

Precise, highly female-biased sex ratios in a social spider

Leticia Avilés*, John McCormack, Asher Cutter and Todd Bukowski

Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA

It has been recognized for some time that the risk of producing maleless clutches should select for a lower than binomial variance in the sex ratio of organisms with female-biased sex ratios, small clutches and breeding groups containing the clutch of a single female. However, to date, precise sex ratios have only been reported for organisms with haplodiploid sex determination, a system which allows direct control of the sex of individual offspring. In contrast, under heterogametic sex determination chance is expected to play a crucial role in determining the sex composition of any one family, in particular when males are the heterogametic sex. Here, we present evidence of precise or underdispersed primary sex ratios in the Neotropical social spider *Anelosimus domingo* Levi. We show that this diploid species with male heterogamety has not only beaten the odds of meiosis by producing mostly daughters, but has also attained relative precision in the proportion of sons and daughters produced in any one clutch. The latter finding suggests the existence of mechanisms that allow sorting of the two types of sperm in this spider species.

Keywords: sex-ratio control; sex-ratio bias; heterogametic sex determination; social spiders; *Anelosimus*

1. INTRODUCTION

With heterogametic sex determination, chance is expected to play a crucial role in determining the sex composition of any one family (Williams 1979; Bull & Charnov 1988; Krackow 1995; Hardy 1997). In humans, for instance, whether a daughter or a son is conceived at any fertilization event depends on whether an X- or Y-bearing spermatozoid first happens to encounter the egg. As a result, the sex ratios across families are binomially distributed, with some families having runs of a few sons or a few daughters.

Binomial sex ratios may be problematic in species that, unlike humans, produce highly female-biased sex ratios. In such species, mating typically takes place within local groups which may contain the offspring of a single mother (Hamilton 1967; Charnov 1982; Hardy 1994). If the clutch size is small, then binomial probabilities could result in a sizeable fraction of maleless clutches and, thus, unmated daughters (e.g. Hardy & Cook 1995; Hardy *et al.* 1998). Selection for a lower than binomial variance would therefore be expected (Green *et al.* 1982; Hardy 1992; Nagelkerke & Hardy 1994; Nagelkerke 1996). As predicted, precise or underdispersed sex ratios have been reported for various organisms with such life histories (Green *et al.* 1982; Nagelkerke & Sabelis 1991; Hardy 1992). However, all cases reported to date correspond to systems with haplodiploid sex determination. Haplodiploidy allows direct control of the sex of individual offspring at the time of fertilization (Green *et al.* 1982; Hardy 1992) or of paternal genome loss (Nagelkerke & Sabelis 1991). In contrast, the probabilistic nature of heterogametic sex determination has been thought to represent a serious barrier to direct sex-ratio control, in particular when males are the heterogametic sex (Williams 1979; Bull & Charnov 1988; Krackow 1995; Hardy 1997).

Here, we report on precise sex allocation in a diploid organism with male heterogamety and highly female-biased sex ratios. *Anelosimus domingo* Levi (Levi 1963) is a social spider that inhabits tropical rainforest areas of northern South America. It forms colonies which may contain from a single female plus her offspring to a few thousand individuals (L. Avilés and P. Salazar, unpublished data). The colony members cooperate in building and maintaining a communal nest, capturing prey on which to feed communally and taking care of the offspring (Rypstra & Tiley 1989). A similar level of social behaviour has been described for another 16 tropical and subtropical species belonging to nine genera and six spider families (for a review, see Avilés 1997). A feature common to most of these species is a highly female-biased sex ratio which in several cases has been demonstrated to represent an overproduction of female embryos (Avilés & Maddison 1991; Rowell & Main 1992; Avilés *et al.* 1999). This sex ratio bias appears associated with a highly subdivided population structure—field and molecular evidence from several species with biased sex ratios has suggested that colony members remain together throughout their lives and mate with each other in order to produce successive generations (reviewed in Avilés 1997). Theoretical studies (Avilés 1986, 1993; Frank 1987) have suggested that selection acting both within and between the isolated colony lineages may be responsible for the equilibrium sex ratio in these spiders.

The sex determination system in spiders is expected to yield equal numbers of sons and daughters as the outcome of meiosis and fertilization. In most species, males have two non-homologous X chromosomes, i.e. X_1X_2O , which segregate together to produce sperm with and without the sex chromosomes. Females have two members of each chromosome type, i.e. $X_1X_1X_2X_2$, and produce gametes with one member of each pair (White 1973). When a sperm lacking the sex chromosomes fertilizes an egg, a son is produced. It remains to be

* Author for correspondence (laviles@u.arizona.edu).

Table 1. *Anelosimus domingo* egg sacs scored for their primary sex ratio

(The overall proportion of males observed (0.097) was used to calculate the expected number of males in each sac given an exact ratio (i.e. embryos scored \times 0.097). The observed variance in the number of males per sac (0.47) was calculated as the sum of the squared deviations/(number of sacs $-$ 1). PQ, Puerto Quito, 0°6' N, 79°5' W, Pichincha Province, Ecuador; Y, Yasuni National Park, 0°41' S, 76°24' W, Napo Province, Ecuador; JS, Jatun Sacha Biological Station, 1°4' S, 77°36' W, Napo Province, Ecuador.)

sac	location/colony	total embryos	total scored	observed number of males	expected number of males	deviation from expected
1	PQ 1.1	13	12	1	1.16	-0.16
2	PQ 1.2	16	13	1	1.26	-0.26
3	PQ 1.2	14	13	1	1.26	-0.26
4	PQ 1.2	15	11	1	1.06	-0.06
5	PQ 1.1	16	10	0	0.97	-0.97
6	PQ 1.2	16	15	3	1.45	1.55
7	Y 1	11	8	0	0.77	-0.77
8	Y 2	9	7	0	0.68	-0.68
9	Y 2	10	9	1	0.87	0.13
10	JS 21	16	15	1	1.45	-0.45
11	JS 21	18	16	2	1.55	0.45
12	JS 21	17	11	2	1.06	0.94
13	JS 3	22	21	2	2.03	-0.03
14	JS 3	17	13	1	1.26	-0.26
15	JS 3	16	12	2	1.16	0.84
total		226	186	18	—	—

discovered how the 1:1 odds of meiosis are beaten in the various social spiders and the one solitary spider (Gunnarsson & Andersson 1992) reported to have a primary sex-ratio bias.

2. METHODS

We prepared 226 out of 232 early developing embryos contained in 15 egg sacs from six *A. domingo* colonies (table 1). The embryos were prepared for chromosome scoring using the technique described by Avilés & Maddison (1991). *A. domingo* females have 24 and males 22 chromosomes (20 autosomes plus four and two sex chromosomes, respectively), so that the sex of an embryo can be determined from its chromosome number (figure 1). The sex ratio of individual clutches can be estimated since the eggs of a clutch are contained within a sealed sac. Individual embryo preparations were examined under \times 1000 magnification and scored blindly with respect to the colony and egg sac they belonged to. Each preparation was scored by two independent scorers. Discrepancies, of which there were 7% after the first round of scores, were resolved by a third scorer who was unaware of the scores of the other two. Four sacs (sacs 1–4, table 1) had been scored for a previous study when the existence of precise sex ratios was not yet suspected (Avilés & Maddison 1991).

We used a Monte Carlo test that allows for incompletely sexed clutches (Avilés *et al.* 1999) in order to compare the variance in the observed number of males per sac with the variance expected given three alternative hypotheses: binomial sex ratios, an exact proportion of males per sac and at least one male per sac. For each hypothesis tested we ran 10 000 Monte Carlo replicates in order to obtain a distribution of the expected variances given the overall proportion of males observed (ρ), the number of egg sacs sampled (n) and the number of embryos present (k_i) and scored (s_i) within each sac (for sacs $i = 1$ to $i = n$). In the actual data and the simulations, we calculated the variance in the number of males per sac by the mean squared error around the expected

number of males in a sac given an exact ratio (table 1) (see also Green *et al.* 1982; Nagelkerke & Sabelis 1991).

Each Monte Carlo replicate simulated the production of n sacs each with a number of males and females adding up to k_i . For the binomial test, we produced males and females in each sac with probabilities ρ and $1 - \rho$, respectively. For the exact ratio test, we produced $k_i\rho$ males and $k_i(1 - \rho)$ females in each sac rounded to the nearest integer. We produced males and females in each sac for the at-least one male test with probabilities ρ and $1 - \rho$, respectively as in the binomial test, but then replaced the last female by a male if no males had been produced. The final step in all three tests involved sampling s_i embryos per sac without replacement in order to accommodate incompletely sexed clutches.

3. RESULTS

We found that egg sacs which contained from nine to 22 embryos had on average one male for every 9.3 females (or a proportion of 0.097 males) (table 1). There was no significant heterogeneity for the sex ratio between the three localities (G -test, $\chi^2 = 1.32$, d.f. = 2 and $p = 0.52$) or between the egg sacs sampled (G -test, $\chi^2 = 8.38$, d.f. = 14 and $p = 0.87$), so we considered them together for subsequent analyses.

We found that the observed variance in the number of males per sac was significantly smaller than expected under binomial probabilities, 0.47 versus an expected 1.16 ($p = 0.02$ replicates with equal or lower variance than the observed variance) (figure 2). Among the sacs for which more than three-quarters of the embryos were sexed, all contained at least one male and only one contained more than two males. *A. domingo* therefore appears to allocate males to its clutches with significantly greater precision than expected by chance.

Our data are consistent with the at-least one male hypothesis of sex-ratio control (expected variance under

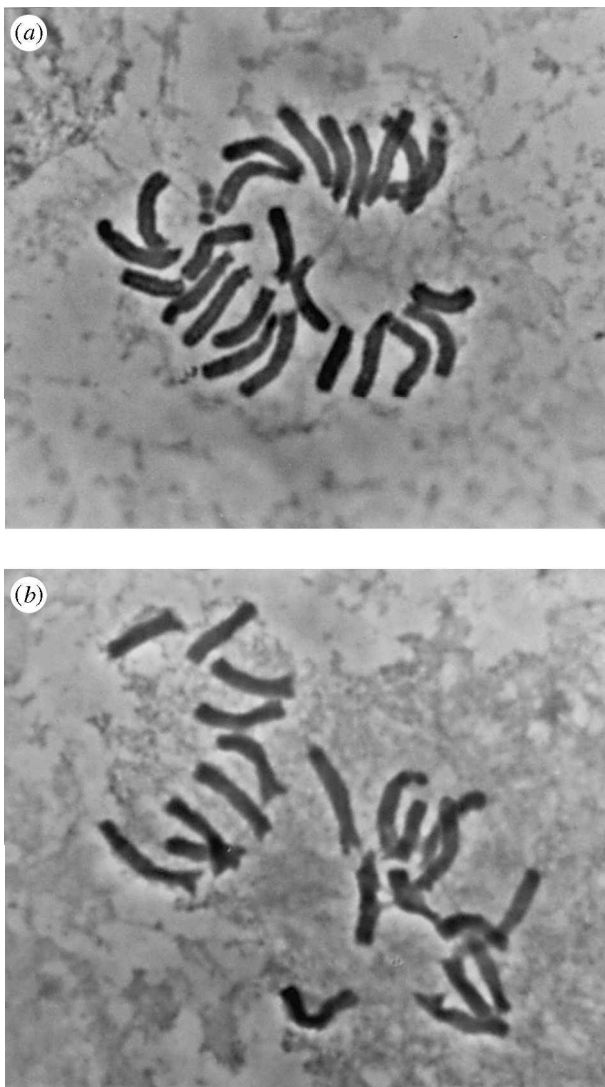


Figure 1. *A. domingo* cytological preparations showing (a) 24 chromosomes for a female embryo, and (b) 22 chromosomes for a male embryo.

the at-least one male hypothesis = 0.9, $p = 0.16$ replicates with equal or lower variance than the observed variance), but not with the exact ratio hypothesis (expected variance under the exact ratio hypothesis = 0.31, $p = 0.045$ replicates with equal or greater variance than the observed variance). However, only one sac (sac 6 with three males) clearly violated the rule of an exact ratio. Rather than representing lack of precision, this sac could represent an individual with a tendency to produce less biased sex ratios. If this sac is excluded from the analysis, there is a much closer match between the observed (0.32) and expected variances (0.24) under the exact ratio assumption ($p = 0.19$ replicates with equal or greater variance than the observed variance). Further studies are thus needed to discriminate more conclusively between our two hypotheses of precise sex-ratio control (with our data, our power to reject the at-least one male hypothesis were the exact ratio hypothesis true is *ca.* 40%).

It should be noted that, out of the 232 embryos contained in the sacs, none were undeveloped or appeared damaged thus ruling out developmental mortality as the proximate cause of the observed bias.

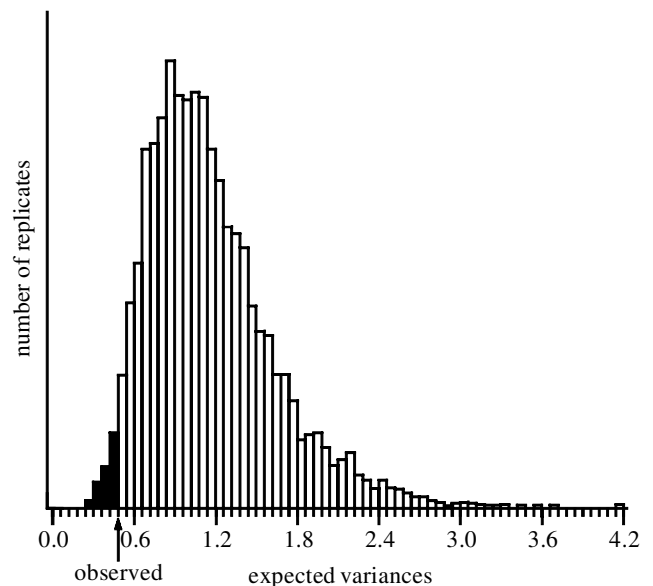


Figure 2. Distribution of the expected sex-ratio variances obtained from 10 000 Monte Carlo replicates under the null hypothesis of binomial sex ratios. The observed variance in the *A. domingo* egg sacs sampled (0.47) is marked with an arrow. The fraction of replicates with equal or lower variance than the observed variance ($p = 0.02$) is coloured in black (note that each division of the x-axis in the figure has a width of 0.06). The sex-ratio variances in the simulations were calculated as described in table 1 for the actual data.

4. DISCUSSION

To our knowledge, this is the first reported case of underdispersed sex ratios in a diplo-diploid organism with male heterogamety. Previously reported cases have involved haplodiploid systems where the sex of individual offspring can be directly controlled by whether or not the egg is fertilized (Green *et al.* 1982; Hardy 1992) or the paternal genome is eliminated (Nagelkerke & Sabelis 1991). In contrast, the probabilistic nature of heterogametic sex determination has been considered a serious barrier to sex-ratio control (Williams 1979; Bull & Charnov 1988; Krackow 1995; Hardy 1997). Recent studies have started to challenge this view. For instance, the repeated derivation of highly female-biased sex ratios in social spiders (reviewed in Avilés 1997) suggests that even diplo-diploid organisms may respond readily to selective pressures for biasing the sex ratio. In vertebrates, where parental condition or rank may influence the fitness of daughters and sons, evidence is beginning to accumulate that individually adaptive biases may evolve (e.g. Austad & Sunquist 1986; Cassinello & Gomendio 1996; Svensson & Nilsson 1996; Heinsohn *et al.* 1997; Komdeur *et al.* 1997; Creel *et al.* 1998; Kilner 1998; Nager *et al.* 1999; Sheldon *et al.* 1999). In birds, in particular, where females are the heterogametic sex, it now does appear that control of meiosis in biasing the sex ratio may be possible (e.g. Svensson & Nilsson 1996; Heinsohn *et al.* 1997; Komdeur *et al.* 1997; Kilner 1998; Nager *et al.* 1999; Sheldon *et al.* 1999). However, in species with male heterogamety it is usually assumed that costly post-fertilization methods, such as differential abortion or infanticide, may be the only available means of sex-ratio control

(Krackow 1995; Hardy 1997). The difficulty of sorting the two types of sperm has continued to appear insurmountable, even for humans applying artificial methods of sperm selection (e.g. Sills *et al.* 1998).

The mechanism used by *A. domingo* and other social spiders to bias the sex ratio remains a mystery. In *Anelosimus eximius*, a close relative of *A. domingo*, spermatids with and without the sex chromosomes are present in equal numbers during the final phases of spermatogenesis, but the sex ratio among early developing embryos is already biased (Avilés & Maddison 1991). This suggests that, at least in this species, the biasing mechanism acts post-meiotically but prior to or during fertilization. The absence of undeveloped or damaged embryos in the present study clearly rules out developmental mortality as the cause of *A. domingo*'s bias. Furthermore, given that spiders fertilize their eggs as they pass through the oviduct on their way to being laid (Foelix 1996), sperm sorting seems to be the only explanation for the observed precision.

It should be noted that precision in sex allocation is not a necessary consequence of highly biased sex ratios in spiders or other organisms (Hardy 1992; Avilés *et al.* 1999). In *Stegodyphus dumicola*, a spider species of a similar level of sociality as *A. domingo*, the primary sex ratio was found to be female biased but binomially distributed across clutches (Avilés *et al.* 1999). A crucial difference between these two species is clutch size, which ranges from nine to 22 in *A. domingo* (table 1) versus 53–100 in *S. dumicola* (Avilés *et al.* 1999). Precise sex ratios become critical when the clutch size is small and mating typically takes place within local groups which may contain the clutch of a single female (Green *et al.* 1982; Nagelkerke & Hardy 1994). In such cases, precise sex allocation ensures that at least one male will be available for fertilizing the daughters while allowing for more extreme biases than would otherwise be possible. Consistent with this observation (although cause and effect cannot be currently distinguished), *A. domingo*'s sex ratios are more acutely biased than those of *S. dumicola*. In *S. dumicola*, with a proportion of 0.17 males and a smallest clutch size of 53, the probability that no males will be produced in a clutch just by chance is 0.00005. In the absence of precise sex-ratio control, maleless clutches in *A. domingo* would be produced with probabilities ranging from 0.40 to 0.11 for clutches of nine to 22 offspring and a male proportion of 0.097. As single-female nests are not an uncommon occurrence in *A. domingo* (L. Avilés and P. Salazar, unpublished data), such probabilities should represent a strong selective pressure for precise sex-ratio control.

In summary, our results suggest that, contrary to common belief (Williams 1979; Bull & Charnov 1988), even systems with chromosomal sex determination and male heterogamety may respond to strong selective pressures by evolving mechanisms for controlling the sex of individual offspring.

The specimens were collected in Ecuador with permission from the Instituto Ecuatoriano Forestal y de Areas Naturales y Vida Silvestre. We thank the Fundación Jatun Sacha and the Department of Biology of the Pontificia Universidad Católica del Ecuador for logistic support and laboratory facilities in Ecuador. We thank J. Cassinello, A. Danielson-Francois, T. Erez, M.

Kidwell, K. S. Powers and two anonymous reviewers for comments on the manuscript. This work was supported by the USA National Science Foundation (DEB grant 9707474 to L.A.) and the University of Arizona (Honors Center small grant to J.M.).

REFERENCES

- Austad, S. N. & Sunquist, M. E. 1986 Sex-ratio manipulation in the common opossum. *Nature* **324**, 58–60.
- Avilés, L. 1986 Sex-ratio bias and possible group selection in the social spider *Anelosimus eximius*. *Am. Nat.* **128**, 1–12.
- Avilés, L. 1993 Interdemic selection and the sex ratio: a social spider perspective. *Am. Nat.* **142**, 320–345.
- Avilés, L. 1997 Causes and consequences of cooperation and permanent sociality in spiders. In *The evolution of social behavior in insects and arachnids* (ed. J. Choe & B. Crespi), pp. 476–497. Cambridge University Press.
- Avilés, L. & Maddison, W. P. 1991 When is the sex ratio biased in social spiders?: chromosome studies of embryos and male meiosis in *Anelosimus* species (Araneae, Theridiidae). *J. Arachnol.* **19**, 126–135.
- Avilés, L., Varas, C. & Dyreson, E. 1999 Does the African social spider *Stegodyphus dumicola* control the sex of individual offspring? *Behav. Ecol. Sociobiol.* **46**, 237–243.
- Bull, J. J. & Charnov, E. L. 1988 How fundamental are Fisherian sex ratios? *Oxf. Surv. Evol. Biol.* **5**, 98–135.
- Cassinello, J. & Gomendio, M. 1996 Adaptive variation in litter size and sex ratio at birth in a sexually dimorphic ungulate. *Proc. R. Soc. Lond. B* **263**, 1461–1466.
- Charnov, E. L. 1982 *The theory of sex allocation*. Princeton University Press.
- Creel, S., Creel, N. M. & Monford, S. L. 1998 Birth order, estrogens and sex-ratio adaptation in African wild dogs (*Lycan pictus*). *Anim. Rep. Sci.* **53**, 315–320.
- Foelix, R. 1996 *The biology of spiders*. Oxford University Press.
- Frank, S. A. 1987 Demography and sex ratio in social spiders. *Evolution* **41**, 1267–1281.
- Green, R. F., Gordh, G. & Hawkins, B. 1982 Precise sex ratios in highly inbred parasitic wasps. *Am. Nat.* **120**, 653–665.
- Gunnarsson, B. & Andersson, A. 1992 Skewed primary sex ratio in the solitary spider *Pityohyphnates phrygianus*. *Evolution* **46**, 841–845.
- Hamilton, W. D. 1967 Extraordinary sex ratios. *Science* **156**, 477–488.
- Hardy, I. C. W. 1992 Non-binomial sex allocation and brood sex ratio variances in the parasitoid Hymenoptera. *Oikos* **65**, 143–158.
- Hardy, I. C. W. 1994 Sex ratio and mating structure in the parasitoid Hymenoptera. *Oikos* **69**, 3–20.
- Hardy, I. C. W. 1997 Possible factors influencing vertebrate sex ratios: an introductory overview. *Appl. Anim. Behav. Sci.* **51**, 217–241.
- Hardy, I. C. W. & Cook, J. M. 1995 Brood sex ratio variance, developmental mortality and virginity in a gregarious parasitoid wasp. *Oecologia* **103**, 162–169.
- Hardy, I. C. W., Dijkstra, L. J., Gillis, J. E. M. & Luft, P. A. 1998 Patterns of sex ratio, virginity and developmental mortality in gregarious parasitoids. *Biol. J. Linn. Soc.* **64**, 239–270.
- Heinsohn, R., Legge, S. & Barry, S. 1997 Extreme bias in sex allocation in *Ecdectus* parrots. *Proc. R. Soc. Lond. B* **264**, 1325–1329.
- Kilner, R. 1998 Primary and secondary sex ratio manipulation by zebra finches. *Anim. Behav.* **56**, 155–164.
- Komdeur, J., Daan, S., Tinbergen, J. & Mateman, C. 1997 Extreme adaptive modification in sex ratio of the Seychelles warbler's eggs. *Nature* **385**, 522–525.
- Krackow, S. 1995 Potential mechanism for sex ratio adjustment in mammals and birds. *Biol. Rev.* **70**, 225–241.

- Levi, H. W. 1963 The American spiders of the genus *Anelosimus* (Araneae, Theridiidae). *Trans. Am. Microsc. Soc.* **82**, 30–48.
- Nagelkerke, C. J. 1996 Discrete clutch sizes, local mate competition, and the evolution of precise sex allocation. *Theor. Popul. Biol.* **49**, 314–343.
- Nagelkerke, C. J. & Hardy, I. C. W. 1994 The influence of developmental mortality on optimal sex allocation under local mate competition. *Behav. Ecol.* **5**, 401–411.
- Nagelkerke, C. J. & Sabelis, M. W. 1991 Precise sex-ratio control in the pseudo-arrhenotokous phytoseiid mite *Typhlodromus occidentalis* Nesbitt. In *The acari: reproduction, development, and life history strategies* (ed. R. Schuster & P. W. Murphy), pp. 193–207. London: Chapman & Hall.
- Nager, R. G., Monaghan, P., Griffiths, R., Houston, D. C. & Dawson, R. 1999 Experimental demonstration that offspring sex ratio varies with maternal condition. *Proc. Natl Acad. Sci. USA* **96**, 570–573.
- Rowell, D. M. & Main, B. Y. 1992 Sex ratio in the social spider *Diaea socialis* (Araneae: Thomisidae). *J. Arachnol.* **20**, 200–206.
- Rypstra, A. L. & Tirey, R. S. 1989 Observations on the social spider *Anelosimus domingo* (Araneae, Theridiidae), in southwestern Peru. *J. Arachnol.* **17**, 368–371.
- Sheldon, B. C., Andersson, W., Griffith, S. C., Örnborg, J. & Sendecka, J. 1999 Ultraviolet colour variation influences blue tit sex ratios. *Nature* **402**, 874–877.
- Sills, E. S., Kirman, I., Thatcher III, S. S. & Palermo, D. G. 1998 Sex-selection of human spermatozoa: evolution of current techniques and applications. *Arch. Gynecol. Obstet.* **261**, 109–115.
- Svensson, E. & Nilsson, J. 1996 Mate quality affects offspring sex ratio in blue tits. *Proc. R. Soc. Lond. B* **263**, 357–361.
- White, M. J. D. 1973 *Animal cytology and evolution*. Cambridge University Press.
- Williams, G. C. 1979 The question of adaptive sex ratio in outcrossed vertebrates. *Proc. R. Soc. Lond. B* **205**, 567–580.