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Sex ratio selection and multi-factorial sex determination in the housefly: a dynamic model

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Abstract

Sex determining (SD) mechanisms are highly variable between different taxonomic groups and appear to change relatively quickly during evolution. Sex ratio selection could be a dominant force causing such changes. We investigate theoretically the effect of sex ratio selection on the dynamics of a multi-factorial SD system. The system considered resembles the naturally occurring three-locus system of the housefly, which allows for male heterogamety, female heterogamety and a variety of other mechanisms. Sex ratio selection is modelled by assuming cost differences in the production of sons and daughters, a scenario leading to a strong sex ratio bias in the absence of constraints imposed by the mechanism of sex determination. We show that, despite of the presumed flexibility of the SD system considered, equilibrium sex ratios never deviate strongly from 1 : 1. Even if daughters are very costly, a male-biased sex ratio can never evolve. If sons are more costly, sex ratio can be slightly female biased but even in case of large cost differences the bias is very small (<10% from 1 : 1). Sex ratio selection can lead to a shift in the SD mechanism, but cannot be the sole cause of complete switches from one SD system to another. In fact, more than one locus remains polymorphic at equilibrium. We discuss our results in the context of evolution of the variable SD mechanism found in natural housefly populations.

Introduction

Sex determination is a fundamental developmental process in animals and plants and one might therefore expect the underlying mechanisms to be conserved. Yet the opposite is true: sex determining (SD) mechanisms vary considerably between closely related taxonomic groups and evolutionary transitions from one system to another seem to occur frequently (Bull, 1983; Marin & Baker, 1998; Werren & Beukeboom, 1998; Kraak & Pen, 2002). Common SD mechanisms are male heterogamety (males XY and females XX, as in nearly all mammals and many insect groups), female heterogamety (females ZW and males ZZ, as in birds, lepidopterans and snakes), haplodiploidy (females diploid and males haploid, as in hymenopterans) and environmental sex determination (as in some reptiles and fish), but there exist a variety of other mechanisms (Bull, 1983).

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It is still far from clear why SD mechanisms are so evolutionarily unstable and what forces are responsible for their rapid turnover rate. Genetic conflict and sex ratio selection might play an important role (Eberhard, 1980; Werren & Beukeboom, 1998). For example, models have been proposed that show how conflicting selection pressures on autosomal genes and cytoplasmic factors may induce transitions from female heterogamety to male heterogamety (Caubet *et al.*, 2000). Despite such theoretical advances, not much empirical progress has been made. In particular, little experimental work has been done (but see Conover & Vanvoorhees, 1990; Conover *et al.*, 1992; Basolo, 1994; Carvalho *et al.*, 1998; Basolo, 2001). One reason for the lack of experiments is presumably that SD mechanisms are usually fixed (or thought to be so) in individual species, although some exceptions are known (Bull, 1983).

The housefly (*Musca domestica*) is such an exception. In this species, several different SD mechanisms have been

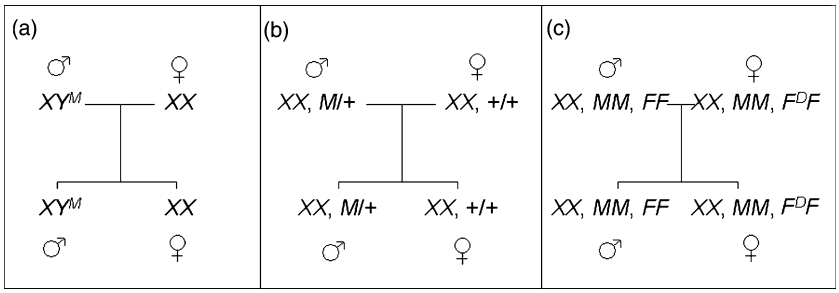

Fig. 1 Common SD mechanisms in natural populations of *Musca domestica*. (a) The standard XY system – male determining factor (*M*) present on the Y chromosome. (b) Autosomal system with male heterogamety – *M* present on one of the autosomes, males and females homozygous for X. (c) Autosomal system with female heterogamety – males and females are homozygous for X and autosomal *M*, sex is determined by presence (females) or absence (males) of the epistatic factor F^{D}. Figure adjusted from Dübendorfer *et al.* (2002).

found to co-exist in field populations (Fig. 1; Franco *et al.*, 1982; Denholm *et al.*, 1985; Tomita & Wada, 1989a). In the so-called standard XY strains, a male-determining factor (*M*) is located on the Y chromosome and males are XY and females XX. The *M* factor blocks the action of an autosomal *F*, which is necessary for female development. In addition to the standard XY system, field populations have been discovered in which an *M* factor is located on one or several of the five autosomes, or even on an X chromosome. These autosomal (more precisely, non-Y) *M* factors seem to have appeared relatively recently and may be spreading, replacing the standard XY system in many locations (see Franco *et al.*, 1982; Tomita & Wada, 1989b). Intriguingly, the frequency of autosomal *M* factors seems to decrease with latitude and altitude: northern and high altitude populations are usually dominated by the standard XY system. Such geographical clines have been found in Europe (Franco *et al.*, 1982), Japan (Tomita & Wada, 1989a), Turkey (Çakir & Kence, 1996) and the USA (Hamm *et al.*, 2005). In most populations with autosomal *M* factors, an additional epistatic factor F^{Dominant} (F^{D}) occurs, which dictates female development, even in the presence of up to three *M* factors (see McDonald *et al.*, 1978; Franco *et al.*, 1982). Presumably F^{D} evolved after the invasion of autosomal *M* factors, instead of vice versa, since populations with F^{D} always have autosomal *M* factors but not the other way around. Some populations with F^{D} appear to be fixed for an autosomal *M*, and in such populations most flies have two X chromosomes, YY genotypes being rare (Franco *et al.*, 1982; Denholm *et al.*, 1983,1985,1990). This has been taken to suggest that YY genotypes may have lower viability, but direct evidence for this is lacking. In addition to SD systems comprising *M* factors and F^{D}, several other mechanisms have been discovered in the laboratory, including a mechanism that induces monogeny (Dübendorfer *et al.*, 2002).

Whatever the causes for the variability and distribution of SD mechanisms in the housefly (more about this in the Discussion), this organism is potentially very suitable for conducting experimental studies on the evolution of sex determination, and we are currently embarking on such studies. However, in addition to carrying out experiments, it is useful to obtain more theoretical insight into the dynamical behaviour of the housefly system. Therefore we present here a study of a three-locus model, with an XY 'locus', an autosomal *M* locus and an autosomal F^{D} locus. We extend an earlier analysis of Jayakar (1987) who studied a similar model but focused on a number of two-locus sub-models, mixing either XY with F^{D} (or mathematically equivalently, *M* and F^{D}) or mixing XY and autosomal *M*. In contrast to Jayakar (1987), who mainly considered the potential effect of meiotic drive, we here investigate the effect of sex ratio selection on the dynamics of the three-locus system. The reason is that the selection for or against biased sex ratios is thought to be, at least theoretically, an important contributing factor in evolutionary transitions between SD systems (Bull, 1983; Wilkins, 1995; Werren & Beukeboom, 1998; Werren & Hatcher, 2000; Kraak & Pen, 2002; Werren *et al.*, 2002). There are various scenarios to explain how natural selection might lead to bias in the primary sex ratio (Hamilton, 1967; Charnov, 1975, 1982; Werren & Taylor, 1984; Reinhold, 1996; Werren & Hatcher, 2000; Beukeboom *et al.*, 2001; Werren *et al.*, 2002; Wade *et al.*, 2003). Here we focus on the most basic mechanism where sons and daughters differ in how much they 'cost' to produce by the parents. Selection will then act on genes affecting the sex ratio to favour overproduction of the 'cheaper' sex (Fisher, 1930; Trivers, 1974).

We aim to achieve three goals with this study. First, our study might contribute to understanding to what extent real-world SD systems constrain the evolution of the sex ratio. This is important because most models of sex ratio evolution assume that the sex ratio is a continuous variable and that any sex ratio is feasible by the underlying genetic system (Pen & Weissing, 2002). Second, we hope that our model sheds some more light on the frequencies of SD factors and sex ratios that have been observed in field populations of the housefly. And

last but not least, we hope that our results will be useful in designing and interpreting future laboratory experiments that will be carried out with houseflies and other organisms.

The model

We model the dynamics of a sex determination system consisting of three gene loci on three different chromosomes, each locus having two possible alleles. The first locus corresponds to the standard XY sex determination system, having an X 'allele' and a Y (male-determining) 'allele'. The second locus has a male-determining *M* allele and a neutral '+' allele. The third locus has an epistatic female-determining F^D allele and a standard *F* allele (we call Y, *M* and F^D the 'focal' alleles at their loci). The total number of possible genotypes is therefore $3^3 = 27$, but we focus on a subset of 18 genotypes, since the nine genotypes with two F^D alleles are not feasible because males never have F^D alleles (Table 1) and hence females are never homozygous for F^D.

A genotype is encoded by a triplet $\mathbf{i} = (i_1, i_2, i_3) = (\#Y, \#M, \#F^D)$, tracking the number of focal alleles at each locus. The sexual phenotype determined by genotype **i** is encoded as a binary variable: $s(\mathbf{i}) = 0$ for females and $s(\mathbf{i}) = 1$ for males. The frequencies of genotype **i** among adult females and adult males are written as $p_f(\mathbf{i})$ and $p_m(\mathbf{i})$ ($\sum_{\mathbf{i}} p_f(\mathbf{i}) = \sum_{\mathbf{i}} p_m(\mathbf{i}) = 1$). Note that for each **i** either $p_f(\mathbf{i})$ or $p_m(\mathbf{i})$ must be zero because the genotype **i** uniquely determines sex.

The conditional distribution of genotype **k** among the offspring of parents with genotypes **i** and **j** is denoted by $T(\mathbf{k} \mid \mathbf{ij})$. Assuming independent assortment of chromosomes, $T(\mathbf{k} \mid \mathbf{ij})$ can be written as:

$$T(\mathbf{k}|\mathbf{ij}) = P(k_1|i_1j_1)P(k_2|i_2j_2)P(k_3|i_3j_3), \qquad (1)$$

where $P(k_n|i_nj_n)$ is the probability that an offspring receives k_n copies of a focal allele at locus *n*, given that the parents have i_n and j_n copies of that allele. Observe that for all *n* $\sum_{k_n} P(k_n|i_nj_n) = 1$. A parent with i_n copies transmits either 0 or 1 copy, with expected value $i_n/2$, assuming 'honest' Mendelian inheritance. The number of copies received by an offspring is therefore distributed according to

$$\begin{aligned} P(k_n = 0|i_nj_n) &= (1 - \tfrac{1}{2}i_n)(1 - \tfrac{1}{2}j_n) \\ P(k_n = 1|i_nj_n) &= \tfrac{1}{2}i_n(1 - \tfrac{1}{2}j_n) + \tfrac{1}{2}j_n(1 - \tfrac{1}{2}i_n) \\ P(k_n = 2|i_nj_n) &= \tfrac{1}{4}i_nj_n. \end{aligned} \qquad (2)$$

The number and viability of offspring may depend on the genotypes of the parents and the genotype of the offspring. In particular, the number of offspring produced by a genotype pair **ij** is denoted by $u(\mathbf{ij})$ and the viability of an offspring with genotype **k** by $v(\mathbf{k})$. We shall use the notation $w(\mathbf{ij}, \mathbf{k})$ as shorthand for $u(\mathbf{ij})\, v(\mathbf{k})$.

Under random mating, the probability that an **i**-female mates with a **j**-male is given by the product of their frequencies, $p_f(\mathbf{i})\, p_m(\mathbf{j})$. Assuming discrete and nonoverlapping generations, the sex-specific genotype frequencies $p'_f(\mathbf{k})$ and $p'_m(\mathbf{k})$ after one round of reproduction and selection are given by the recursions

$$\begin{aligned} S_2 p'_m(\mathbf{k}) &= \frac{1}{\bar{w}} \sum_{\mathbf{ij}} p_f(\mathbf{i}) p_m(\mathbf{j}) T(\mathbf{k}|\mathbf{ij}) s(\mathbf{k}) w(\mathbf{ij}, \mathbf{k}) \\ (1 - S_2) p'_f(\mathbf{k}) &= \frac{1}{\bar{w}} \sum_{\mathbf{ij}} p_f(\mathbf{i}) p_m(\mathbf{j}) T(\mathbf{k}|\mathbf{ij}) [1 - s(\mathbf{k})] w(\mathbf{ij}, \mathbf{k}), \end{aligned} \qquad (3)$$

where

$$\bar{w} = \sum_{\mathbf{k}} \sum_{\mathbf{ij}} p_f(\mathbf{i}) p_m(\mathbf{j}) T(\mathbf{k}|\mathbf{ij}) w(\mathbf{ij}, \mathbf{k}) \qquad (4)$$

is the mean number of surviving offspring, averaged over all pairs, and

$$S_2 = \frac{1}{\bar{w}} \sum_{\mathbf{k}} \sum_{\mathbf{ij}} p_f(\mathbf{i}) p_m(\mathbf{j}) T(\mathbf{k}|\mathbf{ij}) s(\mathbf{k}) w(\mathbf{ij}, \mathbf{k}) \qquad (5)$$

is the sex ratio (proportion males) after viability selection (the *secondary* sex ratio). The *primary* sex ratio (before viability selection) is given by

$$S_1 = \frac{1}{\bar{u}} \sum_{\mathbf{k}} \sum_{\mathbf{ij}} p_f(\mathbf{i}) p_m(\mathbf{j}) T(\mathbf{k}|\mathbf{ij}) s(\mathbf{k}) u(\mathbf{ij}), \qquad (6)$$

where $\bar{u}$ is the mean family size. Where possible, we used analytical methods to analyse eqn (3), but in most cases we had to use numerical iterations. To investigate dependence on initial conditions, for each parameter combination 200 random initial genotype frequencies were sampled.

Table 1 All possible genotypes and their representation in the model

Females				Males			
Genotype			Code (**i**)	Genotype			Code (**i**)
XX	++	*FF*	(0,0,0)	XY	++	*FF*	(1,0,0)
XX	++	FF^D	(0,0,1)	XY	*M*+	*FF*	(1,1,0)
XX	*M*+	FF^D	(0,1,1)	XY	*MM*	*FF*	(1,2,0)
XX	*MM*	FF^D	(0,2,1)	XX	*M*+	*FF*	(0,1,0)
XY	++	FF^D	(1,0,1)	XX	*MM*	*FF*	(0,2,0)
XY	*M*+	FF^D	(1,1,1)	YY	++	*FF*	(2,0,0)
XY	*MM*	FF^D	(1,2,1)	YY	*M*+	*FF*	(2,1,0)
YY	++	FF^D	(2,0,1)	YY	*MM*	*FF*	(2,2,0)
YY	*M*+	FF^D	(2,1,1)				
YY	*MM*	FF^D	(2,2,1)				

Sex ratio selection

To incorporate sex ratio selection in the model, we give all parents the same amount of resources and we let a son cost $0 < c < \infty$ times the (fixed) resource requirements of a daughter. The average cost per offspring is then proportional to $s(\mathbf{ij})c + 1 - s(\mathbf{ij})$, where

$s(\mathbf{ij}) = \sum_{\mathbf{k}} T(\mathbf{k}|\mathbf{ij})s(\mathbf{k})$ is the family sex ratio produced by an **ij** pair. Hence, up to a constant of proportionality, the number of offspring produced by a pair is given by

$$u(\mathbf{ij}) = \frac{1}{s(\mathbf{ij})c + 1 - s(\mathbf{ij})}. \quad (7)$$

If sons are more costly than daughters ($c > 1$), a female-biased sex ratio is selectively favoured. The opposite holds true if daughters are more costly ($c < 1$). Under perfect parental control of the family sex ratio, selection unconstrained by the SD mechanism favours equal allocation of resources (Fisher, 1930), which corresponds to a primary sex ratio of $1/(1 + c)$. We use the Fisherian sex ratio as one of the benchmarks for the sex ratios predicted by our model. In our model, there is no direct parental control of the sex ratio, but rather the genotypes of the offspring determine the sex ratio. Therefore, as a second benchmark we use the optimal sex ratio from the offspring's point of view, when the sex ratio is unconstrained by the SD mechanism. We call this the Triversian sex ratio, since Trivers (1974) first showed that it is given by $1/(1 + \sqrt{c})$ when the relatedness between offspring with the same mother is 1/2. Note that Triversian sex ratios are less biased than Fisherian sex ratios.

Results

No sex ratio selection

As a 'null model' we studied what happens when there are no cost differences between sons and daughters and no survival differences between genotypes [i.e. $w(\mathbf{ij}, \mathbf{k}) =$ constant]. It can be shown analytically (see Appendix) that all equilibria of the system eqn (3) have an even sex ratio, i.e. $S_1^* = S_2^* = \frac{1}{2}$. Numerical iterations showed that the equilibria are reached quite fast, usually within 10 generations (Fig. 2). When introduced at low frequency, F^D and M always persist but never reach appreciable frequencies. Jayakar (1987) studied a model where F^D was introduced into an XY population (without additional autosomal M) and found that F^D always disappears. Apparently, the presence of M is necessary to allow the F^D factor to persist. When M and F^D are introduced at higher frequencies, they can persist at relatively high frequencies, as long as the initial sex ratio does not depart too much from 50 : 50.

Sex ratio selection

Daughters more costly than sons (c < 1)

Under this scenario, male-biased sex ratios are selectively favoured, but, somewhat surprisingly, the equilibrium primary sex ratio was always even. The time required for the system to reach equilibrium depends on the initial genotype frequencies and the strength of selection and may be as long as hundreds of generations when selection is weak (the same applies when $c > 1$, see below). The F^D factor is always removed from the population, regardless of the frequency at which it is introduced (Fig. 3). The logic behind this appears to be that females with an F^D factor always produce at most 50% sons (see Table 2), whereas females without an F^D factor produce at least 50% sons. Since selection favours a male-biased sex ratio, the wild type F allele never has a selective disadvantage (unless the population sex ratio happens to be strongly male-biased, which is at most a transient state) and ultimately goes to fixation. When this happens, the system reduces to a population with a mixture of X, Y and M. It may appear counterintuitive at first sight that such a system cannot produce male-biased sex ratios at equilibrium, since all males with at least two Ms are capable of producing male-biased sex ratios when mated to females without F^D (Table 2). However, in the absence of F^D, YY males are never produced and the same holds true for MM males. A simple argument shows that

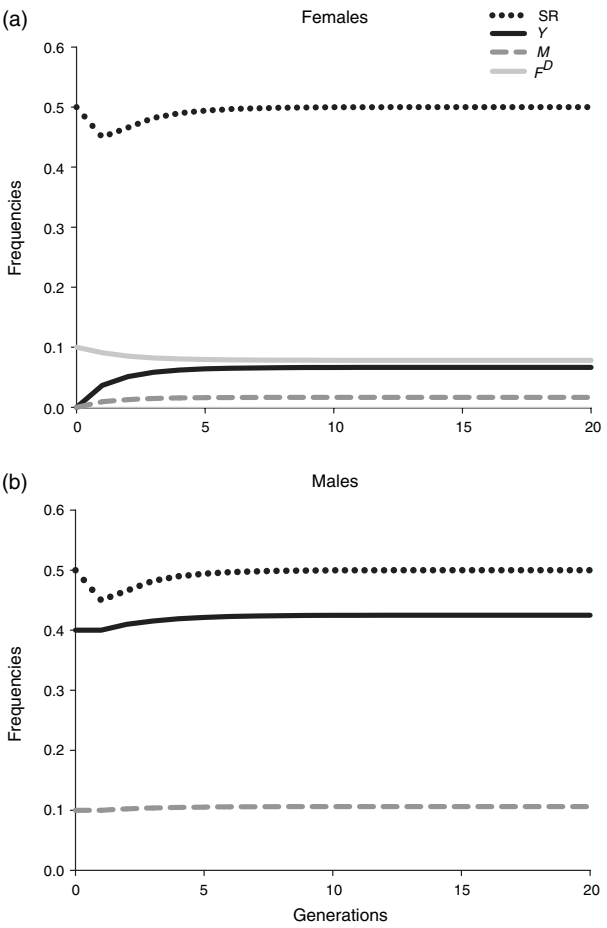

Fig. 2 Example of dynamics of the sex-specific frequencies of the Y chromosome, the autosomal M factor, F^D, and the sex ratio (proportion of sons; SR). Sons and daughters are assumed to be equally costly ($c = 1$). Note that the sex ratio converges to 0.5.

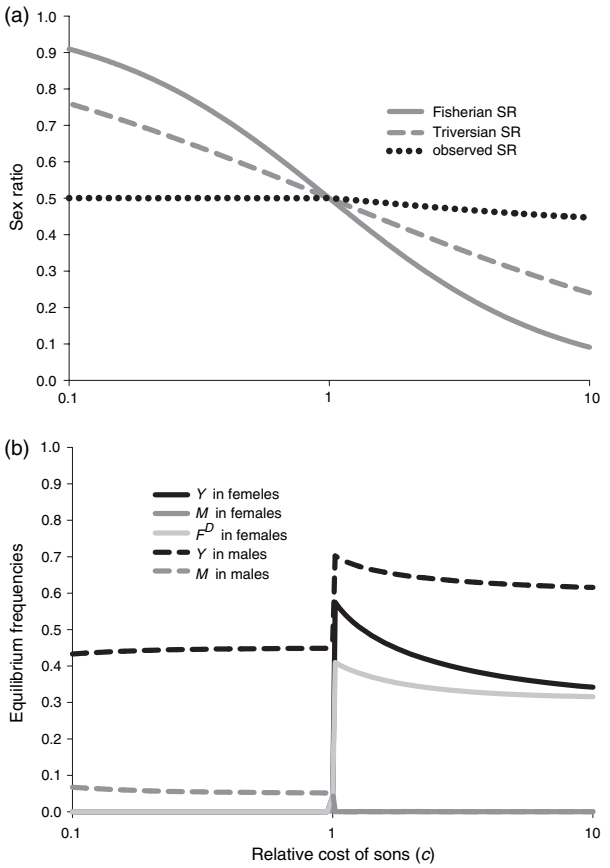

Fig. 3 Equilibrium sex ratio compared to Fisherian and Triversian expectations (a) and equilibrium frequencies of SD factors (b) as a function of the relative cost of sons (c). The outcome depends partly on initial frequencies (see Results), which were here: $p(\mathrm{Y}) = 0.225$, $p(M) = 0.025$, $p(F^{\mathrm{D}}) = 0.025$.

XY/*M*+ males also disappear quickly: XY/*M*+ males produce 75% sons and therefore a family size of

$$\frac{1}{\frac{3}{4}c + \frac{1}{4}} = \frac{4}{3c+1}. \tag{8}$$

XY/++ and XX/M+ males produce 50% sons and a family size of

$$\frac{1}{\frac{1}{2}c + \frac{1}{2}} = \frac{4}{2c+2}. \tag{9}$$

Therefore, in term of family size [see eqn (7)], XY/*M*+ males have a relative advantage to the tune of

$$\frac{2c+2}{3c+1}. \tag{10}$$

For $c < 1$, this advantage is between 1 and 2. On the other hand, the XY/*M*+ males have the disadvantage that only a quarter of their offspring also have the XY/*M*+ genotype. The family size advantage cannot compensate for this and as a consequence the frequency of XY/*M*+ decays at a geometric rate. Thus, the only male genotypes remaining are XY/++ and XX/*M*+, their ultimate frequencies lying on a curve of neutral equilibria (Bull & Charnov, 1977; Jayakar, 1987).

Sons more costly than daughters (c > 1)

Now female-biased sex ratios are expected to be selectively favoured, and this is indeed what we found. The equilibrium sex ratio is always biased towards females and the bias increases with the relative cost of sons, c. For a given c, the equilibrium sex ratio is independent of initial conditions. However, the magnitude of the sex ratio bias is relatively small (<10% from 1 : 1) compared to Fisherian and Triversian optimal sex ratios, even in situations where sons are much more expensive to produce than daughters (Fig. 3).

Surprisingly, only a single *M* can remain in the population. If *M* is introduced at low frequency, it will ultimately disappear. Conversely, if *M* is initially present at a higher frequency than Y, then the latter will disappear. For a given $c > 1$, the equilibrium frequencies of F^{D} and the remaining *M* are independent of the initial conditions. F^{D} never reaches a frequency of 0.5 among females, hence a fully female heterogametic system does not evolve. In fact, with increasing c, the equilibrium F^{D} frequency decreases somewhat (Fig. 3). The explanation seems to be that sex ratio selection maintains polymorphism at the locus with the remaining *M*, due to the fact that heterozygous males produce more daughters than homozygous males (Table 2).

Table 2 Family sex ratios (proportion sons) as a function of maternal (rows) and paternal (columns) genotype

	XY, ++, *FF*	XY, *M*+, *FF*	XY, *MM*, *FF*	XX, *M*+, *FF*	XX, *MM*, *FF*	YY, ++, *FF*	YY, *M*+, *FF*	YY, *MM*, *FF*
XX, ++, *FF*	1/2	3/4	1	1/2	1	1	1	1
XX, ++, FF^{D}	1/4	3/8	1/2	1/4	1/2	1/2	1/2	1/2
XX, *M*+, FF^{D}	3/8	7/16	1/2	3/8	1/2	1/2	1/2	1/2
XX, *MM*, FF^{D}	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
XY, ++, FF^{D}	3/8	3/8	1/2	3/8	1/2	1/2	1/2	1/2
XY, *M*+, FF^{D}	7/16	15/32	1/2	7/16	1/2	1/2	1/2	1/2
XY, *MM*, FF^{D}	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
YY, ++, FF^{D}	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
YY, *M*+, FF^{D}	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
YY, *MM*, FF^{D}	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2

Note that sex ratios produced by mothers with F^{D} are at most 1/2, and those of mothers without F^{D} at least 1/2.

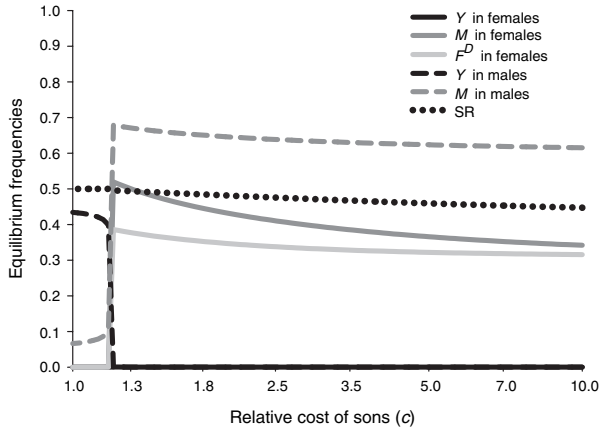

Fig. 4 Equilibrium sex ratio (SR) and equilibrium frequencies of SD factors as a function of the relative cost of sons (c) when relative viability of YY genotypes equals 0.8. Initial allele frequencies: $p(\mathrm{Y}) = 0.225$, $p(M) = 0.025$, $p(F^{\mathrm{D}}) = 0.025$.

Selection against YY

We investigated what happens when YY genotypes have lower survival than other genotypes, which has been offered as an explanation for the scarcity of Y in populations harboring an F^{D} allele (Franco *et al.*, 1982), although direct evidence for lower viability of YY is lacking. The typical outcome of numerical iterations for $c > 1$ is that the Y chromosome disappears from the population and is replaced by autosomal M. When sex ratio selection in sufficiently week (c close to 1) and YY genotypes have sufficiently low survival or for $c < 1$, F^{D} disappears and a stable coexistence of Y and M results (Fig. 4).

Discussion

Sex ratio evolution and constraints on adaptation

We have shown that in the absence of viability differences and cost differences between sons and daughters, the basic three-locus SD system of the housefly always has an even sex ratio at equilibrium (see Appendix). In fact, the analysis shows that under the same assumptions, the result continues to hold true for any number of unlinked SD loci with any number of alleles per locus. Therefore, the result also applies to populations with M factors on multiple autosomes (as have been observed; Wagoner, 1969; Franco *et al.*, 1982; Tomita & Wada, 1989a, personal observations).

Our numerical analysis shows that even when males are 'cheaper' than females, male-biased sex ratios cannot be achieved in equilibrium by the housefly system. Female-biased equilibrium sex ratios are possible, when daughters cost less than sons to produce, but the magnitude of the bias is much smaller than predicted under perfect maternal or offspring control (Fig. 3a). We found this somewhat surprising, since mixtures of genotypes that create strongly biased sex ratios are possible for the housefly system (Table 2) but apparently not stable. A similar lack of flexibility of a genetic SD system in producing biased sex ratios was found by Bull (1983), who studied a one-locus three-allele model, designed to mimic a platyfish SD system, allowing for cost-differences between sons and daughters. Equilibrium sex ratios for this model were biased, but only very weakly so. These results highlight the potential importance of the constraints imposed by genetic mechanisms on the precision and magnitude of adaptation (Shuker & West, 2004).

Offspring sex ratios in natural populations of the housefly have not been studied much, but two studies of several Turkish housefly populations (Çakir & Kence, 1996; Çakir, 1999) found that the vast majority of populations have sex ratios that do not differ significantly from 1 : 1, the few exceptions having slightly male-biased or female-biased sex ratios. Male-biased sex ratios are not predicted by our model; however, it should be noted that very large samples are required to detect weakly biased sex ratios, so more and larger studies are needed to get a reliable picture of housefly sex ratios in the wild.

Maternal-zygote conflict

Werren *et al.* (2002) presented a model that shows how sex ratio selection induces an evolutionary conflict between mothers and their offspring, which in turn may lead to a shift in the SD system. In this model selection for male-biased sex ratios leads to the evolution of female heterogamety by means of a dominant female-determining factor that acts in the zygote, and vice versa that selection for female-biased sex ratios promotes the establishment of a male heterogametic SD system. To some extent this contradicts our results. Although in our model a fully male heterogametic or female heterogametic system never evolves, selection for female-biased sex ratios leads to a system where a large majority of females are heterozygous FF^{D}, whereas males are all homozygous FF, which is close in some sense to a female heterogametic system. The main difference between the two models is that in our model all genes act in the zygote whereas the model of Werren *et al.* (2002) also allows for maternally acting genes to affect the sex of the mother's offspring. In the absence of zygotic SD genes, the maternal genes in Werren *et al.*'s model determine the sex ratio among the mother's offspring, and the result is that the sex ratio evolves towards a Fisherian equilibrium. Since the sex ratio from the offspring's point of view is 'too biased' in this equilibrium (Trivers, 1974), a rare dominant zygotic determiner of the minority sex can invade such a population and in effect establish a new heterogametic SD system. This result is of course limited to situations where the maternal ability to manipulate the sex ratio is sufficiently unconstrained. If genetic or

physiological constraints limit this ability (our model; Pen & Weissing, 2002), then selection may not be able in the long run to produce a sex ratio more biased than the Triversian optimum, in which case a rare dominant zygotic determiner of the rare sex no longer has a selective advantage. Of course one could also argue the other way around and interpret Werren *et al.*'s analysis as providing an evolutionary reason why genetic constraints (e.g. dominant zygotic SD factors) prevent full maternal control of the sex ratio. Interestingly, in the housefly there is clear evidence that maternal genes can affect or even completely determine the sex of the mother's offspring (Vanossi Este & Rovati, 1982; Inoue & Hiroyoshi, 1986; Hilfiker-Kleiner *et al.*, 1994; Schmidt *et al.*, 1997; Dübendorfer & Hediger, 1998), although the latter extreme has only been observed in a laboratory population (Vanossi Este & Rovati, 1982). In flies with the standard XY system, input of maternally produced *F* factor is a necessary condition for female development. It is conceivable that variation in maternally produced *F* can have a quantitative effect on the offspring sex ratio. To determine how this interplay between maternally acting genes and zygotically acting genes affects the co-evolutionary dynamics of SD mechanisms in the housefly remains a theoretical and experimental challenge.

Explaining variability between natural housefly populations

Is sex ratio selection alone sufficient to explain the observed frequencies of *M* and F^D in natural housefly populations? In view of our results this seems unlikely. In most populations with nonstandard SD systems *M* and F^D co-occur, both at high frequencies (Tomita & Wada, 1989a). According to our model (Figs 3 and 4) this should only occur if sons are more costly than daughters and if either YY genotypes are selected against or *M* has a high initial frequency. We already mentioned that there is some evidence that individuals homozygous for Y might have lower fitness (Franco *et al.*, 1982). Occurrence of *M* at high initial frequencies requires, however, presence of additional mechanisms (see below). Most importantly, how likely is it that sons are more costly than daughters in houseflies? Unfortunately, this question is hard to answer at this point due to lack of data. However, since adult females are larger than adult male houseflies (Goulson *et al.*, 1999) and presumably need more food, it seems more likely that daughters, rather than sons, adversely affect family survival, which would make sons the 'cheaper' sex. On the other hand, cost differences are not the only causes of selection for biased sex ratios. Female-biased sex ratios can also be selected for under conditions of inbreeding (Hamilton, 1967) or when females have a greater dispersal tendency than males (Bulmer, 1986; Frank, 1986). We have studied stochastic individual-based simulations of subdivided populations where female-biased sex ratios are selectively favoured (results not shown), and they yielded very similar results as the much simpler cost-based model above, in the sense that male-biased sex ratios never occur at equilibrium and female-biased sex ratios deviate at most only slightly from 50 : 50. There is some evidence that in houseflies local populations might sometimes be small enough to experience some inbreeding (Black & Krafsur, 1986), thus favouring female-biased sex ratios. Variation in local population structure might occur geographically for climatological reasons. Although all this suggests that in the wild the prerequisites might be met to let sex ratio evolution be responsible for the co-occurrence of *M* and F^D at high frequencies, our model cannot explain how initially rare autosomal *M* factors can reach high frequencies in the absence of F^D, as has been observed in several Japanese populations (Tomita & Wada, 1989a), although it is of course possible that frequencies in natural populations are not at equilibrium.

A number of other hypotheses, not mutually exclusive, have been proposed to account for the observed variation in SD systems in field populations of the housefly. The earliest explanations for the emergence of autosomal *M* factors in housefly populations propose that *M* factors 'hitchhike' with genes conferring a fitness benefit. Theoretical models (Bull & Charnov, 1977; Jayakar, 1987) have shown that such hitchhiking may cause transitions between SD mechanisms. Indeed, the first isolation of autosomal *M* factors coincided with the appearance of insecticide resistance in natural populations, as noted by Tomita & Wada (1989a). In some populations, DDT resistance has been shown to be linked with M^{II} or M^{III} (*M* located on the second and third chromosome, respectively; Kerr, 1970; Franco *et al.*, 1982). Geographical clines in *M* frequencies might then be attributed to regional variation in DDT application. However, recent findings shed doubt on the general validity of this hitchhiking hypothesis, since in North American populations no correlation was found between insecticide resistance and the distribution of autosomal *M* factors (Hamm *et al.*, 2005). In addition, the spread of M^X in England (Denholm *et al.*, 1985) is also unlikely to be accounted for by coupling to resistance genes.

Meiotic drive has also been invoked as an explanation for the spread of *M* and F^D. Jayakar (1987) showed with population genetic models that under certain conditions a standard XY system could be replaced by an XX/*M*+ male-heterogametic system if a driving *M* factor is introduced into the ancestral XY population. The XX/*M*+ populations would have male-biased sex ratios allowing the subsequent spread of an F^D factor, ultimately leading to a system with female heterogamety. This explanation cannot be ruled out entirely at the moment; since there is some weak evidence that autosomal *M* factors can sometimes show meiotic drive (Clark, 1999; own observations). However, it is not clear how drive can explain the observed geographical clines.

In our model, we did not consider the interaction between sex ratio selection and other selective forces such as hitchhiking and meiotic drive. Where sex ratio selection alone fails to induce a full shift between different heterogametic SD systems, it seems likely that sex ratio selection in conjunction with other selective forces may easily cause such shifts. A full theoretical analysis of the interaction between sex ratio selection and all possible genotype-specific viability differences in the housefly system would be quite complex. Until more is known about genotype-specific viabilities in the housefly, such analysis is best left to the future. In the mean time, our results including lower fitness of YY genotypes suggest that, even thought detrimental genotypes are removed (as expected: see Bull & Charnov, 1977), final genotype frequencies are affected by the strength of sex ratio selection (Fig. 4).

At the moment it is therefore hard to judge whether sex ratio selection has been an important cause of the remarkable variation in housefly SD mechanisms. However, the housefly can still serve as a useful model organism for experiments on the evolution of sex determination. Our model and future theoretical work will be important for designing and understanding the experiments.

References

Basolo, A.L. 1994. The dynamics of Fisherian sex-ratio evolution: theoretical and experimental investigations. *Am. Nat.* **144**: 473–490.

Basolo, A.L. 2001. The effect of intrasexual fitness differences on genotype frequency stability at Fisherian sex ratio equilibrium. *Ann. Zool. Fenn.* **38**: 297–304.

Beukeboom, L.W., de Jong, T.J. & Pen, I. 2001. Why girls want to be boys. *Bioessays* **23**: 477–480.

Black, W.C. & Krafsur, E.S. 1986. Seasonal breeding structure in housefly, *Musca domestica* L., populations. *Heredity* **56**: 289–298.

Bull, J.J. 1983. *The Evolution of Sex Determining Mechanisms*. Benjamin/Cummings Publishing Co., Menlo Park, CA.

Bull, J.J. & Charnov, E.L. 1977. Changes in the heterogametic mechanism of sex determination. *Heredity* **39**: 1–14.

Bulmer, M.G. 1986. Sex ratio theory in geographically structured populations. *Heredity* **56**: 69–73.

Çakir, S. 1999. Two new sex determining factors (M^V, F^D) in housefly, (*Musca domestica*) pupulations in Turkey. *Turk. J. Zool.* **23**: 73–77.

Çakir, S. & Kence, A. 1996. The distribution of males having XY and XX chromosomes in housefly populations (Diptera: Muscidae) of Turkey. *Genetica* **98**: 205–210.

Carvalho, A.B., Sampaio, M.C., Varandas, F.R. & Klaczko, L.B. 1998. An experimental demonstration of Fisher's principle: evolution of sexual proportion by natural selection. *Genetics* **148**: 719–731.

Caubet, Y., Hatcher, M.J., Mocquard, J.P. & Rigaud, T. 2000. Genetic conflict and changes in heterogametic mechanisms of sex determination. *J. Evol. Biol.* **13**: 766–777.

Charnov, E.L. 1975. Sex ratio selection in an age-structured population. *Evolution* **29**: 366–368.

Charnov, E.L. 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton, NJ.

Clark, M.E. 1999. The Evolution of a neo-Y Chromosome in the Housefly, *Musca domestica*. PhD thesis. University of Houston, Houston, USA.

Conover, D.O. & Vanvoorhees, D.A. 1990. Evolution of a balanced sex ratio by frequency-dependent selection in a fish. *Science* **250**: 1556–1558.

Conover, D.O., Vanvoorhees, D.A. & Ehtisham, A. 1992. Sex ratio selection and the evolution of environmental sex determination in laboratory populations of *Menidia menidia*. *Evolution* **46**: 1722–1730.

Denholm, I., Franco, M.G., Rubini, P.G. & Vecchi, M. 1983. Identification of a male determinant on the X chromosome of housefly (*Musca domestica* L.) populations in South-East England. *Genet. Res.* **42**: 311–322.

Denholm, I., Franco, M.G., Rubini, P.G. & Vecchi, M. 1985. Geographical variation in house-fly (*Musca domestica* L) sex determinants within the British Isles. *Genet. Res.* **47**: 19–27.

Denholm, I., Rubini, P.G., Rovati, C. & Vecchi, M. 1990. Genetic basis of sex determination in two South African strains of house-fly (*Musca domestica* L). *S. Afr. J. Sci.* **86**: 41–43.

Dübendorfer, A. & Hediger, M. 1998. The female-determining gene *F* of the housefly, *Musca domestica*, acts maternally to regulate its own zygotic activity. *Genetics* **150**: 221–226.

Dübendorfer, A., Hediger, M., Burghardt, G. & Bopp, D. 2002. *Musca domestica*, a window on the evolution of sex-determining mechanisms in insects. *Int. J. Dev. Biol.* **46**: 75–79.

Eberhard, W.G. 1980. Evolutionary consequences of intracellular organelle competition. *Q. Rev. Biol.* **55**: 231–249.

Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.

Franco, M.G., Rubini, P.G. & Vecchi, M. 1982. Sex-determinants and their distribution in various populations of *Musca domestica* L. of Western Europe. *Genet. Res.* **40**: 279–293.

Frank, S.A. 1986. The genetic value of sons and daughters. *Heredity* **56**: 351–354.

Goulson, D., Bristow, L., Elderfield, E., Brinklow, K., Parry-Jones, B. & Chapman, J.W. 1999. Size, symmetry, and sexual selection in the housefly, *Musca domestica*. *Evolution* **53**: 527–534.

Hamilton, W.D. 1967. Extraordinary sex ratios. *Science* **156**: 477–488.

Hamm, R.L., Shono, T. & Scott, J.G. 2005. A cline in frequency of autosomal males is not associated with insecticide resistance in house fly (Diptera: Muscidae). *J. Econ. Ent.* **98**: 171–176.

Hilfiker-Kleiner, D., Dübendorfer, A., Hilfiker, A. & Nothiger, R. 1994. Genetic control of sex determination in the germ line and soma of the housefly, *Musca domestica*. *Development* **120**: 2531–2538.

Inoue, H. & Hiroyoshi, T. 1986. A maternal-effect sex-transformation mutant of the housefly, *Musca domestica* L. *Genetics* **112**: 469–482.

Jayakar, S.D. 1987. Some two locus models for the evolution of sex-determining mechanisms. *Theor. Popul. Biol.* **32**: 188–215.

Karlin, S. & Lessard, S. 1986. *Theoretical Studies on Sex Ratio Evolution*. Princeton University Press, Princeton, NJ.

Kerr, R.W. 1970. Inheritance of DDT resistance in a laboratory colony of housefly, *Musca domestica*. *Aust. J. Biol. Sci.* **23**: 377–400.

Kraak, S.B.M. & Pen, I. 2002. Sex-determining mechanisms in vertebrates. In: *Sex Ratios. Concepts and Research Methods* (I. C. W. Hardy, ed.), pp. 158–177. Cambridge University Press, Cambridge.

Marin, I. & Baker, B.S. 1998. The evolutionary dynamics of sex determination. *Science* **281**: 1990–1994.

McDonald, I.C., Evenson, P., Nickel, C.A. & Johnson, O.A. 1978. House fly genetics: isolation of a female determining factor on chromosome 4. *Ann. Ent. Soc. Am.* **71**: 692–694.

Pen, I. & Weissing, F.J. 2002. Optimal sex allocation: steps towards a mechanistic theory. In: *Sex Ratios. Concepts and Research Methods* (I. C. W. Hardy, ed.), pp. 26–45. Cambridge University Press, Cambridge.

Reinhold, K. 1996. Sex-ratio selection with asymmetrical migration of the sexes can lead to an uneven sex ratio. *Oikos* **75**: 15–19.

Schmidt, R., Hediger, M., Nothiger, R. & Dübendorfer, A. 1997. The mutation *masculinizer* (*man*) defines a sex-determining gene with maternal and zygotic functions in *Musca domestica* L. *Genetics* **145**: 173–183.

Shuker, D.M. & West, S.A. 2004. Information constraints and the precision of adaptation: sex ratio manipulation in wasps. *Proc. Natl. Acad. Sci. USA* **101**: 10363–10367.

Tomita, T. & Wada, Y. 1989a. Multifactorial sex determination in natural populations of the housefly (*Musca domestica*) in Japan. *Jpn. J. Genet.* **64**: 373–382.

Tomita, T. & Wada, Y. 1989b. Migration and linkage disequilibrium in local populations of the housefly (*Musca domestica*) in Japan. *Jpn. J. Genet.* **64**: 383–389.

Trivers, R.L. 1974. Parent-offspring conflict. *Am. Zool.* **14**: 249–264.

Vanossi Este, S. & Rovati, C. 1982. Inheritance of the arrhenogenic factor *Ag* of *Musca domestica* L. *Boll. Zool.* **49**: 269–278.

Wade, M.J., Shuster, S.M. & Demuth, J.P. 2003. Sexual selection favors female-biased sex ratios: the balance between the opposing forces of sex-ratio selection and sexual selection. *Am. Nat.* **162**: 403–414.

Wagoner, D.E. 1969. Presence of male determining factors found on three autosomes in house fly, *Musca domestica*. *Nature* **223**: 187–188.

Werren, J.H. & Taylor, P.D. 1984. The effects of population recruitment on sex ratio selection. *Am. Nat.* **124**: 143–148.

Werren, J.H. & Beukeboom, L.W. 1998. Sex determination, sex ratios, and genetic conflict. *Annu. Rev. Ecol. Syst.* **29**: 233–261.

Werren, J.H. & Hatcher, M.J. 2000. Maternal-zygotic gene conflict over sex determination: effects of inbreeding. *Genetics* **155**: 1469–1479.

Werren, J.H., Hatcher, M.J. & Godfray, H.C. J. 2002. Maternal-offspring conflict leads to the evolution of dominant zygotic sex determination. *Heredity* **88**: 102–111.

Wilkins, A.S. 1995. Moving up the hierarchy: a hypothesis on the evolution of a genetic sex determination pathway. *Bioessays* **17**: 71–77.

Appendix

Here we show that without fitness differences (w(**ij**, **k**) = constant), all equilibria of the system eqn (3) produce an even sex ratio. The argument is quite general and holds for SD systems with any number of unlinked loci and any number of alleles per locus.

First we introduce some new notation. Let the sex-specific allele frequencies (of the focal allele) at locus n be denoted by $p_f(n)$ and $p_m(n)$. They are easily calculated from the sex-specific genotype frequencies. Genotype **i** has i_n copies of the focal allele at locus n, hence $i_n/2$ is the relative frequency of the focal allele at locus n for genotype **i**. The frequency of the allele among all females is therefore simply given by

$$p_f(n) = \sum_{\mathbf{i}} p_f(\mathbf{i}) \frac{i_n}{2}. \qquad \text{(A1)}$$

Allele frequencies in males are calculated similarly.

Let p_f^* and p_m^* denote equilibrium frequencies in females and males. Adding the two equations in eqn (3) yields the equilibrium condition

$$S^* p_m^*(\mathbf{k}) + (1 - S^*) p_f^*(\mathbf{k}) = \sum_{\mathbf{ij}} p_f^*(\mathbf{i}) p_m^*(\mathbf{j}) T(\mathbf{k}|\mathbf{ij}), \qquad \text{(A2)}$$

where $S^* = S_1^* = S_2^*$ is the equilibrium sex ratio. Now sum both sides of eqn (A1) over all **k**, weighing each term by $k_n/2$, where k_n is the number of focal alleles at locus n. In view of eqn (A1), this operation transforms the genotype frequencies on the left-hand side of eqn (A2) into the frequencies of the focal allele at locus n:

$$S^* p_m^*(n) + (1 - S^*) p_f^*(n) = \sum_{\mathbf{ij}} p_f^*(\mathbf{i}) p_m^*(\mathbf{j}) \sum_{\mathbf{k}} \tfrac{k_n}{2} T(\mathbf{k}|\mathbf{ij}). \qquad \text{(A3)}$$

Let us first give a heuristic argument why eqn (A3) implies that the equilibrium sex ratio is 1/2. The right-hand side of eqn (A3) is the frequency of the focal allele in the offspring produced by all parents. This ought to be the same as the arithmetic mean of the frequencies in males and females, if mating is at random and segregation is unbiased. In other words: we expect the right-hand side of eqn (A3) to equal $\frac{1}{2}p_m^*(n) + \frac{1}{2}p_f^*(n)$. If this is true, it follows that in equilibrium either $p_m^*(n) = p_f^*(n)$ or $S^* = 1/2$. For a genetic system of sex determination, it is not plausible (although theoretically possible, see Karlin & Lessard, 1986) that the frequency of sex determining factors is, at all loci, the same in both sexes. In fact, we are not aware of any genetic SD system where $p_m^*(n) = p_f^*(n)$ can hold for all alleles at all loci. Accordingly, the sex ratio at equilibrium must always be even in such systems. For example, in the housefly, the frequency of the epistatic F^D allele cannot be the same for females and males, unless the frequency is zero. But if the F^D frequency is zero, then the frequency of M factors cannot be identical in males and females.

Now we shall prove that these heuristic arguments are correct. The rules of Mendelian segregation, as embodied in eqns (1) and (2), imply that

$$\begin{aligned}\sum_{\mathbf{k}} \tfrac{k_n}{2} T(\mathbf{k}|\mathbf{ij}) &= \sum_{\mathbf{k}} \tfrac{k_n}{2} \prod_{l} P(k_l|i_l j_l) \\ &= \sum_{\mathbf{k}} \tfrac{k_n}{2} P(k_n|i_n j_n) \prod_{l\neq n} P(k_l|i_l j_l) \\ &= \sum_{k_n} \tfrac{k_n}{2} P(k_n|i_n j_n) \prod_{l\neq n} \sum_{k_l} P(k_l|i_l j_l) \\ &= \sum_{k_n} \tfrac{k_n}{2} P(k_n|i_n j_n) \\ &= \frac{1}{2} P(k_n = 1|i_n j_n) + P(k_n = 2|i_n j_n) \\ &= \frac{1}{4} i_n + \tfrac{1}{4} j_n.\end{aligned} \tag{A4}$$

The last step follows directly from eqn (2). As a result, the right-hand side of eqn (A3) reduces to

$$\begin{aligned}\sum_{\mathbf{ij}} p^*_{\mathrm{f}}(\mathbf{i}) p^*_{\mathrm{m}}(\mathbf{j}) \sum_{\mathbf{k}} \tfrac{k_n}{2} T(\mathbf{k}|\mathbf{ij}) &= \tfrac{1}{2} \sum_{\mathbf{i}} p^*_{\mathrm{f}}(\mathbf{i}) \tfrac{i_n}{2} + \tfrac{1}{2} \sum_{\mathbf{j}} p^*_{\mathrm{m}}(\mathbf{j}) \tfrac{j_n}{2} \\ &= \tfrac{1}{2} p^*_{\mathrm{f}}(n) + \tfrac{1}{2} p^*_{\mathrm{m}}(n),\end{aligned} \tag{A5}$$

as expected.

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