be more easily propagated to the derived decomposed selection gradients relative to Frequentist approaches. These models are already frequently used in quantitative genetics and behavioural ecology to estimate the genetic covariance between a trait and fitness (Kruuk et al., 2002; Morrissey, 2014; Morrissey et al., 2010; Reid, 2012), and the phenotypic and genetic covariances between traits (Clements et al., 2011; Dingemanse & Dochtermann, 2013; Dobson et al., 2023; Houslay & Wilson, 2017; Reid & Wolak, 2018). Thus, there are many packages available that can run these models, and a number of papers and tutorials outlining how these models are implemented (de Villemereuil et al., 2018; Houslay & Wilson, 2017). While multi-response models are capable of measuring selection gradients (Morrissey et al., 2010), we found very few studies that have used or advocated their use in this context (but see Dingemanse et al. (2021); Mittell et al. (2024); Videlier et al. (2021)). In wild populations with repeated measures across individuals and common environments, we can fit a multi-response model with relative fitness w (relativised by the global mean) and the trait z as two responses, with random intercepts for individual identity and common environments as specified below:

$$w_{ijk} \sim \mu_{w,0} + \beta_{w,d} d_{w,ijk} + u_{w,j} + c_{w,k} + e_{w,ijk}, \quad [12a]$$

$$z_{ijk} \sim \mu_{z,0} + u_{z,j} + c_{z,k} + e_{z,ijk}. \quad [12b]$$

Where global intercepts and random intercepts at each hierarchical level are measured separately for trait *z* and relative fitness *w*, *d* is any variable that may affect relative fitness independent of the trait and $\beta_{w,d}$ is the linear effect of variable *d* on *w*. When measuring β_z using multiple regression (i.e. where *w* is the only response variable), the random intercepts *u* and *c*, and any unexplained effects *e*, are only modelled and estimated for *w*, and *z* is assumed to be free of error. However, multi-response models measure the variances in and covariances between *z* and *w* across hierarchical levels *u* and *c*, and any residual variance and covariance *e,* therefore allowing error in *z* to be explicitly estimated. The random intercepts for *u* and *c*, and residual errors *e*, are distributed assuming a multivariate normal distribution with variance-covariance structures as follows:

$$[u_w, u_z] \sim MVN(0, \Sigma_u \otimes I), \Sigma_u = \begin{bmatrix} \sigma^2_{u_w} & \sigma_{u_{w,z}} \\ \sigma_{u_{w,z}} & \sigma^2_{u_z} \end{bmatrix}, \quad [12c]$$

$$[c_w, c_z] \sim MVN(0, \Sigma_c \otimes I), \Sigma_c = \begin{bmatrix} \sigma^2_{c_w} & \sigma_{c_{w,z}} \\ \sigma_{c_{w,z}} & \sigma^2_{c_z} \end{bmatrix}, \quad [12d]$$

$$[e_w, e_z] \sim MVN(0, \Sigma_e \otimes I), \Sigma_e = \begin{bmatrix} \sigma^2_{e_w} & \sigma_{e_{w,z}} \\ \sigma_{e_{w,z}} & \sigma^2_{e_z} \end{bmatrix}. \quad [12e]$$

Where MVN denotes a multivariate normal distribution and Σ are covariance matrices that link common environment *c* and among individual *u* hierarchical levels, and residual effects *e*, to observations, $\otimes$ is a Kronecker product, and I an identity matrix. From these matrices we can estimate variances and covariances where $\sigma^2_{u_w}$, $\sigma^2_{c_w}$ and $\sigma^2_{e_w}$ are the variances in relative fitness due to differences among individuals, common environments and any residual variance, respectively. $\sigma^2_{u_z}$, $\sigma^2_{c_z}$ and $\sigma^2_{e_z}$ are the variances in the trait due to differences between individuals, between common environments and any variance in the trait left

unexplained by the model, respectively. $\sigma_{u_{w,z}}$, $\sigma_{c_{w,z}}$ and $\sigma_{e_{w,z}}$ are the covariances between trait and relative fitness among individuals, common environments and any residual covariance, respectively.

It is important to note here that a regression coefficient is the covariance between the two variables of interest, standardised by the variance of the explanatory variable. As described by (Phillimore et al., 2010), using the estimates of variances and covariances above, we can therefore calculate a regression coefficient (i.e. a directional selection gradient) akin to a Lande-Arnold model as specified below:

$$\beta_z = \frac{\sigma_{w,z}}{\sigma_z^2}. \quad [13]$$

Total phenotypic covariance is calculated by summing all possible covariances estimated by the model together:

$$\sigma_{w,z} = \sigma_{u_{w,z}} + \sigma_{c_{w,z}} + \sigma_{e_{w,z}}. \quad [14]$$

Total phenotypic variance in the trait is calculated by summing all traits variances estimated by the model together:

$$\sigma_z^2 = \sigma_{u_z}^2 + \sigma_{c_z}^2 + \sigma_{e_z}^2. \quad [15]$$

We can estimate decomposed selection gradients using the variances and covariances estimated at each level as specified below:

$$\beta_u = \frac{\sigma_{u_{w,z}}}{\sigma_{u_z}^2}. \quad [16]$$

$$\beta_c = \frac{\sigma_{c_{w,z}}}{\sigma_{c_z}^2}. \quad [17]$$

$$\beta_e = \frac{\sigma_{e_{w,z}}}{\sigma_{e_z}^2}. \quad [18]$$

When selection is soft, we can correct selection estimates β'_z by not including the covariance between relative fitness and the trait among common environments, nor any variation in trait among common environments, when calculating the directional selection gradient as follows (analogous to Equation 8):

$$\beta'_z = \frac{\sigma_{u_{w,z}} + \sigma_{e_{w,z}}}{\sigma^2_{u_z} + \sigma^2_{e_z}}. \quad [19]$$

4.2 Multi-response animal models

If we have information on relatedness among individuals, we can use a multi-response animal model which involves an additional hierarchical level varying at the level of the individual and linked to the relatedness matrix (A), allowing individual phenotypic effects to be split into additive genetic and non-genetic effects. This uses relatedness information to estimate breeding values, additive genetic variances and covariances. In this model, the previous hierarchical level associated with among individual effects *u* separates to give us additive genetic *a* and permanent environment *pe* hierarchical levels as below:

$$w_{ijk} \sim \mu_{w,0} + \beta_{w,d} d_{w,i} + a_{w,j} + pe_{w,j} + c_{w,k} + e_{w,ijk}, \quad [20a]$$

$$z_{ijk} \sim \mu_{z,0} + a_{z,j} + pe_{z,j} + c_{z,k} + e_{z,ijk}. \quad [20b]$$

From which we can we obtain the following additional variances and covariances

$$[a_w, a_z] \sim MVN(0, \Sigma_a \otimes A), \Sigma_a = \begin{bmatrix} \sigma^2_{a_w} & \sigma_{a_{w,z}} \\ \sigma_{a_{w,z}} & \sigma^2_{a_z} \end{bmatrix}. \quad [20c]$$

$$[pe_w, pe_z] \sim MVN(0, \Sigma_{pe} \otimes I), \Sigma_{pe} = \begin{bmatrix} \sigma^2_{pe_w} & \sigma_{pe_{w,z}} \\ \sigma_{pe_{w,z}} & \sigma^2_{pe_z} \end{bmatrix}. \quad [20d]$$

β_u can be calculated from this model by summing the additive genetic and permanent environment covariances and variances as follows:

$$\beta_u = \frac{\sigma_{a_{w,z}} + \sigma_{pe_{w,z}}}{\sigma^2_{a_w} + \sigma^2_{pe_z}}. \quad [21]$$

4.3 Measuring direct selection on multiple traits

One of the major advantages of the Lande-Arnold method is the ability to model selection on multiple traits simultaneously, allowing pathways of indirect and direct selection gradients for correlated traits to be disentangled and leading to improved understanding of selection. The multi-response model can be expanded to measure decomposed direct selection gradients on correlated traits as follows:

$$w_{ijk} \sim \mu_{w,0} + \beta_{w,d} d_{w,ijk} + a_{w,j} + pe_{w,j} + c_{w,k} + e_{w,ijk}, \quad [22a]$$

$$z_{1_{ijk}} \sim \mu_{z_1,0} + a_{z_1,j} + pe_{z_1,j} + c_{z_1,k} + e_{z_1,ijk}, \quad [22b]$$

$$z_{2_{ijk}} \sim \mu_{z_2,0} + a_{z_2,j} + pe_{z_2,j} + c_{z_2,k} + e_{z_2,ijk}. \quad [22c]$$

Where $z_1$and z_2 are two different traits measured on the same individual, and *d* is any variable that may affect relative fitness independent of either trait. From these we get the following matrices at different hierarchical levels as follows:

$$\Sigma_u = \begin{bmatrix} \sigma^2_{u_w} & \sigma_{u_{w,z_1}} & \sigma_{u_{w,z_2}} \\ \sigma_{u_{w,z_1}} & \sigma^2_{u_{z_1}} & \sigma_{u_{z_1,z_2}} \\ \sigma_{u_{w,z_2}} & \sigma_{u_{z_1,z_2}} & \sigma^2_{u_{z_2}} \end{bmatrix}, \quad [23a]$$

$$\Sigma_c = \begin{bmatrix} \sigma^2_{c_w} & \sigma_{c_{w,z_1}} & \sigma_{c_{w,z_2}} \\ \sigma_{c_{w,z_1}} & \sigma^2_{c_{z_1}} & \sigma_{c_{z_1,z_2}} \\ \sigma_{c_{w,z_2}} & \sigma_{c_{z_1,z_2}} & \sigma^2_{c_{z_2}} \end{bmatrix}, \quad [23b]$$

$$\Sigma_e = \begin{bmatrix} \sigma^2_{e_w} & \sigma_{e_{w,z_1}} & \sigma_{e_{w,z_2}} \\ \sigma_{e_{w,z_1}} & \sigma^2_{e_{z_1}} & \sigma_{e_{z_1,z_2}} \\ \sigma_{e_{w,z_2}} & \sigma_{e_{z_1,z_2}} & \sigma^2_{e_{z_2}} \end{bmatrix}, \quad [23c]$$

Using methods outlined by Bernstein (2009); Froy et al. (2019), we can calculate decomposed direct selection gradients by rearranging these variances and covariances among trait z_1, and z_2 , using the among individual direct selection gradients as examples, as follows.

For each hierarchical level, we first rearrange each matrix into two. The first matrix, A, contains the variance and covariance between traits:

$$\Sigma_{u_A} = \begin{bmatrix} \sigma^2_{u_{z_1}} & \sigma_{u_{z_1,z_2}} \\ \sigma_{u_{z_1,z_2}} & \sigma^2_{u_{z_2}} \end{bmatrix}. \quad [24]$$

The second matrix, B, will contain the covariances between each trait and relative fitness as follows.

$$\Sigma_{u_B} = [\sigma_{\boldsymbol{u}_{w,z_1}} \quad \sigma_{\boldsymbol{u}_{w,z_2}}]. \quad [25]$$

We then can then estimate decomposed direct selection gradients on z_1 and z_2 by multiplying Σ_{u_B} and the inverse of Σ_{u_A} ($\Sigma_{u_B}\ {\Sigma_{u_A}}^{-1}$), this can then be repeated for each level of the multi-response model specified.

5. Empirical example

5.1 Study Population

To illustrate our approach, we provide an empirical example estimating selection on male antler weight using individual-based data from a long-term study of a wild population of red deer (*Cervus elaphus)* living on the Isle of Rum, Scotland (Kruuk et al., 2002; Pemberton et al., 2022). For more a detailed description on the study population see the supplementary material S3. Briefly, males grow and shed antlers each year and these are used as weapons in male-male contests to control groups of females. Males with larger antlers are more likely to win fights and mate with more females (Clutton-Brock et al., 1979; Kruuk et al., 2002; Mittell et al., 2024), suggesting a causal relationship between antler weight and fitness. Previous analysis suggested strong selection on antler weight using the Lande-Arnold method and that antler weight had a large heritable component (Kruuk et al., 2002). Selection on antler weight on the Rum study population may be soft as males compete over a limited

457 resource (females) and would only need larger antlers than their direct competitors. Males
458 regrow antlers each year (Kruuk et al., 2002, Clutton-Brock et al., 1979), and there is
459 evidence that the phenotypic composition of antler weight in the population changes between
460 years (Moyes et al., 2011). Previous studies also found there was no genetic correlation
461 between antler weight and breeding success, and that the phenotypic association was being
462 driven mainly by a permanent environment effect (Kruuk, 2004; Kruuk et al., 2014; Kruuk et
463 al., 2002; Mittell et al., 2024). However, these decomposed selection gradients were not
464 directly compared to each other and therefore not explicitly tested for
465 consistency/inconsistency with causality as laid out above and the results were not discussed
466 in the context of soft or hard selection.
467
468 For our analyses we used data collected between 1974-2020. Annual breeding success was
469 used as our fitness component, which is the number of calves sired by a male each year as
470 determined by paternity assignments. We then relativised annual breeding success by the
471 global mean, that is, the mean across all years. We estimated antler weight by weighing cast
472 antlers on site using an electronic top pan balance accurate to 0.1 g (Kruuk et al., 2002). We
473 then standardised antler weight across years (i.e. the global mean) to a variance of 1 and
474 mean of 0 for analysis.
475
476 ## 5.2 Statistical analyses
477 ### 5.2.1 Measuring selection on antler size using the Lande-Arnold method
478 We ran a linear mixed model as specified in equation [S7], where relative annual breeding
479 success was the response. We fitted standardised antler weight, mean centred age, mean
480 centred age squared and the year annual breeding success was measured as fixed effects. Age
481 and its quadratic term account for variation in annual breeding success due to the ages of

482 males independent of any effects of age on antler weight (Kruuk et al., 2014; Kruuk et al.,
483 2002) while the continuous year fixed effect accounts for an increase in the proportion of
484 calves being assigned paternities over the course of the study as a result of improvements in
485 the proportion of individuals that are genotyped (Walling et al., 2010). We included male
486 identity and year as random effects to account for unexplained variation in relative annual
487 breeding success among individuals and years (Kruuk et al., 2014; Kruuk et al., 2002; Mittell
488 et al., 2024). This model, and all models presented here, were implemented using the
489 MCMCglmm v2.36 package (Hadfield, 2010) in R v4.5.0 (R Core Team, 2025).
490
491 5.2.2 Measuring selection on antler weight using multi-response models
492 We ran two multi-response models, one where we assumed no pedigree information was
493 available, a common scenario for many wild study populations, and one where we used
494 genetic relatedness calculated from the pedigree to allow decomposition of the among
495 individual selection gradient into a genetic and permanent environment gradient as specified
496 in equations [S8]-[S9]. Our response variables were relative annual breeding success and
497 standardised antler weight and the fixed effects of year, age and age^2 were fitted for relative
498 annual breeding success only. We fitted male identity as a random effect either once (in
499 models assuming no pedigree information) or twice for animal models, with one linked to a
500 genetic relatedness matrix to estimate both β_a and β_{pe} in models that used relatedness in the
501 form of a pedigree (Kruuk, 2004) (see the supplementary S4 for details). As males grew
502 antlers and participated in the mating season in the same year, we included year as a random
503 effect and allowed a covariance between relative annual breeding and antler weight to be
504 measured to estimate β_c. We then compared the gradients estimated in the same model and
505 determined whether they were statistically different from each other by subtracting their

posteriors and calculating the posterior mean difference and their 95% credible intervals (95% CIs).

5.3 Results

We do not discuss the estimates of the fixed effects or random effects variances and covariances in detail here as they are not the focus of the study, but they are reported in Tables S1-S2. As expected, the Lande-Arnold derived estimate of β_z for antler weight in the Rum study population was positive (β_z post mean: 0.51, 95% CIs: (0.33 – 0.67), Figure 5, Table S3). Decomposing this selection gradient into among individual, among common environments (years) and within individual selection gradients using a multi-response model with no genetic information suggests that selection on antler weight in the Rum study population was consistent with causality and soft selection. Causality is suggested because the among and within individual selection gradient were similar in both magnitude and direction to each other, and to the Lande-Arnold derived selection gradients, and were statistically different from zero (β_u: 0.64 (0.39 – 0.94), β_e: 0.46 (0.23 – 0.70), Figure 5, Table S3). However, soft selection is suggested because the among common environment selection gradient was not statistically different from zero and was visually removed from the other gradients (β_c: 0.20 (-0.14 – 0.51), Figure 5, Table S3), implying that there was no relationship between the weight of antlers grown in a particular year and the mean relative fitness that year. However, the differences between the posteriors for each gradient do not definitively support this – if selection was soft and consistent with causality, we would expect β_u and β_e not to be statistically different from each other, but for both to be statistically different from β_c. While we found β_u to be statistically different from β_c and not statistically different from β_e, β_c and β_e were not statistically different from each other (Table 1). It is possible that the within individual selection gradient was being biased towards zero by error in the trait

measurement (Dingemanse et al., 2021), but we believe this effect to be minimal as antler weight was measured after casting on digital scales accurate to 0.1g.

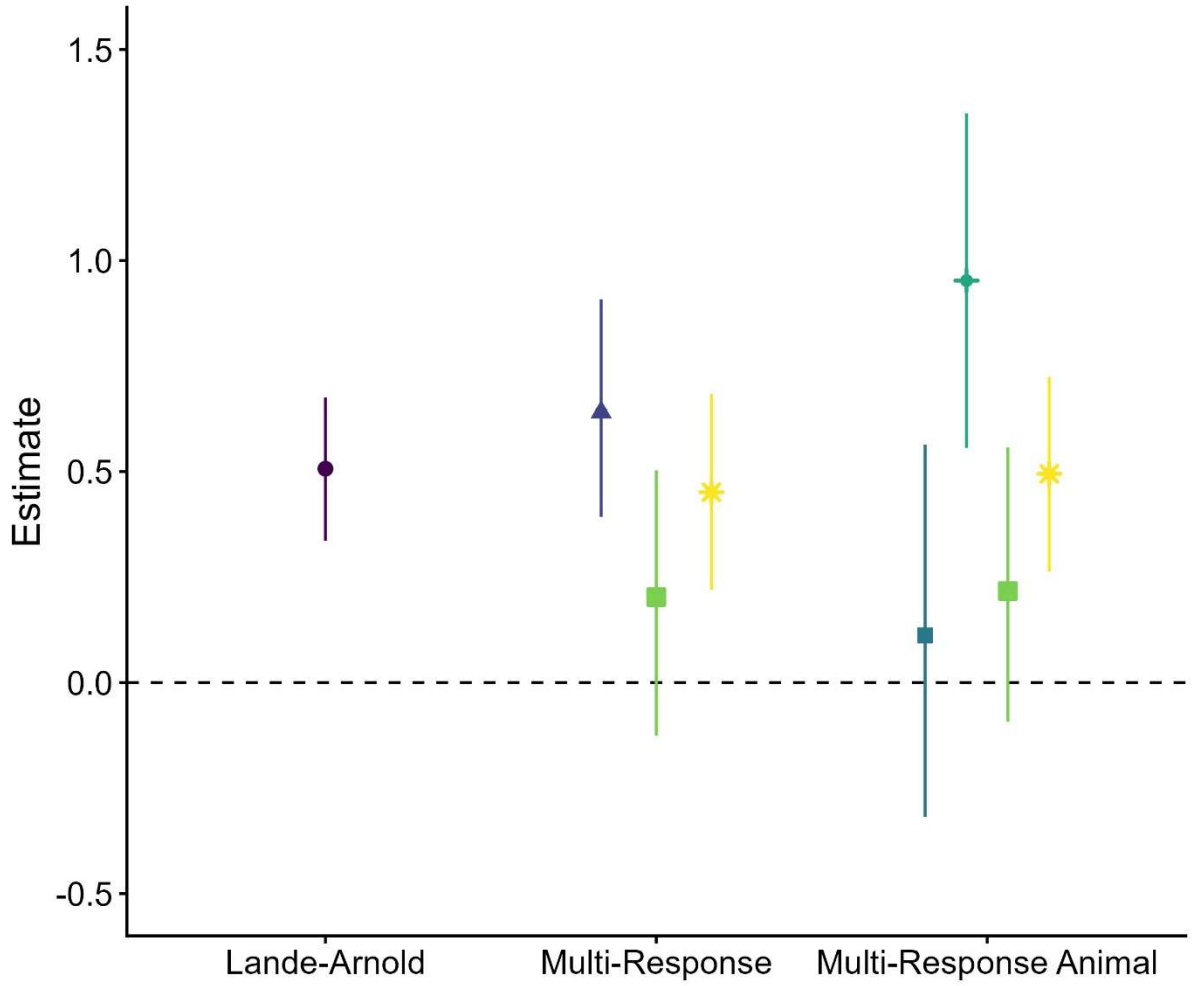

Figure 5: Selection gradient estimates for antler weight in male red deer from the Rum study population derived using the Lande-Arnold method, a multi-response model and a multi-response animal model. The purple circle is the Lande-Arnold derived selection gradient β_z, the dark blue triangle is the among individual selection gradient β_u, the blue small square is the genetic selection gradient β_a, the teal horizontal dash is the permanent environment selection gradient β_{pe}, the green large square is the among common environment (in this case year of measurement) selection gradient β_c and the yellow star is the within individual selection gradient β_e. Points and lines denote the posterior mean estimates and their 95%

credible intervals respectively. 95% credible intervals can be used as an indication of whether the gradients are significantly different from 0.

Table 1: The posterior mean differences and associated 95% credible intervals (CIs) between gradients estimated within the multi-response model and within the multi-response animal model. Where β_u is the among individual selection gradient, β_a is the genetic selection gradient, β_{pe} is the permanent selection gradient, β_c is the among common environment selection gradient and β_e is the within individual selection gradient. Gradients that are significantly different from each other (95% CIs do not overlap 0) are highlighted in bold.

Gradients	Difference between posteriors (Mean + 95% CIs)	
	Multi-response Model	Multi-response Animal Model
$\beta_a - \beta_{pe}$	--	**-0.84 (-1.5 – -0.22)**
$\beta_a - \beta_c$	--	-0.11 (-0.7–0.39)
$\beta_a - \beta_e$	--	-0.438(-0.93 – 0.05)
$\beta_{pe} - \beta_c$	--	**0.73 (0.27 –1.19)**
$\beta_{pe} - \beta_e$	--	**0.46 (0.05 –0.92)**
β_u - β_c	**0.44 (0.09 – 0.87)**	--
β_u - β_e	0.19 (-0.15 – 0.51)	--
β_c - β_e	-0.25 (-0.61 – 0.13)	-0.28 (-0.62 – 0.11)

Decomposing the among individual selection gradient into genetic and permanent environment selection gradients provided further insight. Generally, components that were estimated in both models were similar (Tables S1-S2). Credible intervals were often wider in

556 the multi-response animal model compared to the multi-response model, presumably due to
557 the increase in power required to estimate the genetic components. The genetic selection
558 gradient was not statistically different from zero (β_a: 0.11 (-0.35 – 0.57), Table S3, Figure 5),
559 while the permanent environment selection gradient (β_{pe}: 0.94 (0.57 –1.30), Table S3, Figure
560 5) was larger than the common environment and residual selection gradients (β_c: 0.22 (-0.16
561 – 0.52), β_e: 0.50 (0.24 – 0.72), Table S3, Figure 5). Formal comparison of the posterior
562 estimates shows that the permanent environment selection gradient was significantly greater
563 than every other decomposed selection gradient (Table 1), while the genetic selection
564 gradient was not statistically different from the among common environment nor the within
565 individual selection gradient (Table 1).
566
567 This pattern suggests two possible scenarios:
568 1. Selection on antler weight is soft, indicated by the among common environment
569 selection gradient not being different from 0. Selection is also consistent with
570 causality because both the residual and the permanent environment gradients were
571 different from 0. However, antler weight is genetically correlated with other trait(s)
572 that are selected against, resulting in a genetic selection gradient not different from
573 zero. Additionally, there is the suggestion of a confounding environmental variable
574 that is consistent among individuals, inflating the permanent environment gradient
575 (the point estimate of this gradient is larger than any other; Figure 5). In this scenario
576 the Lande-Arnold selection gradient could be correct, due to simultaneously being
577 biased downwards by the genetic selection gradient and upwards by the permanent
578 environment selection gradient. However, these biases may not be equal and so the
579 Lande-Arnold selection gradient could also be incorrect.

2. Selection on antler weight is inconsistent with causality, and the Lande-Arnold selection gradient is being confounded upwards by variables that are consistent between and within individuals. This is indicated by both the permanent environment and the within individual selection gradients being greater than zero whereas the genetic and common environment selection gradients are not greater than zero. Under this scenario the Lande-Arnold selection gradient is biased upwards by both the permanent environment and the within individual selection gradients.

This approach has helped us narrow this down potential scenario explaining the positive phenotypic association between relative annual breeding success and antler weight. If possible, attempting to account for potential confounding factors that are consistent among individuals could help discern further which of these two scenarios is occurring. Parameter estimates could then be compared among models to assess whether the inclusion of confounding variables reduced the permanent environment selection gradient to be equal in magnitude to the within individual selection gradient or to zero. Considering the Rum red deer population, anecdotal evidence suggests that permanent environment confounding could be driven by differences in where males feed outside the study area through winter and spring prior to the mating season; males that rut in our study area are often opportunistically spotted feeding in the same areas in winter/spring each year. However, as these observations are opportunistic and outside the study area, we do not have the necessary data to explicitly test this hypothesis.

If scenario 1 is correct, and selection on antler weight is causal and soft, selection estimates from the Lande-Arnold approach would be underestimated, but this can be corrected as detailed above using Eq [21]. Applying this correction to the multi-response animal model, we find that our corrected selection gradient estimate did not differ much from the

uncorrected selection gradient derived from the Lande-Arnold method; both credible intervals clearly overlap each other (β_z: 0.51 (0.33 – 0.67), β'_z: 0.56 (0.35 –0.75), Figure 6). To confirm this, we calculated the difference between the posteriors of the selection gradient corrected for soft selection, and the uncorrected selection gradient. We found a non-significant difference ($\beta_z - \beta'_z$: -0.11 (-0.23 – 0.006). This is likely because the effect of the among common environment selection gradient is weighted by the among common environment variance in the trait (Eq [7]), and antler weight did not show substantial among year variance ($\sigma^2_{c_z}$,Table S2).

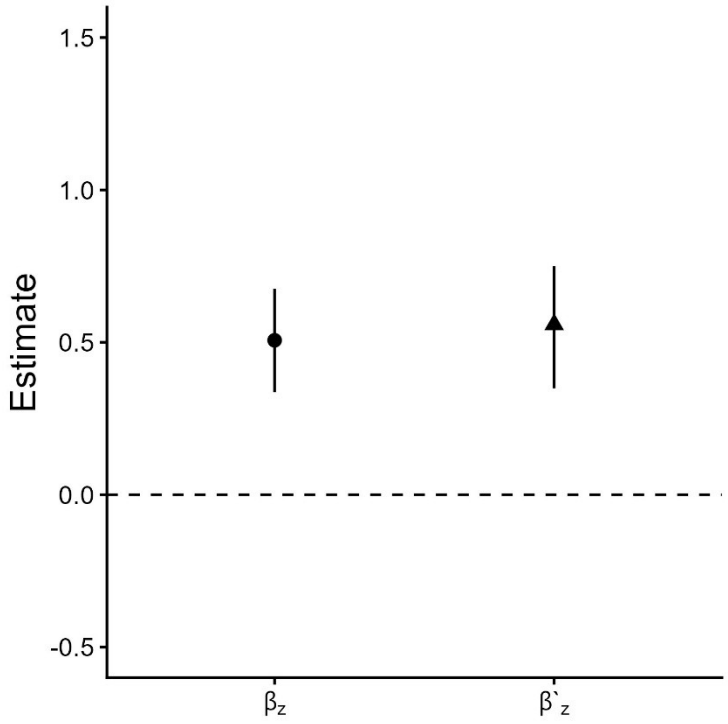

Figure 6: Estimated selection gradients for antler weight in male red deer from the Rum study using the Lande-Arnold method which have not been corrected for soft selection, (left, β_z) and have been corrected for soft selection, right, β'_z, following equation [10] and using data from the multi-response animal model. Points and lines represent posterior mean estimates

and their 95% credible intervals respectively. Note that the credible intervals shown here denote whether these estimates are statistically different from zero, and do not indicate whether they are statistically different from each other.

6. Discussion

We have described how the Lande-Arnold method of estimating selection based on the phenotypic regression of fitness on a trait assumes that selection is causal (see also Rausher (1992); Stinchcombe et al. (2002); Svensson (2023)), and that, when standardising fitness by the global mean, that selection is hard. Importantly we also show that when these assumptions are not met, selection gradients based on the Lande-Arnold method can be biased. We then outline how, when fitness and a trait are measured repeatedly across individuals and common environments, decomposing selection gradients across hierarchical levels can help infer whether patterns are consistent with causality, hard or soft selection, and to correct selection gradient estimates for soft selection. We describe how to decompose selection gradients across hierarchical levels using readily available and well documented multi-response models, and how these models can be expanded to include genetic information and selection on multiple traits. Unlike other methods for determining consistency with causality (Morrissey et al., 2012; Rausher, 1992), when selection is confounded, our method can help identify the source of confounding between trait and fitness, which will help justify and shape future data collection.

While we mostly discuss temporal common environments such as year, we emphasise that other common environments, such as location, can be included in analyses and genetic information is not strictly necessary for the approach we have described. Furthermore, our method can be used to determine consistency with causality not just for selection gradients

(i.e. where the response is a fitness measure of an individual), but any relationship between two traits estimated by linear regression if both traits have been measured repeatedly across individuals and/or common environments. For example, Ravindran et al. (2022) decomposed the correlation between annual measures of telomere length and female reproductive performance among and within individuals and among years in a wild population of Soay sheep (*Ovis aries*) to shed light on the processes underlying their association. However, we note that without genetic information, confounding via permanent environment and genetic correlations with traits under selection cannot be ruled out. Overall, our method is suitable to a large number of studies from wild populations where genetic data is not available but have repeated measures of fitness and trait phenotypes across individuals and common environments, where paternity or fitness related data can be inferred from other sources.

The Lande-Arnold approach models fitness as a Gaussian trait and we followed this approach in our empirical example to facilitate a simpler illustration of the proposed method. However, we recognise that fitness measures are more appropriately modelled assuming other distributions (e.g. annual survival as a binomial trait, or annual reproductive success as a Poisson trait). In cases where fitness is modelled as non-normally distributed, the model will estimate a latent scale gradient, from which a selection gradient on the observed scale can be derived and methods for this have been discussed elsewhere (Morrissey & Bonnet, 2019; Morrissey & Goudie, 2022). Importantly, comparison of decomposed latent scale gradients to test for consistency with causality and hard and soft selection can still be done as we have described

In our empirical example, we added fixed effects (year and age) that account for biases in relative annual breeding success and ecological processes that occur independent of selection,

which otherwise may have biased the variances and covariances estimated at the different hierarchical levels, and therefore the selection gradient estimates (de Villemereuil et al., 2018). However, we recommend that great care is taken that any fixed effects included in these models are not causing selection or are not a part of the causal pathway of selection. This is because if the predictors vary at a particular level, they may affect the variance/ covariances at that level, which would then affect the comparison between different levels. In the latter case, as a part of the selection process is effectively being removed, this would likely cause the selection gradient to be masked or reduced.

While there is considerable scope to apply our methods across a range of different situations, there are some limitations and considerations that should be discussed. Firstly, there are issues of sample size in selection analyses, and many studies on natural populations may lack the sample size required to accurately estimate selection (Hersch & Phillips, 2004). Unfortunately, the multi-response model requires larger sample size than the Lande-Arnold method, as it involves estimating covariances and variances at different hierarchical levels. There are often sample sizes issues in observational studies of wild populations and in Bayesian analyses this can lead the posterior being too heavily dominated by the priors that are set. Estimating the samples sizes required for the multi-response model approach is convoluted and dependent on a number of facets - the strength of selection acting on the trait, the number of traits, the number of hierarchical levels specified, the variation of the trait at different hierarchical levels, the direction and strength of correlations between traits at each hierarchical level, the number of groups in each hierarchical level and the number of samples in each group. Estimating the sample size required for a specific analysis can be done using simulations using packages such as squidSim (Pick, 2025) in R.

Secondly, the main limiting factor for assessing consistency with hard/soft selection in wild population studies is likely the number of common environments (e.g. locations or years) sampled. Keeping individual-based wild studies funded long term or across large locations is challenging (Clutton-Brock & Sheldon, 2010; Festa-Bianchet et al., 2017; Pemberton et al., 2022; Sheldon et al., 2022) and thus the number of years or the locations sampled may not exceed 10 (Hughes et al., 2017; Siepielski et al., 2009). This is an important consideration because the more complex the models become, i.e. more traits or hierarchical levels included, the higher the sample size that is required. Fortunately, there number of long-term individual-based studies that have been running across decades is increasing (Bonnet et al., 2022; de Villemereuil et al., 2020; Sheldon et al., 2022). These studies are likely to have the sample sizes required to make inferences about the relative importance of hard versus soft selection, providing a way forward to utilise currently existing data whilst strengthening the motivation to continue with data collection.

Finally, we note that, as with all purely observational data, correlation is not equivalent to causation and so the patterns suggested by the decomposition of selection into its hierarchical levels may not be those that are actually occurring. For example, in our empirical example the decomposed gradients are consistent with selection on antler weight being soft. However, it is possible that selection on antler mass is instead hard, but a confounding environmental variable is biasing the common environment selection gradient downwards. We argue that, as with all observational data, it is important to use the ecology of the population to infer whether alternative explanations are likely. In the case of our empirical example, hard selection on antler size would appear unlikely as females cannot store sperm across years and so a male's reproductive success in a given year is entirely determined by his competitive ability in that year.

Despite these issues, we argue that decomposing selection gradients is informative. As in our empirical example, qualitative comparison of the magnitude of the different gradients can provide information on likely sources of environmental confounding and potential unaccounted trait correlations even if direct statistical comparisons between different gradients are inconclusive. Additionally, if soft selection is thought to be likely for a trait, our method can be used to investigate if the data is consistent with soft selection and to correct the selection gradient for soft selection.

We have not discussed measuring quadratic, correlational or fluctuating selection in much detail in our paper. Our framework does not allow for selection to vary across common environments and may produce biased selection gradient estimates if selection does vary (Bonnet & Postma, 2018; Westneat et al., 2020). The theory we propose behind decomposed selection gradients does apply to decomposed quadratic and correlational selection. However, we do not recommend using multi-response models to estimate quadratic or correlational selection gradients, as Dingemanse et al. (2021) showed using simulations that quadratic and correlation selection gradients estimated this way were very imprecise and prone to inaccuracies. Instead latent-variable models can be used to estimate quadratic, correlational and fluctuating selection (Dingemanse et al., 2021), but they require more complex statistical programming skills to implement. There are a few packages that are currently available for fitting these models, but few tutorials exist on how to fit them properly (Dingemanse et al., 2021). Therefore, for measuring directional selection where selection does not fluctuate, which is the scope of our paper, multi-response models are an easily accessible and implementable option to decompose selection gradients, with many tutorials available to help fit them.

In conclusion, when trait and fitness are measured repeatedly across individuals or common environments, the association between the two can be decomposed across different hierarchical levels. This approach can provide insight into whether the association between a trait and fitness is consistent with a causal relationship and whether selection on a trait is consistent with hard or soft selection. We demonstrate how this decomposition can be achieved with multi-response models, a readily available and well documented statistical approach. This type of repeated measures data is likely to exist for many wild populations and for associations other than those between traits and fitness. Application of our approach to such data should provide more information about the sources of correlations between traits and between traits and fitness, which can better inform our understanding of the ecology of natural selection and the evolution of traits in wild populations.

Data and Code availability: data and code used in this manuscript can be found at https://github.com/Sazz010101/selection_causal_inference_paper.git

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Supplementary materials

S1 Derivation of equation [9]

The Lande-Arnold selection gradient β_z can be estimated by dividing the total phenotypic covariance between trait and relative fitness ($\sigma_{w,z}$) by the total phenotypic variance of the trait (σ_z^2) as follows.

$$\beta_z = \frac{\sigma_{w,z}}{\sigma_z^2} \qquad [s1]$$

Where $\sigma_{w,z}$ is composed of the covariance between relative fitness and trait among individuals ($\sigma_{u_{w,z}}$), among common environments ($\sigma_{c_{w,z}}$) and within individuals ($\sigma_{e_{w,z}}$).

$$\sigma_{w,z} = \sigma_{u_{w,z}} + \sigma_{c_{w,z}} + \sigma_{e_{w,z}}. \qquad [s2]$$

1002 σ_z^2 is composed of the variance of the trait among individuals ($\sigma_{u_z}^2$), among common

1003 environments ($\sigma_{c_z}^2$) and within individuals ($\sigma_{e_z}^2$).

1004

1005 $$\sigma_z^2 = \sigma_{u_z}^2 + \sigma_{c_z}^2 + \sigma_{e_z}^2 \qquad [s3]$$

1006

1007 Therefore, decomposed selection gradients β_u, β_c and β_e are calculated by dividing the

1008 decomposed covariances by their respective decomposed trait variances as follows:

1009

1010 $$\beta_u = \frac{\sigma_{u_{w,z}}}{\sigma_{u_z}^2} \qquad [s4a]$$

1011 $$\beta_c = \frac{\sigma_{c_{w,z}}}{\sigma_{c_z}^2} \qquad [s4b]$$

1012 $$\beta_e = \frac{\sigma_{e_{w,z}}}{\sigma_{e_z}^2} \qquad [s4c]$$

1013

1014 We rearrange and insert equation [s1] into [s2] to get:

1015

1016 $$\beta_z \sigma_z^2 = \sigma_{u_{w,z}} + \sigma_{c_{w,z}} + \sigma_{e_{w,z}} \qquad [s5]$$

1017

1018 Which we derive to get:

1019 $$\beta_z = \frac{\sigma_{u_{w,z}} + \sigma_{c_{w,z}} + \sigma_{e_{w,z}}}{\sigma_z^2} \qquad [s6]$$

1020 We then rearrange and insert equations [S4a – c] into equation [S6a] as follows:

1021

$$\beta_z = \frac{\beta_u \sigma^2_{u_z} + \beta_c \sigma^2_{c_z} + \beta_e \sigma^2_{e_z}}{\sigma^2_z} \quad [s6a]$$

$$\beta_z = \beta_u \frac{\sigma^2_{u_z}}{\sigma^2_z} + \beta_c \frac{\sigma^2_{c_z}}{\sigma^2_z} + \beta_e \frac{\sigma^2_{e_z}}{\sigma^2_z} \quad [s6b]$$

S2 How different ways of standardising fitness and traits affects selection estimates depending on whether selection on the trait is soft or hard

When explaining how fitness is relativised and traits are standardised when measuring selection, Lande and Arnold (1983) stated that fitness should be relativised by the "mean of the population". Lande and Arnold were considering a single population at a single time point, which allows no way to distinguish hard and soft selection and makes this definition reasonably clear in this context. However, this definition is somewhat vague if a population is measured across multiple environments or time points. Under these scenarios, what constitutes a population becomes more ambiguous and may be context dependent, making hard and soft selection more distinct mechanisms in this context. Importantly, this can result in different ways of relativising fitness and standardising traits that can introduce unintentional biases and error into estimates of selection.

Studies measuring selection can standardise fitness and trait phenotypes either by the global mean of the data or by the mean within common environments (e.g., within each year in populations with annual breeding cycles). A review by De Lisle and Svensson (2017) found that most studies measuring fitness and traits across several environments or 'groups' tended to mostly standardise fitness and traits 'globally' without much justification. If fitness and trait phenotypes are standardised by the global mean selection may be underestimated if

selection is soft. A way of dealing with this would be to standardise fitness and trait phenotypes within common environments (ie. within groups) prior to analysis with the Lande-Arnold method. This would theoretically correct Lande-Arnold derived selection gradients for soft selection because individuals compete with other individuals that exist in the same space and/or time, and this approach relativises their fitness and trait phenotype to the mean of the individuals they compete against. Indeed, this is how (De Lisle & Svensson, 2017) recommend to standardise fitness and trait measurements when soft selection is occurring. However, relativising fitness and trait phenotypes within common environments implicitly assumes soft selection is occurring across those common environments, which can lead to erroneous selection gradients if that is not the case. It can also be difficult to understand in advance of the analysis whether selection on a trait is likely to be soft or hard. For example, when a population occurs across different areas with different environments, it can be difficult to determine how connected these subpopulations are (De Lisle & Svensson, 2017). If soft selection on a trait is occurring and subpopulations are completely isolated, selection would appear soft, but increasing connectivity is likely to give soft selection the appearance of hard selection. For this scenario, relativising fitness and trait phenotypes within common environments would result in the selection gradient being misestimated. Additionally, common environment fitness and trait means are likely to be erroneous, particularly if samples sizes within common environments are small, which may cause underestimation of the error in the means within environments, and therefore, the selection gradient estimates (Lüdtke et al., 2008). Plotting the relationship between a trait and absolute fitness separately for each level of the common environment to determine whether soft or hard selection is occurring may also lead to spurious conclusions being made, as these associations are not tested statistically.

Aside from our method, whether selection is hard or soft can also be explicitly tested for by expanding the Lande-Arnold method to include the within common environment trait mean as a covariate and comparing the slopes (i.e. contextual analysis)(Goodnight et al., 1992), or by including within common environment mean and an individual's deviation from the mean and comparing their slopes (i.e. within and among group centring) (Kreft et al., 1995; Westneat et al., 2020). It is worth noting that these methods are reparameterizations of each other and are consistent with our approach of decomposing selection gradients. However, the statistical implementation of the contextual analysis/ within and among group centring approach differs from our approach, as they suffer from the issues mentioned above when samples sizes within common environments are small.

Our approach of decomposing selection gradients into different hierarchical levels using multi-response models allows for hard vs soft selection to be explicitly tested in a statistically robust framework, while accounting for uncertainty in estimation of the means of common environments with small sample sizes. Our method also simultaneously assesses consistency with causality and allows selection gradient estimates to be corrected when selection is soft without any of the drawbacks associated without having to first assume soft or hard selection is occurring.

S3 Additional information on measurements used in our empirical example of selection on antler weight in male red deer

In the Rum red deer study population, the identity of almost every individual is known from natural markings, collars, ear tags and punches (Pemberton et al., 2022). Females are sexually receptive for 1-2 days each year during the mating season (September – October) and give birth May-June of the following year. Male reproductive success is primarily determined via precopulatory male-male

competition during the mating season, where males will attract females into their harem, which they then mate with once they come into oestrus. Males guard their harem against other males, and contests frequently escalate into dangerous fights to establish dominance and possession of females (Clutton-Brock et al., 1979). We expect selection on antler weight to be consistent with causality because males use their antlers when fighting to gain access to females and defend their harems, and males with larger antlers are likely to win more fights and mate more females (Clutton-Brock et al., 1979; Kruuk et al., 2002; Mittell et al., 2024). Selection on antler weight is likely be soft as males would only need bigger antlers compared to their opponents be able to win fights, and the limiting number of niches to be occupied would be the ratio of females to males during the mating season (Clutton-Brock et al., 1979; Kruuk et al., 2002).

Antler weight has been a major focus in the Rum study population, with much already investigated in terms of both phenology (Clements et al., 2010; Moyes et al., 2011) and its association with male fitness (Clutton-Brock et al., 1979; Kruuk et al., 2014; Kruuk et al., 2002), making it an ideal trait to demonstrate our methods. Antlers start to grow in the spring, encased in blood supplying tissue called velvet which is rubbed off in summer (Clements et al., 2010). Antlers are then cast off the following spring and the cycle restarts (Clements et al., 2010). Cast antlers are collected and measured, and the unique shape of the antler is used to identify which individuals they belonged to (Kruuk et al., 2002).

The paternity of calves born each year were determined via tissue samples collected at birth (Guinness et al., 1982; Pemberton et al., 2022), which were then used to infer male annual breeding success measures as the number of calves males were assigned paternity to each year. The age of males born in the study area were calculated from their date of birth, whereas the ages of immigrant males who were born outside the study area but rutted within the study area were estimated from appearance (Walling et al., 2010). We included males aged 5 and over in our analysis as males are considered to be sexually mature at this age (Pemberton et al., 2022). We then mean centre male age by subtracting mean male age from each male age.

S4 Analysis specifications for our empirical example

Our data comprised of 818 observations from 246 male deer across 44 years. The pedigree used for the animal multi-response model was pruned down to 564 individuals who either had phenotypic data available or where related to individuals who had phenotypic data.

We estimated the Lande-Arnold derived selection gradient for antler weight using the equation specified below:

$$w_{ijk} \sim \mu_0 + \beta_z z_{ijk} + \beta_m m_{ijk} + \gamma_m m_{ijk}^2 + \beta_b b_{ijk} + u_j + c_k + e_{ijk}. \quad [S7]$$

Where w is the relative annual breeding success of a male, μ_0 is the global intercept for relative fitness, z is male antler weight standardised to a variance of 1 and mean of 0, m is the mean centred age of males, m^2 is the mean centred age of males squared, b is the year in which both trait and fitness were measured, u, c and e are identity and year random effects and any residual variation in w not explained by the model. β_z, β_m, γ_m and β_b are the effects of z_{ijk}, m, m^2 and b on w respectively, and β_z is the Lande-Arnold derived directional selection gradient.

We estimated decomposed selection gradients for antler weight with no genetic information using the equations specified below:

$$w_{ijk} \sim \mu_{w_0} + \beta_{w_m} m_{w_{ijk}} + \gamma_{w_m} m_{w_{ijk}}^2 + \beta_{w_b} b_{w_{ijk}} + u_{w_j} + c_{w_k} + e_{w_{ijk}}, \quad [S8a]$$

$$z_{ijk} \sim \mu_{z_0} + u_{z_j} + c_{z_k} + e_{z_{ijk}}. \quad [S8b]$$

Where μ_{w_0} is the global intercept for relative annual breeding success, β_{w_m}, Y_{w_m} and β_{w_m} are the effects of m, m^2 and b on w and u_w, c_w and e_w are identity and year random effects for w and any residual variation in w not explained by the model respectively. μ_{z_0} is the global intercept for

standardised antler weight, and u_z, c_z and e_z are identity and year random effects for z and any residual variation in z not explained by the model respectively.

We estimated decomposed selection gradients for antler weight including genetic information using the equations specified below:

$$w_{ijk} \sim \mu_{w_0} + \beta_{w_m} m_{w_i} + \gamma_{w_m} m_{w_i}^2 + \beta_{w_b} b_{w_i} + a_{w_j} + pe_{w_j} + c_{w_j} + e_{w_{ijk}}, \quad [S9a]$$

$$z_{ijk} \sim \mu_{z_0} + a_{z_j} + pe_{z_j} + c_{z_k} + e_{z_{ijk}}. \quad [S9b]$$

Where a_w and pe_w are genetic and permanent random effects for w and a_z and pe_z are genetic and permanent random effects for z.

We then followed procedures outlined in section 3.1 to estimate variances-covariances matrices and eventually the decomposed selection gradients from both multi-response models.

S5 Additional results and model outputs from our empirical example

Table S1: Fixed effects estimated from models of selection on antler in the rum study population, including a univariate (Lande-Arnold derived) model and multi-response models both with and without genetic information. Effects are the global intercept for relative breeding success (μ_0 or μ_{w_0}), the global intercept for standardised antler weight (μ_{z_0}), the effect of mean-centred age (β_m or β_{w_m}), the quadratic effect of mean-centred age (γ_m or γ_{w_m}) and the effect of year (β_b or β_{w_b}). Estimates are posterior means and 95% credible intervals. Terms are highlighted in bold when pMCMC values are lower than 0.05. Note we do not include estimates of β_z from equation [S1] as these are included in table S3.

	Lande-Arnold derived		Multi-response no genetic information		Multi-response with genetic information	
Outputs	Post.mean (+95 cis)	pMCMC	Post.mean (+95 cis)	pMCMC	Mean (+95 CIs)	pMCMC
Intercept-relative fitness μ_0 or μ_{w_0}	-24.2(-49.9 – 1.72)	0.07	**-44.3 (-80.4 – -8.9)**	**0.001**	-37.4 (-78.9 – 0.74)	0.06

Intercept - Trait μ_{z_0}	--	--	0.02 (-0.18 – 0.24)	0.851	0.11 (-0.15 – 0.35)	0.39
Age- relative fitness β_m or β_{w_m}	**-0.13 (-0.19 – -0.05)**	**0.0008**	**-0.12 (-0.19 – -0.04)**	**0.002**	**-0.13 (-0.2 -0.05)**	**0.002**
Age^2- relative fitness γ_m or γ_{w_m}	**-0.04 (-0.06 – -0.03)**	**0.0008**	**-0.04 (-0.06 – -0.03)**	**0.0008**	**-0.04 (-0.06 – -0.03)**	**0.0008**
Year- relative fitness β_b or β_{w_b}	0.013 (-1.83e-04 – 0.03)	0.06	**0.02 (0.005 – 0.04)**	**0.014**	0.02 (2.4e-04 – 0.04)	0.06

Table S2: Random effect estimates from models of the Lande Arnold derived selection gradient (univariate model) and multi-response models without and with genetic information. Outputs include the variance of relative breeding success among individuals (σ^2_u or $\sigma^2_{u_w}$), permanent environment ($\sigma^2_{pe_w}$), additive genetic variance ($\sigma^2_{a_w}$), among years (σ^2_c or $\sigma^2_{c_w}$) and within individuals (σ^2_e or $\sigma^2_{e_w}$); the variance of standardised antler weight among individuals ($\sigma^2_{u_z}$), permanent environment ($\sigma^2_{pe_z}$), additive genetic variance ($\sigma^2_{a_z}$), among years ($\sigma^2_{c_z}$) and within individuals ($\sigma^2_{e_z}$); and the covariance between relative breeding success and standardised antler weight among individuals ($\sigma_{u_{w,z}}$), permanent environment ($\sigma_{pe_{w,z}}$), additive genetic variance ($\sigma_{a_{w,z}}$), among years ($\sigma_{c_{w,z}}$) and within individuals ($\sigma_{e_{w,c}}$). Estimates are posterior means with 95% credible intervals in parentheses.

	Lande-Arnold derived	Multi-response no genetic information	Multi-response with genetic information
Relative breeding success – Individual σ^2_u or $\sigma^2_{u_w}$	0.75 (0.55 – 0.96)	0.94 (0.67 – 1.22)	--
Relative breeding success – Permanent Environment $\sigma^2_{pe_w}$	--	--	0.8 (0.51 – 1.07)
Relative breeding success – Genetic $\sigma^2_{a_w}$	--	--	0.26 (0.12 – 0.43)

Relative breeding success – Year σ_c^2 or $\sigma_{c_w}^2$	0.07 (0.01 – 0.12)	0.18 (0.09 – 0.29)	0.19 (0.09 – 0.32)
Relative breeding success – Residual σ_e^2 or $\sigma_{e_w}^2$	1.01 (0.89 – 1.13)	1.09 (0.96 – 1.25)	1.1 (0.96 – 1.26)
Antler weight – Individual $\sigma_{u_z}^2$	--	0.46 (0.34 -0.59)	--
Antler weight- Permanent Environment $\sigma_{pe_z}^2$	--	--	0.32 (0.19 – 0.44)
Antler weight- Genetic $\sigma_{a_z}^2$	--	--	0.23 (0.12 – 0.34)
Antler weight – Year $\sigma_{c_z}^2$	--	0.41 (0.24 – 0.66)	0.45 (0.25 – 0.73)
Antler weight – Residual $\sigma_{e_z}^2$	--	0.37 (0.32 –0.42)	0.36 (0.31 – 0.41)
Covariance – Individual $\sigma_{u_{w,z}}$	--	0.3 (0.14 – 0.43)	--
Covariance – Genetic $\sigma_{a_{w,z}}$	--	--	0.03 (-0.08 – 0.14)
Covariance – Permanent Environment $\sigma_{pe_{w,z}}$	--	--	0.29 (0.16 – 0.45)
Covariance – Year $\sigma_{c_{w,z}}$	--	0.08 (-0.06 – 0.23)	0.1 (-0.08 – 0.25)
Covariance – Residual $\sigma_{e_{w,c}}$	--	0.17 (0.08 – 0.26)	0.17 (0.09 –0.27)

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1178 Table S3: selection gradients for antler weight in the Rum study population, derived from a univariate (Land-
1179 Arnold method) model, a multi-response model with no genetic term and a multi-response model with a genetic
1180 term. Outputs are the Lande-Arnold derived selection gradient or the equivalent of a Lande-Arnold derived
1181 selection gradient estimated form a multi-response model (β_z), the among individual selection gradient (β_u), the
1182 genetic selection gradient (β_a), the permanent environment selection gradient (β_{pe}), the among year selection
1183 gradient (β_c) and the within individual selection gradient (β_e). Estimates are extracted from posterior means and
1184 95% credible intervals.

Selection gradient	Lande-Arnold derived	Multi-response no genetic information	Multi-response with genetic information
Lande-Arnold derived/ equivalent β_z	0.51 (0.33 –0.67)	0.44 (0.27 – 0.64)	0.45 (0.27 – 0.62)
Among individual β_u	--	0.64 (0.39 –0.94)	--
Genetic β_a	--	--	0.45 (0.27 – 0.62)
Permanent environment β_{pe}	--	--	0.94 (0.57 –1.30)
Among common environment (year) β_c	--	0.20 (-0.14 –0.51)	0.22 (-0.16 –0.52)
Within individual β_e	--	0.46 (0.23 –0.7)	0.50 (0.24 – 0.72)

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