PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research

Cite this article: Hill MS, Reuter M, Stewart AJ. 2019 Sexual antagonism drives the displacement of polymorphism across gene regulatory cascades. *Proc. R. Soc. B* **286**: 20190660.
http://dx.doi.org/10.1098/rspb.2019.0660

Received: 20 March 2019
Accepted: 12 May 2019

Subject Category:
Evolution

Subject Areas:
evolution, genetics, theoretical biology

Keywords:
sexual antagonism, gene regulation, binding site, transcription factor, regulatory cascade

Authors for correspondence:
Max Reuter
e-mail: m.reuter@ucl.ac.uk
Alexander J. Stewart
e-mail: astewar6@central.uh.edu

Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.4509887.

THE ROYAL SOCIETY PUBLISHING

Sexual antagonism drives the displacement of polymorphism across gene regulatory cascades

Mark S. Hill[1,2], Max Reuter[2] and Alexander J. Stewart[3]

[1]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA
[2]Research Department of Genetics, Evolution and Environment, University College London, London, UK
[3]Department of Biology and Biochemistry, University of Houston, Houston, TX, USA

MSH, 0000-0003-0718-8934; MR, 0000-0001-9554-0795; AJS, 0000-0001-5234-3871

Males and females have different reproductive roles and are often subject to contrasting selection pressures. This sexual antagonism can lead, at a given locus, to different alleles being favoured in each sex and, consequently, to genetic variation being maintained in a population. Although the presence of sexually antagonistic (SA) polymorphisms has been documented across a range of species, their evolutionary dynamics remain poorly understood. Here, we study SA selection on gene expression, which is fundamental to sexual dimorphism, via the evolution of regulatory binding sites. We show that for sites longer than 1 nucleotide, expression polymorphism is maintained only when intermediate expression levels are deleterious to both sexes. We then show that, in a regulatory cascade, expression polymorphism tends to become displaced over evolutionary time from the target of SA selection to upstream regulators. Our results have consequences for understanding the evolution of sexual dimorphism, and provide specific empirical predictions for the regulatory architecture of genes under SA selection.

1. Introduction

Adaptive responses to divergent selection in males and females are hampered by a largely shared genome, which slows or even prevents the evolution of sexual dimorphism, where the two sexes reach their respective phenotypic optima. In this situation, populations can experience the invasion of 'sexually antagonistic' (SA) alleles that are beneficial in one sex, but deleterious in the other [1–4].

Sexual antagonism is increasingly recognized as a taxonomically widespread and evolutionarily important phenomenon. A wealth of empirical evidence for SA fitness variation across a wide range of animal and plant species has now accumulated [5–11]. Sexual antagonism is thought to be a key driver for the evolution of sex chromosomes [12,13] and sex determination [14–16], to play a role in reproductive evolution (by eroding 'good genes' benefits of sexual selection [17]), and to mitigate the evolution of reproductive conflict between the sexes [18]. More generally, sexual antagonism has been predicted to maintain alleles in balanced polymorphism [19] and thus may also contribute to the maintenance of genetic and fitness variation within populations [20,21] and a reduction in the evolvability of both sexes [22].

The conditions that favour emergence and maintenance of SA variation in a population have been explored by a large body of theoretical work. These previous models have captured the fate of SA variation in infinite populations [1,23] under a wide range of dominance effects [24], in the presence of genetic drift in finite populations [25,26], under fluctuating environments [27], and when there is selection on linked SA polymorphisms [19,28]. What they all have in common, however, is that they consider small numbers of allelic variants at one or a small number of loci (often a single bi-allelic locus).

It is important to realize that the abstract concept of the 'locus' in these models imposes limitations on the applicability and generality of their results.

Specifically, the notion of alleles segregating at distinct and unlinked loci makes the implicit assumption that variants with SA fitness effects can arise by simple, individual mutation events. This is appropriate when considering SA selection on protein coding sequences, where non-synonymous substitutions can generate evolutionary relevant phenotypic variation in males and females. However, the assumption of isolated polymorphisms breaks down in the case of regulatory evolution, where the phenotype—and hence fitness—is determined by the match between the sequence of a putative binding site and the motif that is recognized by a transcription factor (TF). Here, it is the combination of sequence states at all positions of a binding site that matters, rather than the state at any individual position. The degeneracy of individual regulatory sequences typifies regulatory evolution but, in the framework of existing models of sexual antagonism, would imply complex effects of linkage and epistasis that cannot be readily analysed. Accordingly, previous models are of limited use to predict SA evolution of gene regulation.

Developing models which allow us to explore the evolution of gene regulation under SA selection is an important goal, because SA selection on regulatory regions is all but inevitable. This inevitability arises because sexual dimorphism requires the differential use, and hence expression, of genes in males and females and therefore can only arise via a period of opposing selection on gene regulation between the sexes [29]. Understanding how sexually dimorphic regulation can evolve, and the constraints that may oppose its evolution, necessitates models that can adequately describe the evolution of regulatory binding sites under sex-specific selection.

To model binding site evolution in this way, we here build on previous work that considers the fitness landscape of sequence states across the entire binding site by integrating the known biophysical properties of TF binding into models of regulatory evolution. These models often make the simplifying assumption that each nucleotide within a binding site contributes equally and independently to that site's binding energy. While in reality this may not always be true [30,31], these models are considered to appropriately capture the evolutionary dynamics of gene regulation [30,32–36].

We extend these models to study the effects of SA selection on cis-regulatory evolution. We explore, via simulation and analysis, the selective conditions that permit invasion and maintenance of SA binding site variants in a population. We then expand our modelling framework to consider regulatory cascades under SA selection, and determine where in a regulatory chain polymorphisms are most likely to arise and persist. We show that regulatory architecture has a fundamental impact on our expectations about the selective conditions, and the positions within a regulatory network, that give rise to SA polymorphisms. We further show that SA selection can lead to ongoing reorganizations in regulatory cascades over evolutionary timescales, including abrupt 'displacement' events, where the location of polymorphism shifts from genes directly under SA selection, to one of their upstream regulators.

2. Material and methods

Here, we describe the details of the biophysical and population genetic model used to generate our results. TF binding sites are typically around 10 nucleotides long in eukaryotes [35], while the population-scaled mutation rate in *Drosophila* is $N_e u \sim 0.01$ (where N_e is the effective population size and u is the mutation rate per nucleotide site [37]) and an order of magnitude lower in humans, placing both species in the weak mutation limit. For simplicity in our simulations (which vary population size, binding site length, and the number of binding sites), we assume a 'standard' binding site of length $n = 10$ and set the per binding site mutation rate at $\mu = 10u$. We then run all of our simulations with $N_e\mu = 0.1$, which keeps all of our simulations in the weak mutation limit [37,38].

(a) Gene expression

The biophysics of TF binding is well approximated by assuming an optimal consensus sequence, such that each nucleotide in a contiguous sequence of n nucleotides can be considered as either 'matched' to the consensus sequence or not. Below we refer to the number of matched nucleotides as k, with a matched nucleotide independently contributing an amount, $\epsilon \sim 1 - 3k_BT$ (absolute energy units) [30,31], to the site's binding energy. The probability π_k that a binding site consisting of k matched nucleotides is bound by a TF protein is given by

$$\pi_k = \frac{P}{P + \exp[\epsilon(n-k)]},$$

where P is the number of TF proteins available to bind to the site. We assume $P = 200$ in our simulations. The rate of transcription (for a fixed decay rate) and the number of translated proteins at the target for a site that up-regulates expression can then be treated, to a first approximation, as proportional to the probability that the binding site is bound. If we define the expression E of the target gene as the number of expressed proteins proportional to the maximum, we have simply

$$E = \frac{P}{P + \exp[\epsilon(n-k)]}. \tag{2.1}$$

This expression assumes that the TF is an activator, meaning stronger binding results in higher expression levels, with expression E varying between a minimum $E \sim 0$ and a maximum $E \sim 1$. Note that the scale here is arbitrary, since we are not directly modelling the process of transcription and translation, we are simply assuming that stronger binding corresponds to higher expression. Similarly, we could consider the case of a repressor, in which case E would decrease with k, and our results apply equally to this type of regulation.

Mutations to binding sites are assumed to occur via single-nucleotide substitutions, such that the probability of increasing the number of matches by 1, from $k \to k+1$, is $u(n-k)/3$ where the factor 3 reflects the fact that only one out of the three possible nucleotide changes will correctly match to the consensus sequence. Similarly the rate of mutations that decrease the number of nucleotide matches by 1, from $k \to k-1$ is uk. We therefore not only have a multi-allele system but one with asymmetric forward and back mutations, which makes analytical treatment difficult in most cases.

Since we are considering diploid organisms each individual carries two alleles, 1 and 2, with two expression levels E_1 and E_2, so that overall expression of the gene in each individual is given by $E = (E_1 + E_2)/2$. This simplifying assumption of additivity is well supported with several studies finding that cis-regulatory alleles tend to have additive effects on gene expression [39,40].

(b) Fitness landscape

To explore SA selection over gene expression in this framework, we assume that the gene under SA selection favours binding that results in maximally high expression ($E \sim 1$) given the TF input P (complete binding) in males and maximally low expression given P (no binding, $E \sim 0$) in females. As we note above however,

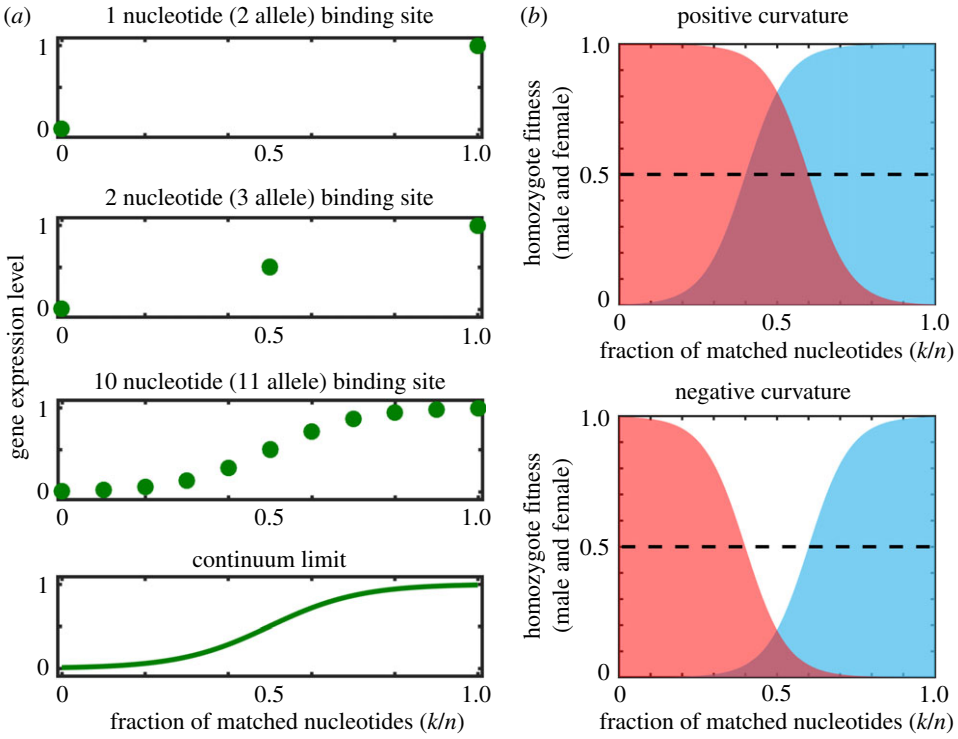

Figure 1. SA selection on gene expression. Regulation of gene expression by TF binding sites is well understood at a mechanistic level, allowing us to construct explicit genotype–phenotype maps. In the case we consider, expression level E increases with the number of nucleotides k correctly matched to a consensus sequence (*a*). Binding site length n is well known to have important consequences for the dynamics of binding site evolution [35,36] generally. However, population genetic models of sexual antagonism typically focus either on a 2-allele system [1,23–26] (corresponding to a binding site of 1 nucleotide in length), or in some contexts on the continuum limit and infinite alleles [42]. Eukaryotic TF binding sites, in contrast, are typically around 10 nucleotides long [35], and vary from as short as 5 nucleotides to more than 20 nucleotides in some cases. By varying the binding site length n and characterizing a binding site by the number of matched nucleotides k, we can generate a system with as few as 2-alleles (*a*, top) to an infinite number of alleles in the continuum limit (*a*, bottom). A realistic eukaryotic TF binding site length of $n = 10$ nucleotides results in 11 alleles at a given locus. We assume that expression is selected to be high in males (blue) and low in females (red) (*b*), and we consider fitness landscapes with different 'curvatures' corresponding to different levels of average fitness at intermediate expression levels.

expression level E is arbitrary under our model and our results correspond to any case where selection favours higher expression in one sex and lower (including non-zero) expression in the other sex. Fitness in both sexes follows a sigmoid function of expression levels:

$$\left.\begin{aligned} w_m(E) &= (1 - s_m) + s_m \frac{1}{1 + \exp[-\sigma_m(E - C_m)]} \\ \text{and} \quad w_f(E) &= (1 - s_f) + s_f \frac{1}{1 + \exp[\sigma_f(E - C_f)]}, \end{aligned}\right\} \tag{2.2}$$

where $w_m(E)$ is male fitness and $w_f(E)$ is female fitness, s defines the overall strength of selection, σ determines the steepness of the sigmoid function, and C determines the position of the threshold—where the contribution of expression to fitness is half its maximum. We can then define

$$c = C_f - C_m, \tag{2.3}$$

as the curvature of the landscape, so that if $C_f > C_m$ the average effect of an allele with intermediate expression $E = 0.5$ will be beneficial compared to alleles with high ($E = 1$) or low ($E = 0$) expression. We note that under positive curvature, the fitness landscape displays beneficial dominance reversal (see [41] for a review of dominance reversals), such that heterozygotes with intermediate expression levels have a net selective advantage relative to homozygotes with either high or low expression. By contrast, while under negative curvature the fitness landscape displays deleterious dominance reversal owing to intermediate expression being deleterious on average compared to high or low expression.

3. Results

(a) A regulatory binding site under SA selection

Gene expression is controlled, to a large extent, by transcription regulation, where TFs bind to characteristic sequences of DNA (binding sites) upstream of a transcription start site. TFs up- or down-regulate gene expression, for example, by aiding or hindering the acquisition of RNA polymerase at the transcription start site. The biophysical properties of TF binding are well understood [30,32–36]. Thus, equation (2.1) describes the expression level, E, of a gene as a function of: (i) the number of nucleotides n in its TF binding site, (ii) the number of nucleotides k within the binding site that match the maximum binding affinity consensus sequence for the binding site, and (iii) the number of TF proteins P available to bind the binding site (figure 1). A gene whose expression is under SA selection experiences conflicting sex-specific pressures on its regulation. Here, we focus on the straightforward case of a somatic gene whose expression is selected to increase in males and decrease in females (the sign associated with the selection pressures operating on each sex is arbitrary and identical results would be obtained for the opposite case). We begin by focusing on a single binding site that up-regulates the expression of its target, meaning that high-affinity binding sites are favoured in males and low affinity sites are favoured in females. Equation (2.1) thus provides us with the basis for an empirically grounded genotype–phenotype map for this system, since it relates the

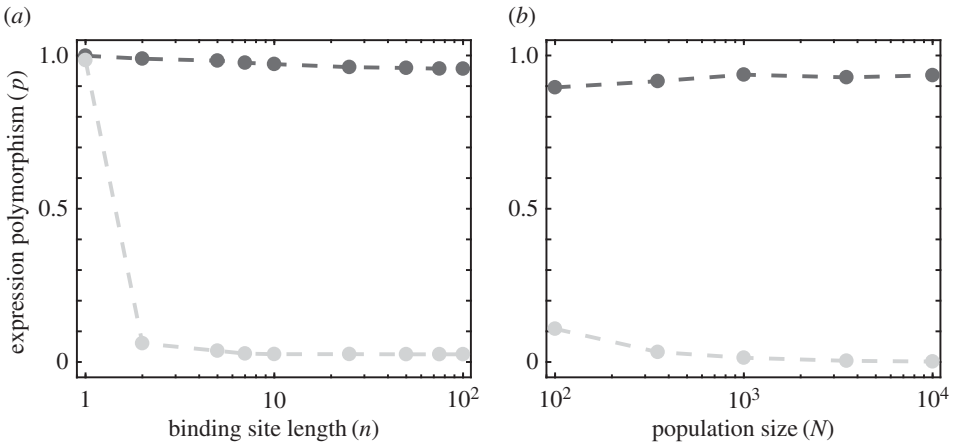

Figure 2. Expression polymorphism at a single binding site. Results of individual based simulations showing the amount of polymorphism in gene expression (p, see electronic supplementary material) as a function of (*a*) binding site length n, where we construct a single binding site with length varying from 1 (below the observed range of real binding site lengths) to 100 nucleotides (well above the observed range of real binding site lengths) and calculate expression from equation (2.1) and (*b*) population size N for landscapes with negative (dark grey) and positive (light grey) curvature. Points show the ensemble average of 10^4 runs at each parameter value. Default population size was fixed at $N = 10^3$ and default binding site length at $n = 10$. Per binding site mutation rates were set to $N_e\mu = 0.1$, selection was assumed to be strong ($s_m = s_f = 0.1$). Curvature was set to $c = \pm 0.2$ and the fitness landscape had steepness $h = 10$ (see Methods). Each simulation was run until 10^6 mutations per binding site had occurred.

nucleotide sequence at the binding site to the expression level of the gene under SA selection. We assume that the level of gene expression E relates to fitness by a sigmoidal function (see Methods, equation (2.2)) which increases from $1 - s_m$ (when $E = 0$) to 1 (when $E = 1$, where E is scaled such that $E = 1$ represents that maximum expression level) in males and decreases from 1 (when $E = 0$) to $1 - s_f$ (when $E = 1$) in females.

The relative steepness of male and female fitness functions has important consequences for the evolutionary dynamics of SA binding site variants. In particular, we must distinguish SA fitness landscapes with *positive* and *negative* curvature, where curvature is determined by average fitness at intermediate expression levels (see Methods). Curvature is said to be positive when the average fitness across males and females of intermediate expression alleles ($E = 1/2$) is greater than the average fitness of maximum or minimum expression alleles ($E = 1$ or $E = 0$) and to be negative when the converse is true (figure 1).

To begin, we use individual-based simulations (see the electronic supplementary material) to determine the equilibrium expression polymorphism at binding sites in SA fitness landscapes with both positive and negative curvature. We explore polymorphism as a function of binding site length n and population size N (figure 2). Because expression is nonlinear in the number of correctly matched nucleotides at a binding site (figure 1*a*), we quantify polymorphism at the expression level rather than at the genetic level. Specifically, we calculate the absolute difference in expression between the two alleles carried by an individual, averaged across all individuals (see the electronic supplementary material). This measure of 'expression polymorphism', p, is maximized ($p = 1$) when one allele of maximum expression and one allele of minimum expression segregate at equal frequencies in the population, resulting in a maximum frequency of heterozygotes (0.5) all of which carry two alleles with maximally different expression. Simulating a wide range of binding site lengths $1 \leq n \leq 100$ (figure 2*a*), we find that landscapes with negative curvature (where intermediate expression is deleterious on average compared to high or low expression) always lead to the evolution of high levels of expression polymorphism. Conversely, high levels of expression polymorphism never evolve in landscapes with positive curvature (where intermediate expression is on average fitter compared to high or low expression), with the notable exception of the limiting 2-allele case ($n = 1$). This result is particularly notable since fitness landscapes with positive curvature tend to generate pairs of alleles that display dominance reversal, which has been found previously to promote maintenance of SA polymorphism [24]. What our results show is that, in the fitness landscapes associated with regulatory evolution, the same circumstances that lead to dominance reversal also lead to a minimization of SA fitness variation via fixation of binding site alleles with intermediate strength. These results hold over a wide range of population sizes $10^2 \leq N \leq 10^4$ (figure 2*b*).

Figure 3 illustrates the intuitive explanation for the effect of fitness landscape curvature, in terms of the selection gradient experienced by mutations that increase or decrease binding affinity in a typical binding site of 10 nucleotides. When curvature is negative, polymorphism is favoured between pairs of alleles with intermediate binding strength, *and* the polymorphisms are subject to divergent selection gradients, with weaker sites favoured to get weaker and stronger sites to get stronger. This results in disruptive selection which generally leads to polymorphism. When curvature is positive, polymorphism can still sometimes be favoured at intermediate expression levels, but there is no disruptive selection and alleles of intermediate binding strength are maintained. This is because sexual antagonism can be reduced to the mutual advantage of both sexes by fixing an allele of intermediate expression that maximizes average fitness across males and females. In the 2-allele case, landscape curvature does not result in these contrasting dynamics. This is because when $n = 1$ binding is a binary function of whether or not the binding site matches the consensus sequence, meaning intermediate binding is not possible. Thus, in this case—even with positive curvature—polymorphism is maintained.

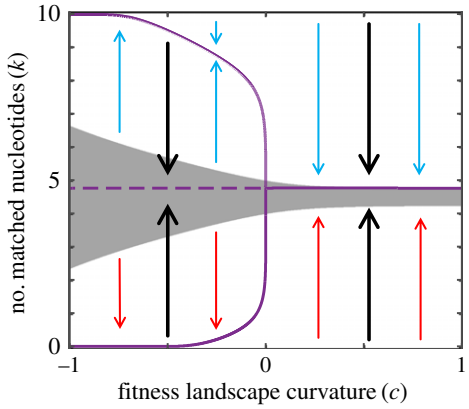

Figure 3. Pairwise invasion plot for a single binding site. We calculated the selection gradient (see the electronic supplementary material) for a 'typical' binding site of 10 nucleotides, assuming weak mutation so that at most two alleles segregate in a population at a given time, as a function of fitness landscape curvature c. We also used a 2-allele approximation to determine whether polymorphism was favoured (see the electronic supplementary material for details), with the polymorphic region indicated in dark grey. Solid purple lines indicate stable monomorphic equilibria that arise due to mutation and drift, while dashed lines indicate unstable equilibria. Blue arrows indicate the direction of the selection gradient on an invading allele that benefits males and red arrows the direction of selection on an invading allele benefiting females. Black arrows indicate the direction of evolution in a monomorphic population under antagonistic selection. Note that when curvature is negative (left-hand side, $c < 0$) the evolutionary dynamics lead to convergence on the intermediate, unstable equilibrium. Once this has been reached, the population experiences disruptive selection, a scenario that results in the emergence and maintenance of SA polymorphism. When curvature is positive (right-hand side $c > 0$) the evolutionary dynamics also converge on the intermediate equilibrium, but since this is stable, with males and females both experiencing high fitness, polymorphism is limited and sexual antagonism is minimal.

(b) Polymorphic displacement in a regulatory cascade

We have focused so far on a single binding site at a single target gene. However, most genes are regulated by multiple binding sites and most regulators are themselves subject to regulation, as part of a wider regulatory network [43,44]. This is particularly true for genes involved in sex determination and sexual differentiation for example, which are frequently arranged in regulatory cascades [45]. In relation to SA selection, this regulatory connectivity creates the potential for polymorphism to arise at multiple points in a regulatory cascade, even if only a single downstream gene is subject to direct SA selection for expression.

In order to investigate the invasion and maintenance of SA polymorphism across regulatory cascades, we once again assume a gene whose expression is under SA selection such that high expression is favoured in males and low expression in females. However, we now assume that this gene (gene 1) is at the bottom of a three-gene regulatory cascade (figure 4*c*, right),where its expression is up-regulated by a second (gene 2) which in turn is up-regulated by a third (gene 3). The third gene further has a binding site that up-regulates its own expression in response to some constant input signal (see the electronic supplementary material).

Under a fitness landscape with negative curvature, SA selection on the expression of gene 1 could potentially lead to polymorphism at any of the three binding sites in the cascade. However, determining precisely where polymorphism will arise is not straightforward, since there is a great deal of epistasis between mutations at different positions in the cascade, meaning that both the ordering of mutations as well as their average fitness effects in males and females becomes important to subsequent evolutionary dynamics [46]. We therefore used simulations to explore the evolutionary dynamics of all three binding sites (figure 4*a*), starting with a three-gene cascade in which all binding sites have high affinity ($k = n$). We observe that a high degree of expression polymorphism initially arises at gene 1, only to subsequently shift towards genes 2 and 3 that sit higher up the cascade.

To understand these dynamics, it is first necessary to evaluate why expression polymorphism should initially arise at the gene directly under SA selection for expression (gene 1). Indeed, we observe that expression polymorphism almost always initially arises at gene 1 (more than 90% of cases, figure 4*b*), and that there is an approximately exponential decline in the frequency of initial expression polymorphism as we move up the cascade to genes 2 and 3. This pattern can be explained by the buffering properties of regulatory cascades, where changes upstream of a focal gene may only result in minimal downstream consequences (as long as binding is strong and proteins are reasonably abundant). Here, this means that mutations to the binding sites of genes 2 and 3 initially generate little variation in expression of the gene directly under SA selection (gene 1). Accordingly, the selection gradients operating on these upstream binding sites are comparatively weak and mutant alleles are unlikely to invade and fix. By contrast, the effects of mutations directly in the binding site of gene 1 are not buffered by the regulatory cascade, resulting in much larger phenotypic changes. This means that the strength of selection on the binding site at gene 1 is strong, making it relatively easier for initial polymorphism to arise here (see the electronic supplementary material).

Regulatory buffering can not only explain the initial emergence of regulatory polymorphism at gene 1, but it is also central to its subsequent displacement. Specifically, displacement is driven by advantageous effects of buffering on fitness in heterozygotes. If gene 1 is polymorphic for highly divergent binding site alleles, such that homozygotes have either expression $E \approx 1$ or $E \approx 0$, heterozygotes will have average expression $E \approx 0.5$ of gene 1. In a fitness landscape with negative curvature, $E = 0.5$ yields the lowest possible fitness and the fitness of heterozygotes is maximally depressed. The emergence of an equivalent polymorphism further upstream in the cascade (gene 2 or gene 3) is then selectively favoured, because it results in heterozygotes expressing at levels $E > 0.5$ or $E < 0.5$ (while homozygote expression remains unchanged at $E \approx 1$ or $E \approx 0$) and alleviates the fitness costs. Thus, the higher overall fitness associated with expression polymorphism at upstream genes will precipitate the upwards displacement of polymorphism, away from gene 1.

Finally, we find that over long timescales, expression polymorphism is most likely to ultimately reside at the very top of the regulatory cascade (gene 3). This pattern is initially surprising because there is no mean fitness advantage for expression polymorphism at gene 3 relative to gene 2 (electronic supplementary material, figure S4). However, we do observe that the sex-specific fitness of males and females is more similar when expression polymorphism resides at gene 3 than at gene 2 (electronic supplementary material,

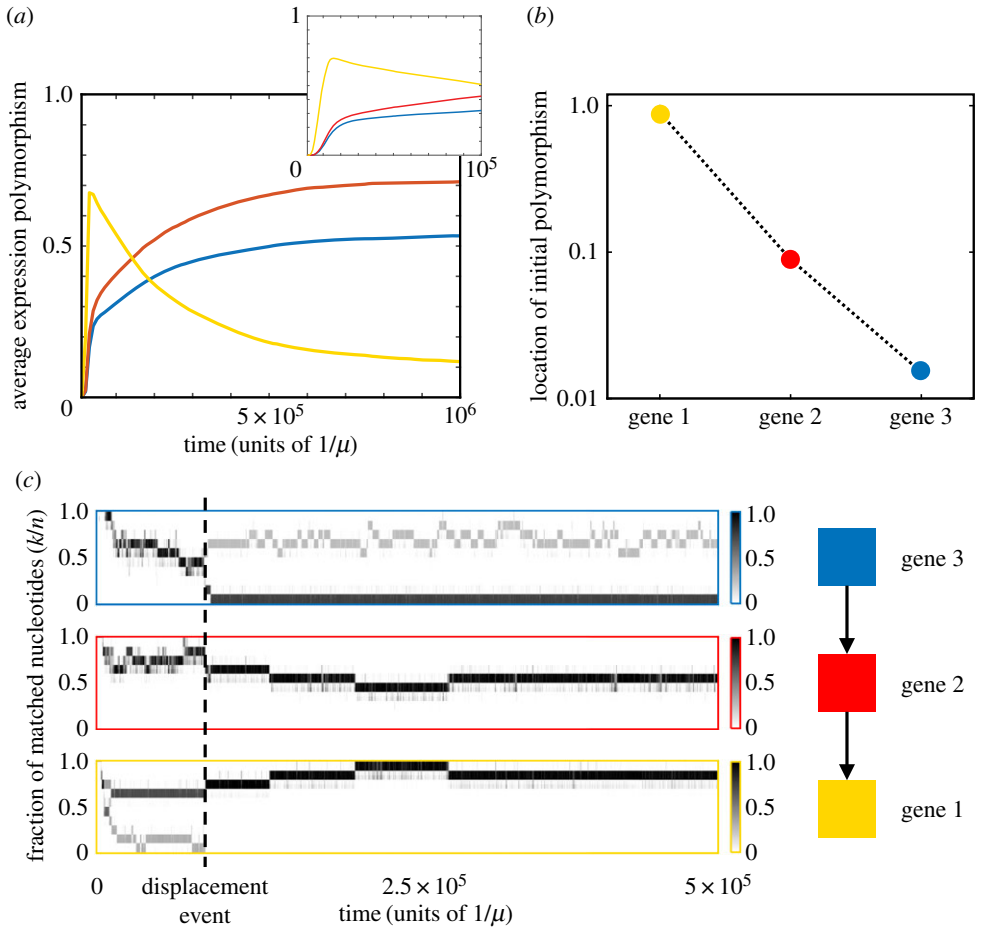

Figure 4. Displaced polymorphism in a regulatory cascade under a fitness landscape with negative curvature. (*a*) We observed the average expression polymorphism for each gene over evolutionary time. The initial phase (inset) sees expression polymorphism arise at gene 1 (yellow) and beginning to pass to gene 2 (red) and gene 3 (blue) higher up the cascade. (*b*) We determined where in the cascade expression polymorphism of $p > 0.5$ first arose. In more than 90% of simulation runs expression polymorphism initially arises at gene 1, with the frequency declining approximately exponentially with position in the cascade. (*c*) A sample path for a single simulation run shows the dynamics of displacement explicitly, with gene 1 quickly acquiring polymorphism until a displacement event shifts the polymorphism up the chain to gene 3. Shading indicates allele frequencies within the population. These individual-based simulations for a cascade of three genes were carried out using the same default parameters given in figure 2.

figures S5 and S6). This suggests that when there is expression polymorphism at gene 2 there is still disruptive selection that can favour the invasion of SA alleles further up in the regulatory cascade. Once expression polymorphism is at gene 3 in the cascade there is greater scope to 'fine-tune' the expression level of gene 1 via the intermediary gene 2, resulting in more balanced male and female fitness, and less opportunity for disruptive selection.

A typical example of polymorphic displacement is shown in figure 4*c*. Here, polymorphism arises quickly at gene 1 before being displaced to gene 3, which remains polymorphic over many generations. As both figure 4*a*,*c* illustrate, displacement takes place over long evolutionary timescales, with binding sites experiencing around 10^5 mutations before any displacement occurs. We are thus describing a slow and ongoing reorganization of regulatory cascades in response to SA selection. We note that this phenomenon is expected to be a general feature of landscapes with negative curvature (see the electronic supplementary material).

4. Discussion

The regulation of gene expression is not only a prime mechanism by which sex-specific adaptation can be achieved, but also an inevitable target for SA selection. By integrating the population genetics of SA variants with a biophysical model of TF binding, our study has generated a number of new predictions for the dynamics of regulatory evolution under SA selection.

First, we show that for binding sites of realistic length, SA polymorphisms will only be maintained when intermediate expression levels are, on average, deleterious compared to high or low expression levels. In this scenario of negative curvature, the fitness landscape generates disruptive selection at intermediate binding that will favour segregating binding site variants of ever-more extreme affinities. By contrast, a fitness landscape with positive curvature will favour a monomorphic, intermediate strength binding site, precluding the maintenance of polymorphism. The requirement of negative curvature for SA polymorphism only vanishes for the extreme (and unrealistic) case of binding site length $n = 1$, the situation captured by standard 2-allele models of sexual antagonism. At this limit, mutational effects on TF binding are so coarse that alleles with intermediate expression cannot arise, and SA polymorphism is predicted even with positive curvature. It is important to note that our definition of expression polymorphism p (see the electronic supplementary material) measures the variation in terminal gene expression resulting from genetic variation at each locus.

This is a highly conservative measure, which can only reach values $p \sim 1$ if there is *both* a high level of heterozygosity *and* the different allelic variants have very different effects on gene expression.

Our prediction that fitness landscapes with negative curvature promote SA binding site variation is in contrast with work focused on 2-allele systems [23,47,48], which find that the 'positive curvature' scenario and dominance reversal [24] promotes polymorphism. In 2-allele models, polymorphism is promoted by effective heterosis, where the fitness of heterozygotes exceeds that of both homozygotes when measured across sexes [23] and heterozygotes are favoured by selection. In the system, we study, by contrast, polymorphism is the result of disruptive selection on binding, where intermediate binding strengths are highly deleterious to both sexes (i.e. deleterious dominance reversal occurs) and heterozygotes are disfavoured by selection. This fundamental difference makes it difficult to directly compare between the two types of model in how restrictive or permissive their conditions for polymorphism are.

Beyond characterizing the conditions under which SA expression variation can occur, our model allows us to gain insight into the distribution of SA polymorphism across regulatory cascades. Thus, we predict that allelic variation will be subject to displacement along the regulatory hierarchy. While polymorphisms are most likely to arise at the target of selection, they can subsequently move to other genes higher up the regulatory cascade. The ultimate location of polymorphism is expected to be that which offers the greatest average fitness to heterozygotes while minimizing the opportunity for disruptive selection (see the electronic supplementary material). In the type of cascade modelled here, this corresponds to the gene at the top of the regulatory chain, where buffering of regulatory effects in heterozygotes results in expression other than $E = 0.5$ at the target gene and an associated benefit compared to heterozygotes with strong and weak binding alleles at the target gene. However, it must be noted that this three-gene cascade is a 'minimal complexity' case, and that in reality regulatory networks involve many more regulators interacting in many more ways. We emphasize the simple case in order to make it clear that the phenomenon of displaced polymorphism arises even here, suggesting that in a real network the location of polymorphism arising due to sexual antagonism will be hard to predict.

It is also important to note that the model we present here, as with many other models of sexual antagonism, focuses on the initial invasion and maintenance of SA alleles. It is widely assumed that over long timescales, sexual antagonism may be resolved by mechanisms that maintain the benefit to one sex while removing the cost to the other [49]—ultimately allowing for the restoration of the optimal phenotype in all individuals. Accordingly, we can only expect to observe polymorphic displacement in real populations if the timescales over which resolution evolves are more substantial than those required for displacement to occur. It is currently difficult to say whether that would be expected to be the case, as we still lack empirical data describing the timescales over which these mechanisms may evolve (although new studies are starting to suggest that the timescales can indeed be substantial [50]). Moreover, it is evident from our simulations that the timing of displacement is highly variable. It is therefore reasonable to suppose that whether resolution or displacement occur first will vary on a case-by-case basis.

We predict that SA polymorphism at the top of a cascade will be most beneficial. However, it is worth noting that this expectation rests on a number of assumptions that do not always hold in real systems. Our simulations show that the displacement of polymorphism is a highly stochastic process. Even when assuming strong selection, the fitness differentials that drive upward displacement rapidly decline along the cascade. Thus, while displacing polymorphism away from the target gene generates significant gains, the location of polymorphisms in the higher echelons of the cascade that we observe in our simulations is largely stochastic and dictated by where suitable mutations first arise. It is likely that this tendency will be exacerbated in real regulatory systems, where regulatory mutations may have significant pleiotropic effects. As a consequence, it will be difficult to make precise predictions about the location of polymorphism, other than that it tends to be above the downstream target gene.

A significant factor that will impact displacement is the structure of a regulatory network. Our simple linear cascade assumes a single target gene under SA selection, yet real-life regulatory networks may feature multiple target genes. In cases where all of these target genes are aligned in terms of the direction of SA selection, such a modular organization may favour and precipitate upward displacement of regulatory polymorphism. This is because, in this case, modularity amplifies the selective benefits of upstream regulatory variants whose effects propagate across all downstream target genes. By contrast, co-regulated target genes may be under different types of selection, for example, some targets may be under SA selection with others under directional/stabilizing selection. Alternatively, multiple targets may be under SA selection, but in opposing directions. In these cases, altered regulation of upstream TFs may generate deleterious pleiotropic effects and prevent polymorphism from being displaced. We may then either see the persistence of SA polymorphism at individual target genes or larger-scale rewiring of gene regulatory interactions to create modules of genes under similar selection (e.g. [51]).

The location of SA polymorphism within regulatory networks has consequences for our understanding of how sexual antagonism may be eventually resolved—and hence how sex-specific development is regulated. The evolution of sex-specific regulation in SA genes is a prime potential mechanism to achieve resolution, certainly in the case that we consider here, where adaptive conflict between the sexes occurs over expression levels (rather than coding sequence) of a gene. As previously discussed, we only expect to observe polymorphic displacement if the timescale for displacement is shorter than that of resolution. In those cases where polymorphic displacement precedes resolution, we would expect a corresponding shift in the level at which eventual resolution may occur. Thus, we would also expect sex-specific regulation to evolve at higher levels of the regulatory hierarchy than would necessarily occur if resolution was faster than polymorphic displacement. Reflecting the arguments on modularity above, this should particularly be the case where genes under SA selection are organized into co-regulated modules. Not only should upwards displacement of polymorphism be more strongly selected in these cases, but also its eventual resolution.

Our work has shown that SA selection acting on gene expression can give rise to counterintuitive evolutionary dynamics across regulatory networks. These are driven by the

conflicting impacts of the inherent robustness of networks, whereby changes to the expression of an upstream regulator are frequently compensated for by others downstream. Such buffering tends to prevent the emergence of initial polymorphism at upstream genes, but once such polymorphism exists at downstream targets, it favours its upward displacement. Over time, we would therefore expect both SA polymorphism and the sex-specific regulation that may arise to resolve it to reside in the upper reaches of regulatory networks. Testing these predictions directly is difficult, as current data on SA loci and sex-specific resolution are relatively sparse. Interestingly, however, parallels exist between sex-specific selection pressures and directional selection in fluctuating environments [52]. It is therefore plausible that evolutionary dynamics analogous to those described here occur in networks governing the response to alternating environmental conditions, allowing the use of microbial evolution for experimental tests of our theory.

Data accessibility. This article has no additional data.

Authors' contributions. M.S.H., M.R., and A.J.S. designed the project; M.S.H. and A.J.S. conducted analyses and produced figures. All authors interpreted the results and contributed to writing of the manuscript.

Competing interests. The authors declare no competing interests.

Funding. This work was supported by a UCL IMPACT PhD Studentship to M.S.H. and a Royal Society University Research Fellowship to A.J.S.

Acknowledgements. We thank two anonymous referees for their constructive comments.

References

1. Rice WR. 1984 Sex chromosomes and the evolution of sexual dimorphism. *Evolution* **38**, 735–742. (doi:10.1111/evo.1984.38.issue-4)
2. Bonduriansky R, Chenoweth S. 2009 Intralocus sexual conflict. *Trends Ecol. Evol.* **24**, 280–288. (doi:10.1016/j.tree.2008.12.005)
3. Cox RM, Calsbeek R. 2009 Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. *Am. Nat.* **173**, 176–187. (doi:10.1086/595841)
4. Pennell TM, Morrow EH. 2013 Two sexes, one genome: the evolutionary dynamics of intralocus sexual conflict. *Ecol. Evol.* **3**, 1819–1834. (doi:10.1002/ece3.2013.3.issue-6)
5. Mokkonen M, Kokko H, Koskela E, Lehtonen J, Mappes T, Martiskainen H, Mills SC. 2011 Negative frequency-dependent selection of sexually antagonistic alleles in *Myodes glareolus*. *Science* **334**, 972–974. (doi:10.1126/science.1208708)
6. Tarka M, Åkesson M, Hasselquist D, Hansson B, Akesson M, Hasselquist D, Hansson B. 2014 Intralocus sexual conflict over wing length in a wild migratory bird. *Am. Nat.* **183**, 62–73. (doi:10.1086/674072)
7. Svensson EI, McAdam AG, Sinervo B. 2009 Intralocus sexual conflict over immune defense, gender load, and sex-specific signaling in a natural lizard population. *Evolution* **63**, 3124–3135. (doi:10.1111/evo.2009.63.issue-12)
8. Rice WR. 1992 Sexually antagonistic genes: experimental evidence. *Science* **256**, 1436–1439. (doi:10.1126/science.1604317)
9. Berger D, Berg EC, Widegren W, Arnqvist G, Maklakov AA. 2014 Multivariate intralocus sexual conflict in seed beetles. *Evolution* **68**, 3457–3469. (doi:10.1111/evo.12528)
10. Barson NJ *et al.* 2015 Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. *Nature* **528**, 1–4.
11. Delph LF, Andicoechea J, Steven JC, Herlihy CR, Scarpino SV, Bell DL. 2011 Environment-dependent intralocus sexual conflict in a dioecious plant. *New Phytol.* **192**, 542–552. (doi:10.1111/nph.2011.192.issue-2)
12. Rice WR. 1987 The accumulation of sexually antagonistic genes as a selective agent promoting the evolution of reduced recombination between primitive sex chromosomes. *Evolution* **41**, 9–11. (doi:10.1111/evo.1987.41.issue-4)
13. Charlesworth D, Charlesworth B. 2005 Sex chromosomes: evolution of the weird and wonderful. *Curr. Biol.* **15**, R129–R131. (doi:10.1016/j.cub.2005.02.011)
14. Haag ES, Doty AV. 2005 Sex determination across evolution: connecting the dots. *PLoS Biol.* **3**, e21. (doi:10.1371/journal.pbio.0030021)
15. Van Doorn GS. 2009 Intralocus sexual conflict. *Ann. NY Acad. Sci.* **1168**, 52–71. (doi:10.1111/j.1749-6632.2009.04573.x)
16. Muralidhar P, Veller C. 2018 Sexual antagonism and the instability of environmental sex determination. *Nat. Ecol. Evol.* **2**, 343–351. (doi:10.1038/s41559-017-0427-9)
17. Pischedda A, Chippindale AK, Bangham J, Rowe L, Gocayne J. 2006 Intralocus sexual conflict diminishes the benefits of sexual selection. *PLoS Biol.* **4**, e356. (doi:10.1371/journal.pbio.0040356)
18. Pennell TM, de Haas FJH, Morrow EH, van Doorn GS. 2016 Contrasting effects of intralocus sexual conflict on sexually antagonistic coevolution. *Proc. Natl Acad. Sci. USA* **113**, E978–E986. (doi:10.1073/pnas.1514328113)
19. Patten MM, Haig D, Ubeda F. 2010 Fitness variation due to sexual antagonism and linkage disequilibrium. *Evolution* **64**, 3638–3642. (doi:10.1111/j.1558-5646.2010.01100.x)
20. Foerster K, Coulson T, Sheldon BC, Pemberton JM, Clutton-Brock TH, Kruuk LEB. 2007 Sexually antagonistic genetic variation for fitness in red deer. *Nature* **447**, 1107–1110. (doi:10.1038/nature05912)
21. Dutoit L *et al.* 2018 Sex-biased gene expression, sexual antagonism and levels of genetic diversity in the collared flycatcher (*Ficedula albicollis*) genome. *Mol. Ecol.* **27**, 3572–3581. (doi:10.1111/mec.14789)
22. Kirkpatrick M. 2009 Patterns of quantitative genetic variation in multiple dimensions. *Genetica* **136**, 271–284. (doi:10.1007/s10709-008-9302-6)
23. Kidwell JF, Clegg MT, Stewart FM, Prout T. 1977 Regions of stable equilibria for models of differential selection in the two sexes under random mating. *Genetics* **85**, 171–183.
24. Fry JD. 2010 The genomic location of sexually antagonistic variation: some cautionary comments. *Evolution* **64**, 1510–1516. (doi:10.1111/j.1558-5646.2009.00898.x)
25. Mullon C, Pomiankowski A, Reuter M. 2012 The effects of selection and genetic drift on the genomic distribution of sexually antagonistic alleles. *Evolution* **66**, 3743–3753. (doi:10.1111/evo.2012.66.issue-12)
26. Connallon T, Clark AG. 2012 A general population genetic framework for antagonistic selection that accounts for demography and recurrent mutation. *Genetics* **190**, 1477–1489. (doi:10.1534/genetics.111.137117)
27. Connallon T, Sharma S, Olito C. 2018 Evolutionary consequences of sex-specific selection in variable environments: four simple models reveal diverse evolutionary outcomes. *Am. Nat.* **193**, 93–105. (doi:10.1086/700720)
28. Úbeda F, Haig D, Patten MM. 2011 Stable linkage disequilibrium owing to sexual antagonism. *Proc. R. Soc. B* **278**, 855–862. (doi:10.1098/rspb.2010.1201)
29. Lande R. 1980 Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* **34**, 292–305. (doi:10.1111/evo.1980.34.issue-2)
30. Gerland U, Hwa T. 2002 On the selection and evolution of regulatory DNA motifs. *J. Mol. Evol.* **55**, 386–400. (doi:10.1007/s00239-002-2335-z)
31. Lässig M. 2007 From biophysics to evolutionary genetics: statistical aspects of gene regulation. *BMC Bioinf.* **8**, S7. (doi:10.1186/1471-2105-8-s6-s7)
32. Buchler NE, Gerland U, Hwa T. 2003 On schemes of combinatorial transcription logic. *Proc. Natl Acad. Sci. USA* **100**, 5136–5141. (doi:10.1073/pnas.0930314100)
33. Bintu L, Buchler NE, Garcia HG, Gerland U, Hwa T, Kondev J, Phillips R. 2005 Transcriptional regulation by the numbers: models. *Curr. Opin. Genet. Dev.* **15**, 116–124. (doi:10.1016/j.gde.2005.02.007)

34. Mustonen V, Kinney J, Callan CG, Lässig M. 2008 Energy-dependent fitness: a quantitative model for the evolution of yeast transcription factor binding sites. *Proc. Natl Acad. Sci. USA* **105**, 12 376–12 381. (doi:10.1073/pnas.0805909105)
35. Stewart AJ, Hannenhalli S, Plotkin JB. 2012 Why transcription factor binding sites are ten nucleotides long. *Genetics* **192**, 973–985. (doi:10.1534/genetics.112.143370)
36. Tuğrul M, Paixão T, Barton NH, Tkačik G. 2015 Dynamics of transcription factor binding site evolution. *PLoS Genet.* **11**, e1005639. (doi:10.1371/journal.pgen.1005639)
37. Lynch M, Conery J. 2003 The origins of genome complexity. *Science* **302**, 1401. (doi:10.1126/science.1089370)
38. Sella G, Hirsh AE. 2005 The application of statistical physics to evolutionary biology. *Proc. Natl Acad. Sci. USA* **102**, 9541–9546. (doi:10.1073/pnas.0501865102)
39. Lemos B, Araripe LO, Fontanillas P, Hartl DL. 2008 Dominance and the evolutionary accumulation of *cis*- and *trans*-effects on gene expression. *Proc. Natl Acad. Sci. USA* **105**, 14 471–14 476. (doi:10.1073/pnas.0805160105)
40. McManus CJ, Coolon JD, Duff MO, Eipper-Mains J, Graveley BR, Wittkopp PJ. 2010 Regulatory divergence in *Drosophila* revealed by mRNA-seq. *Genome Res.* **20**, 816–825. (doi:10.1101/gr.102491.109)
41. Connallon T, Chenoweth SF. 2019 Dominance reversals and the maintenance of genetic variation for fitness. *PLoS Biol.* **17**, e3000118. (doi:10.1371/journal.pbio.3000118)
42. Kimura M, Crow J. 1964 The number of alleles that can be maintained in a finite population. *Genetics* **49**, 725–738.
43. Stewart AJ, Seymour RM, Pomiankowski A. 2009 Degree dependence in rates of transcription factor evolution explains the unusual structure of transcription networks. *Proc. R. Soc. B* **276**, 2493–2501. (doi:10.1098/rspb.2009.0210)
44. Stewart AJ, Plotkin JB. 2013 The evolution of complex gene regulation by low-specificity binding sites. *Proc. R. Soc. B* **280**, 20131313. (doi:10.1098/rspb.2013.1313)
45. Herpin A, Schartl M. 2015 Plasticity of gene-regulatory networks controlling sex determination: of masters, slaves, usual suspects, newcomers, and usurpators. *EMBO Rep.* **16**, 1260–1274. (doi:10.15252/embr.201540667)
46. Shah P, McCandlish DM, Plotkin JB. 2015 Contingency and entrenchment in protein evolution under purifying selection. *Proc. Natl Acad. Sci. USA* **112**, E3226–E3235. (doi:10.1073/pnas.1412933112)
47. Patten MM, Haig D. 2009 Maintenance or loss of genetic variation under sexual and parental antagonism at a sex-linked locus. *Evolution* **63**, 2888–2895. (doi:10.1111/evo.2009.63.issue-11)
48. Connallon T, Clark GA. 2011 The resolution of sexual antagonism by gene duplication. *Genetics* **187**, 919–937. (doi:10.1534/genetics.110.123729)
49. Stewart AD, Pischedda A, Rice WR. 2010 Resolving intralocus sexual conflict: Genetic mechanisms and time frame. *J. Hered.* **101**, S94–S99. (doi:10.1093/jhered/esq011)
50. Ruzicka F *et al.* 2019 Genome-wide sexually antagonistic variants reveal long-standing constraints on sexual dimorphism in fruit flies. *PLoS Biol.* **17**, e3000244. (doi:10.1371/journal.pbio.3000244)
51. Tsong AE, Tuch BB, Li H, Johnson AD. 2006 Evolution of alternative transcriptional circuits with identical logic. *Nature* **443**, 415–420. (doi:10.1038/nature05099)
52. Reuter M, Camus MF, Hill MS, Ruzicka F, Fowler K. 2017 Evolving plastic responses to external and genetic environments. *Trends Genet.* **33**, 169–170. (doi:10.1016/j.tig.2017.01.004)