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STUDIES ON THE NUCLEIC ACIDS AND THEIR CORRELATION WITH ECONOMIC CHARACTERS OF SILKWORM *Bombyx mori* L.

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ABSTRACT

Four pure mulberry silkworm breeds *viz.*, Pure Mysore, Nistari, NB_4D_2 & CSR_2 and two hybrid (Pure Mysore x CSR_2 and Nistari x NB_4D_2) silkworms were selected for the present study. The total DNA and RNA present in the mid gut tissue was estimated during entire fifth instar with a regular interval of 24 h, and the average concentration was estimated. The commercial characters *viz.*, fecundity, larval weight, larval duration, cocoon weight, shell weight, shell ratio, filament length, denier and renditta were selected. The average concentration of DNA and RNA were subjected to regression analysis against selected commercial characters to know the level and kind of correlation between them. The results of regression analysis between DNA and larval duration as well as filament length exhibited moderately high positive level of correlation coefficient. The RNA also exhibited the same trend with larval duration, filament length and denier only. The RNA/DNA revealed moderately high positive correlation with larval duration, shell weight, shell ratio and denier only.

Keywords: Silkworm, *Bombyx mori*, Midgut, DNA, RNA, Commercial Characters

INTRODUCTION

Ever since its inception, Sericulture is playing an important role in the economic life of man **[1]**. To enhance the productivity, many attempts are being made to improve the silkworm stocks through conventional breeding techniques **[2]**. Many researchers have been studied on the morphology, anatomy, cytology, embryology and physiology of *Bombyx mori* when compared to molecular biology aspects of silkworm **[3]**. The DNA content in insect tissue is an index for expressing other biochemical

contents like RNA and protein. The increase in DNA to RNA along with protein suggests the activation of metabolic process like protein synthesis. It also expresses the protein metabolism of silkworm **[4]**. Brindha *et al*. **[3]** reported higher level of DNA, RNA and protein from I to V instar silkworm *Bombyx mori*. Recently, genetic markers have used in animal and plant improvement programmes for varietal and parentage identification, construction of linkage maps and evaluation of polymorphic genetic loci affecting quantitative economic traits. Development of molecular markers is important in the silkworm for construction of linkage map and fingerprinting of strains for breeding **[5]**. A number of reports concerning the correlation aspects of silkworm *Bombyx mori i.e*., among DNA, RNA and proteins **[6]**; between yield and biochemical parameters **[7]**; amylase and larval span, cocoon weight, shell weight, filament length, cocoon color, cocoon shape **[8]**; silkworm proteins **[9]**, amylase **[2]**, succinate dehydrogenase **[10]**, esterase **[11]** and alkaline phoshatase **[12]** with commercial characters have been reported. However, the correlation studies between biomolecules like DNA, RNA and their ratios with commercial characters of silkworm *Bombyx mori* are rather scanty. Therefore, present investigation was undertaken.

MATERIALS AND METHODS

Four pure mulberry silkworm breeds *viz.*, Pure Mysore, Nistari, NB_4D_2 & CSR_2 and two hybrid (Pure Mysore x CSR_2 and Nistari x NB_4D_2) silkworms were selected for the present investigation. The silkworm rearing was conducted in the laboratory following the method described by Krishnaswamy **[13]**. All experimental batches were maintained in triplicate. The midgut tissues was obtained during fifth instar from five larvae daily, with a regular interval of 24 h. till the end of fifth instar by dissecting the larvae in ice cold water and the gut contents were removed. The tissues were thoroughly washed in sterile distilled water. A 10% homogenate was prepared in buffered saline (0.15 M NaCl and 0.15 M sodium citrate, pH 7.0) using mortar and pestle. The homogenate was centrifuged at 8000 rpm for 15 minutes in a cooling centrifuge at 5ºC. The clear supernatant was used for the assay of total DNA and RNA.

The amount of DNA was estimated during fifth instar daily with a regular interval of 24 h. by diphenylamine method **[14]**. Calf thymus DNA was used as standard. The average DNA concentration during 5^{th} instar was calculated. The results were expressed as µg of DNA /mg midgut tissue. The concentration of RNA was estimated during fifth instar daily with a regular interval of 24

h. by orcinol method **[14]**. Yeast RNA was used as standard. The average RNA concentration during 5^{th} instar was calculated. The results were expressed as µg of RNA /mg midgut tissue.

The experimental data were statistically analyzed through SPSS by one way ANOVA **[15]**, Scheffe's post hoc test **[16]** and linear regression analysis **[17]** wherever they were applicable

RESULTS

The summary of the studied commercial characters during pre monsoon, monsoon and post monsoon seasons are presented in the table1. From results **(Table 1)** it is clear that the two bivoltine races are superior for productivity aspects, whereas multivoltines are superior for viability characters. The hybrid silkworms showed mid parental values. The results of one way ANOVA revealed that the variation in all commercial characters among the experimental batches are all significant at 0.1 % ($P<0.001$). The concentrate of DNA in midgut tissue samples showed significant changes in their levels at every 24 hours till the end of fifth instar **(Table 2)**. The results of statistical analysis revealed that the variation among the experimental batches are all found to be significant at 0.1 % ($P<0.001$). The results of quantitative analysis were subjected for regression analysis against selected commercial characters to know the correlation coefficient between them **(Figures 1-9)**. The results of statistical analysis clearly showed that concentration of DNA has positive correlation with denier, filament length, larval duration and single cocoon weight only. The amount of RNA in midgut tissue samples showed significant changes in their levels at every 24 hours till the end of fifth instar **(Table 3)**. The results of statistical analysis revealed that the variation among the experimental batches are all found to be significant at 0.1 % ($P<0.001$). The results of quantitative analysis were subjected for regression analysis against selected commercial characters to know the correlation coefficient between them **(Figures 10-18)**. The results of statistical analysis clearly showed that concentration of RNA has positive correlation with denier, filament length, larval duration, shell ratio, single cocoon weight and single shell weight only. The RNA ratio to DNA (RNA/DNA) showed statistically significant ($P<0.001$) variation among experimental sets **(Table 4)**. The results of quantitative analysis were subjected for regression analysis against selected commercial characters to know the correlation coefficient between them. The results of statistical analysis **(Figures 19-27)** clearly showed that RNA to DNA ratios has positive correlation with denier, filament length, larval duration, renditta,

shell ratio, single cocoon weight, single shell weight except fecundity and larval weight.

DISCUSSION

Of the silkworm digestive system, the midgut is the major organ for food digestion, nutrient absorption and also a barrier for foreign substance. Moreover, midgut has early been recognized as one of the important targets for insect control. Deoxyribonucleic acids and ribonucleic acid are most important biomolecules of the cell as they controls overall metabolism of the cell or organism. Such biochemical growth-rate indicators, such as RNA concentration or the RNA/DNA ratio, are routinely used for estimating growth rates and nutritional condition of larval fish in the field of marine ecology **[18]**. Also, studies on the concentration of DNA are of paramount importance in the breeding program, because the DNA content in insect tissue is an index for expressing other biochemical contents like RNA, Protein, cell division, growth and development. The increase in RNA to DNA along with protein suggests the activation of metabolic process like protein synthesis. It also expresses the protein metabolism of silkworm **[4]**. Singh and Saratchandra **[19]** reported that RNA plays a major role in protein metabolism and morphogenesis. Brindha *et al.* **[3]** in their studies reported that the DNA and RNA content were increased proportionately in the larva as the age progressed. Also they reported that higher level of protein. Our results also correlates with the above mentioned authors as the concentration of DNA showed significant increase in their levels at every 24 hours till the end of fifth instar **(Table 2)**. However, its correlation coefficient with commercial characters was minimum **(Figures 1-9)**. Similar trend was noticed in the case of RNA also, as it showed significant increase in its concentration at every 24 hours till the end of fifth instar **(Table 3)**. The results regression analysis are presented in figures from 10 to 18. From the results majority of the traits showed moderate positive correlation. The RNA/DNA showed statistically significantly variation in their level **(Table 4)**. The regression analysis between commercial characters and nucleic acid ratio clearly indicated the moderate positive correlation with all the traits except fecundity and larval weight **(Figures 19-27)**. Singh and Saratchandra **[20]** reported that within 24 hours of oviposition the RNA/DNA in diapauses and non-diapause increases rapidly but, after 48 hours they reach the same level. Later the RNA/DNA of nondiapauses eggs increases rapidly. This report also suggests that the RNA/DNA indicates the level of metabolism and physiological status of the organism.

CONCLUSSION

The present results clearly indicated that the concentration of midgut DNA showed moderately positive correlation with larval duration, cocoon weight, filament length and denier. The RNA level indicated moderately positive correlation with larval duration, cocoon weight, shell weight and filament length. The RNA ratio to DNA revealed moderate positive correlation with larval duration, cocoon weight, shell weight, shell ratio and denier only. The information gathered from this work will helps to know the kind and degree of correlation between the nucleic acids and commercial characters of silkworm. The information generated from this study may be used to breed new strains of silkworm *Bombyx mori* with better economic characters. In addition it contributes to basic molecular biology of insects in general.

ACKNOWLEDGMENTS

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Table 1: Mean Values ± SD of Nine Commercial Characters in Six Breeds of Silkworm, *Bombyx mori*

Commercial Traits / Silkworm Breeds	Fecundity	Larval Weight (g)	Larval Duration (h)	Cocoon Weight (g)	Shell Weight (g)	Shell Ratio (%)	Filament Length (m)	Denier	Renditta
Pure Mysore	467.22±10.96	2.01±0.06	660±10.39	1.02±0.07	0.12±0.01	12.57±0.49	426.44±19.83	1.77±0.09	11.77±0.82
Nistari	485.11±5.30	2.83±0.06	564.88±10	1.14±0.07	0.15±0.01	13.41±0.87	435.66±17.21	1.78±0.07	13.26±0.24
CSR_2	509.10±16.58	4.07±0.05	578.88±6.45	1.81±0.05	0.43±0.01	24.02±0.18	1011.99±12.3	2.93±0.22	5.78±0.23
NB_4D_2	520.55±16.65	4.16±0.05	576.67±11.1	1.76±0.03	0.35±0.01	20.27±0.15	1020±29.96	2.48±0.06	8.34±0.47
Pure Mysore x CSR_2	466.66±11.52	2.68±0.07	610±11.10	1.67±0.02	0.28±0.01	17.29±0.21	910±18.74	2.75±0.06	7.64±0.12
Nistari x NB_4D_2	490.77±6.81	3.46±0.04	557±10.21	1.47±0.02	0.23±0.01	16.06±0.85	805.99±12.4	1.83±0.02	9.22±0.85

NOTE: Values are the Mean± SD of Pre Monsoon, Monsoon and Post Monsoon Observations; The Variation Between the Races is Statistically Significant at 0.1 % (P<0.001)

Table 2: Concentration of DNA in Midgut Tissue (µg/mg)

SILKWORM BREEDS	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day	8th day	AVERAGE
PURE MYSORE	5.00	8.30	14.60	17.80	19.00	22.30	22.50	23.30	16.60
NISTARI	4.00	7.30	12.60	15.80	19.30	22.10	-	-	6.20
CSR_2	4.20	7.70	12.70	18.50	22.70	25.60	-	-	15.20
NB_4D_2	5.40	9.00	13.30	18.70	23.50	24.50	-	-	15.70
PURE MYSORE x CSR_2	6.50	8.60	14.50	19.10	22.30	24.00	25.00	-	17.10
NISTARI x NB_4D_2	6.50	8.90	17.20	21.00	23.20	25.60	-	-	17.00

NOTE: The Variation Between the Races is Statistically Significant at 0.1 % (P<0.001)

Table 3: Concentration of RNA in Midgut Tissue (µg/mg)

SILKWORM BREEDS	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day	8th day	AVERAGE
PURE MYSORE	8.00	14.00	22.60	27.60	33.30	38.00	41.00	42.00	28.30
NISTARI	6.60	11.60	20.30	28.60	37.00	41.30	-	-	24.20
CSR_2	7.50	13.30	23.60	32.30	39.60	45.60	-	-	26.90
NB_4D_2	9.30	14.30	21.60	31.30	38.60	44.60	-	-	26.60
PURE MYSORE x CSR_2	10.10	14.00	24.80	34.30	38.30	44.60	48.30	-	30.60
NISTARI x NB_4D_2	8.60	13.30	25.60	33.00	39.60	46.00	-	-	27.60

NOTE: The Variation Between the Races is Statistically Significant at 0.1 % (P<0.001)

Table 4: RNA Ratio to DNA (RNA/DNA) in Midgut Tissue

SILKWORM BREEDS	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day	8th day	AVERAGE
PURE MYSORE	1.62	1.68	1.54	1.55	1.74	1.69	1.80	1.79	1.68
NISTARI	1.66	1.59	1.60	1.80	1.90	1.86	-	-	1.73
CSR_2	1.77	1.71	1.85	1.74	1.73	1.77	-	-	1.76
NB_4D_2	1.72	1.59	1.61	1.66	1.64	1.81	-	-	1.67
PURE MYSORE x CSR_2	1.56	1.61	1.70	1.78	1.71	1.85	1.92	-	1.73
NISTARI x NB_4D_2	1.33	1.48	1.48	1.56	1.70	1.78	-	-	1.55

NOTE: The Variation Between the Races is Statistically Significant at 0.1 % (P<0.001)

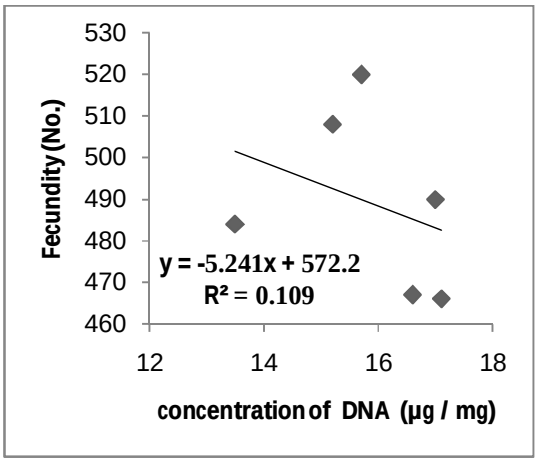

Figure 1: Correlation Between DNA and Fecundity

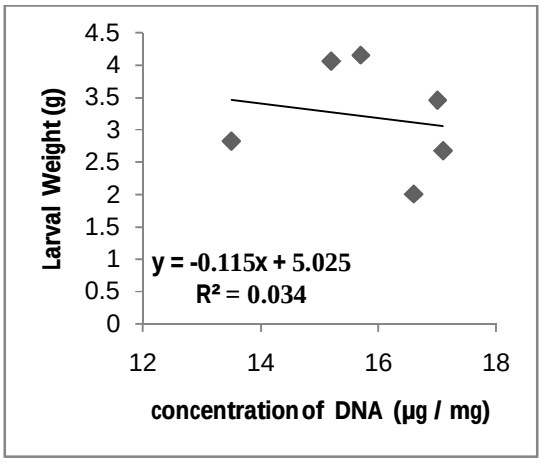

Figure 2: Correlation Between DNA and Larval Weight

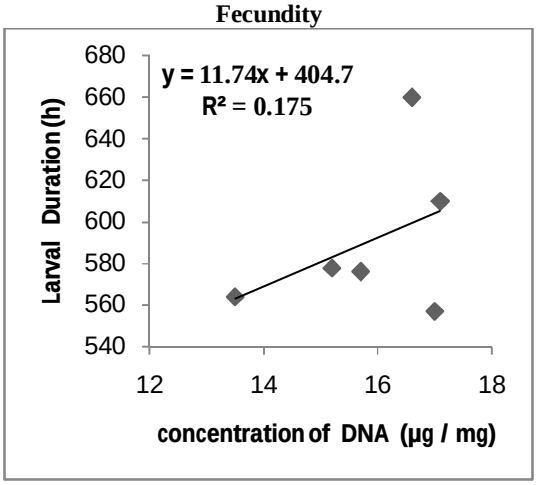

Figure 3: Correlation Between DNA and Larval Duration

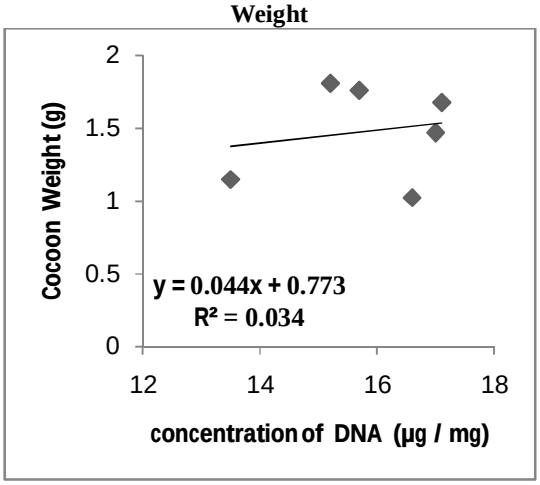

Figure 4: Correlation Between DNA and Cocoon Weight

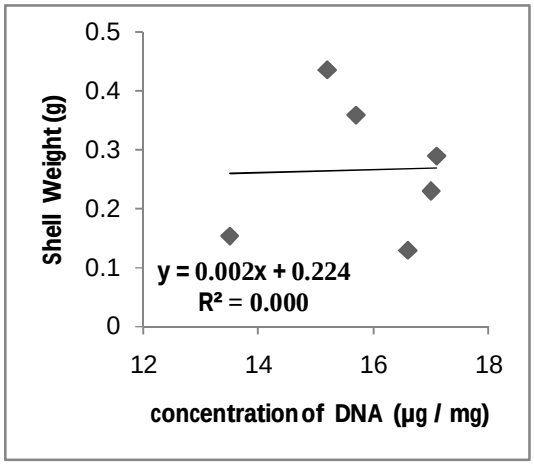

Figure 5: Correlation Between DNA and Single Shell Weight

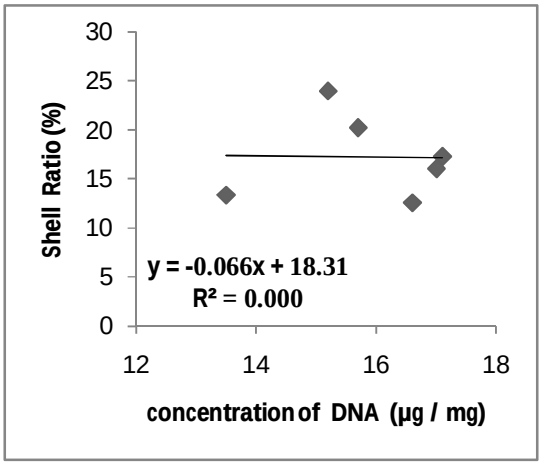

Figure 6: Correlation Between DNA and Shell Ratio

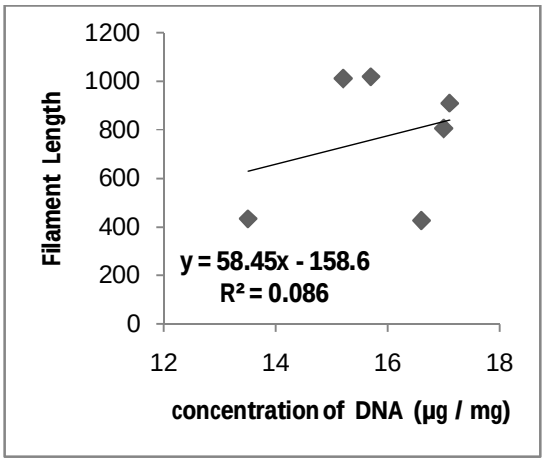

Figure 7: Correlation Between DNA and Filament Length

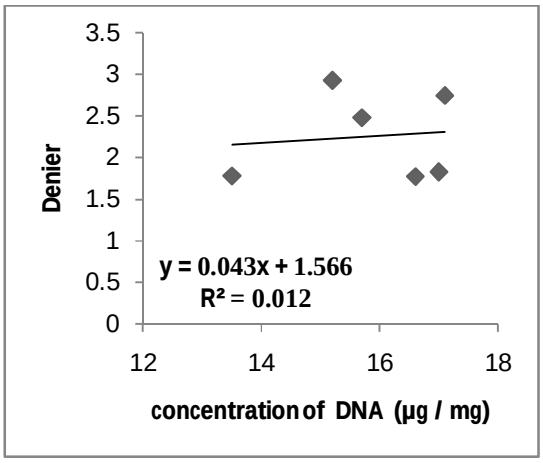

Figure 8: Correlation Between DNA and Denier

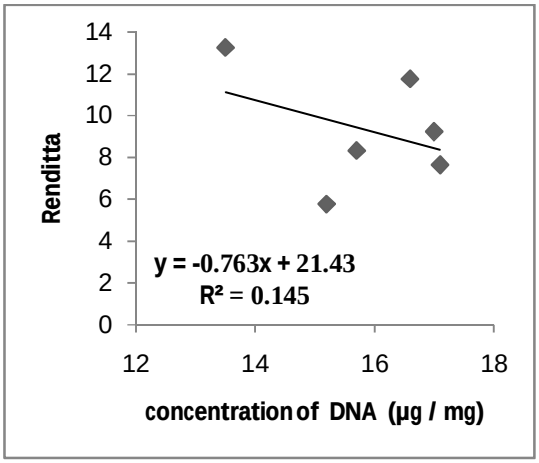

Figure 9: Correlation between DNA and Renditta

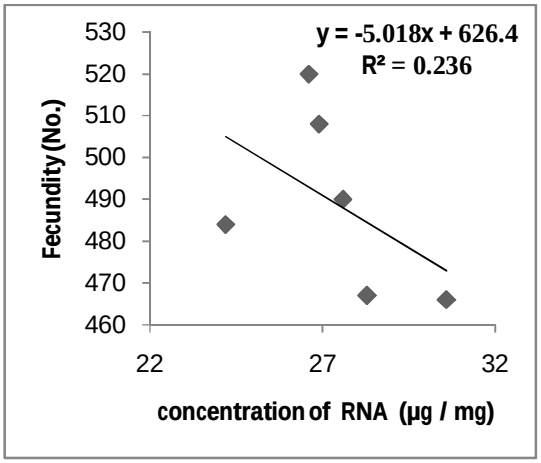

Figure 10: Correlation Between RNA and Fecundity

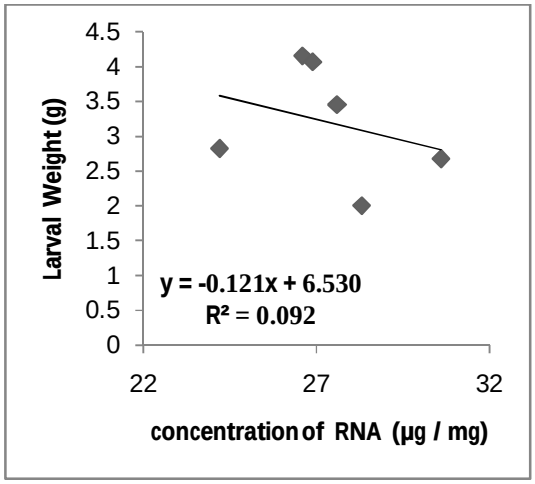

Figure 11: Correlation Between RNA and Larval Weight

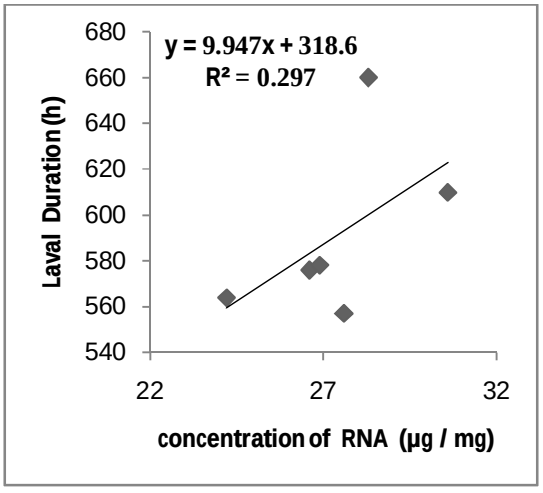

Figure 12: Correlation Between RNA and Larval Duration

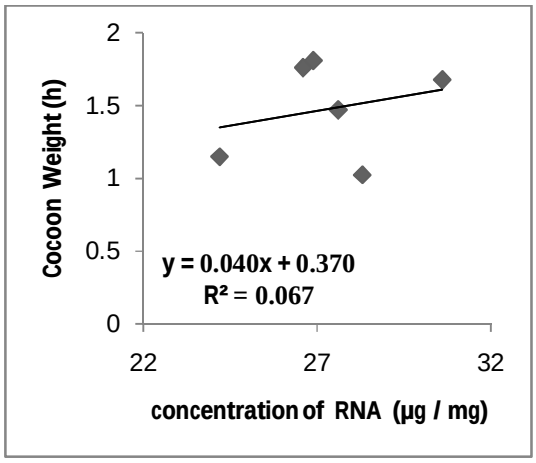

Figure 13: Correlation Between RNA and Cocoon Weight

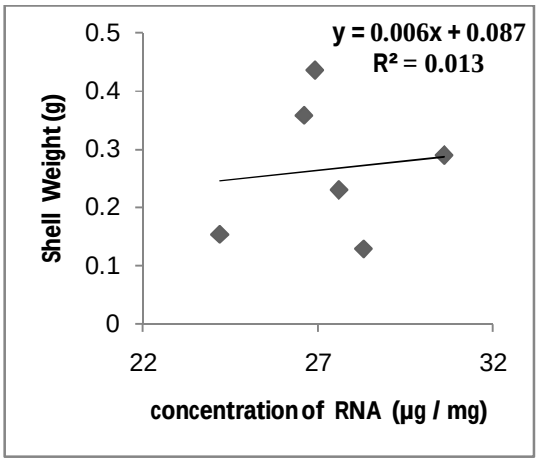

Figure 14: Correlation Between RNA and Shell Weight

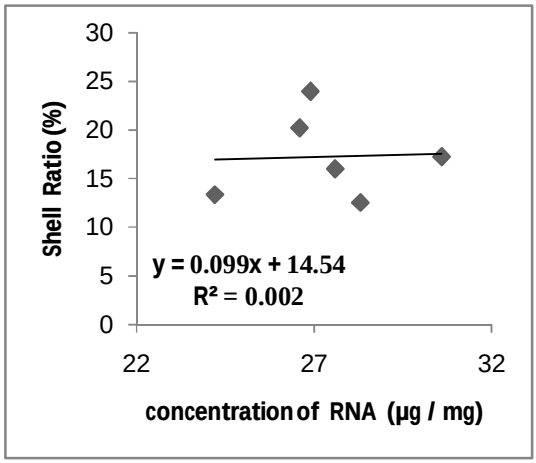

Figure 15: Correlation Between RNA and Shell Ratio

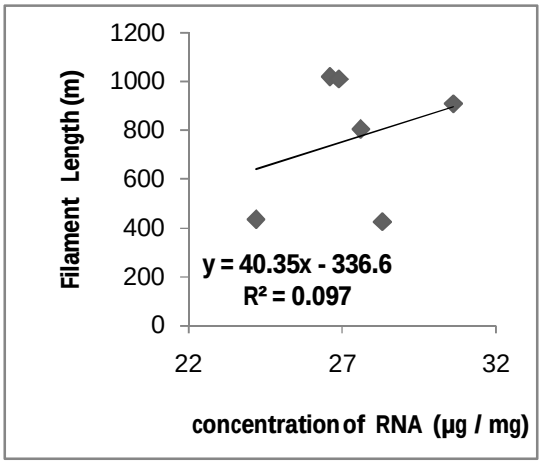

Figure 16: Correlation Between RNA and Filament Length

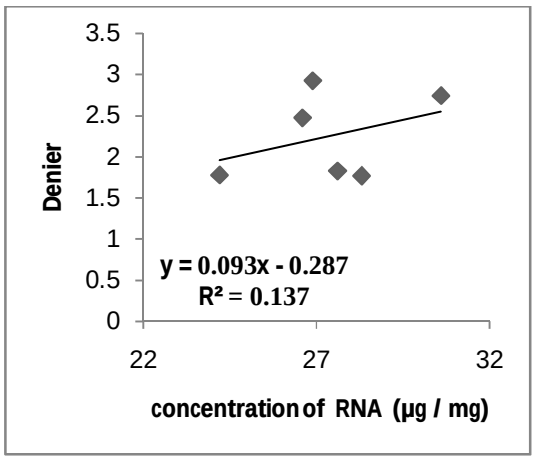

Figure 17: Correlation Between RNA and Denier

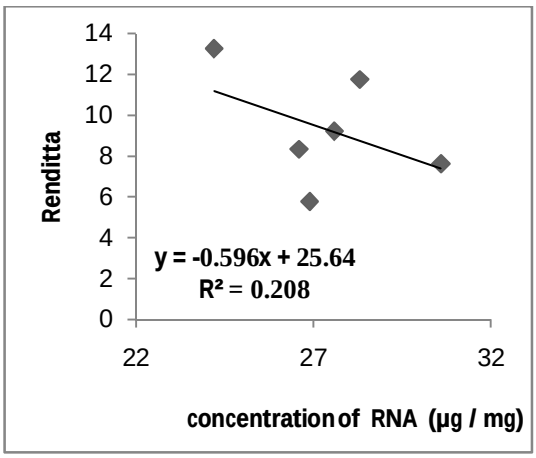

Figure 18: Correlation Between RNA and Renditta

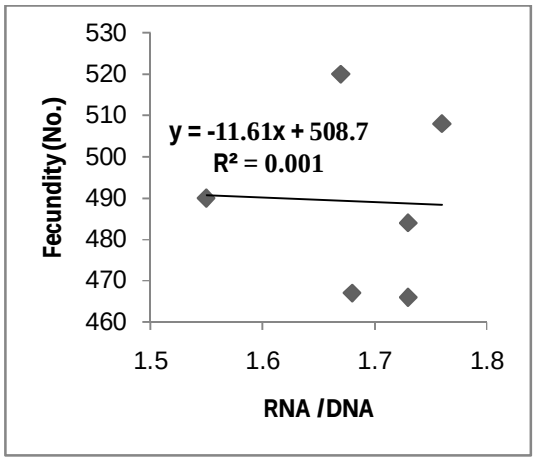

Figure 19: Correlation Between RNA/ DNA and Fecundity

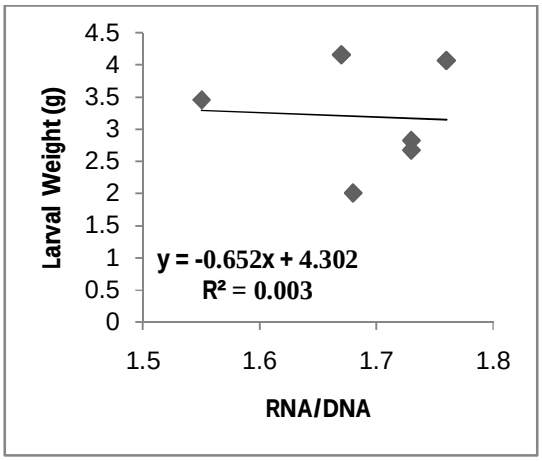

Figure 20: Correlation Between RNA/ DNA and Larval Weight

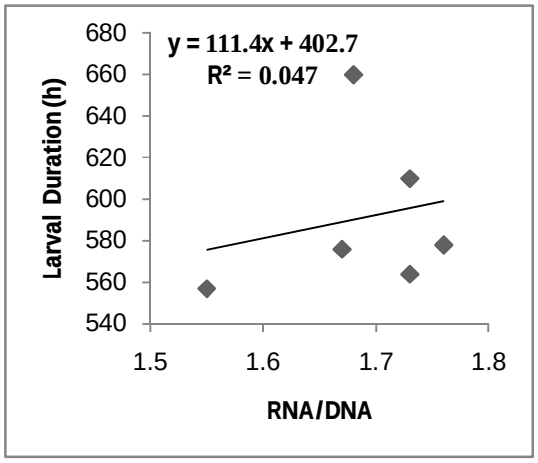

Figure 21: Correlation Between RNA/ DNA and Larval Duration

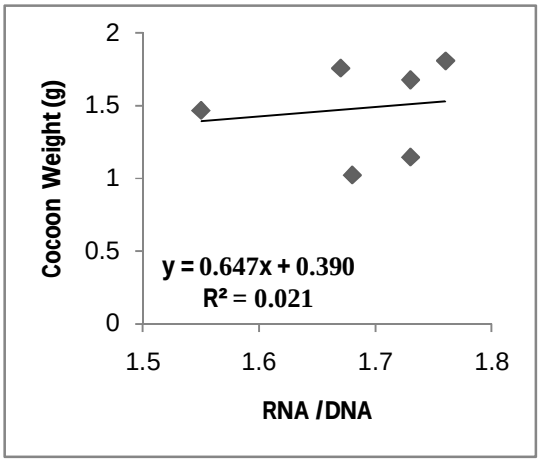

Figure 22: Correlation Between RNA/ DNA and Cocoon Weight

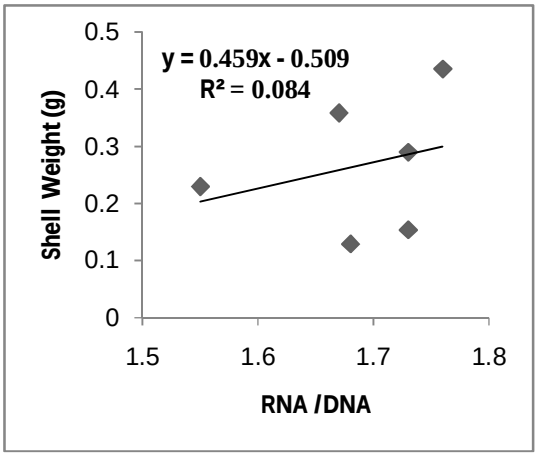

Figure 23: Correlation Between RNA/ DNA and Shell Weight

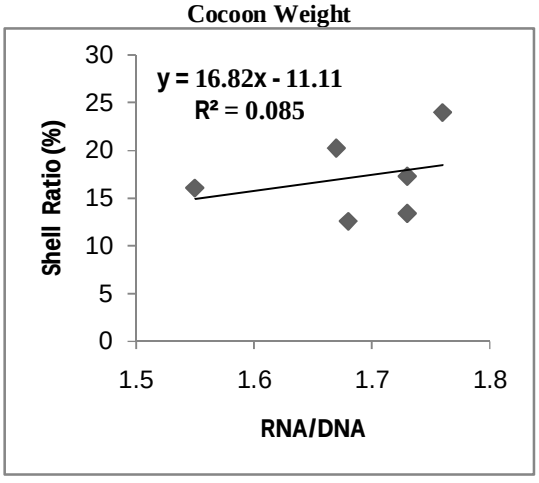

Figure 24: Correlation Between RNA/ DNA and Shell Ratio

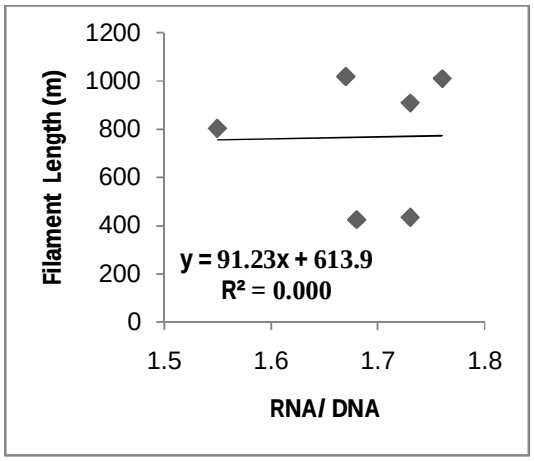

Figure 25: Correlation Between RNA/ DNA and Filament Length

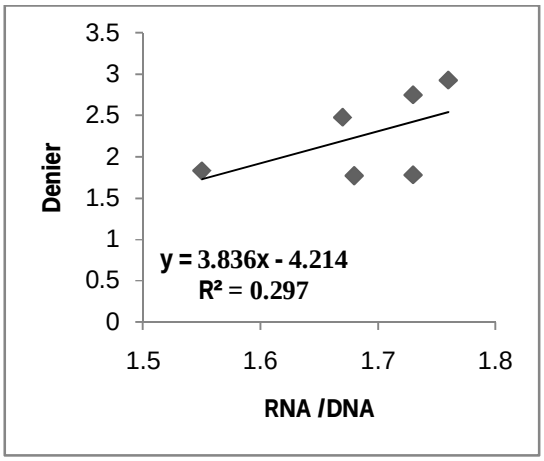

Figure 26: Correlation Between RNA/ DNA and Denier

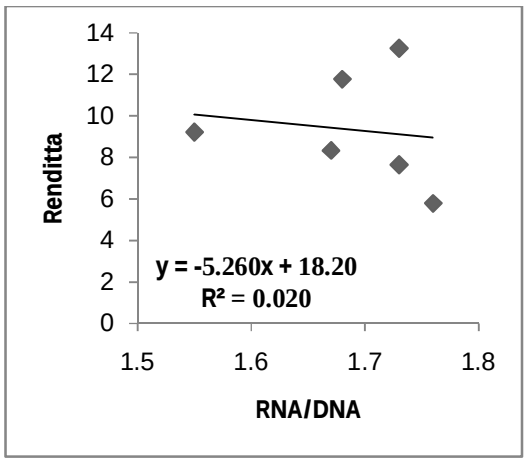

Figure 27: Correlation Between RNA/ DNA and Renditta