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Estimating genetic parameters in natural populations using the 'animal model'

Loeske E. B. Kruuk

Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh EH9 3JT, UK
(*loeske.kruuk@ed.ac.uk*)

Estimating the genetic basis of quantitative traits can be tricky for wild populations in natural environments, as environmental variation frequently obscures the underlying evolutionary patterns. I review the recent application of restricted maximum-likelihood 'animal models' to multigenerational data from natural populations, and show how the estimation of variance components and prediction of breeding values using these methods offer a powerful means of tackling the potentially confounding effects of environmental variation, as well as generating a wealth of new areas of investigation.

Keywords: animal model; restricted maximum likelihood; BLUP breeding values; heritability; microevolution

1. INTRODUCTION

Evolutionary biology aims to explain diversity. Within a population, if individuals have different phenotypes, is this because they have different genotypes, because they have experienced different environments, or because of a combination of both? If it is because they have different genotypes, have these differences arisen by chance, or have the current genotypes been favoured by natural selection in previous generations? Also—a persistent paradox undermining the intuitive appeal of Darwinian natural selection—if selection consistently favours some genotypes at the expense of others, why do we observe any variation? These questions form the basis of the study of evolutionary biology, and although we have firm and well-established answers to some of them, answers to other aspects, for example the last, are more evasive (Barton & Turelli 1989; Roff 1997; Barton & Keightley 2002). For any useful scientific investigation, we need accurate quantitative measures of the relevant factors (Lynch & Walsh 1998). In this review, I consider one field of evolutionary research that focuses on measuring the determinants of biological diversity, quantitative genetics, and show how the recent application of sophisticated analytical techniques to the study of natural populations has facilitated a more powerful analysis of core questions, as well as opening up a range of new hypotheses to be tested.

Phenotypic characteristics such as morphological or life-history traits are likely to be affected by large numbers of genes (Falconer & Mackay 1996; Lynch & Walsh 1998), the genetic basis of which can be quantified indirectly via statistical inferences based on the similarities between relatives in a population. Quantitative genetics, as the subject is known, has a long and successful application in plant and animal breeding. For evolutionary studies of populations in natural environments, the motivation behind estimating the genetic basis of a quantitative trait is ultimately to be able to predict whether natural or sexual selection on the trait will generate a permanent phenotypic change, since a trait must be heritable in order to evolve. Combined with an understanding of the nature of inherited variation in traits, the dissection of patterns of natural selection and microevolution within populations can provide valuable insights into patterns of macroevolution and speciation (Hendry & Kinnison 1999). Recent research suggests that directional selection in particular may be the driving force behind phenotypic diversification (Schluter 2000; Hendry & Kinnison 2001; Rieseberg *et al.* 2002). The results of evolutionary studies may also prove useful for the identification of optimal strategies for the management and conservation of wild populations (Stockwell *et al.* 2003; Coltman *et al.* 2003). Quantitative genetics therefore addresses the question of 'how?' associated with the 'why?' questions posed by the study of adaptation and evolution (Lynch & Walsh 1998).

Natural selection works by weeding out alleles that produce less fit phenotypes, and should therefore reduce genetic variation. By Fisher's Fundamental Theorem of Natural Selection, the rate of change in fitness in a population is equal to the additive genetic variance in fitness (Fisher 1958), so there should therefore be no additive genetic variation in fitness in a population at equilibrium (Kimura 1958; Charlesworth 1987). By corollary, traits closely related to fitness, rather than fitness itself, should also have little or no additive genetic variance in a population at equilibrium, all else being equal (Robertson 1955; Charlesworth 1987; Falconer & Mackay 1996). However, substantial levels of additive genetic variation are consistently reported underlying phenotypic traits known to be under selection (Mousseau & Roff 1987; Roff & Mousseau 1987; Pomiankowski & Møller 1995; Falconer & Mackay 1996; Lynch & Walsh 1998; Merilä & Sheldon 1999, 2001; Stirling *et al.* 2002). Furthermore, artificial selection frequently shows a sustained response to selection, again suggesting that favourable alleles are not quickly fixed in a population (Hill & Caballero 1992; Barton & Keightley 2002). This abundance of polygenic variation underlying quantitative traits under selection

constitutes a central paradox of evolutionary biology, which is not sufficiently resolved by an explanation of continued mutational input (Barton & Turelli 1989; Roff 1997; Barton & Keightley 2002).

In addition, selection on a heritable trait should generate an evolutionary response in the phenotypic distribution of the trait, the exact nature of which will be determined by the magnitude and form of selection and the heritability of the trait (Falconer & Mackay 1996). The continued success of artificial selection in animal and plant breeding testifies to the robustness of these predictions. However, again surprisingly, out of several intensive studies of heritable traits under directional selection in wild populations living in natural environments, remarkably few have demonstrated the microevolutionary response to selection predicted by quantitative genetics theory (Merilä *et al.* 2001*c*).

We are therefore faced with a substantial mismatch between theoretical quantitative genetics and empirical observations from natural populations. Such a mismatch can presumably be attributed to one of two occurrences: (i) the measurements of key parameters are wrong; (ii) the theoretical expectations are too simplistic. I argue here that both of these potential pitfalls (which may not be exclusive) can to some extent be avoided by the use of more sophisticated statistical techniques than have traditionally been employed in the majority of studies of wild populations. The assumption behind (i) is that we are overestimating either the amount of genetic variation underlying a trait, or the selection acting on it, such that our expectations of the transgenerational effects of natural selection are inflated. Regarding point (ii), the simple predictions outlined above assume that each trait is following a solitary trajectory, independent of other associated traits that may also be under selection, evolving under constant environmental conditions. In both cases, careful dissection of the potential impact of environmental heterogeneity within multivariate analyses can provide a much more accurate understanding of the evolutionary dynamics of phenotypic traits evolving in wild populations.

One of the major recent changes in the study of the quantitative genetics of natural populations has been the use of mixed models, in particular the form of mixed model known as the 'animal model', for the estimation of variance components (Lynch & Walsh 1998). In contrast to simpler techniques typically used to estimate heritabilities in studies of wild populations to date, such as parent–offspring regression or sib analyses, these models incorporate multigenerational information from complex pedigrees and allow estimation of a range of causal components of variance. Furthermore, they are not bound by assumptions of no assortative mating, inbreeding or selection, and allow for unbalanced datasets. The animal model has a well-established history in the quantitative genetics of plant and animal breeding, based on a series of key papers by Henderson (1950, 1975, 1984), and significantly advanced by the application of maximum-likelihood techniques (Thompson 1973; Shaw 1987). In the preface to their textbook on quantitative genetics, Lynch and Walsh cite this form of analysis as one of three major recent developments in the subject (Lynch & Walsh 1998, p. xiv). However, for no obvious reason other than possibly computational demands, their use in evolutionary studies outside plant and animal breeding is only recent and still quite restricted. Konigsberg & Cheverud (1992) fitted an animal model, but without referring to it as such, to data from a free-ranging macaque population (see also Cheverud & Dittus 1992), and Knott *et al.* (1995) applied an animal model to data from a laboratory population of bruchid beetles. After this, to my knowledge, the next studies to use the animal model in analysis of data from free-ranging animals were of three ungulate populations (bighorn sheep (Réale *et al.* 1999); red deer (Kruuk *et al.* 2000); and Soay sheep (Milner *et al.* 2000)), since which a handful of other populations has been added to the list (see below). The late arrival of the animal model in studies of the evolutionary genetics of wild species, relative to its ubiquity in plant and animal breeding, is especially surprising given that some of its strongest advantages are in dealing with data typical of the form of natural populations, in particular with the complexities generated by heterogeneous environmental conditions.

The aim of this paper is to provide an introduction to the rationale behind animal models, and to review some of the studies of wild populations that have used them. In particular, I hope to illustrate their potential value to evolutionary ecologists in their ability to provide, first, estimates of a range of different causal components of phenotypic variance, and, second, predictions of individual genetic merit or breeding values. Both forms of information can generate critical insights into the evolutionary ecology of natural populations.

2. METHODS

(a) *The animal model*

The outline of the animal model below is a brief summary introducing its key ingredients, in particular the form of the model and the concept of maximum likelihood (ML); for a more detailed treatment see Lynch & Walsh (1998) and references therein (in particular Henderson 1950, 1984; Shaw 1987; Kennedy 1989; Meyer 1989*b*; Mrode 1996).

The animal model is a form of mixed model, the term used to describe linear regressions in which the explanatory terms are a mixture of both 'fixed' and 'random' effects (see for example Pinheiro & Bates 2000; McCulloch & Searle 2001). Fixed effects are unknown constants that affect the mean of a distribution. Random effects are used to describe factors with multiple levels sampled from a population of possible values, for which the analysis provides an estimate of the variance of the effects rather than a parameter for each factor level. Random effects therefore influence the variance of the trait.

In the case of an animal model, the random effects of interest are the additive genetic value of individual animals. For the simplest form of animal model, the phenotype y of individual i is written as

$$y_i = \mu + a_i + e_i, \tag{2.1}$$

where μ is the population mean, a_i is the additive genetic merit of individual i, and e_i is a random residual error; the model has no fixed effects other than μ. The terminology 'animal model' arises simply because the model is defined at the level of the individual animal—in contrast to, for example, a sire model, in which sires are evaluated based on their progeny records. In common with all mixed models, each random effect is assumed to have originated from a specific distribution with zero mean

and unknown variance that is be estimated: for equation (2.1), the random effects a_i are defined as having variance equal to σ^2_A, the additive genetic variance, the residual errors will have variance σ^2_R and for the model in equation (2.1) the total phenotypic variance in y will be $\sigma^2_A + \sigma^2_R$. Variance components are therefore estimated directly by fitting the respective random effects in a linear model framework, rather than through indirect interpretation from the covariance between relatives (Meyer 1989*a*).

A more general mixed model in matrix form would be given by

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Zu} + \boldsymbol{e}, \tag{2.2}$$

where $\boldsymbol{y}$ is a vector of observations on all individuals, $\boldsymbol{\beta}$ is a vector of fixed effects, $\boldsymbol{X}$ represents a design matrix (of 0s and 1s) relating the appropriate fixed effects to each individual, $\boldsymbol{u}$ is a vector of random effects, $\boldsymbol{Z}$ is a design matrix relating the appropriate random effects to each individual and $\boldsymbol{e}$ is a vector of residual errors. For the simple animal model given in equation (2.1), the matrix form is therefore

$$\boldsymbol{y} = \mu + \boldsymbol{u} + \boldsymbol{e}, \tag{2.3}$$

where $\boldsymbol{X}$ has become a vector of 1s, $\boldsymbol{\beta} = \mu$, $\boldsymbol{Z}$ is the identity matrix, and $\boldsymbol{u}$ is the vector of additive genetic effects. Define $\boldsymbol{G}$ as the variance–covariance matrix for the vector $\boldsymbol{u}$, which can then be derived from the expectations of the covariance between relatives in additive genetic effects. For any pair of individuals i, j, the additive genetic covariance between them is $2\Theta_{ij}\sigma^2_A$, where Θ_{ij} is the coefficient of coancestry, the probability that an allele drawn at random from individual i will be identical by descent to an allele drawn at random from individual j (equal to, for example, 0.25 for parents and offspring, so the additive genetic covariance between parents and offspring is $1/2\ \sigma^2_A$). The variance–covariance matrix $\boldsymbol{G}$ is therefore given by $\boldsymbol{G} = \boldsymbol{A}\sigma^2_A$, where $\boldsymbol{A}$ is the additive genetic relationship matrix with individual elements $A_{ij} = 2\ \Theta_{ij}$. Most models assume that the errors are independent, in which case the corresponding covariance matrix for the vector $\boldsymbol{e}$ is just $\boldsymbol{R} = \boldsymbol{I}\sigma^2_R$ (where $\boldsymbol{I}$ is the identity matrix).

There are two stages to the analysis of an animal model: estimating the variance components and predicting the additive genetic effects (and any other random effects). I will restrict this review to the more common situation in which variance components are estimated using a restricted maximum-likelihood (REML) approach (or, occasionally, ML: Cheverud & Dittus 1992; Konigsberg & Cheverud 1992), but note that other approaches are possible. In particular, variance components can be estimated using a Bayesian framework (Gianola & Fernando 1986; Höschele *et al.* 1987; Sorensen *et al.* 1994), which may have advantages in certain situations (Blasco 2001); I am not aware of any analyses to date of free-ranging populations using a Bayesian animal model. I outline the framework for analysis of a single trait, but one of the great advantages of such models is that they can be readily extended to multivariate analyses of more than one trait (Lynch & Walsh 1998).

(b) *Estimating variance components*

Maximum-likelihood estimation is based on a simple yet powerful logic that can be applied to any form of statistical inference. For a given set of parameters defining a statistical model, their likelihood is defined as the probability of observing the actual data in hand *if* those parameter estimates were true: parameter estimates with low likelihoods are therefore those under which observing the actual data would be a rare event, and so forth. Probability is calculated based on assumptions about the statistical probability distribution of the data, usually that it is multivariate normal. An ML analysis then simply identifies the set of parameters that maximizes the likelihood of observing the actual data (Fisher 1921; Edwards 1972).

To estimate the likelihood of the model in equation (2.2), assume that both the additive genetic effects and the residual errors are normally distributed, and hence that the trait y is also normally distributed (in practice, REML estimators are fairly robust to this assumption; Shaw (1987), Lynch & Walsh (1998)). The vector $\boldsymbol{y}$ has mean $\boldsymbol{X\beta}$ and variance $\boldsymbol{V}$ determined by the variance in both the additive genetic effects (i.e. $\boldsymbol{G}$) and the residuals (i.e. $\boldsymbol{R}$), specifically $\boldsymbol{V} = \boldsymbol{ZGZ}^{\mathrm{T}} + \boldsymbol{R}$. The analysis will then determine ML estimates of $\boldsymbol{G}$ and $\boldsymbol{R}$.

The likelihood of the model in equation (2.2) is calculated from the probability density function for the data $\boldsymbol{y}$ under the normal distribution. It is computationally easier to maximize its natural logarithm, the log-likelihood, given by

$$L = c - \tfrac{1}{2}\ln|\boldsymbol{V}| - \tfrac{1}{2}(\boldsymbol{y} - \boldsymbol{X\beta})^{\mathrm{T}}V^{-1}(\boldsymbol{y} - \boldsymbol{X\beta}), \tag{2.4}$$

where c is a constant that can be ignored in finding the maximum and $|\boldsymbol{V}|$ is the determinant of the matrix $\boldsymbol{V}$. This expression is then used to find the values of $\boldsymbol{\beta}$ and $\boldsymbol{V}$ (or, in the simplest case, σ^2_A and σ^2_R), by differentiating with respect to each parameter and solving for zero to give ML estimates. In practice, this requires an iterative solving procedure, which can make the process computationally highly intensive.

All ML estimates have the undesirable property of being statistically biased, because they fail to account for the degrees of freedom lost in estimating fixed effects (Patterson & Thompson 1971; Shaw 1987). This generates bias even when the only fixed effect being considered is the mean, but the bias can be considerable for larger numbers of fixed effects (Meyer 1989*a*). As a result, an ML approach will underestimate the residual variance. However, the bias can be avoided by considering a REML in which only the likelihood of the part of the data that does not depend on the fixed effects is considered (Patterson & Thompson 1971). To obtain REML estimators rather than just ML for the model in equation (2.2), the likelihood is maximized for a transformed vector $\boldsymbol{y}^*$, where $\boldsymbol{y}^*$ contains the data corrected by a particular transformation matrix $\boldsymbol{K}$ (so $\boldsymbol{y}^* = \boldsymbol{Ky}$), and $\boldsymbol{K}$ depends on the design matrix $\boldsymbol{X}$ such that $\boldsymbol{KX} = 0$. Equation (2.2) therefore becomes

$$\boldsymbol{y}^* = \boldsymbol{KZu} + \boldsymbol{Ke}, \tag{2.5}$$

and the REML estimates are essentially the ML estimates for these transformed variables. Note that because $\boldsymbol{K}$ is determined entirely by $\boldsymbol{X}$, we are no longer considering estimates of $\boldsymbol{\beta}$, only of the variance components.

For the simple model in equation (2.3), the REML analysis will provide estimates $\hat{\sigma}^2_A$ and $\hat{\sigma}^2_R$, from which the heritability can be estimated as $h^2 = \hat{\sigma}^2_A/(\hat{\sigma}^2_A + \hat{\sigma}^2_R)$. The estimates of components of variance provided by the animal model are for a base population from which all other individuals in the population are descended. However, because information in any pedigree rarely dates back to a true base population, the usual assumption is that the first generation of animals with data forms the base population. One important advantage of the animal model is that, because it corrects for the flow of genetic information across subsequent generations, estimates of variance components are unbiased by any effects of finite population size, assortative mating, selection or inbreeding in subsequent

generations (Thompson 1973; Sorenson & Kennedy 1984; van der Werf & de Boer 1990).

The simple model in equation (2.3) is easily extended to include, first, other fixed effects: for example, it may be necessary to correct for an individual's age, sex, date of sampling and so on. Second, additional random effects can also be incorporated to account for correlations between the residual phenotypes while correcting for additive genetic effects: for example, maternal or common environment effects, which I discuss in more detail below, will generate correlations between groups of individuals. Each case involves respective extensions of $\boldsymbol{\beta}$ and inclusion of additional $\boldsymbol{Z}_i\boldsymbol{u}_i$, giving a model such as

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Z}_1\boldsymbol{u} + \boldsymbol{Z}_2\boldsymbol{c} + \boldsymbol{Z}_3\boldsymbol{m} + \boldsymbol{e}, \tag{2.6}$$

where $\boldsymbol{c}$ and $\boldsymbol{m}$ are vectors of the other random effects to be included in the model, each with appropriate design matrix $\boldsymbol{Z}_i$ and corresponding variance to be estimated. The total phenotypic variance is then the sum of the variance components of each random effect, for example for the model in equation (2.6), $\sigma^2_A + \sigma^2_C + \sigma^2_M + \sigma^2_R$. The statistical significance of including additional random effects can be assessed via a likelihood-ratio test, as twice the difference in log-likelihood between two nested models will approximate to a χ^2 distribution (with degrees of freedom equal to the difference in number of variance components estimated). Multivariate analyses of more than one trait can be used to obtain estimates of genetic and environmental covariances: in this case the relatedness matrix also defines a covariance structure for the respective additive genetic effects of different traits (Mrode 1996; Lynch & Walsh 1998).

(c) *Predicting breeding values*

An individual's breeding value for a given phenotypic trait is the total additive effect of its genes on that trait (Falconer & Mackay 1996). Armed with estimates of the variance components that define $\boldsymbol{V}$, we can return to equation (2.1) to make predictions of individual additive genetic effects, or breeding values, and estimates of fixed effects. These are known as BLUPs and BLUEs, respectively: best (because they minimize error variance), linear (they are linear functions of the data), unbiased (their expected mean is equal to what they are estimating), predictors (for random effects) or estimates (for fixed effects). The BLUE of $\boldsymbol{\beta}$ is simply the least-squares estimator:

$$\hat{\beta} = (\boldsymbol{X}^{\mathrm{T}}\boldsymbol{V}^{-1}\boldsymbol{X})^{-1}\boldsymbol{X}^{\mathrm{T}}\boldsymbol{V}^{-1}\boldsymbol{y}. \tag{2.7}$$

The BLUPs for $\boldsymbol{u}$ are then given by

$$\hat{\boldsymbol{u}} = \boldsymbol{G}\boldsymbol{Z}^{\mathrm{T}}\boldsymbol{V}^{-1}(\boldsymbol{y} - \boldsymbol{X}\hat{\beta}). \tag{2.8}$$

For the simple version of the animal model in equation (2.3), $\hat{\boldsymbol{u}}$ is therefore

$$\hat{\boldsymbol{u}} = \boldsymbol{G}\boldsymbol{V}^{-1}(\boldsymbol{y} - \mu)\boldsymbol{A}\sigma^2_A(\boldsymbol{A}\sigma^2_A + \boldsymbol{I}\sigma^2_R)^{-1}(\boldsymbol{y} - \mu). \tag{2.9}$$

Note that for a set of entirely unrelated animals, the additive genetic relationship matrix would just be equal to the identity matrix $\boldsymbol{I}$, so equation (2.9) would reduce to $\hat{\boldsymbol{u}} = h^2(\boldsymbol{y} - \mu)$, where h^2 is the heritability. However, with related animals, an individual's BLUP is determined by the deviation of both its own phenotype from the population mean, and those of all its relatives in the population, each scaled by their relatedness to the given individual. In a multivariate analysis, phenotypic measurements on correlated traits will also contribute information to the prediction of breeding values for a given trait, thus making maximum use of the data available (Mrode 1996).

The prediction of breeding values therefore requires knowledge of $\boldsymbol{G}$ and $\boldsymbol{V}$, for example from the ML analysis. However, finding the inverse of $\boldsymbol{V}$ becomes difficult for large sample sizes, so in practice alternative computational forms, Henderson's mixed-model equations (Henderson 1950), are used, which simultaneously calculate $\hat{\beta}$ and $\hat{\boldsymbol{u}}$ in a more manageable format (Mrode 1996; Lynch & Walsh 1998). The mixed-model equations can also generate predicted error variances for breeding values (Henderson 1975), from which estimates of their accuracy, or the correlation between true and predicted values, can be derived (see Mrode (1996) and Lynch & Walsh (1998) for further details).

(d) *Software*

REML estimation of variance components using an animal model can be fitted within a range of software: for example, ASReml (Gilmour *et al.* 2002), DFREML (Meyer 1989*b*), VCE (Neumaier & Groeneveld 1998) and Genstat. Given information on variance components, breeding values can be predicted using BLUP software such as PEST, which will also generate predicted error variances on BLUPS (Groeneveld *et al.* 1990, 1992).

3. STUDIES OF WILD POPULATIONS

(a) *Datasets*

I review here the use of the animal model in analyses of data from studies of wild animal populations, in which free-ranging animals have been measured in their natural environment. Such studies, which typically involve intensive long-term monitoring of marked individuals, offer unique opportunities to dissect the genetic basis of quantitative traits expressed in natural environments. The ones that I will discuss in particular here are studies of a wild ungulate and a passerine bird population: the red deer (*Cervus elaphus*) population in the North Block of the Isle of Rum, northwest Scotland (Clutton-Brock *et al.* 1982), and the collared flycatcher (*Ficedula albicollis*) population on the island of Gotland, Sweden (Gustafsson 1986; Merilä *et al.* 2001*b*; Sheldon *et al.* 2003). In addition to the free-ranging macaque population mentioned above (Konigsberg & Cheverud 1992), other wild populations for which animal model analyses have been used or are currently underway include a Soay sheep (*Ovis aries*) population in Village Bay on the island of Hirta, St Kilda, northwest Scotland (Milner *et al.* 2000; Coltman *et al.* 2001); a bighorn sheep (*Ovis canadensis*) population at Ram Mountain, Alberta, Canada (Réale *et al.* 1999; Coltman *et al.* 2003); a red squirrel (*Tamiasciurus hudsonicus*) population in southwest Yukon, Canada (McAdam *et al.* 2002; Réale *et al.* 2003*a*,*b*); a great tit (*Parus major*) population in Wytham Woods, Oxford, UK (McCleery *et al.* 2004); a great tit population on the Dutch island of Vlieland (E. Postma and A. van Noordwijk, unpublished data); a long-tailed tit (*Aegithalos caudatus*) population in the Rivelin Valley, Sheffield, UK (MacColl & Hatchwell 2003); three populations of blue tits (*Parus caeruleus*) in the south of France and the island of Corsica (Charmantier *et al.* 2004*a*,*b*); five house sparrow (*Passer domesticus*) populations on islands in northern Norway (Jensen *et al.* 2003); and a transplanted population of Atlantic salmon (*Salmo salar*) in the Sainte-Marguerite River, Québec, Canada (Garant *et al.* 2003).

Many of these study populations share several common features. Individual animals are recognizable by artificial marks (tags or rings) assigned soon after birth, or by natural markings. The bird populations are passerine bird species that generally use nest-boxes; chicks are ringed on the nest, and attendant adults are caught either in the nest-boxes or by mist netting. Wild ungulates can be caught, tagged and measured at birth (red deer: Clutton-Brock *et al.* 1982) or by being rounded up (Soay sheep: Clutton-Brock & Pemberton 2003) or lured into traps (bighorn sheep: Festa-Bianchet *et al.* 2000). In most cases, individuals are monitored from birth to death, throughout all breeding attempts, and morphological and behavioural data are collected in addition to the life-history data, although such an approach is by definition only feasible in certain situations. As an exception to these individually monitored populations, in a study of Atlantic salmon, adults were sampled at the point of release into a new site, and offspring were sampled in subsequent years (Garant *et al.* 2003). Pedigrees are built up based on information on parental identities, detailed below.

Many of the populations considered in such studies are artificial to some degree. This may be due to the use of nest-boxes; because they are a feral population of a domestic breed (the Soay sheep on St Kilda); because they have only recently been released from management by culling (the red deer population on Rum, the Ram Mountain bighorn sheep population); or because of artificial introduction into a new habitat for the purpose of monitoring (Sainte-Marguerite River Atlantic salmon). However, to the extent that they comprise individuals living in a natural environment in which birth and death are naturally regulated, they approach as closely as is feasible to studies of entirely wild populations living under wild conditions.

(b) *Establishing pedigrees*

Construction of a pedigree, or family tree, for a population (from which the relatedness between different pairs of individuals can be calculated) requires knowledge of the parentage of each individual in the population. Parental identity can be established in one of two ways: either through observations made in the field or through genetic data. The former is applied to many studies of birds in which the pair of birds attending a nest are assumed the parents of the chicks in the nest. This can clearly generate errors if there is extra-pair paternity or, less commonly, intraspecific brood parasitism (see § 7). Field observations can also be used to determine maternity in mammal populations, based on suckling observations. In rare cases in mammalian populations, behavioural observations during the mating season can also be used to identify the father of an individual, usually based on back-calculating from an observed birth date to an estimated conception date; this generates reliable paternities in some mating systems (Pemberton *et al.* 1992) but not others (Coltman *et al.* 1999*a*). However, in many mammal studies, paternity is generally more reliably assigned using molecular data such as genotypes at multiple microsatellite loci. Molecular data are also a prerequisite for pedigree construction in systems in which it is not possible to assign parentage of either sex from observations, such as studies of wild fish populations (Garant *et al.* 2003; Wilson *et al.* 2003*a*).

Numerous different analytical techniques and software have been developed for the purpose of parentage assignment using molecular data (see review in Jones & Ardren 2003). For example, the software package CERVUS (Marshall *et al.* 1998) uses a ML approach to identify the most likely father among a set of candidate males, and assigns paternity to that male if, and only if, he is significantly more probable than any rival male. Alternatively, where neither parent is known, relationships may be construed by assigning parents jointly rather than individually, based on their joint likelihood of producing a given offspring genotype (e.g. Duchesne *et al.* 2002), or by identifying sibships (e.g. Thomas & Hill 2000). Molecular data may not always be available for all individuals in a population, or may not be sufficiently powerful to distinguish between alternative candidates. However, one of the advantages of REML estimation is that individuals with, for example, unknown paternity can still be included in an analysis. For example, in pedigrees of both the Rum red deer and St Kilda Soay sheep populations, maternal links outweigh paternal links by factors of 2.7 (Kruuk *et al.* 2002*b*) and 1.2 (Coltman *et al.* 2001), respectively, and Réale *et al.* (1999) present animal model analyses for the Ram Mountain bighorn sheep population based solely on maternal links.

Pedigree data may also be used to provide estimates of individual fitness, and hence of selection on a phenotypic trait, as determined by the association between the trait and fitness (Robertson 1966; Lande & Arnold 1983; Arnold & Wade 1984). It is common practice to define fitness as the total number of offspring produced by an individual, or to consider only a single component of lifetime breeding success, such as survival to adulthood or adult fecundity (see Kingsolver *et al.* (2001) for an indication of the range of fitness components presented in the literature). However, more complex measures accounting for demographic stochasticity and cross-generational effects may ultimately provide better estimates of an individual's genetic contribution to future generations, and hence of selection on different phenotypic traits (see discussion in Grafen 1988; Charlesworth 1994; van Tienderen 2000; Wolf & Wade 2001; Brommer *et al.* 2002; Coulson *et al.* 2004).

4. CAUSAL COMPONENTS OF PHENOTYPIC VARIANCE

(a) *Common environment effects*

The linear model framework of the animal model provides a straightforward means of estimating a range of causal components of variance in phenotypic traits additional to the additive genetic variance. For example, if local environmental conditions affect phenotypes, then individuals sharing the same environment will have similar phenotypes, violating the assumption of standard additive models that residual errors are uncorrelated (Lynch & Walsh 1998). In particular, common environment effects will bias heritability estimation if relatives are more likely than non-relatives to share a local environment.

Common environment effects are fitted in an animal model by including a term identifying the particular

common environment (e.g. nest-box number, or year of birth, or territory) as an extra random effect. Several studies have now shown substantial components of variance due to the effects of the common environment shared by chicks in the same nest. For example, in cooperatively breeding long-tailed tits, parental feeding behaviour has a heritability of 0.43, whereas differences between nests account for a further 43% of the phenotypic variance (MacColl & Hatchwell 2003). In collared flycatcher chicks, differences between nest-boxes account for 49% of the variance in body condition (Merilä *et al.* 2001*b*) and 30% of the variance in tarsus length (Kruuk *et al.* 2001). Similarly, up to 56% and 25% of the variance in body mass and tarsus length, respectively, in blue tit chicks can be attributed to nest-box effects (Charmantier *et al.* 2004*a*).

The exact timing of common environment effects can sometimes be determined by fitting appropriate random effects. For the collared flycatcher morphometric data, by considering chicks that had been experimentally cross-fostered, common environment effects could be split further into: (i) effects acting prior to cross-fostering at 2 days of age, by fitting nest of origin as a random effect; and (ii) effects acting between 2 days and measurement at 14 days, by fitting nest of rearing as a random effect. For tarsus length, nest of origin and nest of rearing explained roughly equal proportions of the variance (Kruuk *et al.* 2001), whereas for body condition, nest of origin effects were negligible, with almost all common environments being due to nest of rearing (figure 1; Merilä *et al.* 2001*b*). Thus, body condition appears to be more sensitive to recent environmental conditions whereas tarsus length, a measure of skeletal size, is affected by more persistent differences generated by nest of origin. The latter may be due to prelaying investment in egg size (see Potti (1999), for an example of maternal effects, which are discussed in more detail below). Alternatively, the increased covariance between full-siblings represented by the nest of origin effect might also indicate dominance genetic variance, as tarsus length shows significant inbreeding depression in the same population (Kruuk *et al.* 2002*a*). Furthermore, in accordance with the above contrast, there is no evidence of inbreeding depression in body condition in this population (Kruuk *et al.* 2002*a*).

Failing to account for the increased covariance between siblings generated by shared environments inflates estimates of heritability: for example, running the same models for the collared flycatcher data without fitting a common environment effect increased estimates of heritability from 0.35 to 0.67 for tarsus length and from 0.30 to 0.76 for condition (L. E. B. Kruuk, B. C. Sheldon and J. Merilä, unpublished data). Such effects may be responsible for the observation that heritabilities estimated using the animal model are generally lower than estimates from parent–offspring regressions or full-sib analyses (table 1). Note, however, that even with the animal model, it may not be feasible to entirely separate additive genetic effects and early common environment or parental effects. For example, estimates of heritability of tarsus length and body condition in collared flycatcher chicks are lower for cross-fostered than for non-cross-fostered individuals (Kruuk *et al.* 2001; Merilä *et al.* 2001*b*, 2004).

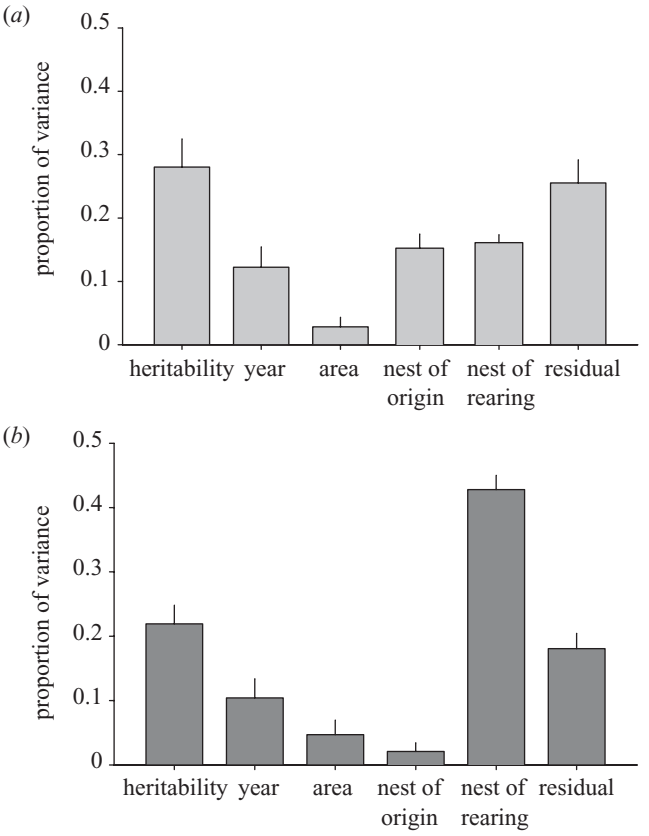

Figure 1. Causal components of phenotypic variance in (*a*) tarsus length and (*b*) body condition in collared flycatcher chicks on Gotland, Sweden. Data are from cross-fostering experiments, with common environment effects split into nest of origin and nest of rearing. Year and area represent variance between different years (1981–1999) and different parts of the study area, respectively. Residual: residual variance. Bars are one standard error. (Data from Merilä *et al.* (2001*b*) and Kruuk *et al.* (2001).)

(b) *Maternal effects*

Maternal effects occur when the phenotype of the mother affects the phenotype of her offspring, in ways additional to the additive effects of the genes she has passed on (Mousseau & Fox 1998). Maternal effects may be either genetically or environmentally determined: in mammals, for example, lactation has a critical effect on offspring growth rate and performance, and may be determined by both the mother's genotype and her environment. Maternal effects were traditionally treated as a thorny statistical issue with the potential to bias estimates of additive genetic variance (e.g. Falconer & Mackay 1996). However, they are increasingly recognized as interesting and substantial sources of phenotypic variance in their own right, as both economically significant effects in animal breeding (Simm 1998) and adaptive evolutionary phenomena (Mousseau & Fox 1998; Wolf *et al.* 1998; Qvarnström & Price 2001). Theoretical work shows that they can have substantial and even counterintuitive effects on the response to selection, such that an understanding of their impact is essential for any accurate representation of the evolutionary dynamics of a trait (Kirkpatrick & Lande 1989; Wolf *et al.* 1998).

In the context of fitting an animal model, the simplest way to quantify maternal-effects variance is simply to

Table 1. Comparison of heritability estimates from parent–offspring regression or sib analysis with estimates from an animal model[a].
(Examples are restricted to cases in which the same trait was studied in the same population, and where the animal model used pedigrees with both maternal and paternal links. All estimates in the parent–offspring column are from parent–offspring (PO) regressions, unless otherwise specified in the previous column. Where the original paper presented results for more than one age class (Garant *et al.* 2004), estimates are given for the 'adult' class.)

			heritability	
species	trait	dataset	parent–offspring	animal model
collared flycatcher (*Ficedula albicollis)*	clutch size	females	0.35 ± 0.08[1]	0.29 ± 0.04[2]
	lay date	females	0.41 ± 0.08[1]	0.19 ± 0.04[2]
	body condition	1993	0.29 ± 0.12[3]	0.30 ± 0.03[4]
		1994	0.35 ± 0.22[3]	
	tarsus length	fledglings	0.65 ± 0.11[5]	0.35 ± 0.02[6]
		males	0.47 ± 0.07[1]	
		females	0.53 ± 0.07[1]	
	forehead patch	males: PO regression	0.39 ± 0.09[7]	0.35 ± 0.05[8]
		males: full-sibs	0.72 ± 0.19[7]	
great tit (*Parus major*)	fledgling mass	—	0.44 ± 0.05[9]	0.24 ± 0.02[10]
long-tailed tit (*Aegithalos caudatus*)	helping behaviour	—	0.59 ± 0.17[11]	0.43 ± 0.07[11]
house sparrow (*Passer domesticus*)	tarsus length	both sexes	0.37 ± 0.10[12]	0.48 ± 0.05[13]
Soay sheep (*Ovis aries*)	parasite burden	males	0.08 ± 0.19[14]	0.11 ± 0.02[15]
	(summer faecal egg count)	females	0.13 ± 0.18[14]	0.13 ± 0.01[15]
red deer (*Cervus elaphus*)	antler spike length	yearlings	0.23 ± 0.25[16]	0.17 ± 0.09[16]

[a] References: [1]Merilä & Sheldon (2000); [2]Sheldon *et al.* (2003); [3]Merilä (1996); [4]Merilä *et al.* (2001); [5]Merilä *et al.* (1998); [6]Kruuk *et al.* (2001); [7]Qvarnström (1999); [8]Garant *et al.* (2004); [9]Gosler & Harper (2000); [10] D. Garant, unpublished data; [11]MacColl & Hatchwell (2003); [12]Jensen (2002); [13]Jensen *et al.* (2003); [14]Smith *et al.* (1999); [15]Coltman *et al.* (2001); [16]P. Wesche and L. E. B. Kruuk, unpublished data.

include maternal identity as an additional random effect. This estimates the component of variance due to differences in the focal trait between offspring of different mothers (additional to additive genetic effects), whether these differences are due to genetic or environmental maternal effects. The model therefore does not explicitly identify the relevant aspects of maternal phenotype, but integrates multiple maternal influences into a single 'maternal performance' character, following the approach of Wilham (1963, 1972).

Maternal effects estimated in this way can contribute significant proportions of phenotypic variance. In a feral population of Soay sheep of St Kilda, maternal identity explained between 10% and 21% of the variance in hind-leg length (Milner *et al.* 2000; Coltman *et al.* 2001), and between 6% and 9% of the variance in parasite resistance, measured by faecal counts of eggs of gastrointestinal nematodes (Coltman *et al.* 2001). Similarly, a study of a range of phenotypic traits in red deer found significant maternal effects for several life-history and morphological traits, for example accounting for 28% and 20% of the variance in male and female birth weight (Kruuk *et al.* 2000). However, in contrast to these results, there was no evidence of significant maternal effects on offspring size in Atlantic salmon (Garant *et al.* 2003).

As for common environment effects, the presence of maternal effects implies that residual errors from the simple animal model are no longer uncorrelated. Omitting maternal effects from a model can therefore seriously inflate estimates of heritability, and simulation studies clearly illustrate the importance of specifying the correct model in the presence of maternal effects (Clement *et al.* 2001). Milner *et al.* (2000) compare models of hind-leg length, body weight and incisor breadth in Soay sheep fitted with and without a maternal effect, and find that estimates of heritability change from anything between 0% and 46%, although, surprisingly, there is no obvious relationship between the magnitude of the maternal-effects variance component and the change in the estimate of heritability (correlation = 0.09, $n = 6$). For red deer, excluding maternal identity from a model of birth weight increased the heritability from 0.17 to 0.54 for males and from 0.28 to 0.48 for females (L. Kruuk, unpublished data; these values are slightly different from those reported in Kruuk *et al.* (2000) because an extended dataset is being used, and the model has included more fixed effects). Thus, as with the common environment effects discussed above, maternal effects have the capacity to generate covariance between siblings that may be mistaken for additive genetic variance unless explicitly modelled, and again may presumably account for the some of the differences in estimates of heritability generated by the animal model and parent–offspring regressions (table 1).

The above examples do not address the extent to which differences between mothers are genetically rather than environmentally determined. Quantifying variance due to maternal genetic effects requires a more complex analysis based on information from more than two generations. Conceptually, a maternal genetic effect can be detected by comparing the performance of the grand-offspring of a given sire, divided into offspring produced by his daughters, which will be influenced by genes he carries for both growth and maternal effects, and those produced by his sons, which will be influenced only by his genes for

growth. Thus if, on average, grand-offspring from daughters are heavier than grand-offspring from sons, the given sire presumably carries genes for high maternal performance (Simm 1998). More generally, a maternal genetic effect can be fitted as an additional random effect within the animal model framework, with an associated variance–covariance matrix determined by the additive genetic-relatedness matrix, exactly as for the additive genetic effect (Mrode 1996; Lynch & Walsh 1998). Animal breeding literature shows widespread evidence of substantial maternal genetic variance in domestic ungulates, but their prevalence remains to be explored for wild populations. From models incorporating a maternal genetic effect, the covariance between it and direct genetic effects (i.e. between the two different random effects) can also be estimated. This direct-maternal genetic covariance will be crucial in determining evolutionary trajectories in response to selection in the presence of significant maternal effects (Kirkpatrick & Lande 1989; Wolf *et al.* 1998). Again, however, such analyses should only be embarked upon given data and pedigrees of sufficient quality and information; see Clement *et al.* (2001) for a comparison of different maternal-genetic models using simulated data of varying complexity.

There can obviously be substantial overlap between maternal effects and the common environment effects discussed in the previous section, as maternal effects will generate common environment effects for a group of siblings. For example, in the models of flycatcher chick morphology, replacing nest identity with maternal and paternal identity accounted for a similar proportion of the variance (Kruuk *et al.* 2001; Merilä *et al.* 2001*b*). It is also worth emphasizing that separating maternal effects (even the simplest environmental form) from additive genetic effects generally requires that there be paternal links in the pedigree, without which maternal effects may appear as additive genetic variance (see discussion in Réale *et al.* 1999, 2003*a*,*b*). (Alternatively, maternal effects can be estimated without paternal information by using cross-fostering experiments: McAdam *et al.* (2002), McAdam & Boutin (2003*a*).)

Finally, note that with this standard quantitative genetics approach, maternal effects are apparent only as variance in a given trait between offspring of different mothers: the assumption is therefore that different mothers provide consistently different levels of maternal care to their offspring. The approach will therefore not detect those maternal effects associated with differential investment between offspring by parents (e.g. Lessells 2002; Badyaev *et al.* 2003), which will generate variance within, rather than between, groups of maternal siblings. Unless modelled explicitly (in the case of, for example, consistent differences between the sexes), such variance will be assigned to the residual variance component, and should not alter estimates of any other variance components. The Wilham model also does not attempt to identify exactly which maternal characteristics are responsible for maternal effects on the offspring (see Kirkpatrick & Lande (1989) for a discussion of the merits and limitations of this approach). This is in contrast to those analyses that treat explicit measures of maternal investment or performance as the phenotypic trait of interest (e.g. parturition date or offspring survival rates; Réale & Festa-Bianchet (2000); McAdam *et al.* (2002)).

(c) *Repeated measures or permanent environment effects*

Long-term studies of individually monitored animals frequently involve repeated measures on the same individual across its lifetime, generating another situation in which the residual errors in a standard animal model are correlated. However, repeated measures can readily be accommodated within the animal model framework by including a further random effect defining the permanent environment common to all observations on the same individual (Lynch & Walsh 1998). This means, first, that all available measurements can be exploited, rather than using a single average value for each animal. More interestingly, it allows a further component of variance to be estimated: the groups of measures on different individuals can be used to quantify permanent between-individual differences, over and above those due to additive genetic effects. For example, the environment that an individual experiences during its early development may generate persistent effects that last throughout adulthood (Kruuk *et al.* 1999; Lindström 1999; Lummaa & Clutton-Brock 2002), as will other factors such as an individual's home range or territory. With multiple observations, these permanent differences between individuals can be partitioned from the residual error, which will then only represent within-individual variance.

In long-lived species, repeated measures may be available from multiple observations on individuals in different years. Using such data, Réale *et al.* (1999) found that permanent environmental effects accounted for 35% and 26% of the variance in, respectively, June and September adult body mass in bighorn sheep. Coltman *et al.* (2001) fitted permanent environmental effects in their analysis of summer parasite burdens in Soay sheep, and found them to account for 11% of the variance in females, but only 4% of the variance in males. Analysis of the size of antlers grown annually by red deer stags showed that 24% of the total variance in mass was due to permanent environment effects (figure 2; Kruuk *et al.* 2002*b*), as was up to 26% of the variance in fluctuating asymmetry (Kruuk *et al.* 2003). However, there was no evidence of any permanent environment effects on either litter size or parturition date in red squirrels (McAdam *et al.* 2002), suggesting much greater effects of current environmental conditions in this system.

Despite its name, the permanent environment effect will also incorporate any non-additive genetic effects such as those due to dominance or epistasis, as these will also contribute to permanent differences between individuals. Thus it is likely that dominance variance is contributing to the permanent environment variance component for parasite resistance observed in Soay sheep (Coltman *et al.* 2001), given the previous evidence for substantial dominance effects on this trait (Coltman *et al.* 1999*b*). Again, permanent environment effects may overlap with maternal effects: if a maternal effect is not fitted in the model, any variation generated by maternal effects may appear as permanent environment effects in an analysis of repeated measures, so care needs to be taken not to overspecify a model. The repeatability of a trait can be calculated from

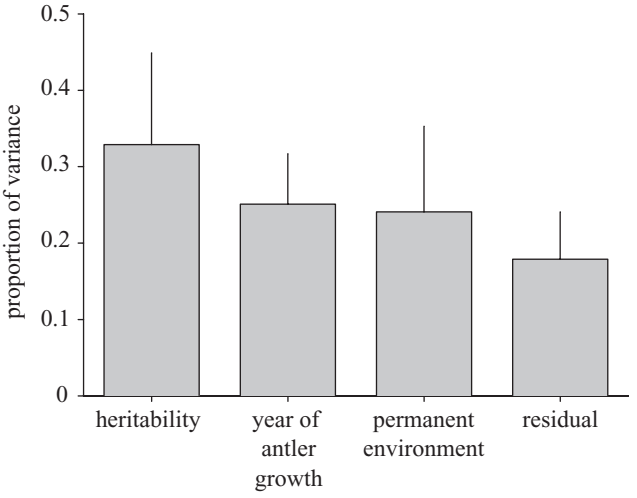

Figure 2. Components of variance in antler mass in red deer: proportion of phenotypic variance in mass of antlers from males aged 5+ years. Bars are one standard error. (Data from Kruuk *et al.* (2002*b*).)

the total sum of the heritability plus the proportion of variance due to permanent environment effects, plus any other random effects, such as common environment or maternal effects that do not change for a given individual (e.g. Milner *et al.* 2000). However, see Dohm (2002) for consideration of unusual situations in which the repeatability may not set an upper limit on the heritability.

Finally, repeated measures on individual animals across their lifetime can be used to explore the quantitative genetics of growth trajectories, through random regression models (Meyer & Hill 1997; Meyer 2000). A random regression model fits the parameters describing an individual's change in phenotype with respect to age as random effects (for application in standard mixed models, see Pinheiro & Bates (2000)). The variance structure of these random regression effects can then be specified in the same way as the additive genetic effects, to give estimates of the additive genetic variance underlying rates of growth. To my knowledge, an animal model random regression has not yet been applied to data from a free-ranging population in a natural environment, but would be a useful tool in describing age-related variation in phenotypic traits, for example to determine the heritable basis of growth trajectories or senescence.

(d) *Using animal models to identify quantitative trait loci*

A further use of animal models is in the identification of quantitative trait loci (QTL). George *et al.* (2000) develop a method of fitting a QTL effect as an additional random effect within an animal model framework, additional to the polygenic effect described by a_i in equation (2.1). The covariance matrix of the QTL effects is then determined by the identity-by-descent matrix, wherein each element is the probability that a pair of alleles is identical by descent based on the pedigree structure. This REML-based analysis has all the advantages of the animal model, in dealing with unbalanced datasets and complex pedigree structures, compared with alternative methods for QTL identification, such as interval mapping by linear regression among half-sibs (George *et al.* 2000). In a rare example of identification of QTLs in a wild population, Slate *et al.* (2002) use the variance-component approach to identify loci with significant effects on birth weight in the Rum red deer population. Their analysis suggests segregating QTL on two linkage groups, of which one is significant at the genome-wide level, with the linear regression analysis identifying a third (Slate *et al.* 2002).

(e) *Comparison of techniques*

Table 1 contains a comparison of heritability estimates of a range of phenotypic traits from different study populations, estimated using either parent–offspring regression or full-sib analysis, versus the animal model. Two general points emerge. First, as noted above, estimates of heritability from an animal model analysis are generally lower than from the parent–offspring regression or full-sib analyses, possibly due to inflation by other sources of variance not accounted for in the simpler techniques. Second, estimates of standard errors are also consistently lower (see below).

5. ANALYSIS OF BREEDING VALUES

The use of the animal model to quantify individual genetic merit or breeding values (Lynch & Walsh 1998) represents a significant departure from previous quantitative genetics methods used in evolutionary ecology. REML variance component estimation is essentially a more efficient and powerful alternative method for estimating heritabilities, whereas in providing estimates of breeding values, the animal model generates a wealth of opportunities for new analyses. Predictions of individual breeding values can be analysed in the same way as individual phenotypes, to quantify temporal trends or selection pressures. These then allow an explicit comparison between trends and associations at the genotypic and phenotypic level, which, as the examples discussed below show, can provide invaluable insights into the evolutionary dynamics of a population.

(a) *Detecting genetic trends*

Evolution involves a change in the genetic composition of a population. To test whether a population is evolving, it is therefore not sufficient to show merely that there has been a change in average phenotypes, since changing environmental conditions could generate such a trend (Hendry & Kinnison 1999). Demonstrating microevolution within a population over a given study period requires evidence of genotypic change, which an analysis of the breeding values can provide.

The parturition date of red squirrels in the Yukon, Canada, has advanced at a rate of 3.7 days per generation between 1989 and 1998 (Réale *et al.* 2003*a*). Part of this change was due to a microevolutionary shift in mean breeding value, which has advanced by 0.8 days per generation, whereas the remaining shift can be explained by phenotypic plasticity generating within-individual change (Réale *et al.* 2003*a*). In the absence of predictions of breeding values, this phenotypic plasticity might have been mistaken for evolutionary change, thus overestimating the response to selection. Similarly, declines in mean breeding values for horn size and body weight in bighorn rams reflect the undesirable impact of trophy hunting in

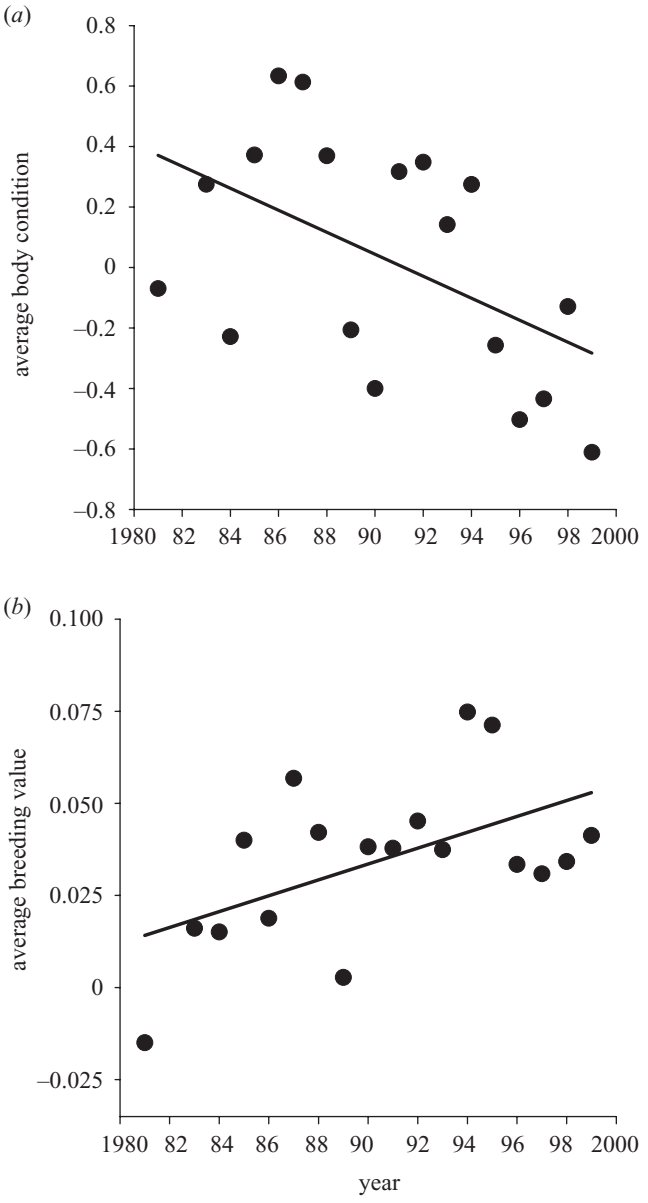

Figure 3. Annual body condition in fledgling collared flycatchers between 1980 and 1999. (*a*) Mean phenotypic value; (*b*) Mean of breeding values. (Data from Merilä *et al.* (2001*a*).)

removing individuals of highest genetic merit (Coltman *et al.* 2003).

In the collared flycatcher population on the island of Gotland, chicks with high body condition index are significantly more likely to survive the winter migration to Africa and return as breeding adults (Merilä *et al.* 2001*a*). Body condition is also heritable (Merilä *et al.* 2001*b*), suggesting that body condition should be increasing. However, deteriorating environmental conditions associated with large-scale climatic variation have resulted in a decline in mean phenotypic values of fledgling body condition over the two-decade study period (figure 3*a*). An analysis of breeding values showed that this environmental change was masking a genetic trend in the opposite direction in the breeding values, which showed a significant increase over the study period (figure 3*b*: increase per year = 0.0022 ± 0.0009; Merilä *et al.* 2001*a*). The existence of opposing environmental trends masking an evolutionary response, or 'counter-gradient variation' (Conover & Schultz 1995), provides an appealing explanation for the lack of an apparent response to directional selection on heritable traits in such cases (Cooke *et al.* 1990; Larsson *et al.* 1998; Merilä *et al.* 2001*c*). Moreover, deteriorating environmental conditions may actually increase the magnitude of selection on a trait (Milner *et al.* 1999; Merilä *et al.* 2001*a*; McAdam & Boutin 2003*b*; Coulson *et al.* 2003), increasing the likelihood of a microevolutionary response to selection.

In the case of the collared flycatcher example, we can show that the observed change was not due to immigration from another population, since there was no change in the breeding values of the offspring of immigrants over this time (change = -0.0006 ± 0.0014; L. E. B. Kruuk, B. C. Sheldon and J. Merilä, unpublished data). Distinguishing genetic trends in a native population relative to immigrants in this way therefore also affords the possibility of quantifying the effects of migration, and in the appropriate situation should provide a means of explicitly demonstrating the impact of gene flow.

(b) *Environmental covariance between a trait and fitness*

Analysis of breeding values can reveal a second situation in which environmental heterogeneity generates misleading impressions of selection dynamics. Selection is measured from the association between a given trait and a measure of fitness. However, in some cases, this association can be generated entirely by an environmental covariance between the trait and fitness—if, for example, prevailing environmental conditions result in an increase in the trait value plus an increase in fitness. The net result is the statistical impression of selection, but no association between the trait and fitness at the genetic level. This theory was discussed by Fisher (1958) and modelled explicitly by Price *et al.* (1988). In terms of measurement, the environmental short-circuiting can be detected by a comparison of selection gradients estimated using phenotypic values versus breeding values (Rausher 1992; Stinchcombe *et al.* 2002).

As an example of this environmental covariance, the size of antlers in male red deer is both heritable (figure 2) and under positive directional selection (Kruuk *et al.* 2002*b*). For the Rum study population, a lack of increase in antler size over the 30-year study period could have been due to worsening environmental conditions as the population density in the study area increased, and antler growth is strongly condition dependent. However, there was no evidence of a genetic trend in breeding values over the study period. Instead, when individual phenotypes were broken down into a breeding value versus an environmental deviation, the only significant selection differential was for the environmental deviation: differences in breeding success were related to the environmental component of antler mass and not the breeding values (Kruuk *et al.* 2002*b*). This may be because environmental conditions affect an individual's nutritional state, and hence both the size of the antlers grown in a given year as well as its fighting ability and mating success in the breeding season that year. The notion of environmental covariance between antler size and fitness is further supported by the lack of any genetic correlation between antler mass and lifetime

breeding success, again suggesting that any association between the two is environmentally determined (Kruuk *et al.* 2002*b*).

The environmental covariance hypothesis thus provides a possible explanation for the maintenance of genetic variation in a trait under strong positive sexual selection, and for a lack of phenotypic response to the apparent directional selection. The analysis also constitutes another example in which environmental variation generates misleading patterns, again highlighting the need to be able to separate phenotypic from genotypic effects.

(c) *Antagonistic selection pressures*

Finally, complex interactions between different traits or different components of fitness may also become apparent through analysis of breeding values. For example, in collared flycatchers, there is strong selection for early breeding in terms of the number of offspring produced from a nest, and breeding time is heritable (Sheldon *et al.* 2003). Predictions of individual breeding values showed that although there was significant selection for breeding values associated with earlier breeding times in terms of the number of recruiting offspring, breeding values for early breeding were also associated with lower adult survival rates. This trade-off, between current fecundity and future survival, at the level of the breeding values is presumably constraining any response to selection, but the patterns of antagonistic selection were not apparent at the phenotypic level.

6. MULTIVARIATE ANALYSES

(a) *Quantifying genetic correlations*

A further valuable aspect of an animal model approach is the ease with which data on multiple phenotypic traits can be analysed. This is important because, unlike artificial selection, natural selection will rarely target only a single trait (Price & Langen 1992; van Tienderen & de Jong 1994). Genetic correlations between traits bind their fates together, such that univariate analyses of evolutionary trajectories are inevitably too simplistic. The existence of trade-offs between traits, acting either through negative genetic correlations or antagonistic selection pressures, may serve to prevent the erosion of genetic variation underlying a trait, if no single genotype is optimal. Intuitively, we might expect genetic correlations between traits under positive directional selection to be negative, simply because alleles that generate positive correlations would presumably have been swept to fixation by selection (Roff 1996, 1997). However, despite the intuitive appeal of this concept, observations of negative genetic correlations between important fitness-related traits are relatively rare: although there is a higher proportion of negative genetic correlations between life-history traits than morphological traits, the majority of genetic correlations in both classes are still positive (Roff 1997).

A multivariate animal model provides a straightforward means for estimating covariances and hence correlations between traits (Mrode 1996; Lynch & Walsh 1998). Results from animal model studies of wild populations to date generally confirm the observation above of a majority of positive correlations between traits. For example, there are positive genetic correlations between three morphometric measures in Soay sheep (Milner *et al.* 2000), and between body mass and parasite resistance (Coltman *et al.* 2001). Body mass and horn size are positively genetically correlated in bighorn rams (Coltman *et al.* 2003), as are tarsus length and body mass in blue tit chicks (Charmantier *et al.* 2004*a*) and two plumage characteristics in male collared flycatchers (Garant *et al.* (2004), although the correlation is not significant among yearlings. There is a significant genetic correlation between large clutch size and early breeding time in collared flycatchers (Sheldon *et al.* 2003), but correlations between breeding time and maternal investment are not significant in either red deer (Coulson *et al.* 2003) or red squirrels (Réale *et al.* 2003*b*). Jensen *et al.* (2003) report evidence of both positive and negative significant genetic correlations between a set of six morphological traits in house sparrows, but in a multivariate analysis of a suite of nine phenological and morphological traits under directional selection in the collared flycatcher, none of the negative correlations is significant (Sheldon *et al.* 2003).

Estimating the genetic correlations between traits requires hefty sample sizes, and standard errors are typically much larger than those associated with heritabilities (Roff 1997; Lynch & Walsh 1998). Furthermore, multivariate analyses will only ever be as informative as the traits that have been measured, whereas it may be that some entirely unmeasured trait is the true vehicle of evolutionary change (van Tienderen & de Jong 1994; Kruuk *et al.* 2002*b*). However, growing appreciation of the evolutionary significance of multivariate evolution should serve to remedy the paucity of estimates of genetic correlations relative to heritabilities, and thereby improve our understanding of the genetic architecture of complex phenotypes (Roff 2003).

(b) *Genotype–environment interactions*

The existence of genotype–environment interactions will alter the expression of genetic variance in different environments. In this case, animal model analyses lend themselves readily to tests for genotype-by-environment interactions, either through fitting a multivariate analysis or through a random regression. In the former, genotype–environment interactions are quantified by defining the trait as expressed in different environments as different 'sub-traits'. Genetic correlations between these sub-traits that are significantly less than unity, or significant differences in the magnitude of additive genetic variance, indicate a genotype–environment interaction (Falconer & Mackay 1996; Hoffmann & Merilä 1999). To date, the estimation of $G \times E$ interactions in this way is an area that remains largely unexplored for wild populations, and it will be interesting to compare the power of such analyses with tests for $G \times E$ interactions using cross-fostering experiments (e.g. Merilä 1997; Kunz & Ekman 2000). Charmantier *et al.* (2004*b*) show evidence of significant genotype–environment interactions on body size of blue tit chicks reared in parasitized and non-parasitized nests, with the presence of parasites reducing heritability estimates from 0.91 to 0.53. Similarly, the causal components of variance in secondary sexual plumage characteristics in the collared flycatcher differ in relation to climatic conditions (Garant *et al.* 2004). In both cases, levels of additive genetic variance were reduced in more stressful

environments, in accordance with previous results from bird populations (Hoffmann & Merilä 1999). To the extent to which the different sexes can be regarded as different environments for the expression of a trait, a multivariate analysis can also be used to quantify genetic covariance and correlations between the sexes, which will be necessary for the prediction of sex-specific evolutionary responses and sexual dimorphism. For example, Jensen *et al*. (2003) report large genetic correlations that were not significantly different from unity between the sexes in morphological traits in house sparrows.

The multivariate approach only allows comparison between discrete environments of different types, with a trait defined as being expressed in one or other environment. Analysis of the effects of a continuous change in environmental conditions, effectively the heritability of reaction norms or individual phenotypic plasticity (Pigliucci & Schlichting 1997), can be achieved through a random regression model similar to those used to describe changes with age (de Jong & Bijma 2002); see Kolmodin *et al.* (2002) and Fikse *et al.* (2003) for applications of this approach in animal breeding. There do not appear to be any studies to date adopting this approach in wild populations, but they should follow as a natural extension of the use of mixed models to explore phenotypic plasticity (Przybylo *et al.* 2000; Brommer *et al.* 2003).

7. DISCUSSION

(a) *Accounting for environmental heterogeneity*

The key distinction between artificial and wild populations is the environmental conditions experienced by the population. These may be constant or at least controlled for in artificial populations, but variable and unpredictable for wild populations. While this variation constitutes one of the most interesting aspects of studies in a natural environment, it also makes teasing out evolutionary patterns considerably more difficult. Thus there are numerous reasons why a heterogeneous environment causes problems for testing evolutionary theory, mainly related to difficulties in obtaining accurate estimates of important parameters. For example, to summarize the examples discussed in the previous sections: (i) common or maternal environment effects can bias estimates of variance components and heritability if not controlled for; (ii) environmental changes can obscure genetic trends; (iii) environmental covariance can generate biased measures of selection; (iv) genotype–environment interactions can affect the expression of variance components. In each case, the effects of environmental heterogeneity can be quantified and controlled for through the appropriate model, and I hope to have demonstrated ways in which this may often be feasible using an animal model approach.

In all cases, substantially more complex analyses are required than when environmental conditions are constant. Larger sample sizes will be required to provide equivalent statistical power given greater variability in the data, and to ensure accurate estimates of environmental effects. These are further reasons for using the most efficient and powerful means of analysis available.

(b) *Advantages of a REML animal model*

The previous sections have given some indication of the advantages of a REML animal model over analyses based on the more traditional parent–offspring regression or full- or half-sib design (see also discussion in Merilä *et al.* 2001*c*). First, in simultaneously considering information from all relatives across all generations, an animal model provides a considerably more efficient use of the available data and hence more powerful statistical tests. (For example, for analysis of the heritability of male antler size in the Isle of Rum study population of red deer, measurements of antler mass were available for only 18 father–son pairs across the 30-year study period. By contrast, incorporating information on both maternal and paternal links plus measurements on siblings in the full multigenerational pedigree through an animal model meant that 126 individuals with known antler measurements could be included (L. E. B. Kruuk, unpublished data).) The multigenerational analysis also avoids individuals being included twice in parent–offspring regressions, as both parents and offspring, or having to average offspring phenotypes. Thus in the few studies that present estimates of heritabilities estimated using both parent–offspring regression and an animal model on exactly the same dataset, standard errors are consistently lower in the latter, by a factor of approximately two (Réale *et al.* 1999; MacColl & Hatchwell 2003). In situations where a study of a given trait in a population has been repeated at a later date, using an animal model, comparisons with earlier results based on parent–offspring regression also reveal substantially smaller standard errors, although the difference will be partly due to larger sample sizes in the more recent analyses (table 1).

Second, in addition to increased efficiency, likelihood estimation also affords considerably greater flexibility than traditional least-squares methodology (Shaw 1987). A REML analysis readily accommodates unbalanced datasets containing missing phenotypic measurements, so that an unmeasured individual can still be included in the pedigree to provide links between measured individuals. A typical example of this would be a sex-limited trait, such as clutch size in female birds: male relatives will be included in the analysis to provide links between relatives, so that covariance between paternal half-sisters or paternal grandmother and offspring can be exploited. Third, there is also no requirement for a balanced design in the pedigree structure, and individuals can be included in the pedigree with as little or as much information on the identity of their relatives as is available. Fourth, a range of fixed effects can be easily incorporated in the model, which is analytically simpler than analysing corrected residuals from a separate model. Fifth, as discussed in detail above, other causal components of phenotypic variance such as maternal effects or common environment effects can be estimated, which might otherwise result in inflated estimates of heritability. Similarly, sixth, repeated measures on the same individual can be included, allowing permanent environment effects or the heritability of individual growth trajectories to be estimated. Finally, because the relationship matrix allows for the flow of genetic information from one generation to the next, estimates of components of variance in the base population are unbiased by any effects of non-random mating,

inbreeding, selection or evolution during the study period, which would bias estimates based on, for example, parent–offspring regressions (Thompson 1973; Sorenson & Kennedy 1984; van der Werf & de Boer 1990; Lynch & Walsh 1998).

(c) *Disadvantages of a REML animal model*

Set against the various advantages of the animal model is the obvious disadvantage of increased computational complexity, with complex numerical methods for the solution of ML equations (Lynch & Walsh 1998) requiring greater amounts of computational time and sophisticated programming tools. Second, as with any analysis, as the complexity of the model increases, so too do the chances of problems due to overspecification, confounding effects and misinterpretation. In particular, including extra random effects may incur a cost of reliability in estimates of variance components, and over-specification can result in a failure of the algorithms to converge.

It is also worth noting that these techniques have been developed for application in animal and plant breeding, where studies typically involve sample sizes of thousands of individuals and entirely reliable pedigree links, guaranteeing a quality of data rarely, if ever, attained in studies of wild animal populations. For the analysis of large, complex pedigrees based on multiple generations and a variety of forms of relatedness, the superiority of the animal model is manifest. However, for simpler datasets, for example in which only parent–offspring data are available, there will be much less advantage over traditional techniques (Knott *et al.* 1995). Similarly, unnecessarily complex analyses should not be used as a foil to disguise lower quality datasets: estimates of genetic parameters are only as good as the data on which they are based (Meyer 1989*a*). It is therefore worth emphasizing the use of caution in the application of the more complex analytical techniques. The ones discussed here are not a panacea for all ills in quantitative genetics, and there is no point in taking a sledgehammer to crack a nut.

Finally, the animal model returns estimates of variance components for a base population, unbiased by effects of selection in subsequent generations. However, if this base population is itself selected, the REML analysis cannot account for this previous selection, nor for selection on any correlated but unmeasured characters not included in the analysis (Schaeffer & Song 1978; van der Werf & de Boer 1990).

(d) *Pedigree errors*

One final point of consideration is the implications of unreliable data. The basis of any quantitative genetics analyses is the pedigree information from which relatedness between individuals is assessed. Errors in a pedigree will generate erroneous estimates of genetic parameters. For field studies of wild vertebrate populations, errors in maternal identity are rare: for example, maternity is determined by field observations with complete reliability for the ungulate populations discussed here (Marshall *et al.* 1998), and there is no evidence of intraspecific brood parasitism among the passerine bird populations discussed (e.g. Kempenaers *et al.* 1995; Sheldon & Ellegren 1999). However, errors in paternal links are considerably more likely, for example due to extra-pair paternity (EPP) in the bird populations. For example, in the collared flycatcher population, paternal links will be wrong because of EPP in 15% of cases (Sheldon & Ellegren 1999); in the blue tit populations in south France, EPP rates range between 14% and 25% (Charmantier & Blondel 2003). However, paternity assignment using genetic data will also be imperfect: for example, levels of statistical certainty in paternity assignment (Marshall *et al.* 1998) in the ungulate studies discussed here involve error rates of 20% (Kruuk *et al.* 2000; Coltman *et al.* 2001) or 5% (Milner *et al.* 2000; Coltman *et al.* 2003).

The paternity error rates are therefore relatively high, if not substantially different from the estimated 10% error rate in paternities in the UK dairy cattle herd pedigrees (Visscher *et al.* 2002). Furthermore, note that in many cases there are substantially more maternal links in the pedigree, so paternities are contributing less than half of the information. However, the net effect of pedigree errors will unquestionably be to reduce estimates of heritability, and the magnitude of such a reduction is an area in need of exploration. Merilä *et al.* (1998) report a 3% increase in the heritability of tarsus length in collared flycatchers on removing known EPPs (16% of individuals) from analyses based on father–offspring pairs. Milner *et al.* (2000) present heritabilities estimated using a pedigree constructed from paternities assigned with 95% confidence, but mention that estimates using 80% confidence were lower, although with fewer paternities the reliability of the models was also reduced. Konigsberg & Cheverud (1992) develop a ML model in which paternal assignment is probabilistic, with all potential candidate fathers assigned as equally likely fathers for a given offspring; using simulated data, they show that the precision of heritability estimates only increased given a very limited number of possible sires. Finally, the results of a meta-analysis showing slightly higher heritabilities in females than in males among bird populations (Jensen *et al.* 2003) may, in part, be due to the effects of EPPs reducing heritabilities estimated from father–son regressions. Simulation studies to explore these effects further would be highly valuable. Note also that we assume that errors are random with respect to phenotype, but again this needs to be tested.

(e) *Future directions*

Despite the long-established history of the animal model in the animal breeding literature, its general application within evolutionary ecology is still relatively recent. Thus, there are numerous elaborations on the simple animal model yet to be explored using data from wild populations, which will generate more sophisticated analyses and allow finer dissection of critical hypotheses. Multivariate analyses, random regressions and analyses of genotype–environment interactions all deserve considerably more attention in studies of wild populations. Given sufficient pedigree information, quantifying the variance in maternal genetic effects and their covariance with direct genetic effects also has the potential to generate a range of new avenues of investigation. The use of mixed models in analysis of non-normal data also remains to be fully exploited for data from natural populations. For example, survival analyses can now be fitted using a mixed model with Bayesian estimation, to quantify the proportion of

additive genetic variance in longevity (Ducrocq & Casella 1996; Ducrocq & Solkner 1998). However, for binomial and especially binary data, results from mixed models can be less reliable than analyses based on normal theory (Breslow & Clayton 1993), such that, if possible, approximation to a normal distribution may be preferable when dealing with such data.

The number of studies of wild animal populations with multigenerational pedigree information is continually increasing, and with it the number of datasets where an animal model analysis can offer substantial benefits. Hopefully, this will result in a widening of the taxonomic range of such studies in the literature, and so break the current near-monopoly of passerine birds or ungulates. A number of methods have been developed recently that allow quantitative genetic parameters to be estimated without explicitly specifying a pedigree, using indirect inferences from molecular marker data (e.g. Ritland 1996; Ritland & Ritland 1996; Mousseau *et al.* 1998), providing obvious advantages over analyses that require prior knowledge of the pedigree structure in a population. However, to date the few available comparisons of alternative approaches (Thomas *et al.* 2001; Wilson *et al.* 2003*b*) suggest that using molecular data to infer familial relationships between individuals (e.g. Thomas & Hill 2000, 2002) will provide more reliable estimates than indirect approaches that do not involve a pedigree. Given the ever-increasing availability of molecular data, such techniques will increase the scope for quantitative genetics analyses in systems in which pedigree construction is otherwise impossible (e.g. Garant *et al.* 2003; Wilson *et al.* 2003*a*). Finally, there will also be much to be gained from a coordinated attack on the genetic basis of phenotypic variation from both ends of the scale, by combining an animal model analysis of a phenotypic trait with genomic analyses aimed at identifying individual quantitative trait loci (Barton & Keightley 2002). Given sufficient pedigree information, QTL mapping is feasible within unmanipulated, wild animal populations (Slate *et al.* 1999), although, with the exception of Slate *et al.* (2002), this has not yet been attempted.

8. CONCLUSIONS

The genetic basis of phenotypic traits is central to the study of evolution and biological diversity. Quantitative genetics provides the statistical means of analysing this basis for continuous characters whose expression is determined by multiple loci. However, two key questions still lie unsolved at the heart of evolutionary quantitative genetics: what maintains genetic variation for phenotypic traits in the face of erosion due to selection pressures (Roff 1997), and why do we rarely see the microevolutionary response to selection expected from theoretical predictions (Merilä *et al.* 2001*c*)? Results from laboratory studies or plant and animal breeding can provide a range of insights into the genetic architecture of phenotypic traits, but in practice components of variance and selection pressures will vary between natural and artificial environments (Hoffmann 2000) such that studies under artificial conditions have only limited relevance for an understanding of evolution in the wild.

Use of the animal model in the analysis of data from long-term, individual-based studies has opened up a range of new weaponry with which to attack these questions for populations in natural environments. In particular, the models have allowed explicit tests of particular hypotheses explaining the maintenance of genetic variation or apparent evolutionary stasis that could previously only have been discussed, and in separating genetic from environmental components of phenotype have revealed underlying patterns that are often markedly different from those suggested by less detailed analyses. As the results discussed here show, simply estimating the heritability of a trait without considering other sources of environmental covariance can generate highly misleading results. Furthermore, interactions between different traits may impose constraints such that univariate predictions are no longer valid. In each case, use of the animal model to estimate variance components and predict breeding values allows significant advances to be made in our understanding of the evolutionary genetics of wild populations. Hopefully the increase in suitable datasets, in accessibility of necessary software, in computational power and in familiarity with mixed models and ML estimation will combine to encourage the use of the animal model in evolutionary ecology.

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