1992. The Journal of Arachnology 20:200–206

SEX RATIO IN THE SOCIAL SPIDER *DIAEA SOCIALIS* (ARANEAE: THOMISIDAE)

David M. Rowell: Division of Botany and Zoology, Australian National University, GPO Box 4, Canberra City 2601 Australia

Barbara York Main: Zoology Department, University of Western Australia, Nedlands, WA 6009 Australia

ABSTRACT. Sex ratio data from embryos and adults are compared in the social thomisid *Diaea socialis*. The relative proportions of males and females do not differ significantly between the two data sets, indicating that a sex ratio bias already exists at the time of fertilization. A statistical comparison with published data for the theridiid *Anelosimus eximius* shows a different ratio but similar pattern for the two types of data. A molecular procedure for determining whether the overproduction of females results from a bias in the sperm or differential success of sperm in fertilization is described.

In those social spiders where the adult sex ratio is known, it is female-biased (Buskirk 1981; Vollrath 1986). However, Fisher's principle (Fisher 1930) predicts that selection will favor an equal parental investment in offspring of both sexes. It would follow, then, that any bias in sex ratio should result from forces acting after dispersal. On the basis of the correlation between sex ratio and social behavior it would seem reasonable to hypothesize that the two phenomena are in some way causally linked. Skewed sex ratios have similarly been reported in colonies of *Diaea socialis* Main, with an observed male:female ratio of 0.2126 (Main 1988).

The life-history and behavior of *D. socialis* were described in Main (1988). Colonies are founded by individual gravid females which use silk to bind together eucalypt leaves to form a nest. The brood spiderlings remain with the mother after hatching, add leaves cooperatively to enlarge the nest, share prey, mature in the nest and mate with siblings. Generally, only mated females migrate from the nest, over the September to November period. The sex ratios reported for this species (Main 1988) have been assessed on adult and preadult morphology only, and consequently the observed bias may reflect differential life span or mortality between the sexes.

Alternatively, it is possible that the sex ratio is skewed from birth as a result of meiotic drive or some other factor. To determine whether such processes are operating requires the ascertainment of gender as soon as possible after fertilization, and preferably before hatching. Using cytological techniques, Avilés and Maddison (1991) have demonstrated a primary female bias in embryos of the social species *Anelosimus eximius* Keyserling and *A. domingo* Levi (proportion of males = 0.08 and 0.09, respectively) which contrasts with a more even sex ratio for non-social species in the genus.

In spiders it is relatively easy to determine the gender of embryos using cytological techniques because of their unusual sex determination mechanism. In 81% of the spider species that have been analyzed there are two sex chromosomes involved in sex-determination (White 1973). Males possess one copy of each of these, while females carry two copies of each (four in all). Consequently, females have two more chromosomes than males, and so can be distinguished simply on the basis of chromosome number. This method of sex-determination is referred to as X_1X_20, and it would appear to be the ancestral condition in spiders (White 1973). This system has been modified to $X_1X_2X_30$ in the Australian huntsman spiders (Rowell 1985) and $X_1X_2X_3X_40$ in the sparassid *Heteropoda sikkimensis* (Datta & Chatterjee 1983). In the majority of the Oxyopidae analyzed, there has been a reduction in sex chromosome number resulting in an XO system similar to that prevalent in the insects (Datta & Chatterjee 1983). Thus, among different species, females may possess one, two, three or four more chromosomes than males.

Over 300 species of spider have been analyzed

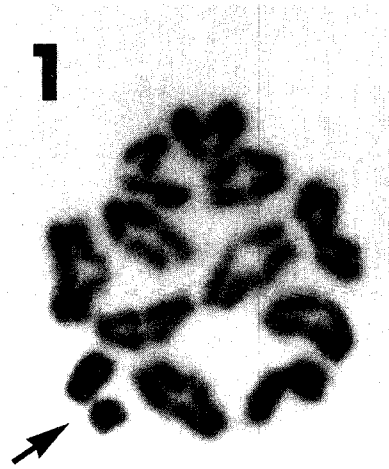


Figure 1.—Male meiosis in *D. socialis* (2N = 24). Note the presence of eleven bivalents plus two heteromorphic X-chromosomes (arrow).

cytologically, and the kind of sex-determination system described above is virtually universal. In only two cases, the sparassid *Delena cancerides* Walckenaer and the salticid genus *Pellenes* Simon, do aberrant systems exist (Rowell 1990; Maddison 1982), both involving rearrangements between the X-chromosomes and autosomes. These modified systems are very distinctive, however, and can easily be identified from male meiotic preparations.

In this study, sex ratios of adult and subadult *D. socialis* are compared to embryonic sex ratios determined by cytological means, to ascertain whether the observed bias arises before hatching, or as a result of differential mortality of the sexes. In addition, the ratios observed are compared with those reported by Avilés and Maddison (1991) from cytological analysis and Avilés (1986) from morphology for the social species *Anelosimus eximius*, to determine whether a particular primary sex ratio may exist in social spiders.

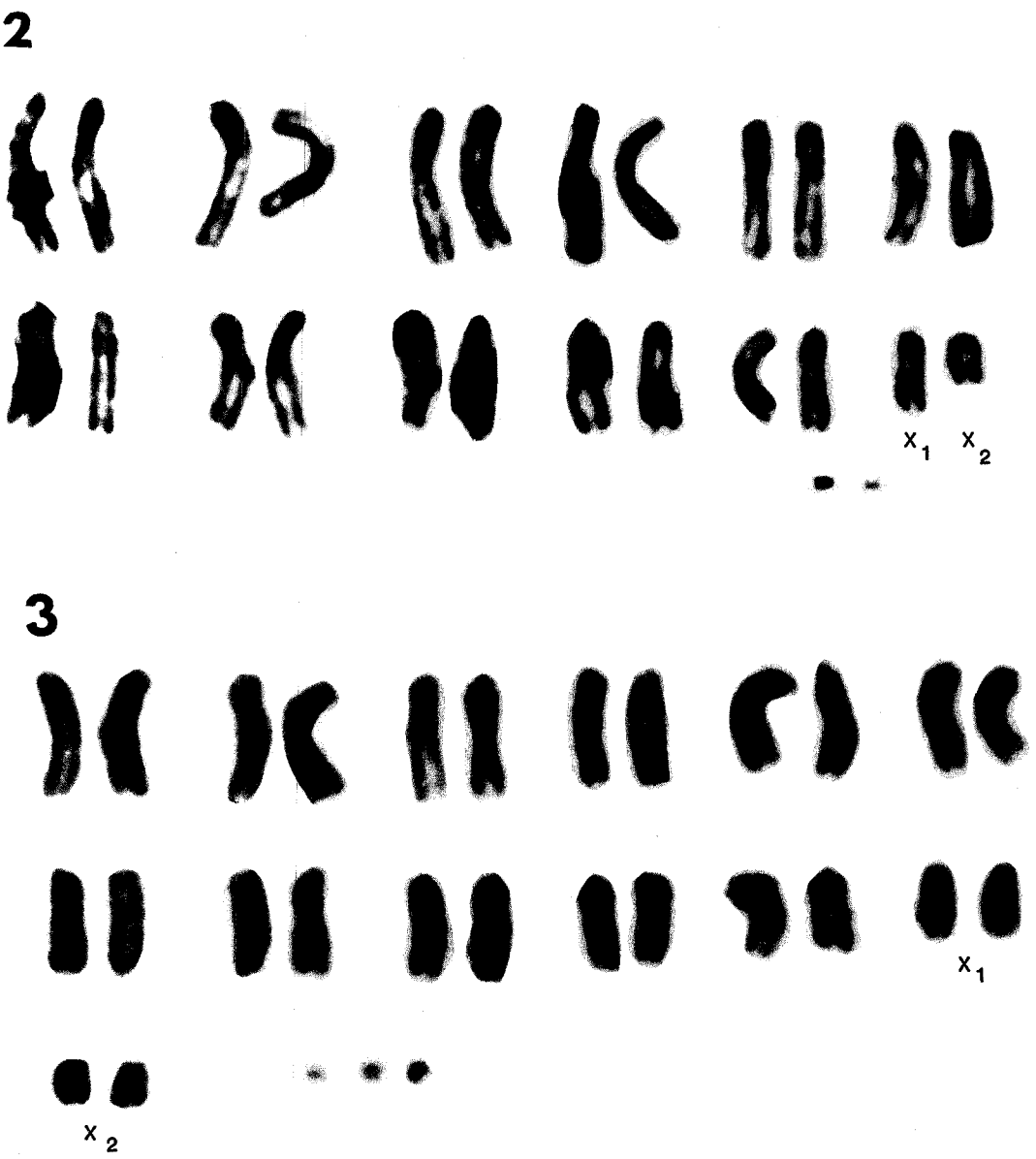
METHODS

All specimens, both eggs and males, used in the cytological analyses were collected by one of us (BYM) from sites in the vicinity of Torbay west of Albany, southwestern Western Australia. The sex ratio was also determined from adult and subadult spiders from colonies collected in the same areas by BYM (subsequently considered by Main (1988)) and from Pemberton by T. A. Evans. In younger colonies at two sites a small proportion of juvenile individuals possessed no characters of either sex. No reliable morphological characters have been found for sexing younger spiders, and the small size of *D. socialis* makes dissection of large numbers of individuals impractical. However, the authors' previous experience with this species has shown that the rate of development within and between the sexes in a colony is concerted (but with males maturing before females). Hence swelling of the palp would have been apparent were these individuals male. Consequently, in colonies possessing a mixture of adults and juveniles, the juveniles were scored as females. The validity of this assumption was borne out in the statistical analysis, where it was shown that the sex ratios within these colonies were not statistically different from those of adult colonies. Colonies in which the majority of individuals were juvenile were not included in the analysis, nor were degenerate colonies with less than five individuals, or those without representatives of both sexes (two colonies qualified for the latter).

Chromosome preparations were made from testes and embryos using the techniques described in Rowell (1985) which differs from that detailed by Avilés and Maddison (1991). Not every embryo yielded spreads which could be

Table 1.—Heterogeneity and deviation from even sex ratio in embryonic data for *D. socialis*. * $P < 0.01$.

Site	Males	Females	Total	Male proportion
Coombes Rd, Torbay	5	13	18	0.28
Coombes/Harding Rds, Torbay	3	8	11	0.27
Coombes/Harding Rds, Torbay	4	12	16	0.25
Coombes/Harding Rds, Torbay	4	9	13	0.31
Total	16	42	58	0.28
Deviation from 1:1 G = 12.08* Heterogeneity G = 0.122 ns.				



Figures 2, 3.—Karyotypes from *D. socialis* embryos: 2, male (2N = 24); 3, female (2N = 26). Note the presence of supernumerary microchromosomes in the two karyotypes.

counted with certainty, but given the marked developmental synchrony within each egg sac, those counted are held to represent a random sample from each colony.

Data were analyzed for deviation from a sex ratio of 1:1 and homogeneity among egg sacs, nests and sites using the G-test of Sokal and Rohlf (1981). This statistic has a chi-square distribution but is more reliable than the traditional chi-square test when sample sizes are small.

The data obtained for *D. socialis* were compared, by G-test, against the data given for *Anelosimus eximius* by Avilés and Maddison (1991) and by Avilés (1986).

RESULTS

Chromosomal analysis.—Both meiotic and mitotic preparations were successfully obtained from testes. Mitotic preparations were examined for 58 embryos from four egg sacs in all.

Table 2.—Summary of data obtained from morphological analysis of adult and subadult *D. socialis* specimens. Colony data, heterogeneity within sites, male proportion and number of colonies with a female bias. Treatment of outliers discussed in text. * $P < 0.001$.

Site	No. of colonies	Female bias: Significant	Female bias: Present	Heterogeneity G value	*n*	Male proportion
Pemberton 26/7–1/8 Area1	10	7	9	8.03 ns	214	0.23
Pemberton 26/7–1/8 Area2	9	8	9	8.17 ns	240	0.15
Puls Rd, Torbay 26/3/89	1	1	1		29	0.28
Puls Rd, Torbay 18/4/90	4	4	4	7.82 ns	104	0.17
Puls Rd, Torbay 23/8/87	1	1	1		34	0.17
Puls Rd, Torbay 14/10 & 5/11/90	8	5	6	43.55*	213	0.26
(without outliers)	5	4	5	3.536 ns	102	0.29
Curinup Rd 3/6/90	6	5	6	11.07 ns	89	0.12
Curinup Rd 14/10/90	2	0	0	0.07 ns	16	1.67
Total (pooled by site)	44	31	39	26.80*	939	
(outliers removed)	39	30	38	12.35 ns	812	0.19

Figure 1 shows male meiosis, which involves 11 bivalents and the X-chromosomes. Of importance here is the fact that two X-chromosomes are visible, indicating that *D. socialis* employs the X_1X_20 system common to most spider species. This was reflected in the mitotic preparations from embryos (Figs. 2, 3), where individuals possessed either 24 or 26 chromosomes. Clearly, the former represent male embryos and the latter females. In many of the preparations a number of chromosome fragments or microchromosomes (ranging from one to three) was visible (Figs. 2, 3).

Table 1 shows the numbers of male and female embryos identified for each egg sac analyzed. In every case, the number of females exceeded the number of males, and the results were statistically homogeneous among the egg sacs. The pooled data deviate significantly from a 1:1 sex ratio, with an overall male proportion of 0.28.

Morphology.—Table 2 shows a summary of the data obtained from morphological analysis of adult and subadult *D. socialis* specimens. The ratios among colonies and sites were quite uniform, with only five deviant colonies (one from Puls Road, 14 October 1990, two from the same locality on 5 November 1990 and both of the Curinup Road, 14 October 1990, colonies). Since these colonies were all collected in mid-October or later, which is towards the end of the dispersal phase, it is probable that they represent the remnants of larger colonies following emigration of many of the spiders. Moreover, two of the colonies were unusual in that they possessed resident clubionid predators. Consequently, the sex ratios in these collections are considered to be non-representative of the adult sex ratio of the population as a whole, and these colonies were removed from further analyses.

In total, 38 of the remaining 39 colonies showed a female bias, and in 33 of these the bias was statistically significant. The overall sex ratio determined from the morphology was not significantly different from the overall embryonic ratio (Table 3).

Comparison with *Anelosimus eximius*.—Table 3 shows the results of comparisons of embryonic and morphological data between *D. socialis* and *A. eximius* from Avilés (1986) and Avilés and Maddison (1991). For both species the embryonic ratios do not differ significantly from those of adult and subadult spiders, but a greater bias is apparent in the morphological data. For both types of data, however, the female bias is significantly greater in *A. eximius*.

DISCUSSION

Karyotype of *Diaea socialis*.—The chromosome number and sex determination mechanism of *D. socialis* differ from those reported for *D. subadulta* Bosenberg & Strand, the only other species of the genus which has been examined karyotypically (Suzuki 1952). *D. subadulta* possesses 26 autosomes and one X-chromosome in the male and consequently a complement of 26 autosomes and two X-chromosomes in the fe-

male can be inferred. Within *D. socialis*, however, the chromosome number and karyotype form are conserved, with identical male complements present in populations from Victoria and the Australian Capital Territory, separated from the Western Australian study site by distances of over 3000 km, with large areas of intervening desert (unpublished data). This contrasts with cytological data from two other wide-ranging spider species, *Delena cancerides* and *Selenops australiensis* Koch which show marked geographical variation in chromosomal number and karyotypic form across their range (Rowell 1990, DMR unpublished).

Sex ratio in *D. socialis* and *A. eximius*.—The embryonic sex ratio bias in *D. socialis* is consistent with the data from adult and subadult spiders. Thus it can be concluded that, as in *A. eximius*, the bias is present before hatching.

The tests for heterogeneity indicate that there is a marked conservatism in the sex ratio of *D. socialis* both between sites and through time, as the data are derived from observations over four years. Moreover, there does not appear to be any relationship between month of collection and sex ratio, except during the period of dispersal, over the October/November period. This is an important observation because such uniformity may not be expected if the ratio is influenced by some environmental factor. For example, if the survival of male embryos or the success of male determining sperm were influenced by temperature, considerable variation in ratio would be expected between years, or even within one season.

The mechanism involved in producing the female bias observed is not apparent; however, some conclusions can be drawn. As in *A. eximius* (Avilés & Maddison 1991), few infertile *D. socialis* eggs were observed, and certainly insufficient to make up the shortfall in males even if it were assumed that every one represented a dead male embryo. The little "infertility" that is apparent in this species is usually associated with egg parasitism (BYM unpublished). In *A. eximius* and *D. socialis*, meiosis proceeds normally through to anaphase II, and so it is possible to narrow down the time at which the mechanism acts in both species to the period between the end of meiosis and fertilization. Since sex is determined by the chromosomal composition of sperm, it follows that male-determining sperm are either present in lower numbers than female-determining sperm, or they compete less well for fertilization of the egg.

Table 3.—G-test comparisons involving morphological and chromosomal data sets from *A. eximius* (Aviles 1986, Aviles & Maddison 1991) and *D. socialis*. * ($P < 0.01$), ** ($P < 0.001$).

Analysis	G Value
A. eximius, heterogeneity among colonies, morphology	24.108 ns
A. eximius, embryos × morphology	0.078 ns
D. socialis, embryos × morphology	2.602 ns
A. eximius embryos × *D. socialis* embryos	10.454*
A. eximius morphology × *D. socialis* morphology	24.496**

To gain any finer resolution would require the use of techniques capable of distinguishing between male and female sperm, such as sedimentation rate or DNA content. Alternatively, if an X-linked probe were available, it might be possible to determine the ratio of the marker to total DNA in males, females and a sperm sample using a technique as simple as dot blotting. If male- and female-determining sperm are present in equal proportions, the ratio of the marker to total DNA in male tissue would equal the ratio in the sperm sample, and be half that found in female tissue (because females possess twice as many X chromosomes as males). If, however, the bias arises from a deficiency of male-determining sperm, X chromosomes would be present in more than half of the sperm; and the ratio would be intermediate between that of male and female tissue.

Hamilton (1967) pointed out that Fisher's principle is based on the assumption of panmixia, and that selection on groups where inbreeding within a single clutch is the norm may favor the production of fewer males, thus minimizing competition between male offspring and the consequent wastage of reproductive effort. This would appear to be a probable explanation here since the life histories of both *D. socialis* and *A. eximius* suggest marked inbreeding. This is further supported by preliminary data from the social spider *Delena cancerides*. This species has been demonstrated to be outbred by the use of enzyme electrophoresis (Rowell 1990), and on the basis of dissection of hatchlings it would appear to have an even sex ratio (Rowell, unpublished).

An alternative explanation discussed in detail by Avilés (in press) is that, in continuously inbreeding lineages of social species where colonies are initiated by individual mated females, selection at the colony level plays a major role. Future success of a given lineage is a function of the number of successful females rather than the absolute number of offspring. Consistent with this is that in the solitary leaf-rolling thomisids of the genus *Cymbacha* L. Koch, which presumably have an even sex ratio, dispersal occurs during the juvenile stage; and, as in many species, there is likely to be a high mortality in the free-living stage of the life cycle in both sexes. Moreover, the chances of free-living females finding a mate rely on a relatively high density of males. In contrast, because only adult females of *D. socialis* (which are assured of a mate within the colony) disperse, the biased ratio increases the probability of a lineage surviving to future generations. Main (1988) noted that only one in five incipient nests survived to maturity, and this does not take into account mortality during emigration prior to leaf-rolling.

Avilés' model is based on the hypothesis of Colwell (1981) which has received some criticism (see, for example, Charlesworth & Toro 1982). Nevertheless the life history of *D. socialis* appears to follow the parameters of the model very closely. Indeed, further study of *D. socialis* may be of particular value in establishing the validity of this model.

It has long been known that a female bias exists in the eusocial Hymenoptera (Hamilton 1967), and in this context it is interesting to note that the presence of nonreproductive females has been demonstrated convincingly only once in spiders (Vollrath 1986). However, a behavioral parallel with true eusociality was also recognized in *D. socialis*, where it appeared that some females forfeited reproduction while still working to the benefit of the colony (Main 1988).

The phenomenon of skewed sex ratio in social spiders is particularly interesting because social behavior has arisen independently in a number of spider families (Burgess 1978). This would imply that either selection for a bias is very strong in social spiders, or that such an alteration in sex ratio is easily achieved. While the mechanism by which this is achieved is obscure, it is probable that it is the same in *A. eximius, A. domingo* and *D. socialis*, because they act at a very precise time in the three species. Moreover, given the observed plasticity in sex ratio among the species, the difference in sex ratio between *D. socialis* and *A. eximius* may imply a difference in the optimal ratio, determined by their life histories. Thus a detailed comparison of the lifestyles of these species may shed light on the particular factors that influence sex ratio.

ACKNOWLEDGMENTS

We thank T. A. Evans for collection and counts of specimens from the Manjimup region. We would also like to thank Ian Scott and Angela Higgins for their help with data analysis and preparation of the manuscript. Leticia Avilés of the University of Arizona provided particularly helpful comments on the manuscript. The Zoology Department of the University of Western Australia provided some of the facilities necessary for this work.

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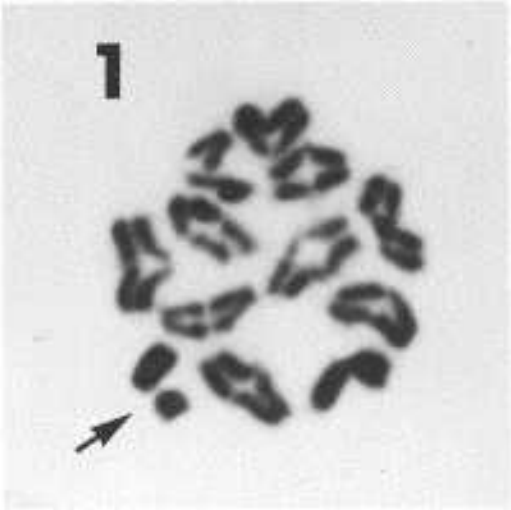

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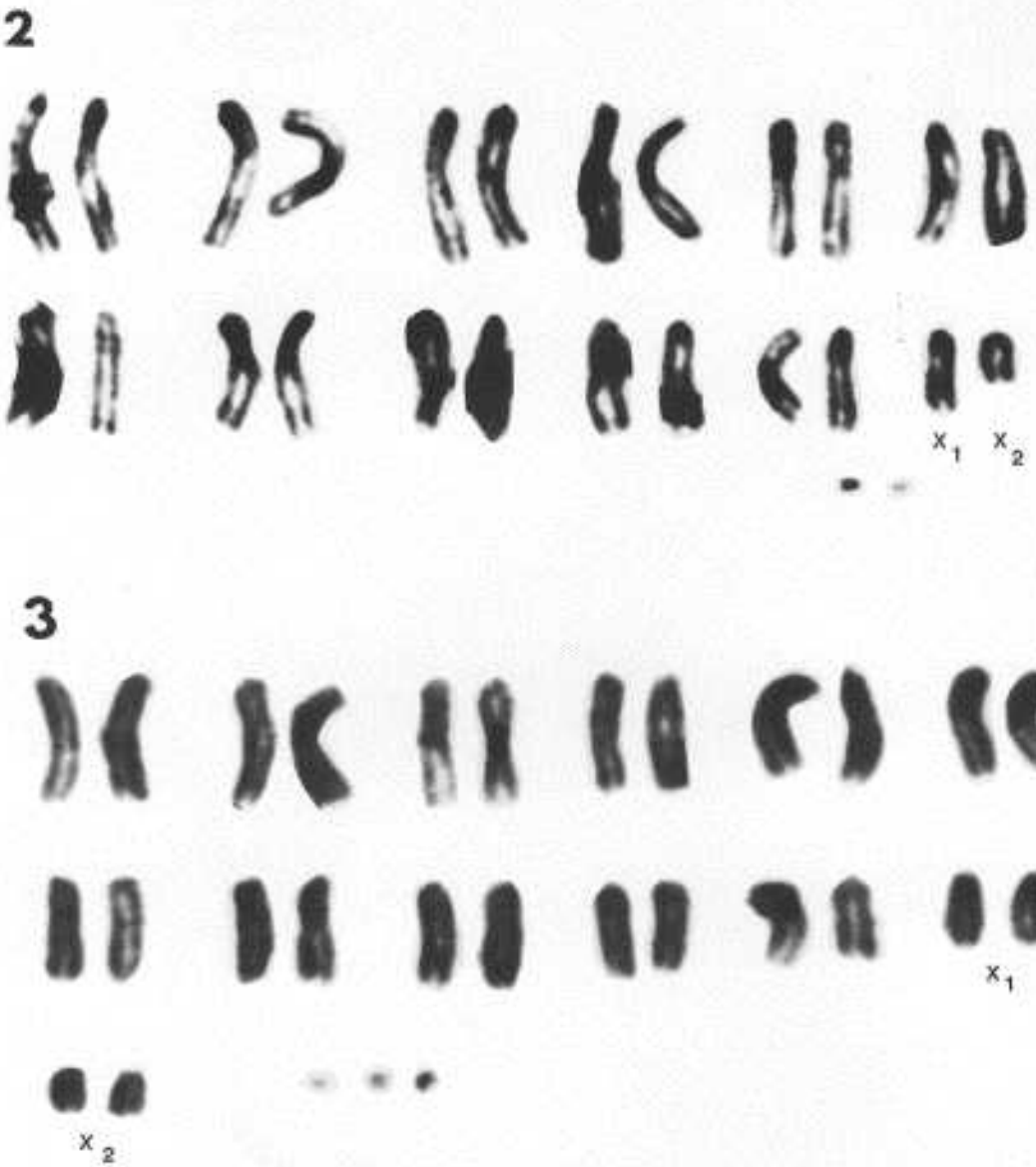

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