

L. N. Trut

Sex ratio in silver foxes: effects of domestication and the *star* gene

Received: 16 June 1995 / Accepted: 26 June 1995

Abstract The course of changes in secondary sex ratio (proportion of males at birth) in silver foxes bred at the fur farm of this Institute was analyzed. Data collected over several years of breeding of a domesticated (experimental) population selected for amenability to domestication and of a commercial (control) were compared. A tendency to increase in secondary sex ratio was demonstrated in both populations. However, the proportion of males at birth was higher in domestic foxes. This proportion, calculated from the combined data for 1978–1993, was 0.538 ± 0.005 and 0.511 ± 0.007 in the selected and commercial populations, respectively. The minimal departure of the observed sex ratio from 0.5 was demonstrated for litters with five pups, which is close to the average litter size in fox populations. The proportion of males increases with both increasing and decreasing litter size. An analysis of secondary sex ratio with respect to maternal age revealed a minimal departure of sex ratio from the expected in offspring from foxes of optimal reproductive age (2–4 years). An effect of the autosomal semidominant coat color mutation *star* on male excess at birth was also found: secondary sex ratio was higher (0.583 ± 0.015) in offspring of mothers heterozygous for the *star* mutation than from standard types of the domesticated population. The increase in secondary sex ratio in the analyzed fox populations is viewed as a correlated response to selection for domestication. The hormonal mechanisms mediating the effects of both this selection and the *star* mutation on sex ratio at birth are discussed.

Key words Secondary sex ratio · Zygotic selection · Hormonal level · Domestication · *Vulpes vulpes*

Introduction

Sex ratio at birth in animal populations remains a topic of genetic, evolutionary and biological interest. Its economic implications are obvious with respect to domestic animals with pronounced sexual dimorphism. While males determine the primary sex ratio of offspring in mammals, certain mechanisms correct this sex ratio so that a secondary sex ratio is formed by the time of birth. The adjustment of sex ratio at birth has been demonstrated for natural and domestic populations of mammals (Clutton-Brock and Jason 1986; Clarke et al. 1992). The departures of sex ratio from the expected are frequently interpreted in terms of adaptation (Trivers and Willard, 1973; Bull 1981; Huck et al. 1987).

Ample evidence indicates that males prevail among the offspring of most domestic animals (Nishida et al. 1976; Gray and Hurt 1979; Gray and Katanbaf 1985). It is relevant that male prevalence has been established in animals with a long history of domestication. Many factors may be implicated as being causative in this male prevalence at birth in such ancient domesticates, and an investigation into variation in sex ratio in the more contemporary domesticates should be helpful in gaining insight into these factors. Consequently, an extensive experiment modeling the process of domestication in the silver fox (*Vulpes vulpes*) has been carried out through selection for behavioral amenability to domestication at this Institute. The major aim was to study domestication as an evolutionary process (Belyaev 1969), an unique population of tame foxes has subsequently been produced as a result of many years of this selection (Figs. 1, 2). The multifaceted effects of continuous selection of foxes for domestic behavior have been considered elsewhere (Belyaev 1979; Belyaev and Trut 1982; Trut 1988), and one of the first correlated effects was observed to be the high frequency of the *de novo* appearance of the *star* (*S*) mutation. The *star* mutation is phenotypically manifested as a depigmented mark (the star) on the head (Fig. 3), and it has been shown to have various pleiotropic

Communicated by G. Wenzel

L. N. Trut
Institute of Cytology and Genetics of the Siberian Department of the
Russian Academy of Sciences, Novosibirsk, Russia

effects (Belyaev et al. 1981). Evidence has been provided indicating that the *star* appears at a high frequency in response to selection pressure on desirable behavioral traits (Belyaev et al. 1981; Trut 1991). Analysis of the transmission of the *star* through successive generations prompted the idea that the *S* gene may be spontaneously and stably activated and inactivated (Belyaev 1979; Belyaev et al. 1981). This report presents an analysis of the effects of domestication and the *star* mutation on secondary sex ratio in offspring from foxes bred at the experimental farm of this Institute.

Populations and procedures

Silver-black foxes (*Vulpes vulpes*) of a domesticated (experimental) and a commercial (control) population were used. All of the foxes of the two populations were maintained under identical conditions and received the same feeding rations. The experimental population has been selected for tameability for more than 30 years, and individuals of this population show responses characteristic of most domestic animals (Fig. 1). Foxes of the control population have not been subjected to selection for tame behavior, and they show the usual vicious, fearful responses to humans (Fig. 2), and their selection was based on economically useful properties, such as fur quality and

reproductive performance. This report is based on data collected at the fur farm from 1978 to 1993. Because handling of the pups on the first day after birth in order to identify their sex combined with subsequent return to the home cage frequently disturbed maternal behavior with a resultant death of offspring, the sex of the pups was mainly identified at the age of 2 weeks and not every year. Only litters in which pups did not die during the first 2 weeks and those in which the sex of the dead pups was identified were included in analysis. Only in 1967 was sex identified on the first day after birth, in 25 litters of the experimental population, when time of eye opening was recorded (Belyaev et al. 1984). Control and domesticated populations were compared in those years when the number of litters in which the sex could be identified was quite high.

It should be recalled that the silver fox is a species with a strictly seasonal reproduction pattern: vixens produce offspring only once each year. A group of more than 200 females of the experimental population, whose offspring was sex-identified for seven successive parities, was set up. The group was analyzed for relation between sex ratio and parity order, and correlations between sex ratio in successive years were calculated. All the vixens were standard silver-black. Foxes heterozygous for the *star* mutation (*Ss*) (Fig. 3) composed a separate group.

For example, litters of three pups in size can have the following sex combinations: 3 males, 2 males and 1 female, 1 male and 2 females, 3 females. Each litter ranging from three to eight was divided by sex combination. On the basis of the observed frequencies of combinations, the expected frequencies were calculated for bimodal expansion (Rokitsky 1973). Comparisons of the observed frequencies with the expected carried out by means of the chi-square test.

Fig. 1 Responses to humans of foxes of the domesticated population that were intentionally selected for tameability.





Fig. 2 Responses to humans of foxes of the control (commercial) population that were intentionally not selected for tameability

Experimental results

Secondary sex ratios (the proportion of males at birth) for both the domestic and control populations are given in Table 1. During the analyzed breeding span, there was an excess of males in both populations. However, if one adds the standard error to the smaller mean sex ratio, (that of the control population), and subtract it from the larger sex ratio (that of the domesticated population), then it is only in 1989 that the sex ratio values of the two populations did not transgress. Nevertheless, the proportion of males in offspring of domestic foxes has clearly increased. The same is true for the secondary sex



Fig. 3 A fox heterozygous for the *star* mutation (*Ss*)

ratio calculated from the combined data: the lowest extent of the standard error interval for the domestic fox ratio was 0.534, while the highest extent of the standard error interval for the control fox ratio was 0.518.

As the data of Table 2 show, sex ratios closest to 0.5 were observed for litters of average size (five pups). With increases and decreases in litter size the proportion of males increased in both the domestic and control populations. With respect to bimodal expansion for litter size, three to eight pups in the domesticated population and five to seven in the control populations, the observed frequencies of sex combinations were compared with those expected. The respective chi-square values are given in Table 3. All the values are significant for the litters of the domestic population with the exception of those with three pups. Two chi-square values are significant in the control population; those for litters with six and seven pups. Maximal chi-square values were calculated for litters with six pups in both the experimental and control populations; the number of such litters was rather high: 331 and 60 in the former and in the latter populations, respectively. The discrepancies between the observed and expected frequencies were very large for the middle combination (three males and three females). As a rule, in litters of different sizes, expected frequencies prevail over the observed for combinations near the middle values and observed frequencies prevail for extreme combinations with large number of males. However, there are some exceptions to this pattern.

Table 1 Sex ratio at birth in foxes of the domesticated (1) and control (2) populations

Years of breeding	Number of litters		Number of pups		Average litter size		Sex ratio	
	1	2	1	2	1	2	1	2
1967	25	–	145	–	5.80 ± 0.25	–	0.498 ± 0.041	–
1978	174	290	991	1477	5.69 ± 0.17	5.09 ± 0.19	0.522 ± 0.016	0.504 ± 0.013
1989	300	393	1567	2046	5.22 ± 0.13	5.20 ± 0.11	0.551 ± 0.012	0.512 ± 0.011
1991–1993	1187	196	6682	1142	5.63 ± 0.14	5.82 ± 0.14	0.538 ± 0.006	0.520 ± 0.014
Combined 1978–1993	1686	879	9240	4665	5.56 ± 0.12	5.30 ± 0.13	0.538 ± 0.005	0.511 ± 0.007

Table 2 Sex ratio at birth in litters of different sizes in foxes of the domesticated (1) and control (2) populations (1991–1993)

Litter size	Number of males		Number of pups		Sex ratio	
	1	2	1	2	1	2
1–3	131	16	209	30	0.626 ± 0.033	0.533 ± 0.091
4	353	39	640	72	0.552 ± 0.020	0.542 ± 0.059
5	756	93	1440	190	0.525 ± 0.013	0.489 ± 0.036
6	1074	185	1986	360	0.541 ± 0.012	0.514 ± 0.026
7	877	175	1659	329	0.529 ± 0.012	0.532 ± 0.028
8–9	417	86	748	161	0.558 ± 0.018	0.534 ± 0.039
Combined	3608	594	6682	1142	0.540 ± 0.006	0.520 ± 0.014

Table 3 Comparison of the observed and expected sex distribution in foxes of the domesticated (1) and control (2) populations

Population	Litter size					
	3	4	5	6	7	8
	chi-square					
1	0.47	17.729*	59.290**	83.15***	23.453*	34.918**
2			6.492	17.155*	16.462*	

*. **. *** Statistically significant at $P = 0.95$, $P = 0.99$ and $P = 0.999$, respectively

Table 4 Sex ratio in litters of the domesticated population by parity order

Parity	Number of litters	Number of pups	Sex ratio
1	28	147	0.558 ± 0.041
2	36	207	0.531 ± 0.035
3	40	230	0.530 ± 0.033
4	33	198	0.510 ± 0.036
5	28	162	0.562 ± 0.039
6	28	124	0.540 ± 0.045
7	15	78	0.577 ± 0.056
Combined	208	1146	0.539 ± 0.015

Table 4 presents the results of analysis of secondary sex ratio as related to parity order of the domesticated females. The proportion of males varied with respect to individual parities, with the lowest sex ratio being observed for parity 4. The male proportion increased in

both the first and latter parities. Correlation coefficients calculated between sex ratios for successive parities of vixens are mainly small and insignificant (Table 5). The correlation coefficient was only significant between the sex ratio for the second and third breeding seasons. It is of interest that the sex ratios in later parities (5, 6, 7) as a rule correlated negatively with those observed in preceding years.

Secondary sex ratio was especially high in offspring of the tame females heterozygous for the autosomal semidominant *star* mutation (*Ss*) (Fig. 3, Table 6). It should be noted that the sex ratio of the newborn was not related to the genotype of the offspring. In matings to standard (*ss*) males, *Ss* females produced both standard (*ss*) pups and pups with the *star* (*Ss*). Secondary sex ratio among the offspring of both genotypes was higher than that among the standard offspring of the standard tame foxes (Tables 1 and 6).

Table 5 Correlations between sex ratio of offspring in successive parities of the same mothers of the domesticated population

Birth	1		2		3		4		5		6		7	
	<i>r</i>	<i>df</i>	<i>r</i>	<i>df</i>	<i>r</i>	<i>df</i>	<i>r</i>	<i>df</i>	<i>r</i>	<i>df</i>	<i>r</i>	<i>df</i>	<i>r</i>	<i>df</i>
1			0.010	90	0.111	63	0.090	44	-0.357	22	-0.432	11	-0.342	8
2					0.195*	108	-0.179	54	-0.017	30	-0.104	15	-0.074	11
3							0.169	67	-0.011	34	-0.001	22	0.164	15
4									0.173	30	0.096	16	0.262	13
5											0.110	23	0.007	15
6													-0.196	14

* Statistically significant at $P = 0.05$

Table 6 Sex ratio in offspring of domesticated foxes heterozygous for the *star* mutation (*Ss*)

Cross	Number of pups	Average litter size	Sex ratio	
			<i>Ss</i> pups	<i>ss</i> pups
♀ <i>Ss</i> × ♂ <i>ss</i>	1021	5.28 ± 0.11	0.610 ± 0.015	0.583 ± 0.015
♀ <i>ss</i> × ♂ <i>Ss</i>	1005	5.47 ± 0.11	0.543 ± 0.015	0.552 ± 0.015

There were virtually no differences in sex ratio at birth between offspring of reciprocal crosses (♀ *Ss* × ♂ *Ss*) and pups from all of the standard tame females mated with standard males (♀ *ss* × ♂ *ss*) (Tables 1 and 6).

Discussion

The data obtained demonstrate that secondary sex ratio departs from the expected 0.5 with male excess in foxes bred at the fur farm of this Institute. The departure was observed both for the control population and the one selected for tame behavior. However, the proportion of males had increased more clearly in the latter. Our results are in agreement with those indicating that males prevail among offspring of many domestic animals (Nishida et al. 1976; Gray and Hurt 1979; Gray and Katanbaf 1985). Evidence for the genetic variation of secondary sex ratio and any alteration thereof under the effect of direct selection is scant (Trivers and Willard 1973; Clutton-Brock and Jason 1986). However, there is some reason for assuming that genetic variation of sex ratio in foxes is correlated with behavioral variation. There is also reason for regarding a modified sex ratio as a correlated response to selection for behavior in foxes of our experimental farm. The domesticated fox population has been under strict direct selection for tameability for more than 30 years (Belyaev 1979; Belyaev and Trut 1982; Trut 1980). The control population under study, like any other population bred for commercial purposes, has never been intentionally selected for behavior. However, foxes have been caged from the beginning of the century, and they have thereby become adapted to a new social environment under natural selection, without any

intentional selection. It may even be suggested that the behavior of foxes of all commercial populations has been genetically reorganized by indirect selection. There is ample evidence that reproductive performance, which is the main selection criterion for commercial fox populations, correlates with behavioral traits (Belyaev and Trut 1964; Trut 1980). There is, thus, reason for suggesting that a hereditary reorganization of behavior has also taken place during the course of selection for reproductive performance of commercial fox populations.

The principal question is: which genetic systems have been affected by this selection for behavior so that they might influence sex ratio at birth? In certain mammalian species there are data indicating that variations among secondary sex ratio correlate with hormonal variation (James 1989, 1992). Various stress factors, for example, those acting on pregnant mothers, alter the sex ratio of pups at birth (Clutton-Brock and Jason 1986; Clarke et al. 1992). Females prevail in offspring from stressed mothers (Trivers and Willard 1973; Pratt et al. 1989a,b). With respect to foxes, it has been reported that sex ratio is related to the social rank of both the mothers and their neighbors (Bakken 1995). This means that hormones of the pituitary-adrenal system, the one involved in adaptation and stress, may be implicated in adjustment of primary sex ratios and formation of secondary sex ratios. This system plays the key role in the adaptation process of captive animals subjected to domestication (Richter 1950; Price 1984). The long-term selection of foxes for domestication has produced significant correlated changes in the various links of the pituitary-adrenal system (Naumenko and Belyaev 1980; Belyaev et al. 1988). The considerable attenuation of the hormonal response to stress is one such important change. To state it otherwise, acting through stress hormones, selection for behavior might have brought about the observed excess of males at birth.

The literature mentions that hormonal levels at conception affect secondary sex ratio. In some species, high levels of estrogens and testosterone correlate with an excess of males (James 1989, 1992). According to a more recent observation, adult female Mongolian gerbils androgenized in utero that gestated between male fetuses produce litters with a significantly greater proportion of sons (Clark et al. 1993); females prevail when the levels

of gonadotropins and progesterone are high. However, it has also been reported that the same hormones can differently affect sex ratio in various species (James 1989, 1992). Our data shows a significantly higher plasma progesterone level after mating in female foxes of the domesticated population and no significant differences in plasma estrogen level between tame and control foxes (Osadchuk and Trut, 1989).

Variations in sex hormone levels with respect to maternal age and their fertility were disregarded in this study. These variations cannot be entirely excluded, and if they do exist the data of Tables 2 and 4, which demonstrate the effect of parity order and litter size on secondary sex ratio in foxes, can be, at least partly, explained by these variations.

It is of importance that the *star* mutation and selection for tameability have a unidirectional effect on sex ratio. It seems possible that the high frequency of the *star* mutation is due to strong selection intentionally applied for behavior (Belyaev 1979; Belyaev et al. 1981). Evidence has also been compiled in favor of the idea that the expression of the major and pleiotropic effects of the *star* gene is related to the photoperiodic environment of the mothers during pregnancy (Trut 1991). All of this suggests that the *star* mutation is relevant to the systems regulating ontogenesis. Our data indicate that the hormonal response of the pituitary-adrenal system to stress in foxes with the *star* mark is suppressed compared to that of standard tame foxes. Other hormonal characteristics relevant to sex ratio in the *star* bearers remain to be study.

Gametic or zygotic selection of a particular sex is thought to be the main mechanism adjusting sex ratio by the time of birth (Trivers and Willard 1973; Howe 1977; Clutton-Brock and Jasen 1986). With reference to the data reported here, there is no explicit evidence for a preference to either zygotic or gametic selection. The data of Table 6 implicitly argue against selective elimination of male gametes: only the maternal gene *S* affects male preponderance, while the paternal *S* is without such an effect. The most plausible mechanism appears to be mortality of female embryos at the preimplantation period. Direct estimates of embryonic losses in domesticated females, standard silver-black and *Ss* heterozygotes, demonstrated that preimplantation embryonic death is somewhat lower ($22.1 \pm 1.3\%$) in the former than in the latter ($27.2 \pm 1.5\%$). Also estimates for early postnatal mortality of pups from domesticated and control females showed that the majority of losses fall during the first 10 days of life and are the same for male and female pups. Thus, 359 females and 362 males from tame mothers and 421 and 434 from controls died during the early postnatal period in 1991–1993. In fact, it has been reported that in certain mammalian species postnatal mortality is higher in males (Trivers and Willard 1973; Clutton-Brock et al. 1985). However, our data did not demonstrate any differences in early postnatal viability between the two sexes in foxes. The view is held that postimplantation mortality is a process ex-

tending to the first days of postnatal life. This means that these are the individuals that should have succumbed after implantation yet managed to survive, only to die early after birth (Evsikov 1987). If this view is accepted it would appear that postimplantation, like postnatal death, is not selective with respect to a particular sex.

Acknowledgments This work was supported by grants RBD000 and RBD300 from the International Scientific Funds and by grant 93-04-06936 from the Russian Fund of Fundamental Research. The author expresses her gratitude to Mrs. A. Fadeeva for translation of the manuscript from Russian into English. The author is also grateful Dr. I. Plyusnina for her help during preparation of the manuscript and E. Omelchenko for technical assistance.

References

- Bakken M (1995) Sex ratio variation and maternal investment in relation to social environment among farmed silver fox vixens (*Vulpes vulpes*) of high competition capacity. *J Anim Breed* (in press)
- Belyaev DK (1969) Domestication of animals. *Science* 5:47–52
- Belyaev DK (1979) Destabilizing selection as a factor in domestication. *J Hered* 70:301–308
- Belyaev DK, Trut LN (1964) Behavior and reproductive function in animals. I. Correlation between behavior and breeding season and fertility (in Russian). *Bull Mosk Obsh Ispit Prirodi* 69:5–19
- Belyaev DK, Trut LN (1982) Accelerating evolution. *Sci USSR* 5:24–29; 60–64
- Belyaev DK, Ruvinsky AO, Trut LN (1981) Inherited activation-inactivation of the *star* gene in foxes. *J Hered* 72:264–274
- Belyaev DK, Plyusnina IZ, Trut LN (1984) Domestication in the silver fox (*Vulpes fulvus* Desm): changes in physiological boundaries of the sensitive period of primary socialization. *Appl Anim Behav Sci* 13:359–370
- Belyaev DK, Oskina IN, Trut LN (1988) Genetics and phenogenetics of the hormonal characteristics of the corticoid function of the adrenals in silver black foxes (in Russian). *Genetika* 24:715–722
- Bull JJ (1981) Sex ratio evolution when fitness varies. *Heredity* 46:9–26
- Clarke IJ, Hemsworth PH, Barnett IL, Tilbrook AJ (1992) Stress and reproduction in farm animals. *Stress Reprod* 86:239–252
- Clark MM, Karpluk P, Galef Jr BG (1993) Hormonally mediated inheritance of acquired characteristics in Mongolian gerbils. *Nature* 364:712
- Clutton-Brock TH, Jason GR (1986) Sex ratio in mammals. *Q Rev Biol* 61:339–374
- Clutton-Brock TH, Albon SD, Guinness FE (1985) Parental investment and sex differences in juvenile mortality in birds and mammals. *Nature* 313:131–133
- Evsikov VI (1987) Genetic and evolutionary aspects of the problem of mammalian fertility homeostasis (mink as a model) (in Russian). *Genetika* 23:988–1002
- Gray E, Hurt VK (1979) Distribution of sexes in cattle. *J Hered* 70:273–274
- Gray E, Katanbaf MN (1985) Sex ratio and distribution of sexes in swine. *J Hered* 76:36–38
- Howe H (1977) Sex ratio adjustment in the common grackle. *Science* 198:744–746
- Huck UW, Labov JB, Lisk PD (1987) Feed-restricting first generation juvenile female hamsters (*Mesocricetus auratus*) affects sex ratio and growth of third-generation offspring. *Biol Reprod* 37:612–617
- James WH (1989) Parental hormone level and mammalian sex ratios at birth. *J Theor Biol* 139:59–67
- James WH (1992) The hypothesized hormonal control of mammalian sex ratio at birth a second update. *J Theor Biol* 155:121–128
- Naumenko EV, Belyaev DK (1980) Neuroendocrine mechanisms in animal domestication. In: Altukhov YP (ed) *Problems in general*

- genetics. Proc 14th Int Cong Genet MIR, book 2. Moscow, pp 12–24
- Nishida SK, Otsuka J, Hayashi H (1976) Sex ratio of offspring in domestic animals: swine. *Jpn J Anim Reprod* 22:106
- Osadchuk LV, Trut LN (1989) Gonad endocrine function female silver foxes under selection for domestic behavior. (in Russian). *J Obshch Biol* 50:189–198
- Pratt N, Huck UW, Lisk RD (1989a) Do pregnant hamsters react to stress by producing fewer males? *Anim Behav* 37:155–158
- Pratt N, Huck UW, Lisk RD (1989b) Effects of social stress during early pregnancy on litter size and sex ratio in the golden hamster. *J Reprod Fertil* 87:763–769
- Price EO (1984) Behavioral aspects of animal domestication. *Q Rev Biol* 59:1–32
- Richter CP (1950) Domestication of the Norway rat and its implications for the stress problem. *Proc Assoc Res Nerv Ment Dis* 29:19–47
- Rokitsky PPh (1973) Biological statistics. *Vischaya shkola* 318
- Trivers RL, Willard DE (1973) Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179:90–92
- Trut LN (1980) Role of behavior in domestic transformation of animals. PhD thesis, Institute of Cytology and Genetics, Novosibirsk
- Trut LN (1988) The variable rates of evolutionary transformations and their parallelism in terms of destabilizing selection. *J Anim Breed Genet* 105:81–90
- Trut LN (1991) Some genetic aspects of piebald spotting in silver foxes (*Vulpes vulpes*) and interrelation between the vector of selection and variability. In: Shumny VK, Ruvinsky AO (eds) *Problems of genetics and theory of evolution* (in Russian). Nauka, Novosibirsk, pp 67–84
- Zhelezova AI (1991) Influence of the *star* gene on potential fertility and embryonic death of silver foxes. In: Trut LN, Osadchuk LW, Borodin PM (eds) *Evolutionary-genetics and genetic-physiological aspects of the fur animals domestication* (in Russian). Institute of Cytology and Genetics, Novosibirsk, pp 171–183