

## REVIEW

# The quantitative genetics of sexual dimorphism: assessing the importance of sex-linkage

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Sexual dimorphism (SD) is a defining feature of gonochorous animals and dioecious plants, but the evolution of SD from an initially monomorphic genome presents a conundrum. Theory predicts that the evolution of SD will be facilitated if genes with sex-specific fitness effects occur on sex chromosomes. We review this theory and show that it generates three testable predictions. For organisms with an XX/XY chromosomal system of sex determination: (1) SD should be associated with X-linked effects; (2) X-linked effects should show strong directional dominance for sexually dimorphic traits favored in males but expressed in both sexes; and (3) SD should be associated with a reduction in the between-sex additive genetic covariance and correlation. A literature review reveals that empirical evaluations of the association between sex-linkage and SD have lagged behind theory. Tests for the presence of sex-linked effects have been

plagued by the need to make simplifying assumptions, such as the absence of dominance or maternal effects, that greatly weaken their discriminatory power. Further, most have used comparisons between species or populations, whereas the correct level of analysis is within populations. To overcome these problems, we derive a novel pedigree design that permits separate estimation of X-linked, dominance and maternal effects. We suggest that the data from such a design would be most appropriately analyzed using the animal model. This novel protocol will allow quantitative evaluation of the above predictions, and hence should spur progress in understanding the role of sex-linkage in the evolution of SD.

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## Introduction

Sexual dimorphism (SD) is the defining feature of organisms in which male and female reproductive organs occur in different individuals (cf. gonochorous animals and dioecious plants). In most organisms, (SD) extends beyond the fundamental differentiation of organs of reproduction to include dimorphisms for body size, shape and color, as well as the presence of specific morphological structures in one sex only (e.g., sex-limited traits such as the antlers of male ungulates and the elaborate coloration of many male birds and teleost fishes; Darwin, 1871; Andersson, 1994; Short and Balaban, 1994). While 'dimorphism' in the strict sense refers to morphology, the term SD is also used for differences between the sexes in physiology, behavior and life history (Daly and Wilson, 1983; Short and Balaban, 1994; Mealey, 2000). In this broader sense, SD includes all aspects of the differentiation of males and females.

SD is presumed to reflect adaptive divergence in response to selection favoring different optimal character states in the two sexes (Hedrick and Temeles, 1989; Fairbairn, 1997; Badyaev and Hill, 2003; Blanckenhorn, 2005). This presents a conundrum for evolutionary

biologists: how does SD evolve given the evolutionary constraints imposed initially by the shared genomes of the two sexual morphs (Lande, 1980, 1987; Reeve and Fairbairn, 1996, 1999, 2001; Fairbairn, 1997; Badyaev, 2002)? In some cases, the sexes can be so disparate as to be unrecognizable as the same species (Darwin, 1871) and yet these highly distinct phenotypes can arise from substantively identical genomes. Chromosomal sex determination is clearly not required, as extreme SD occurs in many animals that lack sex chromosomes (i.e., where sex is determined by environmental cues or in response to age or body size changes; Pieau *et al.*, 1994; Shapiro, 1994; Berc *et al.*, 2005). Nevertheless, evolutionary theory predicts that the evolution of SD will be facilitated if genes with sex-specific fitness effects occur on sex chromosomes or can be translocated there (e.g., Fisher, 1931; Charlesworth and Charlesworth, 1980; Rice, 1984; Charlesworth, 2002). Therefore, sex-linkage may be one solution to the conundrum presented above. This prediction is enticing not only in the context of the evolution of SD but also because it attributes additional functionality to the sex chromosomes beyond their primary role in sex determination.

Although the expectation of an association between SD and sex-linkage has a strong theoretical basis, formal tests of this theory have been limited by lack of appropriate experimental protocols. Here, we describe three predictions that can be derived from the underlying theory and critically review experimental protocols that have been used to test these predictions. We propose

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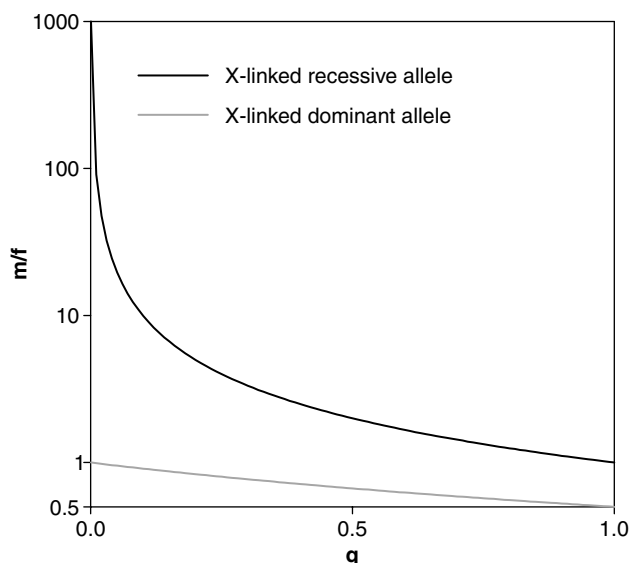
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a new quantitative genetic protocol for separation of X-linked additive variance from confounding maternal and dominance effects. Application of this protocol should permit formal, quantitative evaluation of the association between X-linkage and SD.

## Sex-linkage, sex-limited gene expression and the evolution of SD

For organisms with chromosomal sex determination, genes on the sex chromosomes are responsible for initiating the cascade of developmental processes leading to SD (Lyon, 1994; Marshall Graves, 1994; Mealey, 2000; Bainbridge, 2003). Once the cascade is initiated, the responding genes need not be on the sex chromosomes and evidence from both humans and fruitflies (*Drosophila melanogaster*) indicates that indeed, most are distributed on autosomes (Saifi and Chandra, 1999; Parisi *et al.*, 2003). However, several genetic models predict that the evolution of SD is facilitated by sex-linkage for genes with differential fitness effects in males and females (Fisher, 1931; Charlesworth and Charlesworth, 1980; Rice, 1984; Charlesworth *et al.*, 1987; Reinhold, 1998, 1999; Hurst and Randerson, 1999; Gibson *et al.*, 2002; Reeve and Pfennig, 2003; Parisi *et al.*, 2003; Fitzpatrick, 2004). Such genes are expected to accumulate on the X chromosome (or Z chromosome for organisms with ZZ/ZW sex determination), and to show non-additive allelic interactions. The rationale for this prediction can be illustrated in a simplified fashion by a single-locus model with complete dominance in which females are the homogametic sex (females, XX; males, XY or XO) (Figure 1). In such a system, selection for traits that benefit only males is expected to favor recessive alleles at X-linked loci because these are expressed more often in males, especially when rare. Dominant X-linked alleles are expressed more often in females, but here the differential is small and hence X-linkage is predicted



**Figure 1** The expected ratio of the proportion of males ( $m$ ) and females ( $f$ ) expressing X-linked alleles as a function of the frequency of the recessive allele,  $q$ . For recessive alleles:  $m/f = q/q^2 = 1/q$ . For dominant alleles:  $m/f = p/(p^2 + 2pq) = 1/(1 + q)$ .

primarily for traits favored in males. Similar arguments lead to the prediction that X-linkage will be most strongly favored for traits whose expression is already limited to males (Reinhold, 1999; Andrés and Morrow, 2003).

While one might expect genes coding for traits favored in males to be found preferentially on the Y chromosome in XX/XY systems (e.g., Charlesworth *et al.*, 1987; Chippindale and Rice, 2001; Reeve and Pfennig, 2003), Y chromosomes are often degenerate or even absent and X-linkage is therefore much more common than Y-linkage (Charlesworth and Charlesworth, 2000; Charlesworth, 2002).

Dosage compensation may influence these simple predictions in a number of ways (Charlesworth *et al.*, 1987; Rogers *et al.*, 2003; Carrel and Willard, 2005). The net effect of dosage compensation is to increase the additive genetic variance for X-linked traits in males relative to females, which should favor rapid evolutionary response of X-linked genes favored in males, as predicted (Charlesworth *et al.*, 1987). However, Rogers *et al.* (2003) have suggested that dosage compensation through up-regulation of X-linked genes in males, as in *Drosophila melanogaster*, enhances expression of deleterious X-linked mutations in males, and hence should favor translocation of genes with strong male-specific fitness effects to autosomes. Another complication arises if dosage compensation occurs through inactivation of genes on one X chromosome in females (Rao and Arora, 1979; Carrel and Willard, 2005; Turner, 2006). If this renders females effectively hemizygous at X-linked loci, it will remove dominance effects, and hence remove the advantage of X-linked over autosomal alleles. Thus, although a considerable body of theory predicts an association between SD and X-linkage, our increasing appreciation of dosage compensation adds new complexity, rendering empirical evaluation of this prediction even more critical.

A second genetic mechanism expected to facilitate the evolution of SD is the evolution of sex-specific patterns of expression of autosomal genes (Lande, 1987; Rhen, 2000). This can occur through recruitment of alleles with sex-limited effects at existing loci, as well as through the evolution of new modifier genes and regulatory pathways (Lande, 1980, 1987; Rice, 1984; Rhen, 2000; Badyaev, 2002). Sex-specific gene expression and sex-linkage are likely to be functionally connected because sex-linked genes often exert their phenotypic effects by regulating the expression of autosomal genes (Charlesworth *et al.*, 1987; Marshall Graves, 1994; Chippindale and Rice, 2001; Bainbridge, 2003). Hence, the two mechanisms are not mutually exclusive. Both are expected in organisms with chromosomal sex determination, although their relative importance may vary over the course of the evolutionary trajectory of SD (Lande, 1980, 1987; Rice, 1984).

## Three key predictions

Three key predictions can be derived from the genetic hypotheses described above:

- **Prediction 1:** SD should be associated with X-linked effects. Specifically, the expected contribution of X-linkage should be greatest for traits limited to males, intermediate for sexually dimorphic traits favored in

males but expressed in both sexes, and least for sexually monomorphic traits. In the context of quantitative genetic variance, we would therefore expect the proportion of genetic variance that is X-linked to be positively correlated with the degree of SD. Note, however, that traits limited to females or favored only in females are excluded from these predictions.

- **Prediction 2:** X-linked effects should show strong directional dominance for sexually dimorphic traits favored in males but expressed in both sexes.
- **Prediction 3:** SD should be associated with reduction in the between-sex additive genetic covariance ( $COV_{Amf}$ ) and correlation ( $r_{Amf}$ ), to the limit of  $COV_{Amf} \rightarrow 0$  and  $r_{Amf} \rightarrow 0$  for traits expressed in only one sex. Both sex-linkage and sex-specific expression of autosomal loci are expected to contribute to these effects, but the form and magnitude of the expected changes are difficult to predict. The genetic variance associated with X-linked loci is expected to differ between the sexes, even with most forms of dosage compensation (see above and Lynch and Walsh, 1998), and this can be expected to reduce the between-sex additive genetic covariance ( $COV_{Amf}$ ). Sex-limited autosomal gene expression is also expected to reduce  $COV_{Amf}$  (Reeve and Fairbairn, 2001). Most authors predict a similar trend in  $r_{Amf}$  (Fisher, 1958; Eisen and Legates, 1966; Lande, 1980, 1987; Arnold, 1985; Bonduriansky and Rowe, 2005), but this latter expectation is somewhat more tenuous because  $r_{Amf}$  depends on the additive genetic variances in each sex in addition to the covariance and may not change in parallel with  $COV_{Amf}$  (Reeve and Fairbairn, 2001).

These predictions are derived directly from the genetic models summarized above which assume large or infinite populations and continuous availability of new mutations. An alternative scenario, proposed by Meagher (1992), is that selection for SD could eventually deplete the genetic variance at loci with sex-linked or sex-limited effects, leaving most of the genetic variance segregating at autosomal loci expressed in both sexes. Such a scenario would lead to an increase in  $COV_{Amf}$  and  $r_{Amf}$  as SD evolves (Meagher, 1992), hence reversing prediction 3. It would also eventually reduce the contribution of X-linked effects to the standing genetic variance for dimorphic traits, negating prediction 1. The depletion of genetic variance inherent in Meagher's scenario is not a feature of most genetic models but may be realistic for natural systems if selection is strong and effective population sizes are low. X-linked variance may be particularly at risk, as  $N_e$  is lower for X chromosomes than for autosomes (Gibson *et al.*, 2002). The signature of such an effect would be falsification of prediction 1 combined with a positive rather than negative association between SD, and  $COV_{Amf}$  and  $r_{Amf}$ .

## Previous empirical tests

In this section, we review the conclusions reached from published experiments: we suspend discussion of the limitations of these conclusions resulting from underlying assumptions until the following section in which we review the experimental methods themselves. Much of the research on the genetic mechanisms facilitating the evolution of SD has focused on *D. melanogaster*. This

species has an unusually large X chromosome (Saifi and Chandra, 1999; Parisi *et al.*, 2003; Fitzpatrick, 2004), a characteristic that one might expect would result in unusually high X-linked variance. Nevertheless, empirical evaluations of the prediction that SD should be associated with X-linkage in *D. melanogaster* have yielded conflicting results. Elegant line-cross analyses have revealed strong X-linkage for sexually antagonistic variation in adult fitness (Gibson *et al.*, 2002) as well as for sexually selected behavioral and morphological traits (Reinhold, 1998). An extensive half-sib mating experiment (Cowley *et al.*, 1986; Cowley and Atchley, 1988) uncovered significant additive X-linked variance for 22 of 28 linear morphological measures. These studies indicate strong effects of X-linked genes on the genetic variance of these traits. In contrast, genomic surveys and QTL studies have detected no bias towards X-linkage for genes influencing sexually selected behavioral traits in *D. melanogaster* (Gleason *et al.*, 2002; Fitzpatrick, 2004). Further, a genome-wide survey of genes showing sex-biased expression revealed no tendency for those preferentially expressed in males to be on the X chromosome (Parisi *et al.*, 2003; Ranz *et al.*, 2003). Reconciling these disparate conclusions is difficult: studies partitioning phenotypic and genetic variance for traits (Cowley *et al.*, 1986; Cowley and Atchley, 1988; Reinhold, 1998; Gibson *et al.*, 2002) find significant effects of X-linkage, while those documenting the physical locations of genes for SD traits (Gleason *et al.*, 2002; Parisi *et al.*, 2003; Ranz *et al.*, 2003; Fitzpatrick, 2004) find no bias towards X-linkage. The solution may lie in the distinction between the physical location of a gene (on the sex chromosomes or autosomes) and the relative influence of that gene on trait expression. The results summarized above suggest that genes influencing sexually dimorphic traits are not found disproportionately on the X chromosome, but that those on the X chromosome exert a disproportionate influence on trait expression, particularly in males. Such an effect has been described for X-linked genes in humans (Saifi and Chandra, 1999) and may be at least partly explained by dosage compensation (Cowley *et al.*, 1986; Charlesworth *et al.*, 1987).

Evidence from other organisms also supports the hypothesis that SD is associated with the expression of X-linked genes, particularly for traits exaggerated in or limited to males. Reciprocal line crosses indicate significant sex-linked effects in many insect, bird and mammal species (Barbato, 1991; Reinhold, 1994, 1998, 2002; Wolfenbarger and Wilkinson, 2001). Although maternal effects may inflate these estimates in mammals, this is less likely in birds and insects (Wolfenbarger and Wilkinson, 2001; Reinhold, 2002). Genomic approaches have demonstrated that genes for traits associated with sex and reproduction (SRR traits) in male mammals tend to be X-linked (Saifi and Chandra, 1999; Wang *et al.*, 2001), and in humans, these X-linked genes tend to have high penetrance and strong pleiotropic effects relative to autosomal genes (Saifi and Chandra, 1999).

Although these and other studies support the hypothesis that sex-linked genes play a role in the evolution of many sexually dimorphic traits, there is little doubt that most genes contributing to SD for polygenic traits are autosomal and show some degree of sex-specific expression. The major role played by this mechanism has been

well established by studies of the developmental genetics and ontogeny of SD in species with chromosomal sex determination (Daly and Wilson, 1983; Short and Balaban, 1994; Mittwoch, 1996; Mealey, 2000; Badyaev, 2002; Skelly and John-Alder, 2002; Cox *et al.*, 2005; Emlen *et al.*, 2005; Ketterson *et al.*, 2005). The discovery of similar developmental cascades and hormonal mechanisms in organisms with environmental sex determination (Pieau *et al.*, 1994), including those that are sequentially hermaphroditic (Shapiro, 1994), provides unassailable evidence that sex-linkage is not required for the evolution of even extreme SD. The issue at hand is not whether sex-linked genes are required for the evolution of SD, but rather how much sex-linkage contributes to the standing genetic variance for these traits in organisms with sex chromosomes and whether this is proportional to the degree of SD (e.g., monomorphic vs dimorphic vs sex-limited).

The prediction that  $COV_{Amf}$  should covary negatively with SD (prediction 3) has not been tested empirically, but the predicted negative relationship between  $r_{Amf}$  and SD has recently been confirmed for a series of morphological traits in the fly, *Prochyliza xanthostoma* (Bonduriansky and Rowe, 2005). Other studies from which one can extract both SD and  $r_{Amf}$  for a variety of traits are uncommon (e.g., Cowley *et al.*, 1986; Cowley and Atchley, 1988; Meagher, 1992; Preziosi and Roff, 1998) and of these, only the estimates of  $r_{Amf}$  for *D. melanogaster* (Cowley *et al.*, 1986; Cowley and Atchley, 1988) include possible X-linked effects in males because these are excluded from standard sire estimates from half-sib designs. These data show no correlation between SD and  $r_{Amf}$  ( $R_{26} = -0.12$ ,  $P > 0.50$ ) and the trend is opposite to that predicted. Additional evaluations of the relationship between  $COV_{Amf}$ ,  $r_{Amf}$  and SD are clearly required before any general conclusions can be drawn.

### Experimental protocols: using crosses between populations to detect sex-linked effects

The most frequently used method to detect the influence of sex-linkage is a reciprocal cross between two inbred or selected lines, populations or species (Hagger and Stranzinger, 1992; Reinhold, 1998, 2002; Wolfenbarger and Wilkinson, 2001; Table 1). Males from such crosses differ in their X chromosome but not their autosomes, and differences between males from the two cross types are generally attributed to X-linked genes. However, this assumes that there are no maternal effects, no Y-linked effects in the case of XY males (Carson and Lande, 1984), and no effects of genomic imprinting (Spencer, 2002). The presence of maternal effects can be assessed from the difference between the reciprocal cross lines for the homogametic sex (females; italic font in Table 1). Both sex-linkage and maternal effects can then be estimated if it is assumed that the maternal effects are the same in both sexes. However, the addition of F1 reciprocal backcrosses and F2 reciprocal hybrids permits more robust separation of autosomal, sex-linked and maternal effects (Table 2). Estimates of these effects can then be made using either line-cross analysis (see below) or ANOVA with a priori contrasts (Table 3).

**Table 1** Genomic composition of offspring from reciprocal crosses between two 'lines' (i.e., inbred lines, populations or species), designated as types A and B

Male parent	Female parent	
$\delta_A$	$X_A X_A A A^a$	$X_B X_B A B$
$\delta_B$	$X_A X_B A B$	$X_B X_B B B$
	$X_A 0 A A$	$X_B 0 A B$
	$X_A 0 A B$	$X_B 0 B B$

Within each cell the top line shows the composition of the homogametic sex and the bottom line shows the composition of the heterogametic sex (X0 in this case). Males shown in bold have sex chromosomes derived from different lines and hence demonstrate X-linkage. However, they also differ in the source of their maternal chromosomes. Differences between these males could therefore be due to both sex-linkage and maternal effects. Females shown in italics have the same genomic composition but differ in the source of their maternal chromosomes and hence differences between these females reveal maternal effects.

<sup>a</sup>Source of X chromosomes (e.g.,  $X_A$  from a type A parent) and autosomes (e.g., AB means one half derived from a type A parent and one half from a type B parent).

Line-cross analysis is most frequently used to estimate the contribution of additive and non-additive (maternal, dominance and epistasis) effects using a joint scaling test to determine the most appropriate model (Mather and Jinks, 1982; Lynch and Walsh, 1998). Model fitting is hierarchical, beginning with a simple model, such as the additive model (i.e., the expected mean phenotype of the crosses is a simple linear function of the relative contributions of the parental genomes such that the mean population values of the different crosses fall on a line connecting the two parental means, top graph, Figure 2), and increasing its complexity only if the model fails the joint-scaling test. In the presence of sex-linked effects, the expected mean phenotypic values change (Figure 2), and the line-cross analysis can be expanded to estimate sex-linked, epistatic and maternal effects (e.g., Carbonell *et al.*, 1985; Barbato, 1991; Barbato and Vasilatos-Younken, 1991; Polak and Starmer, 2001; Huttunen and Aspi, 2003).

Parameter estimation from line-cross analyses is made using least-squares regression (Lynch and Walsh, 1998). Models with many parameters may be difficult to distinguish: in particular, the effects of X-linkage can be somewhat confounded with maternal effects. To ameliorate this problem, we recommend testing for X-linked and maternal effects using planned contrast analyses, as shown in Table 3 (e.g., see Wahlsten, 1979; de Belle and Sokolowski, 1987; Huttunen and Aspi, 2003). If X-linked or maternal effects are significant in these planned comparisons, the line-cross analysis can then be initiated with these parameters included in the regression model.

### Experimental protocols: quantifying sex-linked variance within populations

While the reciprocal cross method can detect the presence of sex-linkage, it does not estimate the magnitude of sex-linked effects within populations (i.e., the genetic variances and covariances). As described previously, theoretical studies of the evolution of sex-

**Table 2** Schematic of crosses used to separate hereditary components in two populations (lines or species) in a species with XO males

Cross	Parents <sup>a</sup>		Offspring genotypic ratio(s)				Source of maternal effects
	Dam	Sire	Autosomes <sup>b</sup>		Sex chromosome		
			From dam	From sire	Female (XX) AA:AB:BB	Male (XO) A:B	
<i>Parental population (A × A and B × B)</i>							
1	A	A	1:0	1:0	1:0:0	1:0	A
2	B	B	0:1	0:1	0:0:1	0:1	B
<i>Reciprocal F1 hybrids (A × B and B × A)</i>							
3	A	B	1:0	0:1	0:1:0	1:0	A
4	B	A	0:1	1:0	0:1:0	0:1	B
<i>Reciprocal backcrosses</i>							
5	A	F1 (AB) 3	1:0	1:1	1:0:0	1:0	A
6	A	F1 (BA) 4	1:0	1:1	0:1:0	1:0	A
7	B	F1 (AB) 3	0:1	1:1	0:1:0	0:1	B
8	B	F1 (BA) 4	0:1	1:1	0:0:1	0:1	B
9	F1 (AB) 3	A	1:1	1:0	1:1:0	1:1	(AB)
10	F1 (AB) 3	B	1:1	0:1	0:1:1	1:1	(AB)
11	F1 (BA) 4	A	1:1	1:0	1:1:0	1:1	(BA)
12	F1 (BA) 4	B	1:1	0:1	0:1:1	1:1	(BA)
<i>Reciprocal F2 hybrids (F1 × F1)<sup>c</sup></i>							
13	F1 (AB) 3	F1 (AB) 3	1:1	1:1	1:1:0	1:1	(AB)
14	F1 (AB) 3	F1 (BA) 4	1:1	1:1	0:1:1	1:1	(AB)
15	F1 (BA) 4	F1 (BA) 4	1:1	1:1	0:1:1	1:1	(BA)
16	F1 (BA) 4	F1 (AB) 3	1:1	1:1	1:1:0	1:1	(BA)

<sup>a</sup>Number indicates the cross generating the particular genotype (e.g., 'F1(AB)3' means that this parent is an offspring from the cross in the row labeled '3').

<sup>b</sup>A:B ratio.

<sup>c</sup>Corrects error in Table 1 of de Belle and Sokolowski (1987).

**Table 3** Some possible contrasts to distinguish effects due to autosomal (A), sex-linked (X) and maternal (M) effects in the crosses shown in Table 2

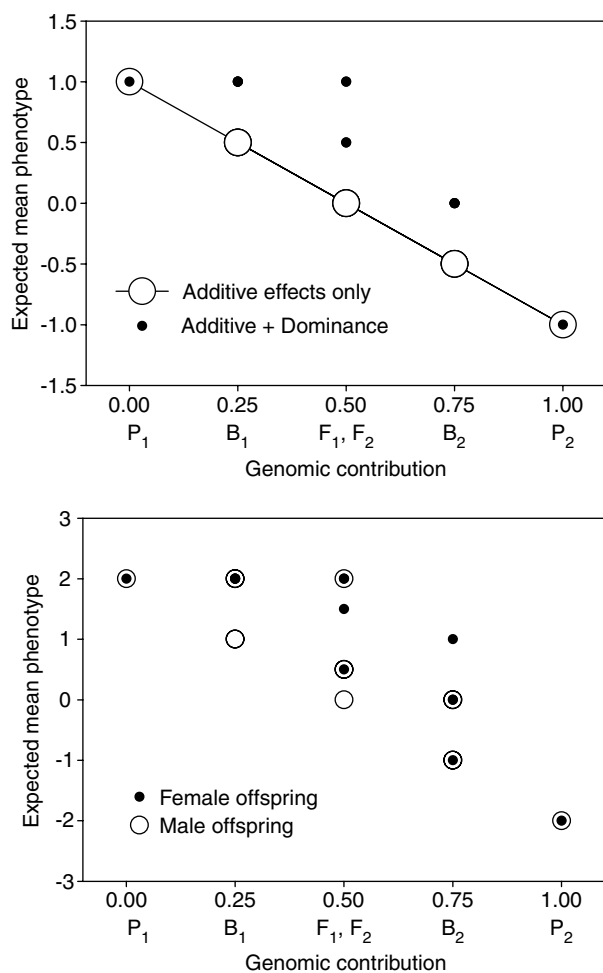
Contrast	Females			Males		
	A	X	M	A	X	M
1 vs 5; 2 vs 8; 9 & 11 vs 13 & 16; 10 & 12 vs 14 & 15	+			+		
1 vs 3; 2 vs 4				+		
3 vs 4			+		+	+
5 vs 6; 7 vs 8; 13 vs 14; 15 vs 16		+				
9 vs 10; 11 vs 12	+	+		+		

'+' indicates that the effect is detected by the contrast. It is assumed that males are XO or that Y-linked effects are negligible.

linkage and SD generate specific predictions with respect to X-linkage, directional dominance and  $r_{Amf}$  at the within-population level. Therefore, to adequately test these predictions, research should be directed at partitioning variances within populations. The three predictions can be addressed by apportioning the phenotypic variance into its genetic and environmental components and then further partitioning the genetic variance into additive, dominance, sex-linked and maternal effects.

One method of estimating X-linked genetic variance utilizes multiple reciprocal crosses between inbred lines, a design known as 'the diallel cross' (Griffing, 1956; Sheridan *et al.*, 1968; Lynch and Walsh, 1998). The diallel method permits the partitioning of trait variance into additive genetic, dominance, reciprocal and environmental variances. However, in most applications of this design, sex-linked and maternal effects are confounded, both being contained within the reciprocal variance.

Further, the method assumes no sex-linked dominance, the key aspect of prediction 2. For example, to estimate sex-linked effects on bristle number in *D. melanogaster*, Sheridan *et al.* (1968) used a complex diallel cross in which they necessarily assumed no sex-linked dominance effects and no maternal effects other than those produced as a result of sex-linkage. Zhu and Weir (1996) solved the latter problem by deriving a method to partition the phenotypic variance ( $V_P$ ) into  $V_P = V_A^A + V_D + V_A^X + V_M + V_E$ , where  $V_A^A$  is the additive genetic variance from autosomal loci,  $V_D$  is the dominance variance from autosomal loci,  $V_A^X$  is the additive genetic variance from sex-linked loci (assuming the male has only a single X chromosome),  $V_M$  is the maternal variance (excluding sex-linked effects) and  $V_E$  is the environmental variance. However, the diallel cross approach remains limited in its application because the production of inbred lines is not practical for most

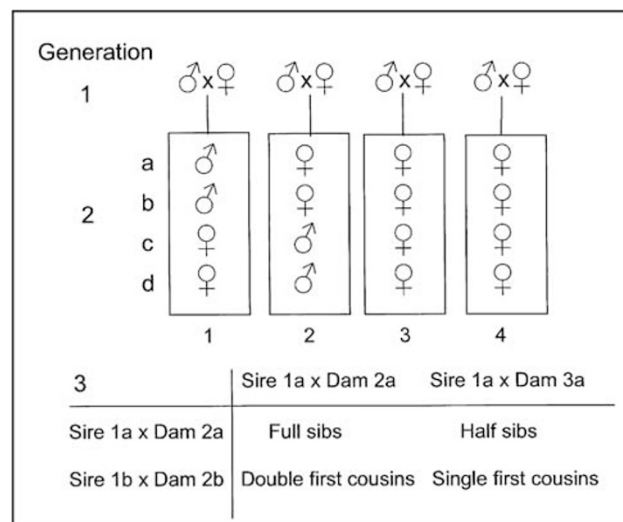


**Figure 2** Schematic illustration of expected mean phenotypes from the line-cross analysis under the assumption of no X-linkage (top panel) and in the presence of X-linkage (bottom panel) with both additive and dominance effects. For simplicity epistasis and maternal effects are ignored. Using notation of Mather and Jinks (1982),  $[d] = [h] = [d_x] = [h_x] = [d'_x] = 1$ , where  $d$  refers to additive effects,  $h$  to dominance effects and the subscript to X-linkage. Genomic contributions are indicated for parental (P<sub>1</sub>, P<sub>2</sub>), F<sub>1</sub> backcross (B<sub>1</sub>, B<sub>2</sub>), and F<sub>1</sub> and F<sub>2</sub> hybrid generations.

animal species: Atchley and Zhu (1997) used the approach on inbred lines of mice but this is the only example we could locate. Further, the method of Zhu and Weir does not remove the problem of having to assume no sex-linked dominance effects.

Cowley *et al* (1986) and Cowley and Atchley (1988) used an alternative approach, a half-sib design, to estimate the sex-linked variance for a series of morphological traits in *D. melanogaster*. As their design utilized only one or two offspring per full-sib family, they had to assume, like Sheridan *et al* (1968), no dominance variance and no maternal effects. As noted above, the contribution of dominance variance is a key prediction of the theory and it should not be assumed to be absent. Maternal effects, if present, will inflate estimates of X-linkage. Hence, it is clearly preferable to estimate them directly, removing this component from the X-linked variance, rather than simply assuming its absence.

We suggest a new pedigree approach that is free from these assumptions. Our proposed design utilizes trait



**Figure 3** Schematic of one breeding set in the proposed three-generation pedigree design. In generation 2, each male mates with the three females on the same row. Relationships are illustrated for four sets of relatives in generation 3 (F<sub>1</sub>). All relationships are shown in Tables 4 and 5.

values from three consecutive generations to derive a full set of covariances among relatives. The basic breeding unit or 'set' is illustrated in Figure 3, and a given experiment would consist of many replicate sets initiated from the same population. (If possible, the number of sets should be determined from a power analysis based on a priori estimates of the (co)variance components.) Within each set, generation 1 consists of eight unrelated individuals that form four pairs of grandparents (GP). In generation 2 (parental generation, P), two full-sib male offspring from each of two GP pairs are used as sires which are mated to three unrelated dams, families 1 and 2 contributing two dams each and families 3 and 4 contributing four dams. This mating scheme produces sets of full sibs, half sibs, and single and double first cousins in generation 3 (F<sub>1</sub>).

To illustrate the procedure and why this novel mating design is necessary to obtain all variance components, we describe the framework for estimating heritabilities. To include sex-linked, maternal and dominance effects we have combined the maternal and sex-linkage models presented by Lynch and Walsh (1998). The covariance between relatives  $x$  and  $y$  is given by  $Cov_{xy} = 2\theta_{xy}^a V_A^a + \Delta_{xy}^a V_D^a + \phi_{xy}^a V_X^a + c_E^m V_E^m + c_A^m V_A^m + c_{Am}^m Cov_A^m$  for males, and  $Cov_{xy} = 2\theta_{xy}^a V_A^a + \Delta_{xy}^a V_D^a + 2\theta_{xy}^x V_X^x + \Delta_{xy}^x V_D^x + c_E^m V_E^m + c_A^m V_A^m + c_{Am}^m Cov_A^m$  for females, where the superscript  $a$  denotes autosomal,  $X$  the X-linked and  $m$  the maternal components, and the subscript  $A$  denotes additive,  $D$  the dominance and  $E$  the environmental variance (e.g.,  $V_A^a$  is the additive genetic variance attributable to autosomal genes,  $V_D^a$  is the autosomal dominance variance,  $V_X^a$  is the additive X-linked variance,  $V_D^x$  is the X-linked dominance variance,  $V_E^m$  is the maternal environmental variance,  $V_A^m$  is the additive genetic maternal variance and  $Cov_A^m$  is the direct maternal additive genetic covariance). The two equations differ because of the possibility of non-additive gene action at X-linked loci in females. Utilizing the phenotypic values of sires and dams permits estimation of both sex-linked and maternal effects within each sex. Values

**Table 4** Coefficients for expressions describing the expected phenotypic covariance between male relatives in the proposed breeding design to estimate variances due to autosomal, sex-linked and sex-specific gene expression

Male–male relationships	Coefficients						
	Autosomal		Sex-linked		Maternal		
	$V_A^a$	$V_D^a$	$V_A^X$	$V_D^X$	$V_E^m$	$V_A^m$	$Cov_A^m$
1. Father–Son	1/2	0	0	0	0	0	1/4
2. Full brothers	1/2	1/4	1/2	0	1	1	1
3. Paternal half brothers	1/4	0	0	0	0	0	0
4. Single first cousins, dams full sisters	1/8	0	3/8	0	0	1/2	1/2
5. Single first cousins, sires full brothers	1/8	0	0	0	0	0	0
6. Single first cousins, opposite sex full sibs	1/8	0	0	0	0	0	1/4
7. Double first cousins, sires full brothers, dams full sisters	1/4	1/16	3/8	0	0	1/2	1/2
8. Double first cousins, opposite sex full sibs	1/4	1/16	0	0	0	0	1/2
9. Paternal uncle–nephew	1/4	0	0	0	0	0	1/4
10. Maternal uncle–nephew	1/4	0	0	0	0	1/2	3/4
11. Paternal grandfather–grandson	1/4	0	0	0	0	0	1/8
12. Maternal grandfather–grandson	1/4	0	1/2	0	0	1/4	5/8

Modified (and corrected) from Tables 23.1, 24.1 of Lynch and Walsh (1998), Eisen (1967) and Grossman and Eisen (1989). Maternal dominance variance is assumed to be zero and environmental maternal effects are assumed to be transmitted only over one generation.

**Table 5** Coefficients for expressions describing the expected phenotypic covariance between female relatives in the proposed breeding design to estimate variances due to autosomal, sex-linked and sex-specific gene expression

Female–female relationships	Coefficients						
	Autosomal		Sex-linked		Maternal		
	$V_A^a$	$V_D^a$	$V_A^X$	$V_A^a$	$V_D^a$	$V_A^a$	
13. Mother–daughter	1/2	0	1/2	0	0	1/2	5/4
14. Full sisters	1/2	1/4	3/4	1/2	1	1	1
15. Paternal half sisters	1/4	0	1/2	0	0	0	0
16. Single first cousins, dams full sisters	1/8	0	3/16	0	0	1/2	1/2
17. Single first cousins, sires full brothers	1/8	0	1/4	0	0	0	0
18. Single first cousins, opposite sex full sibs	1/8	0	1/8	0	0	0	1/4
19. Double first cousins, sires full brothers, dams full sisters	1/4	1/16	7/16	3/16	0	1/2	1/2
20. Double first cousins, opposite sex full sibs	1/4	1/16	1/4	1/16	0	0	1/2
21. Paternal aunt–niece	1/4	0	1/4	0	0	0	1/4
22. Maternal aunt–niece	1/4	0	3/8	0	0	1/2	3/4
23. Paternal grandmother–granddaughter	1/4	0	1/2	0	0	0	1/8
24. Maternal grandmother–grand daughter	1/4	0	1/4	0	0	1/4	5/8

Modified (and corrected) from Tables 23.1, 24.1 of Lynch and Walsh (1998), Eisen (1967) and Grossman and Eisen (1989). Maternal dominance variance is assumed to be zero and environmental maternal effects are assumed to be transmitted only over one generation.

of the coefficients for various relationships are shown in Tables 4 and 5. For males, the relationships indicated by numbers 1, 2, 3, 7, 8 and 12 form the minimum set from which all variance components can be estimated. For females, the minimum set of relationships includes those indicated by numbers 14, 15, 16, 19, 20, 22, 23 and 24. Additional relationships contribute to the precision of the estimation by further limiting the range of possible values.

### Parameter estimation using the animal model

In a design such as the above pedigree analysis, involving several generations, there will often be more than one way to estimate a particular variance component. While one could make separate estimates, a method that combines all the information into a single procedure is clearly more desirable. The analytical method known as ‘the animal model’ accomplishes this (Lynch and Walsh, 1998; Kruuk, 2004). The animal model approach uses each individual as a datum in contrast to more

traditional models, such as the half-sib design, which estimate sire values from the values of their progeny. To illustrate the animal model, consider a simple case in which only additive effects are present: in this case the phenotypic value of all individuals is given by  $\mathbf{y} = \beta + \boldsymbol{\mu} + \mathbf{e}$ , where  $\mathbf{y}$  is the vector of trait values for all individuals,  $\beta$  is the population mean,  $\boldsymbol{\mu}$  is a vector of additive genetic effects and  $\mathbf{e}$  is a vector of residual errors. The advantages of the animal model are that it can accommodate very complex pedigrees and it estimates all variance and covariance components using the entire data set. Further, variance and covariance components are generally estimated using restricted maximum likelihood, which permits the testing of different models using the log-likelihood ratio test. An additional bootstrap approach to estimate parameter values and standard errors is advisable to test the adequacy of the maximum likelihood model: a close correspondence with the restricted maximum likelihood estimates can be taken as evidence that the latter approach is adequate.

Previous applications of the animal model to both non-domestic and domestic animals have assumed maternal effects but no sex-linkage. In the simplest extension of the animal model, maternal effects are fitted by making dam identity a random effect (e.g., Kruuk *et al.*, 2000; Milner *et al.*, 2000). Parameter estimation in domestic breeds has frequently included the maternal additive genetic variance, direct maternal additive genetic covariance and maternal environmental variance (e.g., see Clément *et al.*, 2001) and this model was fitted to the feral population of Soay sheep (Wilson *et al.*, 2005). Additionally, non-additive variances have been estimated for domestic breeds (e.g., Rodriguez-Almeida *et al.*, 1995). The theory for the construction of the relevant matrices for the inclusion of sex-linked effects has been worked out by Grossman and Fernando (1989) and Fernando and Grossman (1990). However, a citation search revealed no citations to this work and, to the best of our knowledge, the animal model has not yet been used to estimate sex-linked variance (personal communication: Loeske Kruuk, University of Edinburgh; William Hill, University of Edinburgh; Denis Reale, Université du Québec à Montréal). Given the proposed wide occurrence of sex-linked effects in SD traits and the bias introduced by use of an incomplete genetic model (Clément *et al.*, 2001), we recommend inclusion of sex-linked effects whenever the traits examined are sexually dimorphic.

The general approach of the animal model is to fit a model with all components and compare this to reduced models using the likelihood ratio test (e.g., Reale *et al.*, 1999; Wilson *et al.*, 2005). For example, although maternal effects are likely to be negligible for most morphological traits (Mousseau and Roff, 1987; Reinhold, 2002), this expectation is insufficient justification for an a priori assumption of no effect. Use of the animal model approach requires no such assumption. We can test for maternal effects by comparing the full model to a model in which maternal effects are assumed to be absent. The same approach can be used to test for sex-linked effects: the full model would be compared to one without sex-linked effects included.

### Using within-population variance components to test the three key predictions

Once the required variance components and covariances have been estimated, as proposed above, the three predictions presented earlier can be recast in a statistical framework and tested. For purposes of illustration and simplicity, we assume an XX/XY or XX/XO chromosomal system, no Y-linked effects, and no epistasis. To test for a positive association between X-linked variance and SD (prediction 1), we are interested in the proportion of the genetic variance attributable to X-linked variance. Males cannot show X-linked dominance variance, so the relevant parameter is  $V_{\tilde{X}}/(V_{\tilde{X}} + V_{\tilde{A}} + V_{\tilde{D}})$  for males, while it is  $(V_{\tilde{X}} + V_{\tilde{D}})/(V_{\tilde{X}} + V_{\tilde{D}} + V_{\tilde{A}} + V_{\tilde{D}})$  for females. The second hypothesis, that X-linked effects should show directional dominance, can be tested by direct estimation of  $V_{\tilde{D}}$ , using a likelihood ratio test to compare a model in which  $V_{\tilde{D}}=0$  for the trait under test with a model in which  $V_{\tilde{D}}$  is free to vary. Since the prediction applies only to traits favored in males but still expressed in both sexes (i.e., not sex-limited), the expectation is that  $V_{\tilde{D}}$  will be

larger for these traits. The final prediction, that the additive genetic covariance and correlation between sexes should decline as SD increases, simply requires standard estimation of  $COV_{Amf}$  and  $r_{Amf}$  (Lynch and Walsh, 1998).

Each of these predictions is relative rather than absolute: we predict an association between the genetic parameter of interest and the degree of SD rather than merely presence/absence of the variance component of interest. Statistical evaluation of the predictions therefore requires estimates for a series of traits differing in SD. Such a series could consist of traits differing in SD within a single species, an approach analogous to the comparative studies of various size traits in *D. melanogaster* (Cowley *et al.*, 1986; Cowley and Atchley, 1988) and *Aquarius remigis* (Preziosi and Roff, 1998). However, if sufficient resources are available, extension of this single-species approach to comparisons of selected lines or closely related species would provide an even stronger test of the hypothesis, particularly if SD varies greatly both among traits and among comparison groups.

### Concluding remarks

In spite of a robust and well-established body of theory, we still know relatively little about the evolutionary genetics of sexually dimorphic traits. We still do not know how much sex-linkage contributes to the standing genetic variance for these traits or whether this is proportional to the degree of SD. We do not know how or if the between-sex genetic covariances and correlations are related to SD, although predictions of both positive and negative associations exist in the literature. Perhaps most elusive of all, we have not yet begun to determine whether sex-linked effects are characterized by directional dominance. Progress on all of these questions has been hampered by lack of robust empirical methodologies for isolating and quantifying sex-linked effects. The hierarchical, pedigree breeding design that we have proposed is one step in addressing this problem. This method is an improvement over previous designs not only because it partitions the additive and dominance components of the sex-linked variance but also because these components are clearly separated from potentially confounding maternal and autosomal dominance effects. The resulting pedigree data can be analyzed using the animal model approach, which provides powerful and flexible hypothesis testing.

The proposed pedigree design should be suitable for many organisms, since it requires only a minimum of three generations of standardized rearing rather than the availability of inbred lines or interfertile species. Ideally, one should estimate sex-linked effects for a suite of traits ranging from sexually monomorphic, through sexually dimorphic to sex-limited within a given population. If resources permit, this approach can be extended to comparisons of the estimated parameters among selected lines or closely related species. This comparative approach would be particularly worthwhile if the range of dimorphisms within populations is small, but among species (or lines) is large. Scaling will be an issue in any of these analyses, as variances are scale-dependent. All measurements should therefore be on the same scale or transformed to a common scale. Strong genetic correlations among traits within a suite may bias statistical



conclusions and limit the power of the comparisons to detect differences between traits. Avoidance of replicate traits within strongly integrated developmental units and *a priori*, statistical removal of integrative variables such as body size may help to ameliorate this problem.

The evolution of SD has long presented a conundrum for evolutionary biologists. In spite of an established body of predictive theory in this area, little empirical progress has been made. We hope that by summarizing the state of the field and providing a feasible experimental protocol for testing the existing hypotheses, we will inspire new research in this area.

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