

Sex ratio of the pupal stage in cocoons with different fluorescence characteristics from silkworm *Bombyx mori* L. breeds and hybrids

Radostina Guncheva

Trakia University, Faculty of Agriculture, Department of Animal Science – Non-ruminant Animals and Special Branches, 6000 Stara Zagora, Bulgaria

E-mail: radostina.guncheva@trakia-uni.bg

Abstract

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Establishing the influence of the sex of individuals of breeds, di- and tetrahybrids of the silkworms *Bombyx mori* L. on the fluorescence of the silk sheaths, when exposed to ultraviolet (UV) rays with a wavelength in the range 254-365 nm was the aim of this paper.

The study was conducted during 2018-2021 at the training experimental station of the Sericulture section of the Faculty of Agriculture at Trakia University – Stara Zagora. In fulfillment of the purpose and tasks, biological material from 8 breeds, 14 dihybrids and 10 tetrahybrids, grown with mulberry leaf was used.

The analysis of the obtained data shows that the sex has significantly proven influence on the character of the silk sheaths fluorescence, shown when exposed to them with UV rays. In fractions with violet fluorescence, the sex ratio is 1.44:1 in favor of the female, and in fractions with yellow fluorescence – 1:1.42 in favor of the male sex.

This gives reason to consider that the established sexual differentiation between the fractions of ultraviolet fluorescence and the influence of the fluorescence of the cocoons on their technological characteristics, proven in the literature, can be used for selection purposes, as well as for increasing the economic efficiency of the sericulture.

Keywords: Bombyx mori L.; breeds and hybrids; sex of the pupa; fluorescence; cocoons; silk sheaths

Introduction

Productivity is an important economic indicator of the efficiency of industrial production. In the silk butterfly *Bombyx mori* L., as in many other farm animals, a difference in the productivity of the two sexes is observed. Comparing the productive capabilities of the two sexes is of great importance from an economic point of view. For example, male individuals are healthier (resilient), easier to breed and consume less food to produce the same amount of silk. Breeding only males can reduce breeding costs by 10%. Male individuals have a higher rate of silk sheath formation with finer and more uniform silk thread thickness (Xu et al., 2004). According to the same author, in some breeds, the cocoons

of the female individuals have a violet or white fluorescence, and the males –yellow.

The difference in hemolymph fluorescence from male and female silkworms is evident from day 4 of instar 5 (Yu et al., 2000). Greater food intake after day 4 also induces the entry of copious amounts of violet and yellow fluorescent pigment into the hemolymph and then into the silk glands (Zhang et al., 2010). In females, it is mainly the violet fluorescent pigment that enters, as most or all of the yellow fluorescent pigment accumulates in the posterior part of the midgut, where it is absorbed by similar collagen, therefore females usually form cocoons with blue-violet fluorescence. Male silkworms form cocoons with mixed fluorescence (yellow or yellow-white) due to the absence of their binding pro-

tein in the midgut (Zhang et al., 2010). In support of this is the statement of Xiaolong et al. (2012), according to which the fluorescent colors of cocoons are identical to that of blood and silk glands. The difference in fluorescence of male and female cocoons may be caused mainly by the difference in the accumulation and transport of fluorescent pigments in the midgut and in silk glands, which they found by analyzing the fluorescent pigments by thin-layer chromatography.

The natural *Bombyx mori* silk has almost no fluorescence. In recent years, fluorescent silk obtained through various methods, such as the genetic modification or dye feeding method, has been applied in a variety of medical fields, such as drug delivery, monitoring surgical and wound healing processes (Lee et al., 2020).

Cheng et al. (2019) report a highly efficient strategy to produce fluorescent silk through directly feeding silkworm larvae with graphene quantum dots or CdSe/ZnS core-shell quantum dots. The obtained quantum dots-reinforced luminescent silkworm silk has superior mechanical strength and durability, stable fluorescence, and good biocompatibility compared with the normal or fluorescent dye-colored silk. In support of this are the results obtained by Liu et al. (2022) who use carbon dots (CDs) made from dried mulberry leaves, which have a strong near-infrared fluorescence with absolute quantum yield of 73% and a full width at half maximum of 20 nm. After feeding on such CDs, silkworms exhibit bright red fluorescence under ultraviolet light, grow healthy, cocooning normally and transform into adult insects finally. Hao et al. (2021) create a series of transgenic silkworms by applying an in vivo method for the selective expression of different fluorescent fusion proteins in silk glands in order to develop a technology for guided production of silk with certain fluorescence. Zhan et al. (2020) report a super-tough composite silk with excellent blue fluorescence intensity and uniformity by feeding silkworms trace (0.0012% in the diet) of hexaphenylsilol (HPS). The obtained uniform fluorescent silks have a number of advantages over ordinary silk and a high potential for application in the field of biomedicine (Liu et al., 2021).

A number of authors have investigated the possibilities for the selection of silkworm breeds in which sex can be differentiated by cocoon fluorescence, establishing a distinct difference between the fluorescence of male and female cocoons. Male cocoons fluoresce yellow and female cocoons violet or white when irradiated with UV light at a wavelength of 365 nm (Chen et al., 1988, Liu et al., 1996, Xu et al., 2004). Thomas & Thomas (2021) are investigating a number of methods, including fluorescence characteristic, to automate the sexing of silkworms. According to Huang (1980), the economic efficiency of breeding can be increased

by 10-15% by rearing only males. So breeding only males benefits both the farmers and the silk industry. On the other hand, more females favor the production of a larger volume of silkworm eggs. From such a perspective, the control and regulation of sex ratio by cocoon fluorescence is of utmost importance and has rightly attracted the attention of researchers for decades.

Material and Methods

The study was conducted during 2018-2021 at the training experimental station of the Sericulture section of the Faculty of Agriculture at Trakia University – St. Zagora. The object of research was the populations of 8 breeds – 1, 1A, 3, 4K, 19, 1013, 20, 1014; 14 dihybrids – 19x20, 20x19, 1013x1014, 1014x1013, 19x1013, 1013x19, 19x1014, 1014x19, 20x1013, 1013x20, Super₁xHesa₂^{*1}, Hesa₂xSuper₁^{*}, Vr₃₅xM₂^{*}, M₂x-Vr₃₅^{*} and 10 tetrahybrid forms of the silkworm *Bombyx mori* L. – (19x20)x(1013x1014), (1013x1014)x(19x20), (19x1013)x(20x1014), (20x1014)x(19x1013), (1013x19)x(1014x20), (1014x20)x(1013x19), (M₂xH₂)x(CH₁xI₁)^{*}, (CH₁xI₁)x(M₂xH₂)^{*}, (B₂xJ₂)x(KKxX₁)^{*}, (KKxX₁)x(B₂xJ₂)^{*}.

The incubation of the eggs and the rearing of the silkworms were carried out in previously prepared specialized rooms under established temperature-humidity conditions and feeding regime (according to Ovesenska & Panayotov, 1991).

The cocoons, obtained by the technology of replaceable bedding were picked on the 9-10th day from the mass climb of the silkworms on the bushes, sorted according to BDS 20-52-76/1977 and dried in an electric dryer under standard conditions.

The cocoons, with a total number of 31 089, were differentiated into three groups – with violet, intermediate and yellow fluorescence (Figure 1). For this purpose, an ultraviolet lamp with a filter passing UV rays in the range 254-365 nm was used. The sex was determined after cutting the cocoons according to the characteristic female and male morphological marks of the pupa stage (Figure 2).

The obtained data were systemized and processed with the respective modules of STATISTICA software of StatSoft and Microsoft Excel 2012.

Results and Discussion

Tables 1, 2 and 3 present the data on the share of individuals of both sexes and their ratio in populations of breeds,

¹ *The specified hybrid forms were created in the Scientific Center on Sericulture, Vratsa



Fig. 1. Cocoons with violet, intermediate and yellow fluorescence

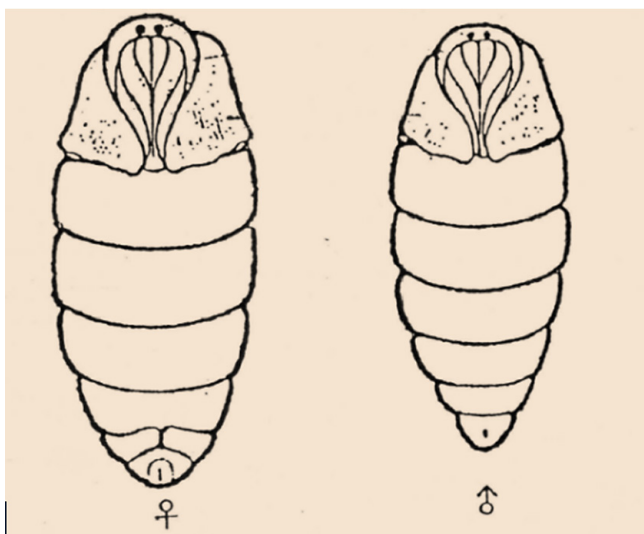


Fig. 2. Female (♀) and male (♂) pupae

di- and tetrahybrids of *B. mori* L. with different fluorescent characteristics of the silk sheaths formed by them.

From the data presented in Table 1, it can be seen that the female dominates the male with high reliability ($p \leq 0.001$) in all populations with violet fluorescence characteristics of the cocoons. The most pronounced sex difference in favor of female individuals was observed in breed 19, dihybrid 20x19 and tetrahybrid (19x1013)x(20x1014) with 25.9%, 32.56% and 23.98%, respectively. While the least pronounced differences between the sexes are in breed 1, dihybrid $M_2 \times Bp_{35}$ and tetrahybrid $(KK \times X_1) \times (B_2 \times J_2)$, where females outnumber males by 9.62%, 7.6% and 8.58%, respectively.

The data of the average value over all breeds show an 18.75% higher relative share of females than males, with a sex ratio of 1.46:1. In dihybrids, in terms of relative share of the entire population, female individuals outnumber males by 18.03%, and in tetrahybrids by 17.36%, with the sex ratio was 1.44:1 and 1.42:1, respectively.

The data presented on the Table 2 show non-unidirectional trends in the ratio between the two sexes in the intermediate fluorescence fractions of the cocoons. In breeds, females outnumbered males by from 1.08% to 11.48% for breeds 1A and 1, respectively. The average value for the all breeds, by which the female significantly exceeds the male sex in terms of relative share of the entire population, is 7.23%, and the sex ratio is 1.16:1 at $p \leq 0.01$. In the majority of dihybrids, the male sex dominates over the female with from 5% to 29.42%, in hybrids 20x19 and $C_1 \times X_2$, respectively. Exceptions are the hybrids 1014x19 and 20x1013, where the female sex exceeds the male by 15.56% and 18.75%. For the average value over the entire group of dihybrids, the results are reliable ($p \leq 0.01$) that the relative proportion of males is 56.57% of the entire population, and females are on average 13.14% less than males, with a sex ratio of 1:1.30. In the group of tetrahybrids, male predominance was observed in 50% of the forms included in the study. The relative share of female individuals varies between 50.35% and 59.00%. In the remaining 50% of the hybrids, the male sex dominates (50.16% – 66.52%), respectively for the individuals with intermediate fluorescence from the tetrahybrids (20x1014) x (19x1013) and $(M_2 \times H_2) \times (CH_1 \times I_1)$. As a result, the difference between the two sexes on average of all tetrahybrids is within narrower limits (47.11% – 52.89%). The dominance of one sex – the female is less pronounced (by 5.78%) and, accordingly, the sex ratio is closer to 1:1.12 compared to dihybrids (Table 2).

In all breeds and hybrids with yellow fluorescence characteristic of the cocoons a distinctly higher percentage of male individuals was observed with high reliability of the results ($p \leq 0.001$) in all analyzed cases (Table 3).

The reported values of the percentage of males in the breeds range from 53.1% to 65.1% at 20 and 4K, respectively, and they are 6.2% and 30.2% higher than females

The average percentage of males of all breeds (58.22%) is 16.44% higher than that of females (41.78%), and the ratio of females to males is 1:1.39.

The percentage of male individuals in the individual dihybrid forms is in the range of 53.85% – 63.83% for $X_{2x}C_1$ and 1013x1014 and is 7.7% and 27.7% higher than that of females, respectively. The averaged values of the dihybrids show that the population of male individuals constitutes 62.09%, and that of females – 37.91% of the entire popu-

Table 1. Relative distribution of sexes in breeds, di- and tetrahybrids silkworm populations with violet cocoons fluorescence

N	Breed/ hybrid	Number			%		Sex ratio
		Female individuals	Male individuals	Total for both sexes	Female individuals	Male individuals	Female:Male
Breeds							
1.	1	479	395	874	54.81	45.19	1.21:1
2.	1A	410	257	667	61.47	38.53	1.60:1
3.	3	93	56	149	62.42	37.58	1.66:1
4.	4K	106	73	179	59.22	40.78	1.45:1
5.	19	948	558	1506	62.95	37.05	1.70:1
6.	1013	985	728	1713	57.50	42.50	1.35:1
	Total for breeds	3021	2067	5088	59.38	40.62	1.46:1 ***
Dihybrids							
1.	19x20	507	292	799	63.45	36.55	1.74:1
2.	20x19	285	145	430	66.28	33.72	1.97:1
3.	1013x1014	718	476	1194	60.13	39.87	1.51:1
4.	1014x1013	213	126	339	62.83	37.17	1.69:1
5.	19x1013	885	667	1552	57.02	42.98	1.33:1
6.	1013x19	610	475	1085	56.22	43.78	1.28:1
7.	19x1014	53	30	83	63.86	36.15	1.77:1
8.	1014x19	72	52	124	58.06	41.94	1.39:1
9.	20x1013	106	71	177	59.89	40.11	1.49:1
10.	1013x20	42	26	68	61.77	38.24	1.62:1
11.	$C_1 \times X_2$	192	139	331	58.01	41.99	1.38:1
12.	$X_{2x} \times C_1$	130	105	235	55.32	44.68	1.24:1
13.	$Bp_{35} \times M_2$	204	159	363	56.20	43.80	1.28:1
14.	$M_2 \times Bp_{35}$	163	140	303	53.80	46.20	1.16:1
	Total for dihybrids	4180	2903	7083	59.02	40.99	1.44:1 ***
Tetrahybrids							
1.	(19x20)x(1013x1014)	91	66	157	57.96	42.04	1.38:1
2.	(1013x1014)x(19x20)	86	59	145	59.31	40.69	1.46:1
3.	(19x1013)x(20x1014)	137	84	221	61.99	38.01	1.63:1
4.	(20x1014)x(19x1013)	126	94	220	57.27	42.73	1.34:1
5.	(1013x19)x(1014x20)	126	78	204	61.77	38.24	1.62:1
6.	(1014x20)x(1013x19)	91	58	149	61.07	38.93	1.57:1
7.	$(M_2 \times H_2) \times (CH_1 \times H_1)$	161	119	280	57.50	42.50	1.35:1
8.	$(CH_1 \times H_1) \times (M_2 \times H_2)$	201	126	327	61.47	38.53	1.60:1
9.	$(B_2 \times J_2) \times (KK \times X_1)$	107	93	200	53.50	46.50	1.15:1
10.	$(KK \times X_1) \times (B_2 \times J_2)$	114	96	210	54.29	45.71	1.19:1
	Total for tetrahybrids	1240	873	2113	58.68	41.32	1.42:1 ***

*** ($p \leq 0.001$)

Table 2. Relative distribution of sexes in breeds, di- and tetrahybrids silkworm populations with intermediate cocoons fluorescence

N	Breed/ hybrid	Number			%		Sex ratio
		Female individuals	Male individuals	Total for both sexes	Female individuals	Male individuals	Female:Male
Breeds							
1.	1	165	131	296	55.74	44.26	1.26:1
2.	1A	140	137	277	50.54	49.46	1.02:1
3.	3	87	73	160	54.38	45.63	1.19:1
4.	4K	90	76	166	54.22	45.78	1.18:1
	Total for breeds	482	417	899	53.62	46.39	1.16:1 **
Dihybrids							
1.	19x20	273	388	661	41.30	58.70	1:1.41
2.	20x19	162	198	360	45.00	55.00	1:1.22
3.	1013x1014	253	276	529	47.83	52.17	1:1.09
4.	1014x1013	112	137	249	44.98	55.02	1:1.22
5.	19x1014	55	73	128	42.97	57.03	1:1.33
6.	1014x19	52	38	90	57.78	42.22	1.37:1
7.	20x1013	38	26	64	59.38	40.63	1.46:1
8.	1013x20	42	49	91	46.15	53.85	1:1.18
9.	$C_1 \times X_2$	84	154	238	35.29	64.71	1:1.83
10.	$X_{2x} C_1$	73	124	197	37.06	62.94	1:1.70
11.	$Bp_{35} \times M_2$	84	108	192	43.75	56.25	1:1.29
12.	$M_{2x} Bp_{35}$	57	103	160	35.63	64.38	1:1.81
	Total for dihybrids	1285	1674	2959	43.43	56.57	1:1.30 **
Tetrahybrids							
1.	(19x20)x(1013x1014)	59	41	100	59.00	41.00	1.44:1
2.	(1013x1014)x(19x20)	47	41	88	53.41	46.59	1.15:1
3.	(19x1013)x(20x1014)	100	97	197	50.76	49.24	1.03:1
4.	(20x1014)x(19x1013)	156	157	313	49.84	50.16	1:1.01
5.	(1013x19)x(1014x20)	73	72	145	50.35	49.66	1.01:1
6.	(1014x20)x(1013x19)	108	93	201	53.73	46.27	1.16:1
7.	$(M_2 \times H_2) \times (CH_1 \times I_1)$	76	151	227	33.48	66.52	1:1.99
8.	$(CH_1 \times I_1) \times (M_2 \times H_2)$	123	156	279	44.09	55.91	1:1.27
9.	$(B_2 \times J_2) \times (KK \times X_1)$	68	84	152	44.74	55.26	1:1.24
10.	$(KK \times X_1) \times (B_2 \times J_2)$	63	88	151	41.72	58.28	1:1.40
	Total for tetrahybrids	873	980	1853	47.11	52.89	1:1.12

** ($p \leq 0.01$)

Table 3. Relative distribution of sexes in breeds, di- and tetrahybrids silkworm populations with yellow cocoons fluorescence

N	Breed/ hybrid	Number			%		Sex ratio
		Female individuals	Male individuals	Total for both sexes	Female individuals	Male individuals	Female:Male
Breeds							
1.	1	303	510	813	37.27	62.73	1:1.68
2.	1A	269	494	763	35.26	64.74	1:1.84
3.	3	59	85	144	40.97	59.03	1:1.44
4.	4K	52	97	149	34.90	65.10	1:1.87
5.	20	855	968	1823	46.90	53.10	1:1.13
6.	1014	870	1202	2072	41.99	58.01	1:1.38
	Total for breeds	2408	3356	5764	41.78	58.22	1:1.39 ***
Dihybrids							
1.	19x20	186	319	505	36.83	63.17	1:1.72
2.	20x19	116	182	298	38.93	61.07	1:1.57
3.	1013x1014	136	240	376	36.17	63.83	1:1.77
4.	1014x1013	138	214	352	39.20	60.80	1:1.55
5.	20x1014	432	538	970	44.54	55.46	1:1.25
6.	1014x20	350	452	802	43.64	56.36	1:1.29
7.	19x1014	108	176	284	38.03	61.97	1:1.63
8.	1014x19	76	95	171	44.44	55.56	1:1.25
9.	20x1013	54	76	130	41.54	58.46	1:1.41
10.	1013x20	25	34	59	42.37	57.63	1:1.36
11.	$C_1 \times X_2$	84	121	205	40.98	59.02	1:1.44
12.	$X_2 \times C_1$	36	42	78	46.15	53.85	1:1.17
	Total for dihybrids	1741	2507	4248	37.91	62.09	1:1.44 ***
Tetrahybrids							
1.	(19x20)x(1013x1014)	28	39	67	41.79	58.21	1:1.39
2.	(1013x1014)x(19x20)	29	49	78	37.18	62.82	1:1.69
3.	(19x1013)x(20x1014)	85	111	196	43.37	56.63	1:1.31
4.	(20x1014)x(19x1013)	76	127	203	37.44	62.56	1:1.67
5.	(1013x19)x(1014x20)	57	84	141	40.43	59.57	1:1.47
6.	(1014x20)x(1013x19)	46	61	107	42.99	57.01	1:1.33
7.	$(M_2 \times H_2) \times (CH_1 \times I_1)$	31	43	74	41.89	58.11	1:1.39
8.	$(CH_1 \times I_1) \times (M_2 \times H_2)$	30	48	78	38.46	61.54	1:1.60
9.	$(B_2 \times J_2) \times (KK \times X_1)$	4	7	11	36.36	63.64	1:1.75
10.	$(KK \times X_1) \times (B_2 \times J_2)$	46	81	127	36.22	63.78	1:1.76
	Total for tetrahybrids	432	650	1082	39.93	60.07	1:1.51 ***

*** ($p \leq 0.001$)

lation. This forms superiority of 24.18% and sex ratio of 1:1.44 in favor of the male sex.

In the tetrahybrids, the proportion of males varies from 63.78% to 56.63% compared to 36.22% – 43.37% for fe-

males, with the most pronounced (with 27.56%) dominance of the male sex over the female is observed at $(KK \times X_1) \times (B_2 \times J_2)$, and the least (with 13.26%) – at $(19 \times 1013) \times (20 \times 1014)$.

From the average sex ratio data in the analyzed tetrahybrids with yellow fluorescent characteristic of the cocoons, it can be seen that the relative share of the male population exceeds the female population by 20.14% highly reliable ($p \leq 0.001$) forming a female:male ratio of 1:1.51.

The presented on the Figure 3 data show the values averaged of all breeds and hybrids for the percentage ratio of both sexes in each fluorescent group. Non-unidirectional trends were observed in the sex distribution, as in the fraction with violet fluorescence the female dominating (with 18.18%), and in the fractions with intermediate and yellow – the male (with 7.54 and 17.42%, respectively). The averaged data for ratio between the two sexes over all breeds, hybrids and fluorescent groups shows a slight difference (0.81%) in favor of the female, or a ratio of 1.02:1, which is close to the expected theoretical distribution.

From the data presented in Table 4, it can be seen that the sex has a significant influence ($P \leq 0.01$) on the presence of

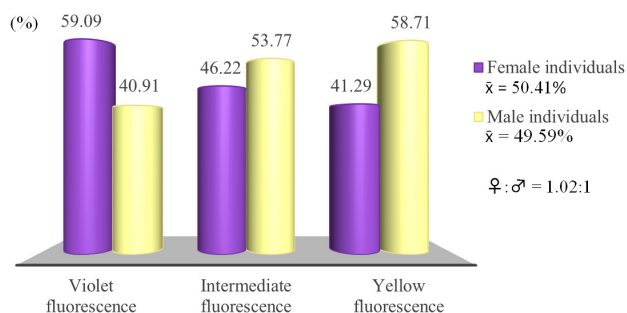


Fig. 3. Average relative share of both sexes of all *Bombyx mori* L. breeds and hybrids (%) in each fluorescent group

Table 4. Analysis of variance of effect of the female and male sex ratio (%) on the fluorescence of the cocoons

№	Sources of variation	df	F	p
1	Violet fluorescence	1	464.69	**
2	Intermediate fluorescence	1	6.23	*
3	Yellow fluorescence	1	467.07	**

** $p \leq 0.01$

* $p \leq 0.05$

violet and yellow fluorescence and a low significant effect ($p \leq 0.05$) on cocoons with intermediate fluorescence.

The analysis of variance for the influence of the sex on cocoon fluorescence (Table 5) shows that the degree of influence and the direction of deviation in the mean values of the percentage of male and female individuals relative to the mean for the model varies within relatively narrow limits, and the least deviation from the model average is observed for cocoons with intermediate fluorescence. Relatively larger differences in the sex ratio demonstrate the individuals with violet fluorescence of the cocoons, where with a positive LS-estimate (9.24) and a correspondingly higher value than the average for the model is the proportion of the female. While for the cocoons with yellow fluorescence, the significant differences in positive direction (with 9.78 of the mean for the model) are in favor of males, which is in confirmation of the results presented above in the present study (Table 5).

The summarized analysis of the results from Tables 1, 2 and 3 shows that there is a certain dependence between the fractions of ultraviolet fluorescence and the sex ratio. This is also confirmed by the data on the table. 4 from which it can be seen reliable influence ($p \geq 0.01$) of sex on the violet and yellow fluorescence.

Our results are in agreement with Chen et al. (1988), Zhang et al. (2010) finding a predominant proportion of female individuals in violet cocoons, and the males – with yellow fluorescence. As a reason for the relationship between sex and fluorescence Xiaolong (2012), point to the difference in the accumulation and transport of fluorescent pigments in the midgut and silk glands, and the relative proportion of silkworm pigments in different sexes (Yu et al., 2000).

The presented on the Figure 1 data show that in the fraction with violet fluorescence the female sex dominates (with 18.18%), and in the fractions with intermediate and yellow fluorescence they are dominant the male individuals (with 7.54 and 17.42%, respectively). The averaged data for ratio between the two sexes over all breeds, hybrids and fluorescent groups shows a slight (0.81%) difference in favor of the female sex, or a ratio of 1.02:1, which is close to the expected theoretical distribution. The slight predominance of the female over the male sex found by us is in dissonance with the findings of Kurbanov & Strunnikov (1982), Strunnikov

Table 5. LS-means and estimates of the influence of the female and male sex ratio (%) on the fluorescence of the cocoons

№	Sources of variation	Violet fluorescence			Intermediate fluorescence			Yellow fluorescence		
		n	LS-mean \pm SE	LS-estimate	n	LS-mean \pm SE	LS-estimate	n	LS-mean \pm SE	LS-estimate
	Mean for the model	60	50.00 \pm 1.28		52	50.00 \pm 1.08		56	50.00 \pm 1.39	
1	Female	30	59.24 \pm 0.61	9.24	26	47.27 \pm 1.46	-2.57	28	40.22 \pm 0.64	-9.78
2	Male	30	40.76 \pm 0.61	-9.24	26	52.57 \pm 1.46	2.57	28	59.78 \pm 0.64	9.78

(1983) – at the egg stage and the early silkworm stage, the sex ratio is close to 50:50. At the pupa stage, however, males outnumber females. According to Kurbanov & Strunnikov (1982), the sex ratio 1:1 is not always optimal, and during certain periods of the phylogeny of a given population, it establishes its own sex ratio optimum.

Conclusions

The results obtained in the present study show that the sex distribution in the studied groups, differing in the fluorescent characteristic of the cocoons, is highly reliable in all analyzed cases with violet and yellow fluorescence. The sex has a reliable influence on the nature of the fluorescence emission of silk sheaths after exposure to ultraviolet rays with a wavelength in range of 254-365 nm. In the fractions with violet fluorescence, the sex ratio is 1.44:1 in favor of the female, and in the fractions with yellow fluorescence – 1:1.42 in favor of the male.

That gives us reason to conclude that the proven relationship between sex ratio and fluorescence can be used to regulate the sex ratio by cocoon fluorescence, which can be used to increase the economic efficiency of sericulture, as well as for selection purposes.

References

- Chen, K. P., Yao, Q. & Lin, C. Q. (1988). Studies on silkworm cocoon fluorescence color. I. The cocoon fluorescence color of different silkworm races, *Acta Sericologica Sinica*, 14, 72-77.
- Cheng, L., Zhao, H., Huang, H., Li, B., Li, R. K. Y., Feng, X.-Q. & Dai, F. (2019). Quantum dots-reinforced luminescent silkworm silk with superior mechanical properties and highly stable fluorescence. *Journal of Materials Science*, 54, 9945–9957 (2019). <https://doi.org/10.1007/s10853-019-03469-w>
- Hao, Z., Long, D., Zhang, Y., Umuhzoza, D., Dai, J., Xua, Z., Zhang, G., Meng, W., Xiang, Z. & Zhao, A. (2021). New insight into the mechanism of in vivo fibroin self-assembly and secretion in the silkworm, *Bombyx mori*. *International Journal of Biological Macromolecules* 169, 473–479.
- Huang, J. T. (1980). Studies on sex control in silkworm (*Bombyx mori* L.). *Hereditas*, 2, 1-5.
- Kurbanov, R. & Strunnikov, V.A. (1982). Artificial sex regulation in the silkworm. Message V. The sex ratio of the silkworm in natural and experimental conditions. *Genetika*, XVIII(1)2, 1966-1975, (Ru).
- Lee, O. J., Sultan, M. T., Hong, H., Lee, Y. J., Lee, J. S., Lee, H., Kim, S. H. & Park, C. H. (2020). Recent Advances in Fluorescent Silk Fibroin. *Frontiers in Materials*, 7 (50), doi:10.3389/fmats.2020.00050
- Liu, G. Q., Yu, Z. C., Cui, Y. M., Yu, D. L. & Duan, Z. H. (1996). Breeding of fluorescence cocoon color sex-limited varieties Yingguang & Chunyu and the preparation of their F1 hybrid. *Acta Sericologica Sinica*, 22, 155-159.
- Liu, Ch., Bai, H., He, B., He, Dr.X., Zhang, J., Chen, Ch., Qiu, Y., Hu, R., Zhao, F., Zhang, Y., He, W., Chau, J. H. C., Chen, S., Lam, J. W. Y. & Tang, B. Zh. (2021). Functionalization of Silk by AIEgens through Facile Bioconjugation: Full-Color Fluorescence and Long-Term Bioimaging. *Angewandte Chemie International Edition*, 60 (22), 12424-12430. <https://doi.org/10.1002/anie.202015592>
- Liu, J., Kong, T. & Xiong, H.-M. (2022). Mulberry-Leaves-Derived Red-Emissive Carbon Dots for feeding Silkworms to Produce Brightly Fluorescent Silk. *Advanced Materials*, 34 (16), 2200152. <https://doi.org/10.1002/adma.202200152>
- Ovesenska, L. & Panayotov, M. (1991). Study aid of Sericulture. Publisher “Earth”, Sofia, ISBN 954-05-0015-X, (Bg).
- Qi, Zh., Fan, S., Wang, D., Yao, X., Shao, H. & Zhang, Y. (2020). Super-strong and uniform fluorescent composite silk from trace AIE nanoparticle feeding. *Composites Communications*, 21, 100414. ISSN 2452-2139, <https://doi.org/10.1016/j.coco.2020.100414>
- Strunnikov, V. A. (1983). Control of Silkworm Reproduction, Development and Sex. *MIR Publishers, Moscow*, 94.
- Thomas, S. & Thomas, J. (2021). A Review on Existing Methods and Classification Algorithms Used for Sex Determination of Silkworm in Sericulture. *Advances in Intelligent Systems and Computing*, 1351, 567–579. https://doi.org/10.1007/978-3-030-71187-0_52
- Xiaolong, H., Renyu, X., Guangli, C., Xing, Z., Yilin, Z., Xiaohua, Y., Yuqing, Z. & Chengliang, G. (2012). Elementary research of the formation mechanism of sex-related fluorescent cocoon of silkworm, *Bombyx mori*. *Molecular Biology Reports*, 39 (2), 1395–1409. <https://doi.org/10.1007/s11033-011-0874-3>
- Xu, A. Y., Li, M. W., Sun, P. J., Zhang, I. H. & Hou, C. X. (2004). Review on Silkworm (*Bombyx mori*) Sex Control in China. *International Journal of Industrial Entomology*, 8 (2), 123-127.
- Yu, Z. C., Gu, Y. Y. & Zhang, F. L. (2000). Studies on difference of fluorescent pigment between female and male larvae of cocoon colour sex distinguished fluorescent silkworm variety. *Acta Sericologica Sinica*, 26, 123–124.
- Zhang, Y. Q., Yu, X. H., Shen, W. D., Ma, Y. L., Zhou, L. X., Xu, N. X. & Yi, S. Q. (2010). Mechanism of fluorescent cocoon sex identification for silkworms *Bombyx mori*. *Science China, Life Sciences*, 53 (11), 1330-1339.

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