

Evidential segregation analysis for offspring sex ratio in rabbit and sheep populations

Joaquim Casellas^{a,*}, Marta Vázquez-Gómez^b, Samir Id-Lahoucine^c, Melani Martín de Hijos-Villalba^a

^a Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, 08193, Bellaterra, Spain

^b Sorbonne Université, INSERM, Nutrition and Obesity: Systemic Approach Research Group, Nutriomics, Paris, F-75013, France

^c Department of Animal and Veterinary Sciences, Scotland's Rural College, Easter Bush Campus, Midlothian, EH25 9RG, United Kingdom

HIGHLIGHTS

- Evidential inference provided a robust paradigm to analyze quantitative traits in livestock populations.
- Segregation analysis could be effectively implemented on offspring sex ratio whatever the origin of the mutation.
- Offspring sex ratio was significantly influenced by inbreeding, dam age, year and season of birth in rabbit and sheep populations.
- A major mutation increasing the percentage of daughters was revealed in the Ripollesa sheep breed.

ARTICLE INFO

Keywords:

Evidential statistics
Inbreeding
Logistic regression
Mutation
Sex ratio

ABSTRACT

Offspring sex ratio has been found to be altered by environmental and genetic distortions in multiple species, against the Mendelian inheritance rules. However, little is known in livestock populations where it is essential to validate whether a polymorphic major gene with relevant effects may segregate in the target population. However, the current analytical tool (model *FREQ*) cannot handle new mutations in non-founder individuals, reducing the chance of detecting them. Our new analytical approach aimed to overcome this limitation in the context of evidential inference, a statistical framework based on the likelihood function as a robust objective measure of the strength of statistical evidence without variation from the sample size. Two field data sets from sheep and rabbit populations were used. Models evaluated environmental and inbreeding effects in both species. Our new approach assumed that the mutation primarily arose in an individual of the analyzed data set (model *MUTj*). Each sire was individually analyzed to determine the most plausible source for the new mutation, if any. The likelihood ratio (*LR*) against a reference parametrization without mutations (model *NULL*) was used to test the statistical relevance of systematic effects ($LR \geq 8$) and models ($LR \geq 32$). Both species revealed relevant departures for offspring sex ratio along the analyzed time frame with strong evidence for the year ($LR = 1.4 \times 10^9$ in Ripollesa sheep and $LR = 85.7$ in MARET rabbits) and season ($LR = 12.6$ in MARET rabbits), although with a fluctuating pattern. The age of the dam reported weak evidence in both species ($LR < 4$). Inbreeding had a relevant linear impact on both sheep ($LR = 60.7$) and rabbit ($LR = 780.4$) populations, whereas the quadratic effect only showed strong evidence in MARET rabbits ($LR = 8.3$). Regarding the segregation analysis, most models showed an almost homozygous pattern of weak or lacking evidence of new mutations influencing offspring sex ratio. The only species showing strong evidence for the *MUTj* model ($LR > 32$) was the Ripollesa sheep, with a ram three to six generations from the founders as the most likely source for a new mutation increasing the odds of daughters. The additive genetic effect of this model for the mutant allele also had strong evidence ($LR = 1,195$). Therefore, the *MUTj* parametrization can be a valuable analytical tool to check for the possibility of new mutations along the pedigree files, not only before the founders.

* Corresponding author.

E-mail address: joaquim.casellas@uab.cat (J. Casellas).

<https://doi.org/10.1016/j.livsci.2023.105354>

Received 9 January 2023; Received in revised form 4 September 2023; Accepted 6 October 2023

Available online 7 October 2023

1871-1413/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Chromosomal mechanisms for sex determination were independently proposed more than a century ago (Stevens, 1905; Wilson, 1905). In almost every mammalian species, sex is determined by the differential inheritance of sex chromosomes (*i.e.*, X and Y chromosomes), with daughters inheriting two X chromosomes and sons inheriting both an X and a Y. According to Mendel's (1866) inheritance rules, this would anticipate a 1:1 sex ratio in the offspring of each sire, although with substantial variability inherent to the binomial distribution pattern of this biological outcome (Toro et al., 2006). However, both environmental (King, 1927; Trivers and Willard, 1973; Roche et al., 2006) and genetic distortions (King, 1918; Weir, 1976; de la Casa-Esperón et al., 2000) have been reported in multiple species. There exist some mice strains with systematic departures in offspring sex ratio (Cook and Vlcek, 1961; Schlager and Roderick, 1968; Casellas et al., 2010). This opens the door to scanning for genetic mutations affecting the sex ratio in livestock, although little is known about this topic in domestic species (Kennedy and Moxley, 1978; Skjervold, 1979; Skjervold and James, 1979).

Genetic laboratory technologies allow genome-wide screening for polymorphisms at a reasonable cost. However, prior to this economic investment, it is crucial to validate whether a polymorphic major gene with relevant effects on the phenotype of interest may be segregating in the target population. This preliminary survey could be carried out through segregation analysis, as originally developed by Elston and Stewart (1971) and Morton and Maclean (1974) in humans, and later adapted to livestock by Janss et al. (1995). This analytical approach explored field and pedigree data and has been applied to livestock (Janss et al., 1997; Walling et al., 2002; Argente et al., 2003), pet (Janutta et al., 2006) and laboratory species (Casellas et al., 2010), where some major mutations were suggested. It is important to note that the standard parametrization assumed a single mutation that arose some time ago in the population and currently segregated among founder individuals of the analyzed data set at a given frequency. Although this could be viewed as a reasonable assumption, the emergence of new mutations in non-founder individuals could not be properly accommodated unless they arose in a founder individual itself. This limitation precluded a proper analysis of all relevant mutations segregating in the population (*i.e.*, both arising before and after founder individuals) and reduced the chance of detecting them.

Segregation analyses in livestock populations have been previously implemented through the two main statistical frameworks of inference, frequentist (van Arendonk et al., 1989; Knott et al., 1992) and Bayesian (Janss et al., 1995, 1997; Casellas et al., 2010). Both rested on solid decision-theoretic foundations. The frequentist null hypothesis was rejected depending on a *p*-value falling below a fixed significance level that strictly controlled type-I error (Bickel, 2012). Bayesian posterior probabilities led to a measure of belief regarding how much one would wager on its truth (*i.e.*, prior knowledge) given the available data and model (Jeffreys, 1948). Attempts have been made to adapt them to scenarios looking for the strength of evidence favoring one hypothesis over another rather than rejecting one hypothesis in a black-or-white choice. However, those attempts were controversial and highlighted evidential statistics as an appealing alternative focused on the objective quantification of statistical evidence (Edwards, 1972). This principle stated that all the statistical evidence was contained in the likelihood function once the data and the analytical model were accounted for. Within this context, the likelihood function must be viewed as a robust measure of the strength of statistical evidence that did not vary from one researcher to another (objectivity criterion) and has the same practical interpretation whatever the sample size of the data set (interpretability criterion; Bickel, 2012).

This research focused on generalizing segregation analysis to new mutations arising in founder individuals and subsequent generations within the context of evidential inference (Bickel, 2012). The developed

analytical approach for sex ratio data was tested in two different livestock data sets such as sheep and rabbit populations. Moreover, model fit was compared to the standard segregation analysis approach (Janss et al., 1995) to elucidate the most plausible source for new mutations, if any (*i.e.*, the new mutation appeared (1) before founder individuals, or (2) from founders onward).

2. Materials and methods

Animal Care and Use Committee approval was not necessary for this study because analyses were performed on existing field data obtained under standard farm management.

2.1. Sex data from livestock populations

The *Servei de Granges i Camps Experimentals* of the Universitat Autònoma de Barcelona (Bellaterra, Spain) has kept a sheep flock of ~100 Ripollesa ewes since its foundation in 1986. It is a medium-sized sheep breed (rams, 75 to 90 kg of live weight; ewes, 45 to 65 kg) characterized by white animals with black or dark brown marks on head and legs, and semi-fine wool with closed fleece. The Ripollesa breed is native from Catalonia (a north-east region of Spain) and should be considered as a representative example of the meat-type sheep industry from the Mediterranean basin, producing *pascual*-type lambs (22 to 24 kg of live weight at slaughter; Esquivelzeta et al., 2011). The analyses included sex data at birth (both born alive and dead) from 3731 lambs (males, 50.3 %) born between 1988 and 2022 from 49 sires and 561 ewes. Each ram contributed between two and 229 lambs (mean, 76.1 lambs; Fig. 1a) distributed between one and four lambing seasons. This flock was maintained under standard semi-intensive management practices, with lambings concentrated in late summer and fall until 2006

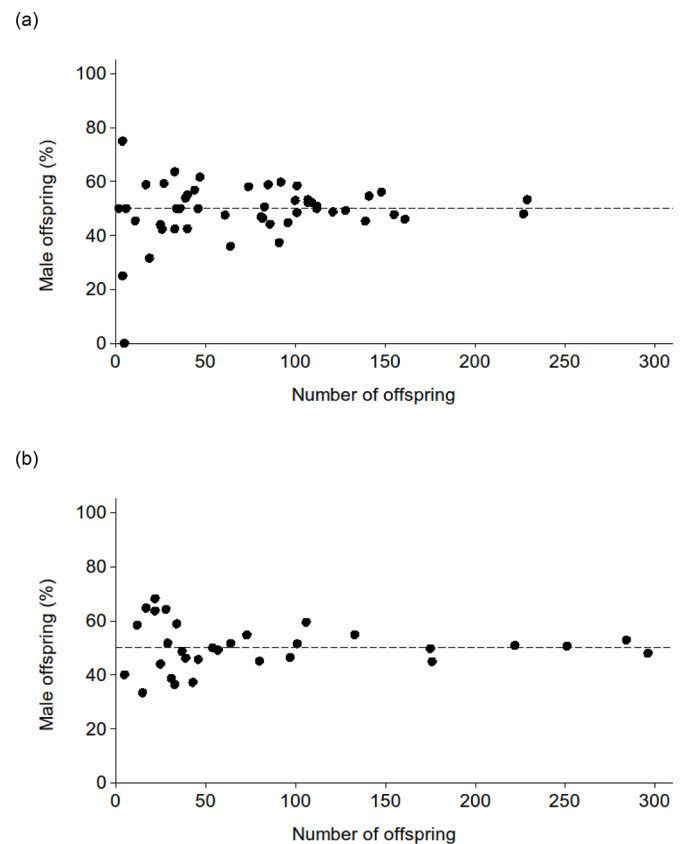


Fig. 1. Distribution of the offspring sex ratio against the number of offspring for each sire of the Ripollesa sheep breed (a) and the MARET rabbit population (b).

and in spring from 2008 onward (no lambings in 2007).

On the other hand, the MARET rabbit population was founded between 1992 and 1999 from four unrelated rabbit farms located in the Girona province (Catalonia, Spain) that contributed with one doe and four bucks (Casellas et al., 2011). This rabbit population was maintained under standard farm conditions (Sant Gregori, Spain) to produce rabbits for the slaughterhouse. Its data set contained sex data from 2607 rabbits at weaning (males, 50.2 %) from 400 litters born between 2002 and 2022. The pedigree included 36 bucks and 39 does, where 31 males sired between five and 296 offspring with relevant sex data (Fig. 1b). The remaining five bucks contributed relevant pedigree relationships, although without available offspring sex data.

All relevant productive and reproductive data were systematically registered in both livestock populations by the stockbreeders who made it available to this study.

2.2. Analysis of binomial offspring sex data

Sex is determined by the sex chromosome inherited from the sire as a dichotomous variable with two possible outcomes, male and female, although some additional genetic and environmental distortion factors have been reported (King, 1918, 1927; Weir, 1976; Roche et al., 2006; de la Casa-Esperón et al., 2000). These factors may influence offspring sex ratio at several biological levels (e.g., fertilization success of X- and Y-bearing gametes [Sweeney and Barr, 1978], differential implantation [Cameron, 2004] and differential survival during embryo or fetal and neonatal periods [McMillen, 1979]) and cause departures from the expected 1:1 ratio at different stages between fertilization (primary sex ratio), birth (secondary sex ratio; Berry et al., 2011), at puberty or among sexually mature individuals (tertiary sex ratio), and among individuals that can no longer expect to produce offspring (quaternary sex ratio; Ancona et al., 2017). Within this context, the offspring sex ratio can be appropriately analyzed based on each sire and the sex of its progeny (Toro et al., 2006). Sex of a given offspring i can be viewed as a phenotypic record (y_i) with two possible values, $y_i = 1$ (female) or $y_i = 0$ (male). This was an independent Bernoulli trial with female (male) probability π_i ($1 - \pi_i$) as typically defined for logistic regression model (Berkson, 1944),

$\pi_i = 1/[1 + \exp(-\mathbf{x}_i\mathbf{b})]$, where \mathbf{b} was the vector of fixed effects and \mathbf{x}_i was an appropriate vector of incidences. Random infinitesimal additive genetic effects were discarded, as previously suggested by Toro et al. (2006). The joint probability of n observed data ($\mathbf{y}^T = [y_1 y_2 \dots y_n]^T$) as a function of \mathbf{b} and the chosen statistical model (i.e., the likelihood) generalized to $\text{top}(\mathbf{y}|\mathbf{b}) = \prod_i [y_i \pi_i + (1 - y_i)(1 - \pi_i)]$

This logistic regression model was fitted by maximum likelihood estimation implemented through a gradient descent algorithm (Courant, 1943).

2.3. Operational models

The analysis of lamb data accounted for three discrete effects and two continuous covariates. The model included the population mean (a single level influencing all records), the year of lambing (34 levels from 1988 to 2022), and the age of the dam at lambing (<3 years, 3–4 years, 5–6 years, 7–8 years, and >8 years) as discrete effects following in part Arun Kumar et al. (2021), and the linear and quadratic effect of inbreeding as continuous covariates. Only 15 out of 49 sires (30.6 %) were inbred with inbreeding coefficients fluctuating between 1.6 % and 12.5 %. These sires contributed with 1.132 lambs (30.3 % of the lambs under study).

The model for MARET rabbit data evaluated four discrete effects, such as the populational mean, the season of birth (four levels), the year of birth (10 levels grouping years by pairs to guarantee a minimum of 50 offspring per year), the age of the doe at parturition (<12 months, 12 to 24 months, and >24 months), and two regression coefficients involving the linear and quadratic effect of inbreeding. In this case, only three

bucks (9.7 %), were not inbred, they contributing 10 % of offspring. Remaining breeders had an inbreeding coefficient between 18.2 % and 74.7 %. In the preliminary analysis we found that there was a high degree of collinearity among inbreeding coefficients (i.e., offspring-sire-dam). Their joint inclusion in the same analytical model led to severe convergence issues and, therefore, could not be applied.

The statistical relevance of each fixed effect of the model was tested within the context of evidential inference (Edwards, 1972). This is a well-known paradigm relying on the likelihood function as a mathematical structure that encompasses all statistical evidence from the data relevant to the analytical model assumed (Birnbaum, 1962). Within this context, a given effect of the model (θ) could be tested by calculating the ratio (LR) between the likelihood of the full model (numerator model) against the likelihood of a reduced model without θ (denominator model; Hacking, 1965). This ratio advocated for the superiority of the numerator model ($LR_\theta > 1$) or the denominator one ($LR_\theta < 1$), also accounting for an intermediate scenario where both models could not be properly discriminated ($LR_\theta = 1$). Small departures from $LR_\theta = 1$ provide weak evidence in favor of either hypothesis, and Royall (1997) proposed a benchmark of $LR_\theta > 8$ (or $< 1/8$) for a simple hypothesis (this roughly corresponded to a frequentist type-I error of 0.05 as demonstrated by Blume [2002]). Within this context, both data sets were analyzed with the full model accounting for all sources of variation, and effects with $LR_\theta < 8$ were removed from the final model (model NULL) used as the reference for further segregation analyses. Confidence interval-like estimation could also be used in evidential statistics by properly defining likelihood intervals at $1/8$ (LI_8) such as the set of θ values with higher than $1/8$ likelihood ratio when comparing against the maximum likelihood θ estimate (Royall, 1997).

2.4. Segregation analysis by evidential inference

Model NULL expanded to segregation analysis by including two additional linear covariates (i.e., additive and dominance) inherent to an ungenotyped major gene that potentially segregates in the population (Janss et al., 1995). Assuming only two alleles with null (wild allele) and non-null effects (mutant allele) on offspring sex ratio, the additive covariate accumulated the number of mutant copies in sires' genome, whereas the dominance covariate was 1 for heterozygous sires and 0 otherwise (Falconer and Mackay, 1996). Nevertheless, genotypes must be updated as unknown parameters of the model by Kerr and Kinghorn (1996).

The original parametrization of the segregation analysis (Janss et al., 1996) assumed that the mutation arose some time ago in the population and was currently segregating in the founder individuals of the analyzed data set at a given allelic frequency (model $FREQ$). Neither recurrent (i.e., wild to mutant) nor reverse (i.e., mutant to wild) mutations were allowed.

Alternatively, the segregation analysis could assume that the new mutation primarily arose in the j th individual of the analyzed data set (model MUT_j); i.e., this individual was arbitrarily fixed as heterozygous, and remaining genotype probabilities were calculated following Kerr and Kinghorn (1996). Additional recurrent and reverse mutations were not allowed again. An independent analysis was required for each sire contributing offspring sex ratio data with model MUT_j to determine the most plausible source for the new mutation, if any. Given that the analysis focused on a sire-linked trait, this approach could not discriminate between a sire with offspring sex data and its dam when trying to elucidate the real origin of the mutation unless the dam had more than one son with similar departures in their offspring sex ratio. As for model NULL, both model $FREQ$ and MUT_j were solved by a gradient descent algorithm (Courant, 1943), although with the peeling approach developed by Kerr and Kinghorn (1996) to update genotype probabilities.

Comparisons between models NULL, $FREQ$, and MUT_j were carried out by the ratio of their likelihood probabilities (Hacking, 1965). The

total number of pairwise comparisons was equal to the triangular number of $s + 1$ (T_{s+1}), such as

$T_{s+1} = (s + 1)(s + 2)/2$, where s was the number of sires with offspring sex ratio data. In order to account for multiple comparisons, the benchmark for strong evidence favoring the numerator (denominator) model was raised to $LR > 32$ ($LR < 1/32$) as proposed by Royall (1997) and Blume (2002). This value equaled 15 deciban units, a base-10 logarithmic unit that measures information and entropy (Good, 1979).

3. Results

3.1. Environmental and genetic factors influencing sex ratio

Both species revealed relevant departures for offspring sex ratio along the analyzed time frame with strong evidence for the year effect in the Ripollesa sheep ($LR = 1.4 \times 10^9$), and both season ($LR = 12.6$) and year ($LR = 85.7$) effects in MARET rabbits (Table 1). Although odds ratios (i.e., the ratio of the odds of having a daughter in a given level of the fixed effect of the model to the odds of it occurring in the reference level of the same effect) for the year effect did not show a clear tendency across the parametric space in the Ripollesa breed, they fluctuated around the unity and even several *LI8* did not include this estimate by excess (years 1995, 1996, 2005, 2006, 2009 and 2013) or shortage (year 1997; Fig. 2). It is important to note that *LI8* characterized the range of estimates guaranteeing a likelihood estimate of (at least) 1/8 of the likelihood probability at maximum likelihood estimates, and where roughly comparable to the 95 % confidence intervals (Blume, 2002). A similar fluctuating pattern was revealed in MARET rabbits, although a top estimate in the first (i.e., reference) level generated odds ratios lower than one for the remaining levels and a positive odds ratio estimate for the population mean of 1.20 (*LI8*, 1.11 to 1.31). On the other hand, the season effect suggested a fluctuating pattern one-year around, with the top odds ratio for daughters during spring (1.19; *LI8*, 1.13 to 1.29), and the lowest in autumn (0.84; *LI8*, 0.75 to 0.97; Fig. 3).

The age of the dam reported weak evidence in both species, with *LR* values lower than the relevant benchmark ($LR < 8$), although larger than the unity (Table 1). Inbreeding had a relevant linear impact on the offspring sex ratio of both sheep ($LR = 60.7$) and rabbit ($LR = 780.4$) populations, whereas the quadratic effect only over-passed the evidence boundary in MARET rabbits ($LR = 8.3$).

As shown in Fig. 4, the linear regression coefficient for inbreeding depression in the Ripollesa sheep breed was 0.25 (*LI8*, 0.07 to 0.42), which increases the odds for daughters when increasing inbreeding. Similarly, inbreeding depression in the MARET rabbit population revealed a positive linear coefficient of 0.32 (*LI8*, 0.24 to 0.41) and a negative quadratic coefficient of -0.24 (*LI8*, -0.38 to -0.11). Likelihood intervals discarded negative odds (i.e., male offspring) for low and intermediate inbreeding coefficients until an inbreeding of 62.7 % (Fig. 4).

3.2. Segregation analysis

Most pairwise model comparisons did not discriminate among

Table 1
Statistical relevance for each fixed factor involved in the analysis of offspring sex ratio data (i.e., likelihood ratio (*LR*) between the full model against the model discarding the factor itself).

Ripollesa sheep data set		MARET rabbit data set	
Factor	<i>LR</i>	Factor	<i>LR</i>
Population mean	1.1	Population mean	1.5×10^5
Year	1.4×10^9	Season	12.6
Ewe age	3.5	Year	85.7
Inbreeding (linear)	60.7	Doe age	1.7
Inbreeding (quadratic)	1.3	Inbreeding (linear)	780.4
		Inbreeding (quadratic)	8.3

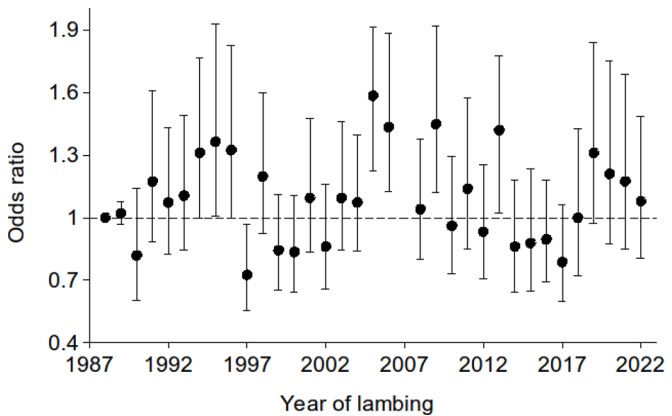


Fig. 2. Odds ratio estimates (black dot) and their 1/8 likelihood intervals (whiskers) for year of lambing effect on the analysis of offspring sex ratio in the Ripollesa sheep breed (upper-than-one estimates indicated an increase of female offspring).

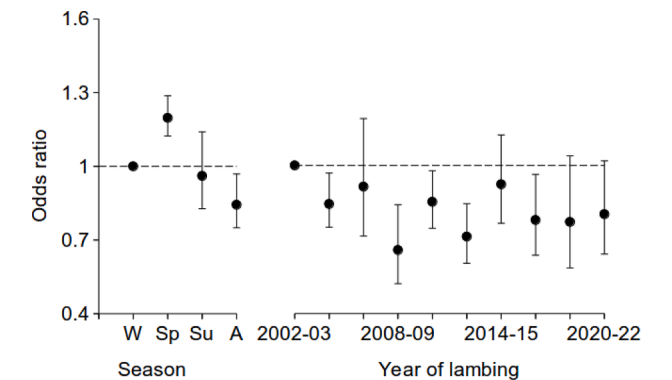


Fig. 3. Odds ratio estimates (black dot) and their 1/8 likelihood intervals (whiskers) for season (W, winter; Sp, spring; Su, summer; A, autumn) and year of birth effects (years in pairs from 2002 to 2003 to 2018 and 2019 and a final estimate for 2020 to 2022) on the analysis of offspring sex ratio in the MARET rabbit population (upper-than-one estimates indicated an increase of female offspring).

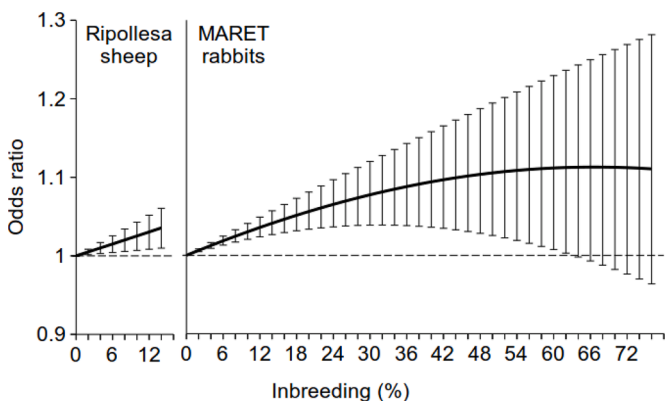


Fig. 4. Odds ratio estimate (black line) and its 1/8 likelihood intervals (whiskers) for inbreeding depression on offspring sex ratio (upper-than-one estimates indicated an increase of female offspring).

competing parametrizations. Indeed, the NULL model must be viewed as the reference one. The heatmap shown in Fig. 5 provided an almost homozygous pattern of weak or even lacking evidence of new mutations influencing offspring sex ratio. The only species showing strong

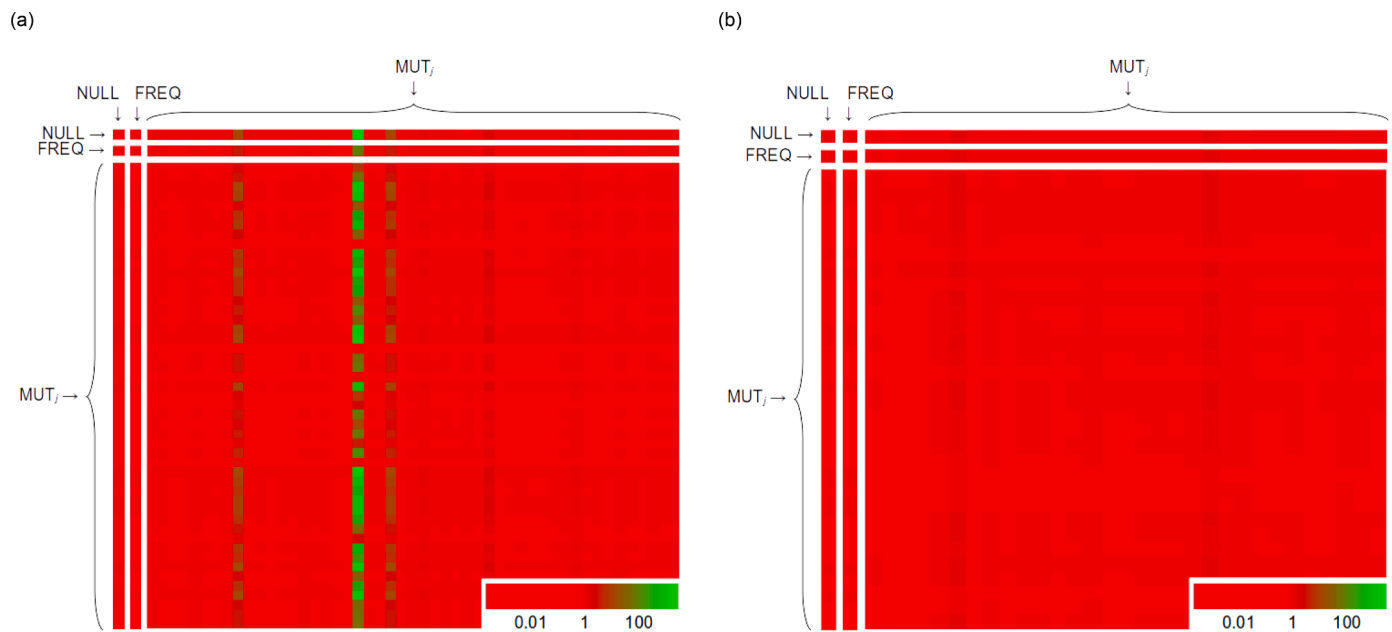


Fig. 5. Heatmap for the likelihood ratio (columns, numerator model; rows, denominator model) of segregation models on offspring sex ratio data of the Ripollesa sheep breed (a) and MARET rabbit population (b). Comparisons included the model without mutations (NULL), the model with a mutation already segregating in the founder generation (FREQ), and models with a new mutation arising in the j_{th} individual (MUT_j).

evidence for the MUT_j model ($LR > 32$) was the Ripollesa sheep, where a ram born in 1997 was revealed as the most probable source for a new mutation increasing the percentage of daughters. This model had a likelihood of 183.1 and 102.5 times greater than the ones from the NULL and FREQ models, respectively. Moreover, this model outperformed remaining MUT_j models with an $LR \geq 27.6$, except for direct comparisons with its father ($LR = 2.7$) and two grandsons ($LR = 2.9$ and 9.7) who generate the dark green columns flanking the fair green one in Fig. 5a.

Focusing on the MUT_j model with the highest likelihood in the Ripollesa breed, there was strong evidence supporting the additive genetic effect for the mutant allele ($LR = 1195.0$) and null evidence for the dominance effect ($LR = 1.0$). The additive genetic effect reached an odds ratio of 2.23 for daughter offspring ($LI8$, 1.43 to 3.53), which is consistent with the departed 50:50 ratios shown in Fig. 5a for some sires. This specific MUT_j model highlighted a ram born in 1997 as the first carrier of a new mutation departing offspring sex ratio (Fig. 6), with 57 daughters (62.6 %) and 34 sons (37.4 %). Three out of its four male sons/grandsons retained for breeding purposes inherited the mutation with a higher-than-0.5 probability, although those lineages did not contributed rams since 2013 (Fig. 6). Unfortunately, MARET rabbits did not suggest relevant segregation patterns influencing offspring sex ratio (Fig. 5b).

4. Discussion

4.1. Analytical framework

The segregation analysis model developed in this manuscript (MUT_j) must be viewed as a relevant example where the mutation source must be evaluated along the pedigree file with multiple hypotheses to be compared in terms of probability instead of being accepted or discarded. This relied on an evidential inference approach (Birnbaum, 1962) as opposed to both Bayesian and frequentist inferential frameworks. Bayesian inferential procedures failed to satisfy the objectivity criterion because of the inclusion of researcher-specific *a priori* distributions to obtain the relevant joint posterior distribution (Bickel, 2012). On the other hand, the interpretability of frequentist tests was impaired due to the invariable boundary for type-I errors, characterizing the probability of observing misleading evidence. This parameter must tend to zero with

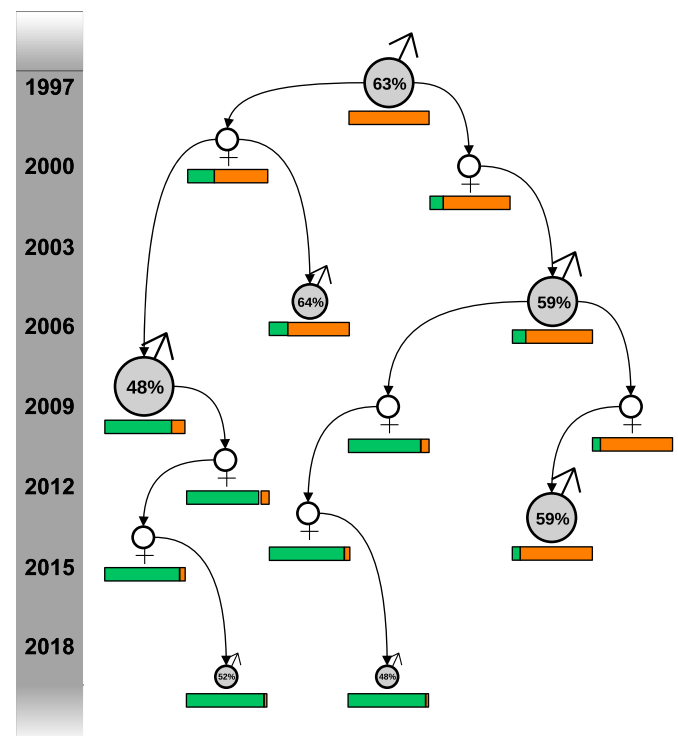


Fig. 6. Maximum likelihood predicted genotypes (green, homozygous wild-type; orange, heterozygous wild-type/mutant) under the MUT_j model with maximum likelihood probability in Ripollesa data set (i.e., the model assumed a new mutation arising in a non-founder sire placed on the top of the figure). Note that the size of the σ symbol characterized the number of offspring (from 39 to 109 offspring; not applicable to ϕ) and included the percentage of daughters. Only descendants from the sire carrying the first mutation (on the top) were included.

increasing the sample size, whereas it is typically fixed to 0.05, whatever the amount of data involved in the analysis (Bickel, 2012).

4.2. Factors influencing offspring sex ratio

Environmental factors inherent to year and/or season of birth influenced the offspring sex ratio in both species, as previously reported in sheep (Kent, 1996; Thiruvengadan et al., 2008; Hossein-Zadeh, 2016; Kumar et al., 2021) and discarded in rabbits, although under research maintenance conditions (Sawin and Gadbois, 1947). Indeed, seasonal departures were also described in other mammalian species such as seals (Stirling, 1971). These variations were previously linked to resource availability and the Trivers and Willard's (1973) hypothesis, an argument that suggested higher chance to produce sons from mothers in superior body condition, and vice-versa. The same rationale must apply to the age of the dam effect, which slightly increased the percentage of daughters from young and old females, and males from intermediate ages. These results were previously reported in sheep (Kent, 1996) and pigs (Toro et al., 2006).

Inbreeding was also revealed as a relevant source of variation for offspring sex ratio. The higher inbreeding, the higher chance of daughter offspring as reported in Irish Holstein-Friesian cattle (Mc Parland et al., 2007), pygmy hippopotamus in zoological gardens (Graczyk et al., 2015), eastern bongo (*Tragelaphus eurycerus isaaci*; Malo et al., 2019), Arabian oryx (*Oryx leucoryx*) and red wolf (*Canis rufus*; Frankham and Wilcken, 2006). These results could be due to sex-linked deleterious recessive alleles, although other genetic influences could not be discarded.

4.3. New parametrization for segregation analysis

Janss et al. (1995) assumed a previous mutation already segregating in the founder individuals of the analyzed data set, and Casellas et al. (2010) adapted the original segregation analysis (Elston and Stewart, 1971; Morton and Maclean, 1974) to a dichotomous trait. The current research takes a step ahead to generalize the analysis to a new scenario where the new mutation on the binary trait arose in one individual of the analyzed population. One main criticism would focus on the lack of reference to the very low chance of new mutations in the genome, typically around 10^{-8} per base pair (Lipson et al., 2015). The odds of a new mutational event in the subset of analyzed sires departing their offspring sex ratio would be minimal, if any. Nevertheless, the same low chance must apply to the recent ancestors of founder individuals under Janss et al. (1995) approach, where both mutation and genetic drift played a key role. It must be highlighted that both parametrizations took the same assumption about the mutational event, conditioning all the subsequent analysis to the premise of a new mutation that happens with full probability in the j_{th} individual (model MUT_j) or some recent ancestor (model FREQ; Janss et al., 1995). As a consequence, this canceled in likelihood functions and did not bias the LR.

Model FREQ did not reveal statistical evidence favoring a mutation already segregating neither in the Ripollesa breed nor in the MARET population when compared against the NULL model ($LR = 3.2$ and 1.7 , respectively). Nevertheless, the MUT_j model succeeds in the Ripollesa breed with an LR of almost 200 compared to NULL and FREQ. It is important to highlight the contradictory behavior of FREQ and MUT_j models on the same data set. One would expect some absorption of the new mutation in the founders' generation when analyzed under the FREQ model. Nevertheless, the new mutant shown in Fig. 6 was between three and six generations away from the founders, and its father and grandfathers had offspring sex ratios between 47 % and 52 % with 70 to 134 offspring. It is important to note that current analytical approaches cannot discriminate between this ram and its mother as the first known carrier of the segregating mutation. Unfortunately, this paternal lineage was unconsciously removed from the UAB experimental flock after the 2021 breeding season, by culling the two rams born in 2019 (Fig. 6).

Further efforts must focus on active ewes descending from the 1997 ram. Predicted genotype probabilities must be viewed as a key tool to identify descendants with the highest heterozygote probabilities, they being target dams to provide a new generation of rams to be tested in terms of offspring sex ratio.

The whole data set involved deep pedigree files rooted more than three decades ago and with a minimum of ten generations (results not shown). Within this context, the MUT_j parametrization must be viewed as a relevant analytical tool to check for the possibility of new mutations along the pedigree files, where Janss et al. (1995) model would fail due to the inherent restriction about the mutational event happening before founders.

4.4. Mutation in offspring sex ratio

Scientific literature about sex ratio departures was scarce and controversial in livestock species (Sawin and Gadbois, 1947; Kennedy and Moxley, 1978; Skjervold, 1979; Skjervold and James, 1979), whereas they were more frequently reported in laboratory rodents (Weir, 1960, 1976; Cook and Vlcek, 1961; Beamer and Whitten, 1991; de la Casa-Esperón et al., 2000; Lee, 2002), and experimental invertebrate strains (Sweeny and Barr, 1978; Cazemajor et al., 1997; Tao et al., 2007a,b). The additive mutation increasing the odds of female offspring in the Ripollesa sheep breed must join this small collection of mammalian populations with sex ratio departures. Indeed, this female-biased pattern agreed with deviations previously reported in rabbits (Sawin and Gadbois, 1947) and laboratory mice (Weir, 1960, 1976; Cook and Vlcek, 1961; Casellas et al., 2010). Our analyses suggested a major autosomal locus, although the lack of homozygous wild-type individuals in Fig. 6 did not completely discard contributions from the sex chromosome X. Indeed, research in mice over the last few years has found sex chromosome-linked gene families associated with sex ratio distortions (*Prssly*, *Sly* & *Slx/Slx1*; Kruger et al., 2019 and Hughes et al., 2022), mainly from the chromosome X. These genes have been related to sperm production and release and may be critical for male fertility.

5. Conclusions

Segregation analysis on binary traits can be performed within the context of evidential inference, which involves the degree of statistical evidence regarding each mutational source. This statistical tool must be viewed as useful to check for new mutations affecting traits of interest in livestock populations and laboratory species. The MARET rabbit population did not provide evidence supporting offspring sex ratio departures due to new mutations. In contrast, the Ripollesa sheep breed revealed a new mutation increasing the odds of female offspring. This mutation arose in an intermediate generation (year 1997) of the pedigree file and segregated with high probability until 2013.

CRedit authorship contribution statement

Joaquim Casellas: Conceptualization, Data curation, Software, Writing – original draft. **Marta Vázquez-Gómez:** Methodology, Writing – review & editing. **Samir Id-Lahoucine:** Methodology, Writing – review & editing. **Melani Martín de Hijas-Villalba:** Methodology, Writing – review & editing.

Declaration of Competing Interest

There are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgments

The authors gratefully acknowledge the staff of Can Quim Maret farm (Sant Gregori, Spain) and Servei de Granges i Camps Experimentals of the Universitat Autònoma de Barcelona (Bellaterra, Spain) for field data collection.

References

- Ancona, S., Dénes, F.V., Krüger, O., Székely, T., Beissinger, S.R., 2017. Estimating adult sex ratio in nature. *Phil. Trans. R. Soc. B* 372, 20160313.
- Argente, M.J., Blasco, A., Ortega, J.A., Haley, C.S., Visscher, P.M., 2003. Analyses for the presence of a major gene affecting uterine capacity in unilaterally ovariectomized rabbits. *Genetics* 163, 1061–1068.
- Beamer, W.G., Whitten, W.K., 1991. Do histocompatibility genes influence sex-ratio (percent-males). *Reprod. Fertil. Dev.* 3, 267–276.
- Berkson, J., 1944. Applications of the logistic function to bio-assay. *J. Am. Stat. Assoc.* 39, 357–365.
- Berry, D.P., Kearney, J.F., Roche, J.R., 2011. Evidence of genetic and maternal effects on secondary sex ratio in cattle. *Theriogenology* 75, 1039–1044.
- Bickel, D.R., 2012. The strength of statistical evidence for composite hypotheses: inference to the best explanation. *Stat. Sin.* 22, 1147–1198.
- Birnbaum, A., 1962. On the foundations of statistical inference. *J. Am. Stat. Assoc.* 57, 269–306.
- Blume, J.D., 2002. Tutorial in biostatistics: likelihood methods for measuring statistical evidence. *Stat. Med.* 21, 2563–2599.
- Casellas, J., Farber, C.R., Verdugo, R.A., Medrano, J.F., 2010. Segregation analysis of a sex ratio distortion locus in congenic mice. *J. Hered.* 101, 351–359.
- Casellas, J., Vidal-Roqueta, D., Flores, E., Casellas-Vidal, D., Llach-Vila, M., Salgas-Fina, R., Casellas-Molas, P., 2011. Epistasis for founder-specific inbreeding depression in rabbits. *J. Hered.* 102, 157–164.
- Cameron, E.Z., 2004. Facultative adjustment of mammalian sex ratios in support of the Trivers–Willard hypothesis: evidence for a mechanism. *Proc. R. Soc. B* 271, 1723–1728.
- Cazemajor, M., Landre, C., Montchamp-Moreau, C., 1997. The sex-ratio trait in *Drosophila simulans*: genetic analysis of distortion and suppression. *Genetics* 147, 635–642.
- Cook, M.J., Vlcek, A., 1961. Sex-ratio in mice. *Nature* 191, 89.
- Courant, R., 1943. Variational methods for the solution of problems of equilibrium and vibrations. *Bull. Amer. Math. Soc.* 49, 1–23.
- de la Casa-Esperón, E., Pardo Manuel de Villena, F., Verner, A.E., Briscoe, T.L., Malette, J.M., Rosa, M., Jin, W.H., Sapienza, C., 2000. Sex-of-offspring-specific transmission ratio distortion on mouse chromosome X. *Genetics* 154, 343–350.
- Edwards, A.W.F., 1972. *Likelihood*. Cambridge University Press, Cambridge, United Kingdom.
- Elston, R.C., Stewart, J., 1971. A general model for genetic analysis of pedigree data. *Hum. Hered.* 21, 523–542.
- Esquivelzeta, C., Fina, M., Bach, R., Madruga, C., Caja, G., Casellas, J., Piedrafita, J., 2011. Morphological analysis and subpopulation characterization of Ripollés sheep breed. *Anim. Genet. Res.* 49, 9–17.
- Falconer, D.S., Mackay, T.F.C., 1996. *Introduction to Quantitative Genetics*, 4th ed. Longman, Essex, United Kingdom.
- Frankham, R., Wilcken, J., 2006. Does inbreeding distort sex-ratios? *Conserv. Genet.* 7, 879–893.
- Good, I.J., 1979. Studies in the history of probability and statistics. XXXVII A.M. Turing statistical work in World War II. *Biometrika* 66, 393–396.
- Graczyk, M., Cwiertnia, P., Borowska, A., Barczak, E., Szwaczkowski, T., 2015. Inbreeding and offspring sex ratio in the pygmy hippopotamus (*Cheropsis liberiensis*) population kept in zoological gardens. *Folia Biol. (Krakow)* 63, 35–42.
- Hacking, I., 1965. *Logic of Statistical Inference*. Cambridge University Press, Cambridge, United Kingdom.
- Hossein-Zadeh, N.G., 2016. Threshold analysis of genetic effects for secondary sex ratio in domestic sheep using Bayesian approach via Gibbs sampling. *Small. Rumin. Res.* 144, 197–204.
- Hughes, J.F., Skaletsky, H., Nicholls, P.K., Drake, A., Pyntikova, T., Cho, T.-J., Bellott, D. W., Page, D.C., 2022. A gene deriving from the ancestral sex chromosomes was lost from the X and retained on the Y chromosome in eutherian mammals. *BMC Biol.* 20, 133.
- Janss, L.L.G., Thompson, R., VanArendonk, J.A.M., 1995. Application of Gibbs sampling for inference in a mixed major gene-polygenic inheritance model in animal populations. *Theor. Appl. Genet.* 91, 1137–1147.
- Janss, L.L.G., VanArendonk, J.A.M., Brascamp, E.W., 1997. Segregation analyses for presence of major genes affecting growth, backfat, and litter size in Dutch Meishan crossbreds. *J. Anim. Sci.* 75, 2864–2876.
- Janutta, V., Hamann, H., Distl, O., 2006. Complex segregation analysis of canine hip dysplasia in German shepherd dogs. *J. Hered.* 97, 13–20.
- Jeffreys, H., 1948. *Theory of Probability*. Oxford University Press, London, United Kingdom.
- Kennedy, B.W., Moxley, J.E., 1978. Genetic and environmental factors influencing litter size, sex-ratio and gestation length in pig. *Anim. Prod.* 27, 35–42.
- Kent, J.P., 1996. Birth sex ratios in sheep over 10 lambing seasons: year 10, the effects of litter size, seasonal factors and ageing. *Irish J. Psychol.* 17, 60–69.
- Kerr, R.J., Kinghorn, B.P., 1996. An efficient algorithm for segregation analysis in large populations. *J. Anim. Breed. Genet.* 113, 457–469.
- King, H.D., 1918. Studies on inbreeding. III. The effects of inbreeding, with selection, on the sex ratio of the albino rat. *J. Exp. Zool.* 27, 1–35.
- King, H.D., 1927. Seasonal variations in fertility and in the sex ratio of mammals, with special reference to the rat. *Wilhelm Roux Arch. Entwickl. Mech. Org.* 112, 61–111.
- Knott, S.A., Haley, C.S., Thompson, R., 1992. Methods of segregation analysis for animal breeding data: a comparison of power. *Heredity (Edinb)* 68, 299–311.
- Kruger, A.N., Brogley, M.A., Huizinga, J.L., Kidd, J.M., de Rooij, D.G., Hu, Y.C., Mueller, J.L., 2019. A neofunctionalized X-linked ampliconic gene family is essential for male fertility and equal sex ratio in mice. *Curr. Biol.* 29, 3699–3706.
- Kumar, A., Misra, S.S., Sharma, R.C., Gowane, G.R., 2021. Genetic parameters for sex ratio in an organised sheep farm. *Indian J. Small Rumin.* 27, 31–36.
- Lee, J.T., 2002. Homozygous Tsix mutant mice reveal a sex-ratio distortion and revert to random X-inactivation. *Nat. Genet.* 32, 195–200.
- Lipson, M., Loh, P.-R., Sankararaman, S., Patterson, N., Berger, B., Reich, D., 2015. Calibrating the human mutation rate via ancestral recombination density in diploid genomes. *PLoS Genet.* 11, e1005550.
- Malo, A.F., Gilbert, T.C., Riordan, P., 2019. Divers of sex ratio in the eastern bongo: lower inbreeding increases the probability of being born male. *Proc. R. Soc. B* 286, 20190345.
- McMillen, M.M., 1979. Differential mortality by sex in fetal and neonatal deaths. *Science* 204, 89–91.
- McParland, S., Kearney, J.F., Rath, M., Berry, D.P., 2007. Inbreeding effects on milk production, calving performance, fertility, and conformation in Irish Holstein–Friesians. *J. Dairy Sci.* 90, 4411–4419.
- Mendel, G., 1866. Versuche über Pflanzen–Hybriden. *Verhandlungen des naturforschenden Vereines in Brünn (Abhandlungen)* 4, 3–47.
- Morton, N.E., Maclean, C.J., 1974. Analysis of family resemblance. 3. Complex segregation of quantitative traits. *Am. J. Hum. Genet.* 26, 489–503.
- Roche, J.R., Lee, J.M., Berry, D.P., 2006. Pre-conception energy balance and secondary sex ratio – partial support for the Trivers–Willard hypothesis in dairy cows. *J. Dairy Sci.* 89, 2119–2125.
- Royall, R.M., 1997. *Statistical Evidence: A Likelihood Paradigm*. Chapman and Hall, London, United Kingdom.
- Sawin, P.B., Gadbois, D.S., 1947. Genetic influences upon the sex ratio in the rabbit. *Genetics* 32, 286–302.
- Schlager, G., Roderick, T.H., 1968. Secondary sex ratio in mice. *J. Hered.* 59, 363–365.
- Skjervold, H., 1979. Causes of variation in sex-ratio and sex combination in multiple births in sheep. *Livest. Prod. Sci.* 6, 387–396.
- Skjervold, H., James, J.W., 1979. Causes of variation in the sex-ratio in dairy cattle. *J. Anim. Breed. Genet.* 95, 293–305.
- Stevens, N.M., 1905. Studies in spermatogenesis with special reference to the “accessory chromosome”. *Carnegie Inst. Washington Publ.* 36, 1–33.
- Stirling, I., 1971. Variation in the sex ratio of newborn Weddell seals during the pupping season. *J. Mammal.* 52, 842–844.
- Sweeney, T.L., Barr, A.R., 1978. Sex ratio distortion caused by meiotic drive in a mosquito, *Culex pipiens* L. *Genetics* 88, 427–446.
- Tao, Y., Araripe, L., Kingan, S.B., Ke, Y., Xiao, H., Hartl, D.L., 2007a. A sex-ratio meiotic drive system in *Drosophila simulans*. II: an X-linked distorter. *PLoS Biol.* 5, e293.
- Tao, Y., Masly, J.P., Araripe, L., Ke, Y., Hartl, D.L., 2007b. A sex-ratio meiotic drive system in *Drosophila simulans*. I: an autosomal suppressor. *PLoS Biol.* 5, e292.
- Thiruvankadan, A.K., Chinnamani, K., Muralidharan, J., Karunanithi, K., 2008. Effect of non-genetic factors on birth weight of Mecheri sheep of India. *Livest. Res. Rural Develop.* 20, 1–4.
- Toro, M.A., Fernández, A., García-Cortés, L.A., Rodríguez, J., Silió, L., 2006. Sex ratio variation in Iberian pigs. *Genetics* 173, 911–917.
- Trivers, R.L., Willard, D.E., 1973. Natural selection of parental ability to vary the sex ratio of offspring. *179*, 90–92.
- van Arendonk, J.A., Smith, C., Kennedy, B.W., 1989. Method to estimate genotype probabilities at individual loci in farm livestock. *Theor. Appl. Genet.* 78, 735–740.
- Walling, G.A., Bishop, S.C., Pong-Wong, R., Gittus, G., Russel, A.J.F., Rhind, S.M., 2002. Detection of a major gene for litter size in Thoka Cheviot sheep using Bayesian segregation analyses. *Anim. Sci.* 75, 339–347.
- Weir, J.A., 1960. A sex ratio factor in the house mouse that is transmitted by the male. *Genetics* 45, 1539–1552.
- Weir, J.A., 1976. Allosomal and autosomal control of sex-ratio in PHH and PHL mice. *Genetics* 84, 755–764.
- Wilson, E.B., 1905. The chromosomes in relation to determination of sex in insects. *Science* 22, 500–502.