Dissecting the complex genetic basis of mate choice

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Abstract | The genetic analysis of mate choice is fraught with difficulties. Males produce complex signals and displays that can consist of a combination of acoustic, visual, chemical and behavioural phenotypes. Furthermore, female preferences for these male traits are notoriously difficult to quantify. During mate choice, genes not only affect the phenotypes of the individual they are in, but can influence the expression of traits in other individuals. How can genetic analyses be conducted to encompass this complexity? Tighter integration of classical quantitative genetic approaches with modern genomic technologies promises to advance our understanding of the complex genetic basis of mate choice.

Sexual selection
Occurs when individuals of one sex have differential success in gaining matings with the other sex.

Mate choice in many species results from a series of complex interactions between males and females, which can be highly context dependent. Multiple signals and displays are often assessed by individuals during mate choice[1], and many such traits have complex phenotypes (FIG. 1). In addition, theory predicts that male signals and displays could be associated with immunocompetence[2,3], and energetic or other life-history costs[4,5], indicating that the genetic basis of mate choice will be more complex than a univariate measure of a single trait. The genetic analysis of mate choice has consequently been considered a particularly difficult empirical task[6,7].

The genetic basis of mate choice in natural populations has been investigated in two main contexts. First, the genetic basis of reproductive and sexual isolation[7–9] and the reinforcement of reproductive isolation among closely related taxa[10] have recently received substantial attention. Many of these studies take advantage of conspicuous differences in morphology or behaviour between two taxa, and have been successful to some extent in characterizing the molecular genetic basis of these phenotypic differences. Second, the characterization of genetic variation associated with mate choice that segregates within populations is usually conducted using classical quantitative genetic approaches[11,12]. These two contrasting approaches have little overlap in the current empirical literature, despite the fact that the potential for sexual selection to result in reproductive isolation has been a major theory of speciation since Darwin[13]. The integration of quantitative descriptions of the genetic basis of mate choice and associated behaviours with a mechanistic understanding of the molecular genetic basis of phenotypic variation in mate choice, within and among populations, is therefore a major challenge in evolutionary and behavioural genetics[6,14].

Here we focus on the complexity of mate choice within populations and the recent progress made in the genetic analysis of mate choice using quantitative genetic and molecular approaches. Some effort has been devoted to the genetic analysis of interspecies differences in male sexually selected traits and female preferences[15,16]. We do not review these studies here, however, as although such studies have been successful in characterizing the genetic basis of interspecific differences, they might not inform us directly about the evolution of these traits within populations as a consequence of sexual selection[11]. For example, it has recently been demonstrated that genes underlying interspecific differences in mating signals might not be the same genes that are responsible for intraspecific variation in the same traits[17,18].

We consider both male sexually selected traits and the female mating preferences for them. Although it is clear that in many species males can exert sexual selection on females, most genetic analyses have focused on female preferences for male traits, and we restrict our discussion to this aspect of mate choice. First, we outline experimental and analytical approaches that allow the multiple phenotypes involved in mate choice to be determined and subjected to genetic analysis. Second, we review progress in the characterization of genes underlying the genetic variance in single traits that are known to be associated with mate choice. Finally, we address the difficulties that are associated with multivariate genetic approaches, and highlight the role that integration of quantitative genetics with genome-wide molecular approaches might have in a comprehensive genetic analysis of mate choice.

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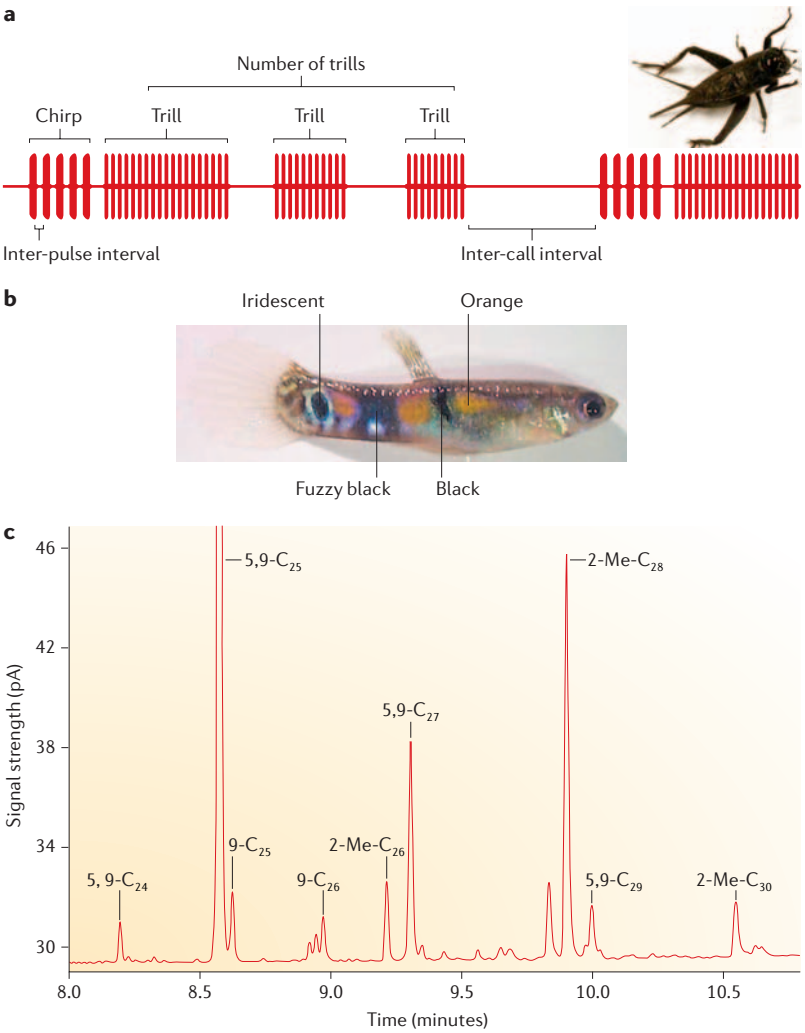


Figure 1 | **The complexity of male sexually selected traits**. The complexity of mate choice not only arises as a consequence of a number of different signalling mechanisms and/or behaviours contributing to attractiveness, but also stems from each type of mechanism often having a multi-component phenotype. Three examples are shown in which sexual selection operates on a combination of the components of a single mechanism. **a** | The acoustic signal of male *Teleogryllus commodus*. Four individual traits are highlighted here: inter-pulse interval, inter-call interval, chirp length and number of trills. A fifth trait, dominant frequency, is not shown. **b** | Visual signals in male *Poecilia reticulata*. Four individual colouration traits are highlighted: orange, black, fuzzy black and iridescent spots. Body size and tail size are also often considered important sexually selected traits in guppies. **c** | Chemical signals in male *Drosophila serrata*. Here, nine individual hydrocarbons that have been implicated in various aspects of mate choice are identified on a trace from a gas chromatograph. The concentration of each hydrocarbon can be calculated from the area underneath each peak. Images in panels **a**,**b** courtesy of J. Hunt and R. Brooks, respectively, University of New South Wales. Gas chromatograph trace in panel **c** courtesy of M. Higgie, University of Queensland.

Male sexually selected traits

Identifying the traits. Determining which male traits are under sexual selection is an important first step in a genetic analysis of mate choice, but direct evidence for which traits are involved in mate choice is lacking from many genetic studies[7]. Typically, single, highly sexually dimorphic male traits are chosen as the subject of genetic analysis. Although the extent of sexual dimorphism is often associated with a role in mate choice, such traits might not be the only traits that are under sexual selection, or could be sexually dimorphic as a consequence of natural, rather than sexual, selection. The identification of traits under sexual selection in the laboratory can be achieved through mate choice trials that enable sexual selection to be quantitatively assessed (BOX 1).

Male sexually selected traits often form part of a display, and can therefore be phenotypically plastic. For example, there could be changes in the chemical composition of a pheromone, the intensity of male colouration or male trait size in response to the presence of a female during the act of displaying. In such situations, it is not only the genes that males carry that can contribute to phenotypic variation among males (direct genetic effects), but the genes that females carry can also influence male displays[19] (indirect genetic effects). Both these sources of genetic variance have the potential to influence the evolution of mate choice.

Assessing direct genetic variance. The genetic basis of male sexually selected traits has received considerable theoretical and empirical attention, primarily in attempts to understand why females have evolved mating preferences for these traits[20–22]. Although the ultimate reason for the evolution of female mating preferences is the subject of fierce debate, which we will not enter into here, a prerequisite for the operation of many models of sexual selection is the presence, and more importantly the maintenance, of genetic variance in male traits. With constant sexual selection, genetic variance in male traits is expected to be depleted, resulting in the lek paradox. Generally, however, this does not seem to be the case, and the level of genetic variance in male sexually selected traits is on average considerably higher than in other types of trait[12].

The maintenance of high levels of genetic variance in male sexually selected traits has been attributed to the evolution of condition-dependent trait expression; male trait attributes such as size or colour intensity depend on the general condition of the individual[4,23]. By becoming condition-dependent, the number of loci that contribute to the trait is thought to be vastly increased because of the many factors that contribute to condition, thereby 'capturing' genetic variance in condition and maintaining genetic variance in the male trait under sexual selection. Although the observed levels of genetic variance in male traits are consistent with this hypothesis, direct evidence for condition-dependent trait expression is generally lacking[24], and has been sought in few examples. When it has been studied, the presence of genetic covariance between male traits and condition[25–27] indicates at least a partially shared genetic basis to some male traits. However, more targeted genetic analyses that search for pleiotropic loci contributing to these traits, or for direct evidence for substantial numbers of loci contributing to male trait expression, are rare (although see REF. 28).

Assessing indirect genetic variance. Another potential source of genetic influence on male sexually selected traits are indirect genetic effects, which occur when the genes of one individual influence the phenotype of another. A common form of indirect genetic effects are maternal

Direct genetic effects
Contributions to the phenotype that are the consequence of an individual's genotype.

Box 1 | **Identification of traits under sexual selection**

Here we use an example of female mate choice for male colour in guppies to demonstrate how trait combinations under selection can be identified. The attractiveness of 251 male guppies was assessed by presenting them to females (data kindly supplied by R. Brooks; experimental details can be found in REF. 1). Single females were allowed to choose to spend time in the proximity of 6 males presented simultaneously to them, and male attractiveness was scored as the time a female spent with each male. In this species the time a female spends in the proximity of a given male can be used as a proxy for his mating success. Four areas of male colour were measured for each male (FIG. 1): black, fuzzy black, iridescent and orange (this trait order is maintained in the examples below).

The strength of directional (linear) sexual selection (w) can then be quantified using linear regression[118] (equation 1):

$$w = \alpha + z^{T}\beta \quad \beta = \begin{pmatrix} 0.069 \\ -0.000 \\ 0.154^{*} \\ 0.115 \end{pmatrix} \tag{1}$$

where α is the y-intercept, z is a vector of the traits on which selection is being measured, T represents matrix transposition and β contains the partial regression coefficients and is called the vector of linear sexual selection gradients. The significant selection gradient for iridescence (at $P<0.05$, indicated by an asterisk) indicates that this trait is under sexual selection when the effects of the other traits have been controlled for. Methods for determining the significance of individual selection gradients can be found in REF. 119.

Sexual selection might not only be linear in form, but might also be stabilizing (selection for decreased variance) or disruptive (selection for increased variance) in nature. The extent of such nonlinear sexual selection can be quantified using second-order polynomial regression (equation 2):

$$w = \alpha + z^{T}\beta + z^{T}\gamma z \quad \gamma = \begin{pmatrix} 0.016 & & & \\ -0.016 & 0.000 & & \\ -0.028 & 0.066 & -0.011 & \\ 0.103 & -0.131^{*} & -0.099 & 0.030 \end{pmatrix} \tag{2}$$

where γ is the matrix of quadratic and cross-product terms that indicates the extent of nonlinear selection on the set of traits. Here, there is a single cross-product coefficient between fuzzy black and orange that is significant, indicating negative correlational selection on these two traits. To facilitate the interpretation of how the traits interact to result in mating success, further analysis of this matrix using canonical rotation can find the major axes of nonlinear selection or, in other words, those combinations of traits which are under the greatest nonlinear selection[120,121] (equation 3):

$$w = \alpha + y^{T}\theta + y^{T}\Lambda y \quad \Lambda = \begin{pmatrix} 0.132^{**} & & & \\ & 0.006 & & \\ & & -0.038 & \\ & & & -0.064 \end{pmatrix} \quad \theta = \begin{pmatrix} 0.046 \\ 0.094 \\ 0.151^{*} \\ 0.088 \end{pmatrix} \tag{3}$$

where individual traits (z_i) have been replaced by the new major (or canonical) axes (y_i), θ is a vector of linear selection gradients, and the eigenvalues of γ found along the diagonal of Λ now replace the quadratic coefficients in γ as measures of the strength of nonlinear selection along the canonical axes represented as the rows of the matrix M (equation 4). The double asterisk indicates significance at $P<0.01$.

$$\mathrm{M} = \begin{pmatrix} 0.390 & -0.467 & -0.389 & 0.692 \\ 0.846 & 0.467 & 0.256 & -0.018 \\ 0.011 & -0.487 & 0.861 & 0.149 \\ -0.363 & 0.572 & 0.206 & 0.706 \end{pmatrix} \tag{4}$$

The significant canonical coefficient in Λ indicates the presence of disruptive selection along the major axis of the individual fitness surface that integrates all four colours. Significant linear selection along the third axis (that is, the third row of M) is indicated in θ, representing selection on a combination of iridescent and fuzzy black spots.

Indirect genetic effects
Contributions to phenotype that are the consequence of another individual's genotype.

Lek paradox
The conundrum that female preference should deplete genetic variance in male sexually selected traits, but females continue to choose. Thought to be resolved by the evolution of condition-dependent expression of male traits.

Genetic covariance
A quantitative measure of the extent to which two phenotypes are affected by the same genes.

Pleiotropic loci
Loci that affect more than one phenotypic trait.

Eigenvalue
The eigenvalue is the scale factor with which the eigenvector length changes.

Individual fitness surface
The relationship between a trait (or traits) and fitness for individuals of a population using second-order polynomial regression.

Maternal effects
The effect of the maternal genotype or environment on the phenotype of the offspring.

Contact pheromones
Non-volatile pheromones that are sampled by individuals of the other sex by touching.

effects, but when traits influence the outcome of social interactions (male display traits are a good example), the genes of completely unrelated interacting individuals can have a pronounced effect on the expression of such traits. That is, the genes of a female could influence the expression of the sexually selected trait in the males that she is choosing among. There are two important implications that arise with the presence of indirect genetic effects. First, a trait needs to be investigated in the appropriate context when the animals are displaying to females to capture the relevant phenotypes and their genetic basis. Second, theoretical models of indirect genetic effects[19] indicate that these can influence the response to selection of the affected trait, and might even allow traits with no direct genetic variance to evolve.

The detection of indirect genetic effects can be accomplished within standard quantitative genetic experimental designs (BOX 2). For example, in *Drosophila serrata*[29], genetic covariance between female genes for body condition and male contact pheromones was detected. In other words, genes that control female condition were implicated in changing the expression of a male's pheromone profile. Although it is unclear whether such indirect genetic effects are widespread, the complex interactions between males and females during mate choice in many species indicate that male sexually

selected traits are likely to be influenced by indirect genetic effects. Although the evolutionary implications of indirect genetic effects are being investigated in other social contexts[30–32], their effect on the evolution of sexual display traits remains to be empirically tested.

Mating preferences

Mating preferences are of fundamental importance to the study of mate choice, representing the combined processes of perception of signals from potential mates, and assessment and response to those signals. When considering mating preferences, the distinction has been made between 'choosiness' and 'preference functions', which refer respectively to the amount of effort that an individual invests in mate assessment and the order in which an individual ranks potential mates depending on signal level[33]. Here we refer primarily to the genetic basis of preference functions.

Although most work has focused on female mating preferences, it is important to note that both sexes can exhibit mating preferences, even within species with conventional sex roles[34,35]. The existence of mating preferences in both sexes can complicate genetic analyses, owing to the possibility of sexual dimorphism for preference[36] and genetic constraints between the sexes that are manifested as genetic correlations between male and female preferences. For simplicity, we refer to female preferences throughout, but all discussions and techniques are equally applicable to the analysis of male mating preferences.

Measuring female preference functions. Female preference functions are commonly estimated at two levels of resolution[37] (BOX 3). Population-level analyses provide information about the average of all individual mating preferences within a population in terms of the sexual selection that they generate on the opposite sex. Alternatively, individual-level preferences describe the specific preferences of an individual female. Although both types of mating preference could potentially be used in a genetic analysis, individual preference functions are more desirable. They potentially allow the genetic basis of preferences within a population to be fully characterized, as the different preferences that might be present in the population can be identified and the genetic relationships among them determined.

Unfortunately, accurate measurement of individual-level female mating preferences is often extremely difficult[37]. This is particularly the case when preferences cannot be measured without allowing mating to occur (as in many insect systems), thereby changing the motivation of females before multiple measures can be taken. However, the individual preference function approach can be applied in these cases by using a panel of inbred lines, a common approach in behavioural genetics that provides convenient access to genetically identical males and females. Therefore, multiple females from a line can be exposed to a range of male signals or behaviours by performing a series of either one- or two-stimulus choice tests, allowing the calculation of the within-line female preference function. It should be noted that these types of experiment often limit the previous experience of females, and female preferences can change with observations of the choice of other females (copying) and/or other aspects of learning. It is an open question as to the genetic association between naive female preferences and those that are exhibited after multiple interactions.

Assessing genetic variance. Female mating preferences exhibit significant additive genetic variance within some, but not all, populations[11]. Given the inherent difficulties in measuring female preferences, manipulative evidence for the presence of genetic variance in preference measures has been a major goal. Variation among natural populations has indicated that female preferences might evolve in response to varying environmental

Box 2 | Detecting indirect genetic effects

The short-term univariate response to selection of a trait, z_1, which is influenced by direct genetic variance only, is given by the evolutionary quantitative genetic version of the classic breeder's equation (equation 5):

$$\Delta z_1 = V_A \beta \quad (5)$$

where V_A is the additive genetic variance and β is the selection gradient as defined in BOX 1. If a trait in one individual (for example mate choice or z_1) depends on the expression of a heritable trait (such as a male display trait or z_2) in another individual, an indirect genetic effect can occur and the response to selection of z_1 will now be as shown in equation 6 (REF. 19):

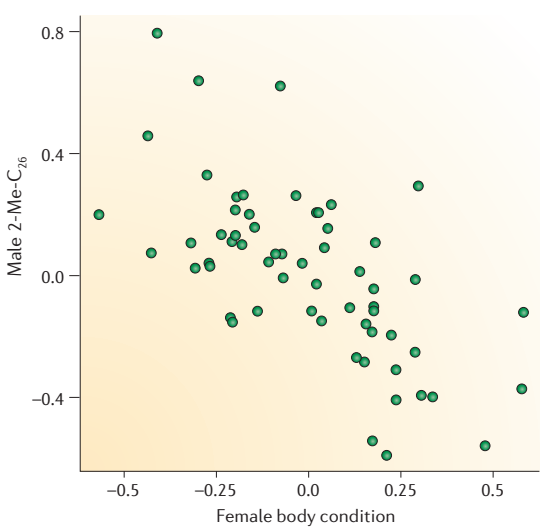
$$\Delta z_1 = (V_{11}\beta_1 + Cov_{12}\beta_2) + \Psi_{12}(Cov_{12}\beta_1 + V_{22}\beta_2) \quad (6)$$

where the first term on the right hand side represents the response to selection of z_1 that is caused by direct selection on z_1 and selection on z_2 transmitted through the genetic covariance between z_1 and z_2 (Cov_{12}). The second term is the indirect genetic effect, in which the interaction parameter ψ_{12} quantifies the importance or the extent of the effect of z_2 on z_1. More complicated equations are required to represent the response in the presence of reciprocal interactions[19].

Estimating indirect genetic effects can be accomplished by exposing unrelated target males to females of known parentage from a breeding design. After the mate choice interaction has occurred, the phenotypes of the target males are then subjected to genetic analysis as if they were from the pedigree.

The example in the figure below shows a plot of the best linear unbiased predictors for 60 sires (data from experiment 3 in REF. 29) for body condition of females from the breeding design, and the expression of the cuticular hydrocarbon 2-Me-C_{26} of unrelated males exposed to the females during mate choice. This relationship indicates that the genes controlling female body condition influence the expression of the male pheromone profile.

The key interaction parameter ψ_{12} has yet to be estimated in any experiment, but can readily be obtained by using a set of inbred lines[19]. For example, males are presented to females from a series of inbred lines that vary in body condition. The slope of the regression of males displaying the phenotype on ranked female body condition from the inbred lines would estimate ψ_{12}.



conditions[38], and experimental confirmation of this has recently been reported[39]. In addition, artificial selection has been used to determine whether preferences within a species have a genetic basis[11,33]. However, even when selection experiments have been successful in changing female preferences, later experiments have sometimes been unable to successfully select for preferences using the same population, for example in ladybirds[40,41], or in different populations of the same species, as observed in guppies (*Poecilia reticulata*)[42–44].

In one example in which this approach was successful, Wilkinson *et al.*[45] used the Malaysian stalk-eyed fly,

Box 3 | **Measuring preference functions**

Population-level preference functions

Population-level preference functions are the most commonly used method to analyse mate preference[37]. Typically, a group of females within a population are tested only once for their response to a range of male signal levels and a statistical model is fitted relating female response as a function of male signal[36]. For genetic analysis this approach has limited value because variation in preference within and among females is unaccounted for. Therefore, a population-level function can in fact contain multiple female preference phenotypes.

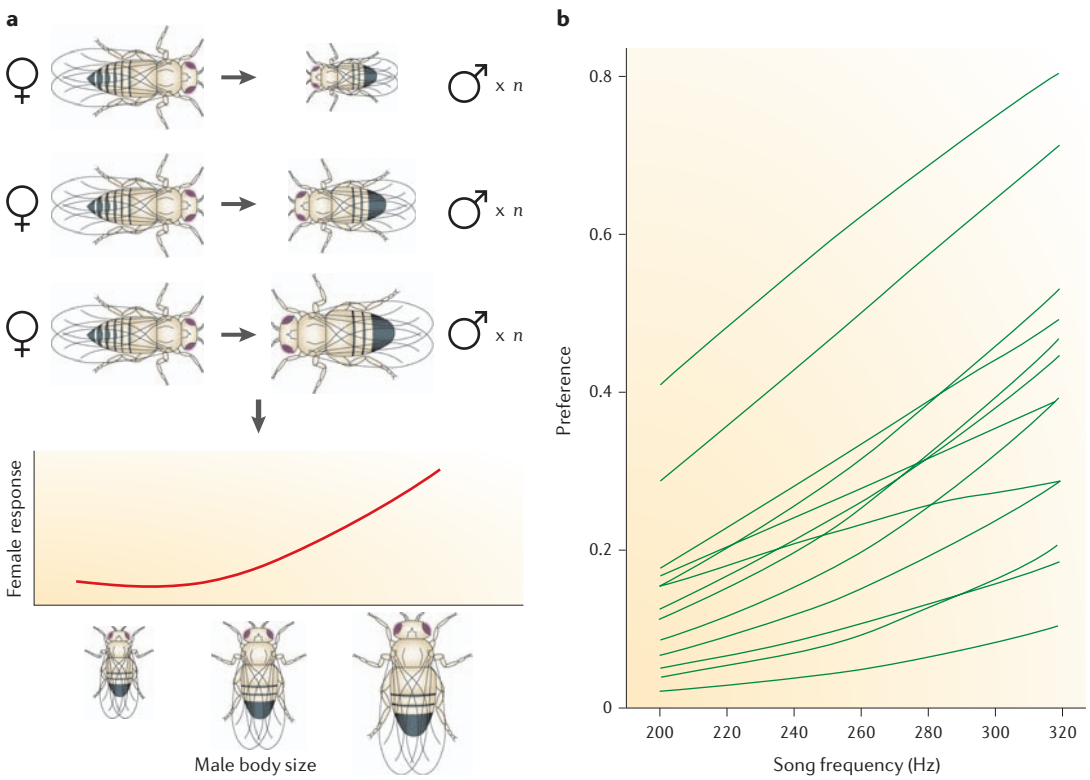
Individual preference functions

Individual preference functions measure a female's response to an array of male signal levels. Typically, a single female is repeatedly tested for her response to a randomized series of male signal levels and preference functions are estimated using either polynomial regression or cubic spline methods[56]. It is important that females are tested multiple times for their response to the same stimulus to allow variances to be calculated around mean response levels and variation among individuals to be compared. By extending this technique Ritchie *et al.*[122] calculated family-level female preference functions for male wing song in *Drosophila montana*. Multiple individual females were tested from each of 27 F1 families for their response to synthetic male wing songs. Preference functions were estimated at the family level, allowing an estimate of genetic variation in female preference functions. The variation among family preference functions is, in this case, analogous to broad-sense heritability because full-sib families were assayed[123].

Part **a** in the figure below shows a hypothetical experimental design for the analysis of individual female preference functions for male body size in a fly. A female is tested for her response to males of different size with *n* trials conducted for each male size class. This example is an absolute preference function, as females were not given a choice between males. Many variations on this design to accommodate relative preferences can be found in REF. 37.

In the example in part **b**, genetic variation in female preference functions for male song was analysed in *D. montana* (after Ritchie *et al.* REF. 122). Each green line is the female preference function for an individual family. Note that although many functions are overlapping, there is significant variation among genotypes within this population.

The graph in part **b** is modified with permission from REF. 122 © (2004) Elsevier Science.



Artificial selection
Selection by the researcher of a proportion of individuals, based on phenotype, that will contribute to the next generation. Usually repeated for 10 or more generations.

Cyrtodiopsis dalmanni, and applied bidirectional artificial sexual selection on male eye span, a sexually selected trait in this species. Both increases and decreases in eye span were observed in selected lines after 13 generations of selection. An analysis of female mating preferences in the selected lines showed a correlated response to artificial selection on eye span[46], indicating a genetic correlation between female preferences and male eye span. Without additional genetic analyses, artificial selection experiments provide no details about the underlying genetic architecture of sexually selected traits. Other genetic approaches, such as line crosses, QTL analysis or microarray analysis, need to be used in combination with artificial selection to exploit its full potential, as we discuss below.

Characterizing the underlying polymorphisms

Once the existence of genetic variance for either a sexually selected trait or a mating preference has been established, attention often turns to determining the number, genomic distribution and effect-sizes of the contributing genetic variants. Depending on the organism, this might involve anything from a simple series of line crosses to determine the degree of sex linkage, to a parallel analysis of genome-wide transcriptional patterns during mate choice. Although advanced genomic tools have so far largely been the domain of model organisms such as *Drosophila melanogaster,* an increasing number of studies use these techniques in non-model organisms that have well-studied mating behaviours.

Sex linkage. Because the strength of sexual selection on individual traits often differs between the sexes, there are a number of reasons why genes that are associated with such traits might become sex linked[47–49]. Sex linkage provides one mechanism by which homologous traits in males and females can evolve independently towards their sex-specific optima. Reciprocal line crosses between divergent populations or artificial selection lines have been the mainstay of analyses of sex linkage for both sexually selected traits and female mating preferences[50–54].

Ritchie[55] used line crosses to examine the genetic basis of both male song and female preference functions for song in the bushcricket, *Ephippiger ephippiger*, using line crosses. This species uses a system of acoustic signals for mate recognition, with natural populations that are divergent for both calls and preferences[56]. Reciprocal crosses among populations that are divergent for song and preference indicated that the X chromosome had a disproportionate control over male song, accounting for 25% of the parental difference. By contrast, there was no evidence for sex-linked control of female preference functions, with 75% of the significant variation in mating preferences among populations being attributed to the autosomes.

In another example, parent–offspring regression techniques were sufficient to demonstrate sex-biased inheritance of female preferences in the arctiid moth, *Utethesia ornatri*[57]. In this case, inheritance was paternal (males are homogametic, with a ZZ genotype, in Lepidoptera), with preference genes residing on the Z chromosome. Line crosses and parent–offspring regression techniques have also been used to demonstrate that genes for sexually selected colour patterns in guppies are situated on the non-recombining Y chromosome[58].

However, the observation of sex linkage of sexually selected traits is by no means universal. Model organisms offer an alternative bioinformatic approach for analysing the chromosomal distribution of sexually selected genes. Using the complete list of annotated genes within the *D. melanogaster* genome, Fitzpatrick[28] used functional criteria to develop a list of candidate sexually selected genes. Analysis of the chromosomal distribution of these candidates demonstrated that there was no bias towards sex linkage. Furthermore, studies of genome-wide sex-biased transcription in *D. melanogaster* have reported no bias towards sex linkage in genes that are preferentially expressed in males[59,60]. However, the link between sexual selection and sex-biased gene expression remains to be experimentally validated.

Chromosomal distribution — QTL analysis. Although sex linkage can be an important component of the genomic distribution of genes underlying sexually selected traits and preferences, the techniques described above are obviously limited in their ability to provide information on the nature of effects within sex chromosomes and across the rest of the genome. QTL analysis is the next step, often providing details of the location, number and effect of the polymorphisms responsible for phenotypic variation. Approaches to QTL analysis for the dissection of a range of biological traits have been widely applied and reviewed[61–64] and are not discussed in detail here. So far, intraspecific QTL mapping studies have focused on sexually selected traits rather than female mating preferences. Although QTLs for sexually selected traits have been found for a range of species, including fish[65] and non-drosophilid flies[66], the vast majority of effort has focused on *Drosophila*.

Male wing song is a target of sexual selection in many species of *Drosophila* and has received intensive genetic investigation[18]. A QTL analysis of inter-pulse interval, a key component of courtship song in *Drosophilia melanogaster* that is targeted by female choice, found evidence for three QTLs explaining 54% of the genetic variance among inbred lines[67]. QTLs have also been mapped for other wing song components in *Drosophilia virilis*, with eight QTLs detected for pulse trains and a further four detected for pulse-train length, predominantly mapping to the third chromosome[68]. An important insight from the study of *Drosophila* wing song QTLs is that the genomic locations of QTLs often do not coincide with candidate genes that have been identified using single gene mutagenesis[17,67,69]. These discrepancies highlight the fundamental difference in the information provided by mutagenesis and QTL analyses. The ability of a mutagenic allele to affect trait expression might not demonstrate that allelic variation at that locus generates naturally occurring genetic variance in the trait[70].

Few studies have mapped QTLs for female preference functions within species, possibly due to the complexity of female preference functions as traits. Unlike sexually

Reciprocal line crosses
Males and females of both lines are crossed to allow the contribution of the sex chromosomes to a trait to be determined.

Parent–offspring regression
The association of parental phenotypes with offspring phenotypes using linear regression to enable an estimate of heritability.

Inter-pulse interval
The time interval between sound components of a song.

Pulse trains
A string of sound components of a song.

selected signals, which are more easily quantified within and among individuals, female preference functions are essentially a reaction norm representing a female's predicted response to a range of male signal levels. A quantitative genetic theory exists for the study of reaction norms[71,72] and QTL analysis has been successfully applied to such function-value traits[73]. Nevertheless, the primary challenge for the genetic analysis of female preference functions within species is the measurement of preferences themselves as a quantitative trait, as discussed earlier. For example, measuring preference functions in a set of recombinant inbred lines (RILs) provides an estimate of the variation among lines, which gives an estimate of the genetic variance in preference functions, and, with adequate replication, QTLs could be mapped for these functions. The inbred-line approach opens up the possibility of studying the genetic basis of individual preference functions in species for which male signals cannot be readily synthesized. This is because the replicate male signal levels that are required to estimate preference functions can be supplied from inbred lines for experimental use.

Identifying the responsible genes. After QTL identification, it remains a difficult process to establish which candidate gene(s) might be associated with a specific QTL. This is a particularly important step, however, as QTL analysis will generally only result in the identification of large genomic regions that might affect the trait of interest. In model systems for which the genome has been sequenced, QTL analysis enables the assembly of a list of candidate genes that are contained within the genomic region that has been identified. Unfortunately, for non-model organisms that lack a comparative linkage map to a fully sequenced organism, the identification of QTLs can supply only limited information on the genetic basis of complex traits.

In an extension of the standard QTL technique, Moehring and Mackay[74] performed an analysis of male mating behaviour components (courtship occurrence, courtship latency, copulation occurrence and copulation latency) in *D. melanogaster*. They found four major QTLs using a panel of 98 RILs. To increase genomic resolution, deficiency complementation mapping[75] was used, which showed evidence for the involvement of seven candidate genes. Interestingly, none of these genes had previously been implicated in mating behaviour.

Many species of insect, including *D. melanogaster*, rely on a pheromonal system of cuticular hydrocarbons (CHCs) for mate recognition and mate quality assessment[76–79]. These sexually selected compounds have undergone detailed genetic analyses using both mutagenesis and mapping techniques, and several genes have been cloned that affect CHC biosynthesis. One well-studied example involves the two tightly linked desaturase genes *desat1* and *desat2* in *D. melanogaster*. These loci were originally cloned in laboratory stocks by Wicker-Thomas *et al.*[80]. Subsequently, Coyne *et al.*[81] found that a naturally occurring CHC polymorphism in female *D. melanogaster,* involving alternate production of the dienes 7,11-heptacosadiene and 5,9-heptacosadiene, which are known to affect male courtship, mapped to one of these loci (*desat2*). Dallerac *et al.*[82] then showed that this polymorphism was a consequence of the segregation of two naturally occurring alleles.

Recently, two mutagenesis studies confirmed the role of *desat1* in the production of CHCs[83] and mating discrimination[84] in *D. melanogaster*. An interesting pleiotropic effect was observed for *desat1* mutants: male mating discrimination was also affected, with males losing the ability to discriminate between the sex and CHC phenotype of other flies[84]. Tissue-specific analyses of *desat1* expression indicated that the gene was expressed not only in the oenocytes (the main tissue of CHC production in *D. melanogaster*), but also in the antennae and proboscis, indicating a pleiotropic role for *desat1* in both pheromone production and reception. This example highlights the power of the complementary use of mapping from naturally occurring genetic variation and mutagenesis, with mutagenesis providing the necessary manipulative validation of the role of a given locus.

A potentially useful approach that builds on the power of mutagenesis to identify a causal link between candidate gene polymorphism and trait expression is association mapping. Once candidate genes are identified, samples from natural populations can be simultaneously phenotyped and assayed for naturally occurring sequence polymorphisms in the gene of interest. This approach has proven powerful in dissecting model quantitative traits such as wing shape[85] and bristle number[86] in *D. melanogaster*, and could be useful for those studying mating behaviour in natural populations of species for which candidate genes are available. One drawback of the approach, however, is that large sample sizes are required to detect even modest associations between functional nucleotide polymorphisms and phenotypic variance[87].

Transcriptional profiling approaches to genetic architecture and gene identification. QTL analyses unfortunately remain relatively imprecise[88,89] and are biased towards identifying genes of large phenotypic effect[62], making it difficult to empirically determine the distribution of the effect sizes and the number of allelic effects that respond to sexual selection. Similarly, molecular genetic studies use approaches such as mutagenesis, chromosomal introgression and deletion mapping to analyse the developmental and genetic basis of traits, but it is often difficult to relate this genetic basis to naturally occurring genetic variation[70]. Microarray technology offers the promise of being able to characterize the genetic difference between populations and/or treatments by determining the genome-wide expression differences that exist between them[90]. Although transcriptional profiling potentially allows expression changes of relatively small effect to be detected, it does not generally address the issue of the number of independent genetic changes that underlie a phenotypic difference.

In one of the first microarray-based studies to consider mating behaviour, Mackay *et al.*[91] used a combination of artificial selection and transcriptional profiling to examine

Reaction norm
A function that describes the response of a single genotype to a gradient in the environment.

Recombinant inbred lines
A set of lines that are formed by crossing two inbred strains, followed by 20 or more consecutive generations of brother–sister matings.

Chromosomal introgression
The placement of an entire chromosome of a donor parent in the genetic background of a recipient parent.

the genome-wide transcriptional response to artificial selection for mating speed in *D. melanogaster*. Selection lines were founded from a natural population and selection was applied to mating pairs for either fast or slow mating (that is, time to copulation) for 29 generations. The phenotypic response in mating speed was functionally related to changes in female receptivity. Whole-genome transcriptional profiling was used to assay the expression changes associated with this selection regime in both males and females. The breadth of response was surprising: approximately 25% of the genome showed differences in transcript abundance between the selected lines.

One way to find the underlying regulatory loci responsible for expression changes is to apply QTL approaches to the expression phenotypes[92]. Although this approach will potentially allow individual expression phenotypes to be associated with chromosomal regions, QTL analysis has not been particularly useful for large numbers of phenotypes[93] and determining the pleiotropic associations among QTLs. Furthermore, only regulatory regions of large effect are likely to be identified, as with other classes of phenotype, and concordance between QTLs and changes in expression levels at candidate genes might not be seen for several reasons[94]. It will be important in future studies to establish how many regulatory polymorphisms actually generate the large-scale responses in transcription that were observed in the studies described above. The complexity of changes in transcription indicated by studies such as REF. 91 implies that we will need more sophisticated experimental designs and statistical tools to make sense of such patterns.

Genetic analysis of multiple traits

The complexity of mate choice is one of the key problems associated with its genetic analysis. Complexity can arise as a consequence of one trait involved in mate choice having multiple components (FIG. 1), such as the constituent acoustic properties of a single call[95,96], multi-component pheromones[79], visual signals[1,97] or courtship behaviours[98]. In addition, mate choice can involve any combination of these different types of cue. Although the presence of multiple types of trait is generally acknowledged as a limitation of most studies[6], the multi-component nature of single traits is often overlooked in genetic analyses.

Multivariate quantitative genetics and sexual selection. Given the potential complexity of mechanisms contributing to male attractiveness, it is important to be able to establish how the genetic basis of a single mechanism (or a set of mechanisms) equates to the genetic basis of attractiveness itself. Although selection analysis (BOX 1) indicates the phenotypic associations between attractiveness and a suite of sexually selected traits, such associations are likely to be affected by shared environmental influences as well as a common genetic basis. One way to determine the importance of a mechanism of mate choice in a genetic analysis is to determine how much of the genetic variance in a holistic measure of male attractiveness is explained by genetic variance in the mechanism. For example, in guppies, approximately 37% of genetic variance in male attractiveness can be explained by a combination of colour traits and tail size[99], indicating that other sexually selected traits that genetically covary with male attractiveness were not included in the analysis.

When more than one trait is under sexual selection, the genetic associations among the traits become important, as the response to sexual selection will not only be influenced by the genetic basis of each individual trait, but also by the genetic covariance among them. Recent work in *D. serrata*[27,100] has shown that single-component genetic analyses of multi-component male sexually selected traits can be misleading to the extent that when all component traits are considered together, virtually no genetic variance exists in the direction of sexual selection. This occurs despite the fact that all individual component traits display the high levels of genetic variance commonly found in studies of single traits, and is a consequence of the distribution of genetic covariance among individual components. These analyses indicate that sexual selection could be sufficiently strong to deplete genetic variance in male sexually selected traits, as predicted by the lek paradox. From the point of view of genetic analysis, these results indicate that alleles segregating in natural populations for sexually selected traits might not be those that are highly favoured during mate choice.

Pleiotropic QTL mapping. The genetic covariance that exists between components of male sexually selected traits indicates that pleiotropic loci should affect combinations of these components, and this has been partially supported in at least one study of male mating behaviour[74]. Furthermore, if a number of sexually selected traits are condition-dependent, pleiotropic loci that mediate their effects through condition are likely to be an important part of the genetic basis of these types of trait. Although multiple traits are often measured in QTL studies, multivariate analysis is seldom performed[93]. Pleiotropic QTL mapping is most often implemented by either transforming a set of traits into new linear combinations before genetic analysis[101–103] or, alternatively, mapping individual traits and assessing whether the confidence intervals for QTLs overlap for some combinations of traits. True multivariate approaches that implement joint mapping of multiple traits have been developed[104]. Of particular interest are methods based on factor-analytic modelling of complex pleiotropic relationships among QTLs[105,106], an approach we now discuss more fully in relation to microarrays.

Gene expression and the integration of classical quantitative genetic approaches. Microarray experiments are usually conducted within experimental designs that allow an estimate of experimental error, so that differences among treatments can be identified with confidence[107,108]. It is important to note that although microarray experiments measure gene expression, such data are simply phenotypes; that is, both genetic and environmental

Factor-analytic modelling
Multivariate statistical method for fitting underlying latent factors to high-dimensional data.

sources of variation contribute to gene expression profiles, and microarray experimental designs do not usually include a genetic component[109–111]. By conducting a microarray experiment within an appropriate breeding design, not only can experimental error be estimated, the variation in expression profiles that has a genetic basis (as distinct from variation caused by other sources in the experiment) can be identified[109,110]. Therefore, differences between groups in expression profile can be investigated at the genetic level, rather than simply at a phenotypic level, as is commonly the case. In this way, the underlying genetic variation in the regulation of a large number of expression profiles could be characterized.

Conducting microarray experiments within quantitative genetic experimental designs will require large numbers of arrays (which is becoming possible, at least for species closely related to model organisms), and the application of mixed model approaches to analysis[112,113]. Factor-analytic modelling has been adopted as a way of fitting genetic factors that allows direct hypothesis tests of the number of genetic dimensions required to explain genetic covariation among traits within a restricted maximum likelihood (REML) framework[114–116] (BOX 4). For transcriptional profiling experiments, this means that the many gene expression profiles that are generated by such experiments could be reduced to a

Box 4 | Factor-analytic modelling of genetic data

Factor-analytic modelling of genetic data, to fit fewer underlying latent variables to high-dimensional data, can be conducted within any mixed model that describes a breeding design[116]. At the genetic level of interest, a reduced-rank genetic covariance matrix $\hat{G}$ that contains genetic factors of lower dimension can be found using equation 7:

$$\hat{G} = \Lambda\Lambda^{T} \quad (7)$$

where T is the transpose of a matrix, and Λ (p x m) is a lower triangular matrix of constants that represent the factor loadings of the m latent variables (or factors) for the p original traits. A series of nested hypothesis tests can be conducted to determine how many genetic dimensions are required to explain the observed patterns of genetic covariance. A full model is first fit ($m = p$), and factors are sequentially dropped until the model achieves a significantly worse fit compared with the previous model (in which it is nested), indicating that the amount of variation accounted for by the tested factor is sufficient for the factor to be retained.

In this example, eight cuticular hydrocarbon traits of *Drosophila serrata* (FIG. 1) were measured on males from a standard half-sib breeding design (data from Blows *et al.*[100]), and the factor-analytic covariance structure is modelled at the paternal level. The original $\hat{G}$ matrix from the paternal level (equation 8):

Z,Z-5,9-$C_{25:2}$	0.061	0.068	0.044	0.043	0.071	0.023	0.052	0.015
Z-9-$C_{25:1}$		0.128	0.066	0.049	0.080	0.047	0.032	0.046
Z-9-$C_{26:1}$			0.053	0.043	0.071	0.029	0.059	0.017
2-Me-C_{26}				0.149	0.073	0.099	0.116	0.074
Z,Z-5,9-$C_{27:2}$					0.142	0.041	0.085	0.023
2-Me-C_{28}						0.065	0.071	0.049
Z,Z-5,9-$C_{29:2}$							0.051	0.041
2-Me-C_{30}								0.036

(8)

was found to be best represented by a reduced-rank genetic covariance matrix $\hat{G}$ with 2 dimensions (equation 9):

$$\begin{pmatrix} 0.037 & 0.021 & 0.036 & 0.040 & 0.073 & 0.024 & 0.045 & 0.014 \\ & 0.012 & 0.020 & 0.023 & 0.042 & 0.014 & 0.026 & 0.008 \\ & & 0.035 & 0.051 & 0.069 & 0.033 & 0.050 & 0.020 \\ & & & 0.155 & 0.076 & 0.109 & 0.106 & 0.075 \\ & & & & 0.144 & 0.046 & 0.087 & 0.026 \\ & & & & & 0.077 & 0.072 & 0.053 \\ & & & & & & 0.085 & 0.048 \\ & & & & & & & 0.037 \end{pmatrix} = \begin{pmatrix} 0.193 & 0 \\ 0.110 & 0.000 \\ 0.184 & 0.039 \\ 0.206 & 0.336 \\ 0.379 & -0.007 \\ 0.126 & 0.246 \\ 0.234 & 0.173 \\ 0.072 & 0.178 \end{pmatrix} \begin{pmatrix} 0.193 & 0 \\ 0.110 & 0.000 \\ 0.184 & 0.039 \\ 0.206 & 0.336 \\ 0.379 & -0.007 \\ 0.126 & 0.246 \\ 0.234 & 0.173 \\ 0.072 & 0.178 \end{pmatrix}^{T} \quad (9)$$

It can be concluded that there is evidence for two independent genetic traits that underlie these eight phenotypes that are represented by the eigenvectors of $\hat{G}$ (equation 10):

$$\begin{pmatrix} 0.232 & 0.319 \\ 0.132 & 0.182 \\ 0.255 & 0.213 \\ 0.536 & -0.436 \\ 0.449 & 0.642 \\ 0.363 & -0.362 \\ 0.430 & -0.014 \\ 0.239 & -0.293 \end{pmatrix} \quad (10)$$

The first eigenvector has contributions from all eight phenotypes in a similar manner (possibly representing condition), whereas the second contrasts the three methylalkanes with the other hydrocarbons, indicating that the levels of expression of these three hydrocarbons share a common genetic basis.

Mixed model
A linear statistical model that contains both fixed and random sources of variation.

Restricted maximum likelihood
An iterative-based approach used for the estimation of variance components.

Reduced-rank genetic covariance matrix
A covariance matrix that has fewer dimensions than traits.

Half-sib breeding design
A breeding design in which a number of sires are each mated to a number of dams, and the resulting offspring are phenotyped.

Eigenvector
A linear combination of original traits that are measured. A set of eigenvectors are orthogonal.

far smaller number, corresponding to the number of genetic factors that represent the underlying genetic variation in regulation. QTL mapping of these major regulatory control regions would then be possible.

One potentially useful genetic experimental design for microarray studies in general is the inbred-line approach[94]. Factor-analytic modelling of expression profiles collected from inbred lines has the potential to simplify the interpretation of the large number of up and downregulated genes into causal, independent genetic traits. For example, in the context of the genetic analysis of mate choice, it might be possible to discern mechanisms such as acoustic, chemical and behavioural cues as genetically independent factors from a set of inbred lines that have been classed as having high and low attractiveness. This 'topping and tailing' approach greatly reduces the number of genetic lines that are required to be subjected to expression profiling, while at the same time creating a treatment effect of interest at which the factor-analytic covariance structures are modelled. In relation to artificial selection experiments such as that conducted by Mackay *et al.*[91], factor-analytic modelling has the potential to determine how many genetic changes underpinned the vast number of expression profiles that differed between selection treatments.

Conclusion

There are three general aspects that future studies of the genetic basis of mate choice need to address. First, the complexity of male traits and female preferences as traits that are to be subjected to genetic analysis needs to be appropriately accounted for. Although there has been some success in demonstrating that genetic variance exists for male sexually selected traits and female preferences, and the identification of single genes that might contribute to these traits, most studies have relied on simplifying mate choice to a single trait or preference. Mapping of single male traits, or the female preferences for them, is unlikely to lead to a comprehensive understanding of the genetic basis of mate choice in many species.

Second, as a consequence of the multivariate nature of mate choice in many species, tighter integration of current approaches to determining the genetic basis of traits needs is required. Classical quantitative genetic approaches have lacked the ability to characterize specific gene effects, QTL analyses can only detect genes of major effect in many cases, and transcriptional profiling experiments have generally lacked any genetic component to their experimental design. The combination of modern genomic approaches with classical experimental designs encompassed by quantitative genetics provides a way to address these limitations[117].

Finally, many of the genetic approaches outlined here are associative in nature, and do not supply direct manipulative evidence for the genetic effects they identify. Deletion mapping and selection experiments have been used in some cases to establish the importance of the role of particular genes uncovered by associative studies. Surprisingly however, the process of sexual selection that underlies mate choice has been experimentally manipulated in few genetic studies. Given the difficulties in identifying the correct sets of traits and preferences to study, manipulating the entire process of sexual selection in multigenerational experiments to supply direct evidence for the role of specific genetic effects in mate choice has considerable appeal.

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Competing interests statement
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