



Canonical Correlation of Physiological Traits and Growth Parameters of Two Biotypes of *Anopheles stephensi*: intermediate and mysorensis

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ABSTRACT

Introduction: Malaria, transmitted by *Anopheles* mosquitoes, remains a significant public health concern. The aim of this study is to evaluate the canonical correlation between physiological traits and growth parameters of two *Anopheles stephensi* biotypes: intermediate and *mysorensis*.

Methods: A total of 25 males and 25 females were reared under controlled laboratory conditions ($27 \pm 2^\circ\text{C}$, $75 \pm 5\%$ relative humidity, 12:12 light-dark cycle), and parameters such as egg lifespan, larval stages, pupal duration, and adult longevity were examined. Growth parameters, including the finite rate of population increase, life expectancy, net reproductive rate, and average generation time, were analyzed separately.

Results: Canonical correlation analysis revealed that only the first canonical variable was significant, explaining 87.24% of the data fit. The first canonical variable exhibited the highest cumulative percentage (87.32), canonical square (0.94), and eigenvalue (23.59). Among growth parameters, life expectancy had the highest canonical correlation coefficient ($r = 0.942$). Significance tests indicated that Wilks' Lambda produced the highest eigenvalues, while other methods yielded Hotelling-Lawley Trace: 0.38, Pillai's Trace: 1.43, and Roy's Greatest Root: 37.40.

Conclusion: Findings indicate that growth parameters, such as net reproductive rate and average generation time, are directly correlated. A higher net reproductive rate corresponds with a longer average generation time, leading to an increased intrinsic rate of population growth and life expectancy. These insights into *Anopheles stephensi* population dynamics may contribute to improved vector control strategies.

Keywords:

An. stephensi, life expectancy, larval duration, canonical variable, Wilks' Lambda.

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Introduction

Malaria, a deadly disease caused by the *Plasmodium* parasite, is spread to humans through the bites of infected female *Anopheles* mosquitoes. It's particularly prevalent in Africa and parts of Asia (1). In 2022, malaria impacted an estimated 249 million individuals worldwide, resulting in 608,000 deaths. Despite over a century of global research and efforts to combat malaria, its toll on health remains significant (2). *Anopheles stephensi* is a leading malaria vector across regions like the Middle East, Indian subcontinent, Iran, Iraq, Bangladesh, South China, Myanmar, Thailand, and Ethiopia. Egg morphometric analysis identifies three biological forms: *mysorensis*, intermediate, and type (3).

Canonical correlation analysis is a highly

recognized method in multivariate analysis. Its primary aim is to uncover simultaneous relationships between two distinct sets of variables, referred to as X and Y. Essentially, this analytical method focuses on evaluating the strength of relationships among various traits—be they physiological, morphological, or chemical—observed in plants or animals. It can be advantageous to perceive one set as containing independent variables while the other contains dependent variable. Canonical correlation analysis is applicable across a diverse range of fields, including plant science, biology, chemistry, meteorology, demography, artificial intelligence, cognitive science, political science, sociology, psychometrics, educational research, economics, and management science. This technique allows

researchers to explore and analyze multidimensional relationships between various sets of independent and dependent variables.(4) In the management and control of biological pests, it is necessary to identify the growth rate parameters of insect populations. In fact, by determining these parameters, population growth can be predicted. The most important parameters are the net reproductive rate, intrinsic rate of natural increase, finite rate of increase, and mean generation time. The most important growth parameter is the intrinsic rate of natural increase, which is defined as the maximum rate of increase for a species under specific biological and physical conditions (5). In the context of canonical correlation analysis, the variable sets (commonly identified as the X and Y sets) are combined to maximize the correlation between them. Given that multiple variables exist within each set, a variety of combinations can emerge. As a result, the number of possible ways to recombine the variables is equal to the number of variables in the smaller set (6).

The aim of this study was to investigate the canonical correlation between physiological traits and growth parameters, especially the intrinsic rate of natural increase, in order to determine the canonical relationship between these traits. The equations obtained from the canonical relationship between physiological traits can provide a model for evaluating any growth parameter of the *Anopheles* mosquito.

Materials and Methods

Rearing *Anopheles stephensi*

Two biotypes of *Anopheles stephensi* (intermediate and mysorensis) were reared under laboratory conditions at the National Insectarium of Iran at Pasture institute. For the study of growth parameters, 25 males and 25 females were released into breeding cages and provided with a 10% sucrose solution for feeding. Throughout the study, mosquitoes were maintained at a temperature of $27 \pm 2^\circ\text{C}$, relative humidity of $75 \pm 5\%$, and a 12:12 light-dark cycle.

For each biotype, 2-liter water containers were used (Figure 1). To estimate life table parameters, newly hatched larvae were observed. Male and female mosquitoes were then transferred to breeding cages (Figure 2), where key growth factors including egg lifespan, first to fourth instar larval stages, pupal stage, and adult lifespan of male and female mosquitoes were recorded.



Figure 1: Rearing *Anopheles* mosquito larvae under laboratory conditions



Figure 2: Rearing cages for adult *An. stephensi* mosquitoes

Data Analysis

To obtain important parameters of the life table, such as the finite rate of population increase, life expectancy, net reproductive rate, and average generation time, the number of surviving mosquitoes each day, as well as the number of eggs and larvae produced, are first counted. Using this data, the survival probability from birth to the beginning of age x , and the average number of offspring produced per mosquito per day at age x , can be determined for the table. To calculate the intrinsic rate of population increase (r_m), the following formula is used.

$$\sum_{x=0}^y L_x m_x e^{-r_m x} = 1$$

To calculate the Gross Reproductive Rate

(GRR), the following formula is used.

$$\sum_{x=0}^y m_x$$

The Net Reproductive Rate (R_0) is the ratio of the population size in the next generation to the population size at the beginning of the previous generation, and it is obtained from the following formula.

$$R_0 = \sum_{x=0}^y L_x m_x$$

To calculate life expectancy (e_x), the following formula is used.

$$e_x = \frac{T_x}{L_x}$$

To calculate the finite rate of population increase (λ), the following formula is used.

$$\lambda = e^{rm}$$

In these equations, L_x is the survival number between two generations, T_x is the average length of a generation, and m_x is the average number of female offspring in each age group (7).

Statistical analysis of the data was performed using the SAS statistical software, version 9.1.0. The determination of the number of canonical variables and the selection of the appropriate canonical correlation were based on the adjusted

canonical correlation values and the Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace, and Roy's Greatest Root tests.

Results of Canonical Correlation Analysis

The results of the canonical correlation analysis between physiological traits and growth parameters of the two biotypes of *An. stephensi* show that only the first canonical variable was significant, while the second and third variables were not significant (Table 1). The first canonical variable had the highest eigenvalue of 23.59, while the second and third canonical variables had eigenvalues of 1.3 and 0.42, respectively.

The analysis of the canonical tables indicated that the first canonical variable had the highest cumulative percentage, canonical square, and eigenvalues, with values of 87.32, 0.94, and 23.59, respectively, and should be placed at the center of the correlation between physiological traits and growth parameters.

The canonical variables for the physiological traits explained 92% of the total variance of the growth variables, while the growth variables explained 87% of the variance in the physiological traits. These results suggest that the physiological traits in *An. stephensi* mosquitoes can more accurately reflect the correlation between traits.

Table 1: Canonical Correlation and Significance Level of Physiological Traits and Life Table Parameters

Canonical variable	Canonical correlation	Square of canonical correlation	Special values	Percentage	Accumulative percentage	P value
1	0.95	0.94	23.59	87.32	87.32	0.01
2	0.42	0.16	1.32	9.25	96.57	0.14
3	0.25	0.07	0.27	3.42	100	0.67

The correlation coefficients assess the relationship between canonical variables and main variables, which are generally referred to as structural coefficients (8). In evaluating the correlation coefficients, the first canonical variable of physiological traits explained 92.43% of the traits. In the first canonical variable, the highest correlation coefficient was observed for third instar larvae ($r = 0.91$), followed by the duration of adult female mosquitoes, male mosquitoes, second instar larvae, fourth instar larvae, egg, pupae, and first instar larvae, in that order.

The first canonical variable of physiological

traits showed a positive correlation with other physiological traits, except for pupal duration. Therefore, it can be concluded that the closer we move to the first canonical correlation, the increase in one physiological trait leads to an increase in other physiological traits, except for the pupal duration. In canonical correlation, an increase in the pupal duration leads to a decrease in the duration of other traits. This can be attributed to the dependency of *An. stephensi*'s pupal duration on juvenile hormones, which have the most significant impact on environmental physiology during the pupal stage. In the two biotypes studied, the shortest growth durations

were associated with the *An. stephensi* myorensis biotype, and the longest growth durations were found in the *An. stephensi* intermediate biotype.

Therefore, it can be concluded that the biotype type affects the growth duration. Since the second

and third canonical variables were not significant, they were not considered for further analysis. There is only one canonical correlation between physiological variables and life table parameters.

Table 2: Canonical Correlation of Physiological Traits with the Three Significant Canonical Variables of Physiology

Physiological traits	Canonical variables	
	First canon	Second canon
Egg Duration period	0.85	0.32
First Instar Larval Duration	0.78	0.14
Second Instar Larval Duration	0.88	0.15
Third Instar Larval Duration	0.91	0.53
Fourth Instar Larval Duration	0.87	0.26
Pupal Duration	0.72	0.41
Male Adult Life Span	0.90	0.48
Female Adult Life Span	0.90	0.47
Variance of Canonical Variables	92.43	3.27

The first canonical variable for growth parameters explained 24.87% of the data (Table 2). The highest canonical correlation coefficient in the first canonical variable for growth parameters was related to life expectancy ($r = 0.942$). The first canonical variable for growth parameters showed a positive correlation with the finite rate of population increase, net reproductive rate, and generation time. Therefore, it can be concluded that as we move closer to the first canonical correlation, an increase in any of the

growth parameters leads to an increase in the other growth parameters.

Among the biotypes studied, the *An. stephensi* intermediate biotype had the highest growth parameters (Table 3). In the second canonical variable, the correlation coefficients were low, and therefore the parameters were not considered for further analysis. Based on the results, it can be concluded that the first canonical variable better explains the correlation between growth parameters.

Table 3: Canonical Correlation of Growth Parameter Traits with Two Significant Canonical Variables of Growth Parameters

Growth parameters	Canonical variables	
	First canon	Second canon
Life expectancy	0.94	0.17
Finite rate of population increase	0.91	-.14
Net Reproductive Rate	0.82	.12
Average Generation Time	0.90	.08
Variance of canonical variables	87.24	1.23

The results in Table 4 for the analysis of standardized canonical variable equations show that the first canonical variable is the most important for examining the correlation between physiological traits and growth parameters of the two biotypes. However, the second and third equations cannot be considered with different variables to determine the canonical correlation between these variables. By examining the $PHYT_1$ equation, it appears that $PHYT_1$ is an equation

used to compare X_1 (the duration of the 3rd instar larval stage) with other variables X . Thus, $PHYT_1$ represents the larval duration of the two biotypes, *An. stephensi* intermediate and *mysorensis*. On the other hand, $PARA_1$ has positive coefficients for all four growth parameter variables. It seems that the biotype with the longest third instar larval stage has the highest intrinsic population growth rate, life expectancy, net reproductive rate, and average generation time.

Table 4: Equations of Standardized Canonical Variables

Equation	Formula
$PHYT_1$	$+0.96X_1+0.94X_2+0.85X_3+0.82X_4+0.78X_5+0.73X_6+0.69X_7+0.65X_8$
$PARA_1$	$+0.86Y_1+0.84Y_2+0.79Y_3+0.72Y_4$

The results in Table 5 for testing the significance of canonical correlations of physiological traits showed that the highest eigenvalues were obtained for the correlation test using Wilks' Lambda method. The eigenvalues for the other correlation tests were as follows:

Hotelling-Lawley Trace = 0.38, Pillai's Trace = 1.43, and Roy's Greatest Root = 37.40. In all the statistical tests mentioned, the canonical correlation for physiological traits and growth parameters was found to be significant.

Table 5: Significance Tests of Canonical Correlations for Physiological Traits and Growth Parameters of the Two Biotypes of *An. stephensi* mysorensis and intermediate

Tests	Special values	F	Freedom degree	P value
Wilks' Lambda	42.54	231.2	16	<0.0001
Pillai's Trace	1.43	8.73	16	0.0005
Hotelling-Lawley Trace	0.38	39.28	16	<0.0001
Roy's Greatest Root	37.40	151.39		0.0001

Discussion

The study of mortality parameters for analyzing insect population mortality is very useful for determining the key factors responsible for the highest mortality in a population. These parameters are determined by two types. In addition, various mathematical formulas have been presented for assessing appropriate fertility tables, stable age distribution, and life expectancy. The life expectancy of beneficial insects can be calculated by predicting natural factors, especially at a specific age when the highest pest mortality occurs. This can be used for biological control programs and to plan for timely pest management. (9)

In canonical correlation studies, the structural relationship between physiological traits and growth parameters provides different results compared to standardized canonical coefficients. These differing results may be due to collinearity in the data and the low sample size, which affects the stability of the canonical coefficients (10). Overall, a significant correlation between some physiological traits and growth parameters suggests that indirect selection of certain physiological traits could be used in control programs for *Anopheles* mosquitoes.

In Medical Entomology, the study of canonical correlation between physiological traits and growth parameters between the two biotypes of *An. stephensi*, intermediate and mysorensis, has been addressed for the first time. In this study, the relationship between canonical correlation coefficients in physiological traits and growth parameters showed that with an increase in each of the physiological traits (larval duration, pupal duration, and the lifespan of adult males and females), life expectancy, the finite rate of

population increase, net reproductive rate, and mean generation time also increase.

The results of this study also indicated that the level of each growth parameter, such as the net reproductive rate and average generation time, had a direct relationship with each other. In fact, it can be stated that the higher the net reproductive rate of *Anopheles* mosquitoes, the longer the average generation time, which in turn can lead to an increase in the intrinsic rate of population growth and life expectancy.

Conclusion

Anopheles stephensi mosquitoes have two important biotypes, mysorensis and intermediate. Understanding the physiological traits and growth parameters of these biotypes is crucial for studying the biology of this disease vector. By examining the canonical correlation between physiological traits and growth parameters, it was found that an important factor in the biology of this vector is the larval stages, especially the third instar larval stage. This may be due to the onset of sexual organ formation and fertility potential, which is influenced by nutrition and growth during this developmental phase.

In growth parameters, based on canonical studies, the lifespan of this vector was found to be significant. The longer the life expectancy of a disease vector, the more individuals it can infect. Therefore, by understanding the important factors of a vector insect, targeted control programs can be developed to reduce the health issues within a community.

Conflicts of interest

The authors declare that they have no conflict of interest.

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