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# Characteristic of newly created $F_1$ tetrahybrids of the silkworm *Bombyx mori* L. by basic biological traits reared with artificial diet

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## Abstract

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The aim of this paper was characteristic of newly created $F_1$ tetrahybrids of the silkworm *Bombyx mori* L. reared with artificial diet throughout the larval stage on basic biological traits, which are essential for determining their receptivity to the food.

The study was conducted during 2019-2020 at the Training Experimental Station of the Sericulture section of the Faculty of Agriculture at Trakia University. Object of the study were the tetrachybrid forms of the silkworm (*Bombyx mori* L.) Vraca63 x Line22/Nig2 x Merefa6 (1st experimental group) and Nig2 x Merefa6/Vratsa63 x Line22 (2nd experimental group). The new, but already established in practice tetrahybrid I1 x VB1/N2 x HB2 was used for the control. Silkworms were reared with artificial diet containing 30% powder of dried mulberry leaf produced at Scientific Center on Sericulture – Vratsa and prepared by methods, developed by the manufacturer. The receptivity of the analyzed hybrids to artificial diet was determined based on the results of the survival rate and duration of the larval development during the first and the last instars of larval stage.

The results obtained showed that the studied tetrachybrids are characterized by very high survival rate (> 90%) and relatively short duration of development (average 291 and 293 hours for the first and 309 and 308 hours for the last instars of larval stage, respectively for the individuals from the 1st and 2nd experimental group), in some cases even exceeding, though insignificant, the control. Analysis of variance of effects of hybrid on the duration of larval development shown that the influence is a highly significant ($p \leq 0.001$) during the first instars.

The established tendencies give grounds to consider that the newly created tetrahybrids have very good receptivity and have a high biological potential of the parental forms, allowing their improvement through the methods of selection and their establishment as specialized for rearing with artificial diet.

*Keywords: Bombyx mori* L.; hybrids; survival; duration of larval development; artificial diet; receptivity

## Introduction

Artificial diets provides great opportunities for precise research in many areas, incl. related to general biological significance and application. According to Madyarov & Khamraev (2013), the artificial diet, in addition to the needs of sericulture, can be used in the future in the field of biotechnology for the production of high quality proteins, lipids, carbohydrates, biologically active substances and alternative medicine.

The main one issue in the area of application of artificial diet for silkworm *Bombyx mori* L. rearing is determining their receptive to such a diet and the creation of hybrid forms adapted to rearing with artificially prepared food (Horie, 1981). Silkworm rearing with artificial diet can be applied only in the first instars of larval stage or during the entire de-

velopment (Ito et al., 1974; Ito & Kobayashi, 1978; Maura, 1983; Iwanami, 1985; Matsubara et al., 1988a, 1988b).

According to Nair et al. (2013) mulberry leaf, especially in tropical conditions, does not always contain the necessary balanced nutrients to meet the needs of young silkworms during the year. The nutritional requirements of the silkworms from the first instars of the larval stage are specific and require the creation and maintenance of specialized mulberry plantations. To overcome this problem, it is to some extent recommended to rearing silkworms in the firs instars of their development with artificial diet.

In the countries with developed sericulture intensive work is carried out for making recipes for artificial mixtures. According to their type (synthetic or semi-synthetic), they contain chemical compounds or chemical compounds and mulberry leaf powder (Ito, 1961; Horie & Watanabe, 1969; Kim, 1979; Horie, 1981; Furuyama et al., 1985; Machida, 1987; Kato, 1988). Mulberry leaf powder is the main ingredient of artificial diet. It is contain components irreplaceable for the silkworms without which they refuse to take in the food. As additional protein sources are used soya meal, casein, egg albumin, gluten, sein, bovine albumin, gelatin, etc. (Ito, 1978). According to Ito & Arai (1965) ascorbic acid deficiency affects growth and survival depending on age development. They used an artificial diet to establish the relationship between the content of ascorbic acid in food and in silkworms and whether ascorbic acid biosynthesis takes place in their body. In 2009-2010 Tsenov & Georgiev (2010) developed for the first time in Bulgaria artificial diet suitable for silkworm nutrition during the entire larval period and cocoon production. Silkworms, reared with artificial diet have shorter larval development at age 5 and throughout the rearing period.

Azizov & Gulamova (1982), as a result of long studies, found, that is need for selection of breeds and creation of hybrids susceptibility to artificial diet rearing was realized. When rearing silkworms with artificial diet containing 40 and 50% of mulberry powder, better results are obtained with hybrids compared to the initial breeds (Genova, 1991).

According to Petkov (1995), efforts to create and use $F_1$ hybrid forms in Bombyx mori L. have intensified to such an extent that all production of industrial cocoons in developed silkworm countries is on a hybrid basis. In the development of artificial diets the main goal is to replace mulberry in the diet of industrial hybrids (Ito, 1981).

The larval development duration is an important biological and technological feature of silkworms and is of paramount economic importance in terms of the labor costs and resources for silkworms breeding. It was found, that in late autumn silkworm rearing with artificial diet has more favourable effect on the speed of development compared rearing with mulberry leaf (Panayotov, 1995).

The survival rate and the larval development duration are the basic biological characteristic of silkworms *Bombyx mori* L. determining their receptivity to artificial diet, which focused our efforts to the objective of the present study.

## Material and Methods

The study was conducted at the Training Experimental Station of the Sericulture section of the Faculty of Agriculture at Trakia University. Object of the study were the tetrachybrid forms of the silkworm (*Bombyx mori* L.) Vraca63 x Line22/Nig2 x Merefa6 (1st experimental group) and Nig2 x Merefa6/Vratsa63 x Line22 (2nd experimental group). The new, but already established in practice tetrahybrid I1 x VB1/N2 x HB2 was used for the control group. The necessary silkworm eggs for the present study were obtained and stored until the moment of their incubation by the method of early wintering (Tsenov et al., 2009). Silkworms were reared with artificial diet containing 30% powder of dried mulberry leaf produced at Scientific Center on Sericulture – Vratsa and prepared by methods, developed by the manufacturer, whereby 250 g dry substance and 675 ml of distilled water are homogenized using a mixer. Placed in a box with a lid, the mixture is treated thermally in MW for 10' at ~800 W.

Of all breeds and hybrids object of this study, it was formed groups in 3 reps of 400 normal silkworm eggs previously disinfected with a 2% formalin solution. Incubation of the silkworm eggs, rearing and feeding the silkworms was done in sterile petri dishes, in thermostats prepared in advance for the purpose to established temperature-humidity conditions, according to which in 1st, 2nd, and 3th instar air temperature was 28-30°C and air humidity – 80-85% and in 4th and 5th instar – 24-26°C and 70-75%.

In each repetition of the three experimental groups, immediately after the 3rd sleep, from the initially laid and surviving the 4th instar silkworms, 80 individuals were selected, equalized in their development and set for rearing until to the cocoons formation. This also explains the higher average

**Table 1. Scheme of the experiment**

| Group | Hibryd | Mulberry powder, % | First instars (n) | Last instars (n) |
|---|---|---|---|---|
| Control | I1 x VB1<br>N2 x HB2 | 30 | 3x400 | 3x80 |
| 1 | Vratsa63 x Line22<br>Nig 2x Merefa6 | 30 | 3x400 | 3x80 |
| 2 | Nig2 x Merefa6<br>Vratsa63 x Line22 | 30 | 3x400 | 3x80 |

survival rate of some of the hybrids in the last instars than in the first (Table 1).

The receptivity of silkworms to artificial diet can be established at the initial of the larval stage, but for greater reliability and more complete characterization of the newly created $F_1$ tetrahybrids of the silkworm (*Bombyx mori* L.) in the present study the survival rate and duration of development were controlled in the first instars (1st, 2nd, and 3rd) and in the last instars (4th and 5th), covering the entire larval stage. To determine the survival rate it was recorded the number of hatched viable specimens and the number of silkworms after mass awakening from the first sleep calculated in percentages according to the following formula:

$$\text{Survival (\%)} = \frac{N}{n} \times 100,$$

where *N* is number of viable silkworms in the relevant instar; *n* is total number of silkworms laid.

Data analysis has been performed on the basis of the criteria set out in Table 2 defining the degree of receptivity of larvae to artificial diet.

**Table 2. Criteria for assessing the degree of receptivity of silkworms to artificial diet**

| Degree of receptivity | Survival, % |
|---|---|
| Very high | Over 90 |
| High | 85 – 90 |
| Normal | 70 – 85 |
| Low | 60 – 70 |
| Very low | Under 60 |

In order to establish the duration of larval development, date and time for the beginning and end of the respective physiological states (instar and sleep), as of the individual instars of the larval stages, as well as the whole period of development, were determined individually for each repetition.

To process the data obtained we used the relevant modules from the STATISTICA software package of StatSoft and Microsoft Excel 2010.

## Results and Discussion

Nutrition is an important growth regulatory factor in silkworm. Significant advances in the research of silkworm nutrition began with development of artificial diets (Meeramaideen et al., 2017). The survival rate is one of the basic biological characteristic that determined the receptivity of silkworms to artificial diet. Table 3 presents data on the survival rate of individuals from the tetrahybrids analyzed in the present study during their age development, summarized as mean values for the first and the last instars.

From the data presented in Table 3 it can be seen that in general the average values of the survival rate vary within narrow limits during the first (93.85÷97.97%) and the last (from 82.50% in 2nd to 100% in control and 1st experimental group) instars of the larval stage. All analyzed hybrids are characterized by a very high average survival rate (>90%), respectively with a very good degree of receptivity (>90%), throughout the larval stage. The obtained results are in confirmation of the established by Guncheva et al. (2016) superiority in terms of survival rate of hybrid forms (> 90%) compared to parental ones.

The high survival ratio is indicative of the good receptivity of the individuals of the two experimental groups to artificial diet, despite the finding by Dong et al. (2018) metabolic system response and immune resistance of the species when switching from fresh mulberry leaf feeding to an artificial diet.

The data presented in Table 4 shows that the hybrid did not have a significant effect on the variation of the survival rate, both in the first and in the last instars.

The larval development duration, as of the individual instars of the larval stages, as well as the whole period of

**Table 4. Analysis of variance of effects of hybrid on the survival rate during the first and the last instars of larval stage**

| Sources of variation | df | First instars | | Last instars | |
|---|---|---|---|---|---|
| | | F | P | F | P |
| 30% mulberry powder | 2 | 44.97 | – | 1.20 | – |

**Table 3. Mean values of the survival (%) in $F_1$ hybrids during the first and the last instars of the larval stage**

| Group | Hybrid | n | First instars | | Last instars | |
|---|---|---|---|---|---|---|
| | | | x̄±SE | min-max | x̄±SE | min-max |
| Control | I1 x VB1<br>N2 x HB2 | 3 | 97.22±0.63 | 95.97-97.97 | 97.92±1.10 | 96.25-100 |
| 1 | Vratsa63 x Line22<br>Nig 2x Merefa6 | 3 | 96.00±1.34 | 93.85-98.46 | 98.75±0.72 | 97.50-100 |
| 2 | Nig2 x Merefa6<br>Vratsa63 x Line22 | 3 | 96.30±0.72 | 94.97-97.47 | 91.67±4.64 | 82.50-97.5 |

**Table 5. Mean values of the development duration (h) in $F_1$ hybrids during the first instars of the larval stage**

| Group | Hybrid | n | I instar | II instar | III instar | Total I-III |
|---|---|---|---|---|---|---|
| | | | x̄±SE | x̄±SE | x̄±SE | x̄±SE |
| Control | I1 x VB1<br>N2 x HB2 | 3 | 103±0 | 94±0.66 | 115±0.33 | 312±0.57 |
| 1 | Vratsa63 x Line22<br>Nig 2x Merefa6 | 3 | 97±0 | 93±0 | 101±0.66 | 291±0.66 |
| 2 | Nig2 x Merefa6<br>Vratsa63 x Line22 | 3 | 98±0 | 97±0.66 | 101±1.00 | 293±0.33 |

development, is an important biological and technological feature of silkworms and is of paramount economic importance in terms of the labor costs and resources for silkworms breeding. The duration of development in the first (Table 5) and in the last (Table 6) instars of the larval development of the analyzed hybrids also varies within narrow limits.

**Table 6. Mean values of the development duration (h) in $F_1$ hybrids during the last instars of the larval stage**

| Group | Hybrid | n | IV instar | V instar | Total IV-V |
|---|---|---|---|---|---|
| | | | x̄±SE | x̄±SE | x̄±SE |
| Control | I1 x VB1<br>N2 x HB2 | 3 | 145±0.33 | 169±0.33 | 314±0 |
| 1 | Vratsa63 x Line22<br>Nig 2x Merefa6 | 3 | 145±0.33 | 163±0.33 | 309±0.67 |
| 2 | Nig2 x Merefa6<br>Vratsa63 x Line22 | 3 | 150±5.00 | 158±2.19 | 308±2.85 |

The hybrids from the two experimental groups show a different trend in terms of the duration of the individual stages of development. In the first instars (Table 5) the larvae from the 1st experimental group have a shorter duration, while in the last instars (Table 6) – those from the 2nd group. As a result, the two hybrids has not differ in the duration of the entire larval stages. In all cases, about the whole period, the development of control individuals lasted the longest (Tables 5 and 6). According to the selection goals in Bulgaria, the desired duration of the larval period is 27-29 days, and the survival rate of the silkworms – not less than 90% (Petkov et al., 2006). Patil et al. (2017) research the growth and development of the mulberry silkworm when rearing with a mixture of leaves of mulberry cultivars in different ratio. The obtained results show a variation in the average values of the duration of larval development within 28.24-29.67 days. In this regard, it can be argued that all tetrahybrids of the present study have a shorter larval development (25-26 days) than that found for the mulberry silkworm.

**Table 7. Analysis of variance of effects of hybrid of development duration during the first and the last instars of the larval stage**

| Sources of variation | df | First instars | | Last instars | |
|---|---|---|---|---|---|
| | | F | P | F | P |
| 30% mulberry powder | 2 | 389.8 | *** | 30.3 | – |

***$p \leq 0.001$

The hybrid has not a one-way effect on the variance of the development duration. In the first instars it has highly significant ($p \leq 0.001$), and in the last it has unreliable (Tables 7 and 8).

The average LS-estimates of the experimental groups deviate in a negative direction from the model average in the first instars by 8 and 6 and at the last instars by 1 and 2 at the 1st and 2nd experimental groups, respectively. Therefore, growing the hybrids of the first and second experimental groups with 30% participation of mulberry flour in the diet combined with an appropriate breeding program would lead to an improvement of the trait. According to Dong et al. (2017) an important point for expanding the application of sericulture in agriculture is the increase in the values of bio-

**Table 8. LS-means and estimates of the influence of the hybrid on development duration during the first and the last instars of the larval stage (h)**

| Group | Source of variation | n | First instars | | Last instars | |
|---|---|---|---|---|---|---|
| | | | LS-means±SE | LS-estimates | LS-means±SE | LS-estimates |
| | Means for the model | 9 | 299±3.30 | | 310±1.25 | |
| Control | И 1 x ВБ 1<br>Н 2 x ХБ 2 | 3 | 312±0.58 | 13 | 314±0 | 4 |
| 1 | Vratsa 63 x Line 22<br>Nig 2 x Merefa 6 | 3 | 291±0.67 | -8 | 309±0.67 | -1 |
| 2 | Nig 2 x Merefa 6<br>Vratsa 63 x Line 22 | 3 | 293±0.33 | -6 | 308±2.85 | -2 |

logical and technological traits, which is why their improvement continues to be the main goal of breeding programs.

In general, as a result of hybridization between breeds and hybrids with established potential for rearing with artificial diet (Guncheva et al., 2016), the obtained $F_1$ tetrahybrid forms of *Bombyx mori* L. show a very good degree of receptivity, expressed by the levels of the basic biological traits survival and duration of larval development. Confirmation of the obtained results is the established of Singh et al. (2012), according to which the survival and duration of larval development are among the traits characterized by a high degree of heterosis.

## Conclusions

The results obtained showed that the studied tetrachybrids are characterized by very high survival rate (>90%) and relatively short larval development duration (average 600 and 601 hours for the individuals from the 1st and 2nd experimental group, respectively), in some cases even exceeding, though insignificant, the control group, which mean that the studied tetrachybrids have very good degree of receptivity (>90%) to the artificial diet (30% mulberry powder), throughout the larval stage.

During in the first instars the hybrid has a highly significant influence ($p \leq 0.001$) on the duration of the larval development.

The average LS-assessments of the experimental groups deviates in a negative direction from the average for the model, which is an indication of shortening the development duration compared to those of the control group and therefore their use for the production of cocoons and silk would be beneficial to the economic efficiency of the rearing process.

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