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Wonder Findings of Number of Cells in a Body Including Sexual Cells, Remedy of Corona Virus, Increase of Memory, And other Cells by Couple System

By Nirmalendu Das

Abstract- It is difficult to calculate fixed data of a number of cells in a body. It is varying on time and age of life. The time increasing that cells are increasing, though we can estimate the number of cells in various nerves in a body, brain, sexual platform, etc through a couple system. The coupling system is a new system of the finding of peculiar series of numbers [1], which is applies to many fields. In the cases of Medical Science, it has been observed by calculation that due to disturbing of the couple caused different difficulties in the body. A smooth Coupling cell may produce a healthy body, and it is possible to increase memory by adding particular cells number in a loss position. The coupling system is interesting that, can explain the real mechanism of every cell. There are many types of cells in a living body. Almost all cells follow a couple of system. The coupling system performs coupling between two (say, A, 1st party & B, 2nd party) with keeping relation as 3rd party, denoted by R (Relative Number) mathematically.

Keywords: number of cells related to couple system, sexual activity, corona virus, cancer cells, neurons of humans & animals, cause of diseases, memory cells.

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Wonder Findings of Number of Cells in a Body Including Sexual Cells, Remedy of Corona Virus, Increase of Memory, And other Cells by Couple System

Nirmalendu Das

Abstract- It is difficult to calculate fixed data of a number of cells in a body. It is varying on time and age of life. The time increasing that cells are increasing, though we can estimate the number of cells in various nerves in a body, brain, sexual platform, etc through a couple system. The coupling system is a new system of the finding of peculiar series of numbers [1], which is applying to many fields. In the cases of Medical Science, it has been observed by calculation that due to disturbing of the couple caused different difficulties in the body. A smooth Coupling cell may produce a healthy body, and it is possible to increase memory by adding particular cell number in a loss position. The coupling system is interesting that, can explain the real mechanism of every cell. There are many types of cells in a living body. Almost all cells follow a couple of system. The coupling system performs coupling between two (say, A, 1st party & B, 2nd party) with keeping relation as 3rd party, denoted by R (Relative Number) mathematically. This system applies to finding the series of Pi [1], ½ values [2], Searching of Properties of Mind, Activity of number of Cells in a brain [3]; Determination of relative numbers by using couple system and its application to the atomic fields and quark coupling strength of the LHCb collaboration [4]. We can determine the atomic number, electron, proton, neutron, splitting of quark from one point to other, etc. So, we can call this system to give birth to all systems. It requires more study and searches in every case, couple system in medical science a touch of light that can bring a revolutionary change to keep fit body from various virus effects.

Keywords: number of cells related to couple system, sexual activity, corona virus, cancer cells, neurons of humans & animals, cause of diseases, memory cells.

I. Introduction

The process of the birth of the body is a natural system. Cell forms accordingly by nature. It has seemed that all processes of birth probably follow one relation to the next relation by coupling each other. Figure 1[5], is indicating that system here.
The coupling system is related to the above birth mechanism. The obtained different numbers are indicating a new era of cell number of how it forms for every life. Why this number almost fixed to the body? How we can determine these numbers in series, which will become apply to the human body and animals. We can treat the number as day, month, hour, second, mile, km, kg, etc where needed. Again the number is the only number may apply to cells as cell number, two bodies coupling periodically. If a couple destroys somehow by the effect of the virus or by any way, the coupling will disturb. As a result, various disease attacks in a body. If we keep coupling steady in a uniform process by medicine or by such equipment, we can protect ourselves.

Let us go through the Couple System a new process:
Application of "No-1 Formation": [1, 2]

Problem (1):

The well known equation \( (r + M)^2 = r^2 + 2rM + M^2 \). Similarly, \( (r + M)^3 = r^3 + 3r(rM + M^2) + M^3 \). Likewise, \( (r + M)^4, (r + M)^5, (r + M)^6 \) etc. but new function derived in the form of:

If \( (r + M)^3 \rightarrow r^3 + 3r(rM + M^2) + M^3 \) and let \( M = 1 \), then

\( (r + 1)^3 \rightarrow r^3 + 3r(r + 1)^2 + 1^3 \rightarrow r^3 + 3r(r + 1) + 1 \) ……. Known fact.

The middle part of the above equation, \( 3r(r + 1) \) means 3 times of the factor \( [r(r + 1)] \). Let, \( r = A \), \( 1 = B \) and \( R = (r + 1) \). Naturally, \( R \) interrelated to \( A \) and \( B \) shown here in the form of:

\[
\begin{array}{c}
\text{Fig. 2}
\end{array}
\]

So, the equation,

\( (r + 1)^3 = r^3 + 3rR + 1^3 \) is representing the equation of \( (r + 1)^3 \rightarrow r^3 + 3r(r + 1)^2 + 1^3 \rightarrow r^3 + 3r(r + 1) + 1 \) (known).

If the figure 1 is extended as:

\[
\begin{array}{c}
\text{Fig. 2}
\end{array}
\]

No. 1, Formation

Reaction of couple:
**Description:**

1) D is related to A and C is related to B. But A and C are respectively the value of B and D. D' in L.H.S. means that it is the end of the reaction of problem to form relative number (R) acting with C^+ in R.H.S.

2) The original value of D is C; therefore, D reacts with A and forms CA. Similarly, C is original value of D, but the original value of B is A. So, C reacts with B and forms CA.

3) Total couple reaction with respect to R is 2CA.

**No. 2, Formation**

When the couple will increase to one step towards E & F (Fig. – 4) from the coupling zone of C & D (Fig- 3) respectively, the positive (+) & negative (-) sign will play as the reverse function of “No. 1, formation” that is, F to F^+ on L.H.S. of the couple & E to E^+ on R.H.S. of the couple. If D remains unchanged & C^+ turns to C^- (1st negative row).

**Fig. 4**

**No. 2, Formation**

The couple reaction with respect to R as follows:

\[
\begin{align*}
& \longrightarrow [E . C + (-E . C^-) ] + [ C . A + (-C^- . A^-) ] \\
& \longrightarrow 2 E^- . C + 0 \\
\text{Hence: } & \ R \longrightarrow 2 \ E^- . C
\end{align*}
\]

When the couple will increase to one more step towards G & H (Fig.- 5) from the couple zone of E & F (Fig. 4) respectively, then the end of the couple will play as a function of “No. 1 formation” and if F^+ remains unchanged and E^- turns to E^+, then another positive row will be formed (Fig.- 5). If the couple proceeds to another next step, the end of the couple will follow “No. 2, formation” and again one negative row will be formed (Fig. – 5).

**Fig. 5**
If the process continues as above alternately as +ve, -ve, +ve, -ve, +ve, -ve.........rows will produced successively and middle part of the couple will be zero after the couple reactions (vide problems). This process is applicable to determination of value of Pi. [1, 2]. Wonder Findings of Number of Cells in a Body Including Sexual Cells, Remedy of Corona Virus, Increase of Memory, and Other Cells by Couple System.

To follow the figure – 3,
Let us an example, \((r + 1)^2 = r^2 + 2r.1 + 1^2\)
Middle term is \(2r.1\) and may represent by \(R\). then according to fig. – 3 we can draw a figure as,

![Diagram](image)

**Fig. 6**

The equation \((a + b)^2, (a + b)^3, (a + b)^4, (a + b)^5\), etc are very well known simple equation. If we consider middle term of this equation represented by \(R\) and put in coupling system to find relative numbers, then we can get a new series of numbers which may apply to some fields. The obtained new equation is:

\[
(r \pm M)^N \rightarrow r^N \pm M^{[1 + 2(N - 2)]}N \times (N - 1)r \pm M^N
\]

And middle part of this equation is \(M^{[1 + 2(N - 2)]}N \times (N - 1)r\)
The process is given here step by step.

We known the equation \((r + 1)^3\), it will turns to \((r + 1)^3 = r^3 + 3r.2r + 1^3\) using couple system.

If \(M = 2,3,4,5, \ldots\) Then, we can get the following series.

\[
(r + 2)^3 \rightarrow r^3 + 2^3.3r.2r + 2^3
\]
\[
(r + 3)^3 \rightarrow r^3 + 3^3.3r.2r + 3^3
\]
\[
(r + 4)^3 \rightarrow r^3 + 4^3.3r.2r + 4^3
\]
\[
(r + 5)^3 \rightarrow r^3 + 5^3.3r.2r + 5^3
\]
\[
\vdots \rightarrow \vdots
\]
\[
(r + M)^3 \rightarrow r^3 + M^3.3r.2r + M^3----------------------------------------------- (a)
\]

This equation (a) will satisfy by only 0 & 1.

When, \(r\) and \(M = 0\), then result brings 0, but, when \(r = M = 1\), then, on putting this value in L.H.S., \((r + M)^3 = (1 + 1)^3 = 2^3 = 8\) and for R.H.S., \(r^3 + M^3.3r.2r + M^3 = 1^3 + 3^1 \times (2x1) + 3^3 = 1 + 6 + 1 = 8\). This equation satisfying Binary Numbers as 0 & 1 only. This equation will not satisfy others numbers like 2,3,4, etc, in this case numbers of L.H.S. and R.H.S. will defer, for example, if \(r = 1 & M = 2\), the we get,

\[
(r + M)^3 \rightarrow r^3 + M^3.3r.2r + M^3
\]
\[
\vdots \rightarrow \vdots
\]

L.H.S is known equation, R.H.S. is unknown.

Here we can say, relative number of 27 is 57. The difference between is 30. We considered numbers as 1 & 2 for \(r\) and \(M\). Now \(1 + 2 = 3, \) so, \(30 - 3 = 27\), this similarity we have from this relation using the number 1 & 2 only. From the above deduction, we can arrange the equation, \((r + 1)^3 = r^3 + 3r (r + 1) + 1\) to \((r + 1)^3 \rightarrow r^3 + 3r.2r + 1^3\) \(\rightarrow (r + 1)^3 \rightarrow r^3 + 1^3.3r.2r + 1^3\). Similarly, on putting next odd number 5, then, we observed that:
Problem (2):
When, \((r + M)^5 \rightarrow r^5 + 5r (r^3 + 2r^2 + 2r + 1) + 1^5\), then, \(R = r^3 + 2r^2 + 2r + 1\), if \(A, B, C, D\) represents the corresponding values of \(r^3, 2r^2, 2r, 1\) (Since, these are the real values of \((r + 1)^5\)) respectively. Then, \(R\) will relate in Couple Systems as follows:

\[
R \rightarrow (D^+.2r + 1.C) + (C^+.2r^2 + 2r^+.B) + (B^+.2r^3 + 2r^2.A)
\]
\[
R \rightarrow (1.2r + 1.2r) + (2r.2r^2 + 2r.2r^2) + (2r^2.2r^3 + 2r^2.r^3)
\]
\[
R \rightarrow 1.(2r + 2r) + (2r.2r^2 – 2r.2r^2) + (2r^2.2r^3 – 2r^2.r^3)
\]
\[
R \rightarrow 4r + 0 + 0
\]
\[
R \rightarrow 4r
\]

Therefore,
\[
(r + 1)^5 \rightarrow r^5 + 5r.4r + 1^5 \hspace{1cm} \text{(b)}
\]

If \(M = 2,3,4,5,\ldots\) [Vide Problem (1)], then,
\[
(r + 2)^5 \rightarrow r^5 + 2^7.5r.4r + 1^5
\]
\[
(r + 3)^5 \rightarrow r^5 + 3^7.5r.4r + 1^5
\]
\[
(r + 4)^5 \rightarrow r^5 + 4^7.5r.4r + 1^5
\]
\[
(r + 5)^5 \rightarrow r^5 + 5^7.5r.4r + 1^5
\]

Therefore, the real formation of \((r + 1)^5\) will \(r^5 + 1^7.5r.4r + 1^5\)

Problem (3):
When, \((r + M)^7 \rightarrow r^7 + 7r (r^6 + 3r^4 + 5r^3 + 5r^2 + 3r + 1) + 1^7\), then, \(R\) will related to bellow as:
R → (F . 3r + 1 r + . E) + (E r + 5 r^2 + 3 r^3 . D) + (D r + 5 r^2 + 5 r^3 . C) + (C r + 3 r^4 + 5 r^5 . B) + (B r + 3 r^6 . A)
R → (1 . 3r + 1 . 3r) + ((3r . 5r^2 + 3 r^3 . 5r^2) + (5r^2 . 5r^3 + 5 r^3 . r^4)) + (3r^4 . 3r^4 + 3 r^4 . r^5)
R → (3r^4 . 3r^4 + 3 r^4 . r^5) + (3r^5 . 3r^5)

(r + 1)^7 → r^7 + 1^7 . 7r . 6r + 1^7 and if M = 2, 3, 4, 5 ...., then,
(r + 2)^7 → r^7 + 21^7 . 7r . 6r + 2^7
(r + 3)^7 → r^7 + 31^7 . 7r . 6r + 3^7
(r + 4)^7 → r^7 + 41^7 . 7r . 6r + 4^7
(r + 5)^7 → r^7 + 51^7 . 7r . 6r + 5^7
...
(r + M)^7 → r^7 + M1^7 . 7r . 6r + M^7

"No – 1 formation" is only for the series of odd numbers as 1, 2, 3, 5 ...., if the series increases, then the process of couple will increase.

Application of "No – 2 formation" for even number. [1]

Problem (1):
(r + 1)^4 → r^4 + 4r . (r^2 + [3/2] x r + 1) + 1^4 = r^4 + 4r . (R) + 1^4

Note:
(a + b)^4 = a^4 + 4a^3b + 6a^2b^2 + 4ab^3 + b^4 = a^4 + 2ab (2a^2 + 3ab + 2b^2) + b^4.

(a + 1)^4 = a^4 + 4a^3 + 6a^2b^2 + 4ab^3 + b^4, when b = 1, then,
(a + 1)^4 = a^4 + 4a . (a^2 + [3/2] x a + b) + b^4, where b = 1, then,

Similarly,
(r + 2)^4 → r^4 + 2^5 . 4r . 3r + 2^4
(r + 3)^4 → r^4 + 3^5 . 4r . 3r + 3^4
(r + 4)^4 → r^4 + 4^6 . 4r . 3r + 4^4
(r + 5)^4 → r^4 + 5^7 . 4r . 3r + 5^4

(r + M)^4 → r^4 + M^8 . 4r . 3r + M^4

For power 6, we get,
(r + 1)^6 → r^6 + 1^9 . 6r . 5r + 1^6

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(r + 2)^6 \rightarrow r^6 + 2^6.6r.5r + 2^6
(r + 3)^6 \rightarrow r^6 + 3^6.6r.5r + 3^6

...............(r + M)^6 \rightarrow r^6 + M^6.6r.5r + M^6

“No – 2, formation” is only for the series of powers of (r + M), when integrates acts as a function of, 2,4,6,8 ….. When the series increases, then the process of couple increases.

Hence the series:

(r + M)^2 \rightarrow r^2 + M^2.2r.1r + M^2
(r + M)^3 \rightarrow r^3 + M^3.3r.2r + M^3
(r + M)^4 \rightarrow r^4 + M^4.4r.3r + M^4
(r + M)^5 \rightarrow r^5 + M^5.5r.4r + M^5
(r + M)^6 \rightarrow r^6 + M^6.6r.5r + M^6
(r + M)^7 \rightarrow r^7 + M^7.7r.6r + M^7

...............(r + M)^N \rightarrow r^N + M^1 + 2(N – 2) .Nr.(N – 1)r + M^N………………..(A)

(r + M)^N \rightarrow r^N + M^2 .Nr.(N – 1)r + M^N…………………………………….(B)

When, Z = [1 + 2(N – 2)] & N = 2,3,4.....

In the case of negative functions, this equation will turn to:

(r - M)^N \rightarrow r^N - M^1 + 2(N – 2) .Nr.(N – 1)r - M^N .........................(C)
(r - M)^N \rightarrow r^N - M^2 .Nr.(N – 1)r - M^N .............................(D)

Relative Numbers (R):

The middle part of the equation (A) or (B) and (C) or (D) is same. We have the Relative number as [Nr.(N – 1)r] which connected to M^1 + 2(N – 2) .Nr or M^2, when Z = [1 + 2(N – 2)] of (r + M)^N or (r - M)^N. so, we may write the general equation in the form of:

(r ± M)^N \rightarrow r^N ± M^1 + 2(N – 2) .Nr.(N – 1)r ± M^N .........................(E)

And middle part of this equation is

M^1 + 2(N – 2) .Nr.(N – 1)r  ..............................................(F)

When, N = 1, 2, 3, 4, 5 ….. we get relative numbers 1r, 2r, 3r, 4r, 5r etc both of even and odd numbers. The equation (A) obtained by the couple system and is applicable in forming relative numbers with respect to Z of which numbers become odd in series, when N = 2, 3, 4, 5 ….of the equation, M^1 + 2(N – 2) .Nr.(N – 1)r. On changing the number of Z as Z = [2 + 2(N – 2)], we get,

M^2 + 2(N – 2) .Nr.(N – 1)r \rightarrow M^2 .2r.1r, when N = 2
M^{2 + 2(N – 2)} .Nr.(N – 1)r \rightarrow M^4 .3r.2r, when N = 3, where, \((r + M)^3 \rightarrow M^3.3r.2r, when, N = 3, due to change of Z, power changes as:

When, Z = [1 + 2(N – 2)], Z = - 3, when, N = 0 (not satisfying).
Z = [1 + 2(N – 2)], Z = - 1, when, N = 1 (not satisfying).
Z = [1 + 2(N – 2)], Z = 1, when, N = 2 (satisfying). It shows N > 1

If, Z = [2 + 2(N – 2)], we get,
Z = [2 + 2(N – 2)], Z = - 2, when, N = 0 (not satisfying)
Z = [2 + 2(N – 2)], Z = 0, when, N = 1 (satisfying), because, M^2 = M^0 = 1
Z = [2 + 2(N – 2)], Z = 2, when, N = 2 (satisfying), N > 1
At the time of changing of Z, let, Nr.(N – 1)r will change to Nr.(N – 2), then, we get a series as:

\[ M^{2 + 2(N - 2)}.Nr.(N - 2)r + M^0.0 \times r.(0 - 2)r = 0, \text{ when } N = 0 \]

\[ M^{2 + 2(N - 2)}.Nr.(N - 2)r \rightarrow M^1.1r.(1 - 2)r = M^1 x 1r x 1 = - M^1 \vee N = 1 \]

\[ M^{2 + 2(N - 2)}.Nr.(N - 2)r \rightarrow M^2.2r.0x r = 0, \text{ when } N = 2 \]

\[ M^{2 + 2(N - 2)}.Nr.(N - 2)r \rightarrow M^3.3r.1r, \text{ when } N = 3 \text{ etc., } N > 2 \]

When this equation turns to \[ M^{2 + 2(N - 2)}.Nr.Nr \ldots \ldots (G), \] when, (N – 1)r treated as Nr, then we will get even numbers (Z) of M of the series. So,

\[ M^{2 + 2(N - 2)}.Nr.Nr \rightarrow M^2.2r.2r, \text{ when, } N = 2, N > 1, \text{ if, } M = r = 1, M^2.2r.2r = 400 & 400/2 = 200. \]

\[ M^{2 + 2(N - 2)}.Nr.Nr \rightarrow M^4.3r.3r, \text{ when, } N = 3 \]

\[ M^{2 + 2(N - 2)}.Nr.Nr \rightarrow M^6.4r.4r, \text{ when, } N = 4 \text{ etc.} \]

Therefore, the deduction (F) and (G) finds,

\[ M^{2 + 2(N - 2)}.Nr.(N - 1)r \rightarrow M^1.2r.1r, \text{ when, } N = 2, Z = 1 \text{ of power of M, odd number.} \]

\[ M^{2 + 2(N - 2)}.Nr.Nr \rightarrow M^2.2r.2r, \text{ when, } N = 2, Z = 2 \text{ of power of M, even number.} \]

\[ M^{2 + 2(N - 2)}.Nr.(N - 1)r \rightarrow M^3.3r.2r, \text{ when, } N = 3, Z = 3 \text{ of power of M, odd number.} \]

\[ M^{2 + 2(N - 2)}.Nr.Nr \rightarrow M^4.3r.3r, \text{ when, } N = 3, Z = 4 \text{ of power of M, even number.} \]

From the above deduction, we have the following results as:

i) When, \( N = 0 \), the equation (F) yields \( M^3.0r.(-1).r \)

ii) \( " \ N = 0 \), \( " \ (G) \ " \ M^2.0r.0r \)

iii) \( " \ N = 1 \), \( " \ (F) \ " \ M^1.1r.0r \)

iv) \( " \ N = 1 \), \( " \ (G) \ " \ M^0.1r.1r \)

Therefore, when N has tendency to proceed in negative direction, i.e, \( N = -1, -2, -3, -4 \ldots \) then the deduction (F) & (G) will give results, the yielded values are listed here in a table (Zr).

**Relative Numbers obtained by Couple System.**

**Table (Zr):**

\[ M^2 \times [r_2 \times r_1] \quad M^2 \times [-r'_2 \times -r'_1] \]

R.H.S.(Relative No.) (Relative No.) L.H.S.

\[ M^0 \times 1 \times 1 = 1 \text{ (M}^0 =1^0 = 1, \text{ when, } M = 1 \text{ and relative number, } 1 \times 1 = 1 \]

\[ M^1 \times 2 \times 1 \quad M^1 \times 1 \times 0 \quad \text{Now, } 2/0 = 2 \]

[Relative number = 2 (r_2 \times r_1) = 2 \times 1 = 2]. On the other hand,

\[ (1^1 \times 2 \times 1 = 10 \times 2 \times 1 = 20 \text{ and } 20/10 = 2, \text{ and } 1^1 \times 1 \times 0 = 0.1 \times 1 \times 0 = 0. \text{ so, } 2/0 = 2). \]

\[ M^2 \times 2 \times 2 \quad M^2 \times 0 \times 0 \quad \text{Now, } 4/0 = 4 \]

\[ M^3 \times 3 \times 2 \quad M^3 \times 0 \times -1 \quad \text{Now, } 6/0 = 6 \]

\[ M^4 \times 3 \times 3 \quad M^4 \times -1 \times -1 \quad \text{Now, } 9/1 = 9 \]

\[ M^5 \times 4 \times 3 \quad M^5 \times -1 \times -2 \quad \text{Now, } 12/2 = 6 \]

\[ M^6 \times 4 \times 4 \quad M^6 \times -2 \times -2 \quad \text{Now, } 16/4 = 4 \]

\[ M^7 \times 5 \times 4 \quad M^7 \times -2 \times -3 \quad \text{Now, } 20/6 = 3.333 \]

\[ M^8 \times 5 \times 5 \quad M^8 \times -3 \times -3 \quad \text{Now, } 25/9 = 2.777 \]

\[ M^9 \times 6 \times 5 \quad M^9 \times -3 \times -4 \quad \text{Now, } 30/12 = 2.5 \]
When the coupling zones presented by +1 in the form of object & image, then it may treat as 1st, 2nd, 3rd, 4th coupling zones are shown in fig-11.

In this way, if coupling series increases with respect to 1, the reacting results to be 2. This is also applicable to No. 1, Formation. The sum of Fig.-11 is given below for example:

\[
R \quad \cdot \quad [(+1^+ \times +1^+) + (+1^+ \times +1^+)] + [(+1^1 \times +1^+) + (+1^1 \times +1^+)]
\]

\[
R \quad \cdot \quad [(+1 \times 1^1) + (-1 \times +1^1)] + [(+1 \times +1^+) + (-1 \times +1^+)]
\]
These formations of relative numbers are most important to find the different types of cells of the brain and other parts of a body. R.H.S. of these formations brings the total number of cells, if we consider M as base1, that is (According to Calculator, $1^{\text{EXP}} - 0 = 1$, & same is applies to all $M^n$, where, $n = 0, 1, 2, 3, 4, 5, \ldots$). $M^0 \times 1 \times 1 = 1^0 \times 1 \times 1$ number ($1^0 = 1$), here we can assume, $1^0 \times X \times Y = 1$ sex cell for male and for female $1^0 \times X \times X = 1$ sex cell. Ten is representing as the relation between two as $1^0 = 1$, which one is commending as love or agrees to meet in sexual functions.

**Applications of most basic formation Application:**

**Sex cells and chromosomes:**

$M^1 \times 2 \times 1 = 1^1 \times 2 \times 1 = 20$ numbers ($1^1 = 10$) and so on. Here we observed that coefficient of $M^1$ is 2 & 1 as relative number and if we add $2 + 1 = 3 \& 3$ to $20 = 23$ numbers. We may treat these 23 numbers as sex cells and $23 + 23 = 46$ of human body.

**Sex cells and chromosomes [6]**

Human body cells each contain 23 pairs of chromosomes. Parents pass on their genes to their offspring in their sex cells. Female sex cells are called egg cells or ova. Male sex cells are called sperm.

**Process of fertilization:**

Sex cells only contain one chromosome from each pair. When an egg cell and sperm cell join together, the fertilized egg cell contains 23 pairs of chromosomes. One chromosome in each pair comes from the mother, the other from the father.

The pair of chromosome is random. Due to this different child in the same family gets a different combination. This is why children in the same family look a little like each other and a little like each parent but are not identical to them.

Again, $[M^2 \times 2 \times 2] = [1^2 \times 2 \times 2 = 10 \times 2 \times 2 = 400$, when $M = 1]$. 1/10th of 400 are 40 numbers, if we consider, the coefficient of $M^1$ is 2 and 2, then, $2 + 2 = 4$. Then, $40 + 4 = 44$. Then, $44/2 = 22$ cells plus with 1 chromosome, then brings 23 cells. But 44 cells + 2 chromosomes = 46 cells of XX and XY sex cells.

$\text{Again, ratio of } \frac{[M^2 \times 2 \times 2]/10}{M^1 \times 2 \times 1} = \frac{[1^2 \times 2 \times 2]/10}{1^1 \times 2 \times 1} = \frac{40}{20} = 2$

Here, 2 (two) means for any birth, two couples need to meet together.

**Birth of Baby:**

When power of M increases 1 to 6, then the equation, $M^6 \times 4 \times 4 = 1^6 \times 4 \times 4 = 16000000 = 1.6 \times 10^7 = 16$ million and if we treated as seconds this number, then, $16000000$ sec $= 185.185$ days or 6.17 months. As so, 6 month baby is unmeasured. If we multiply by the factor $3/2, j = 1 + \frac{1}{2} \& j = 3$, in the case of atomic stage, $j$ used as angular quantum number) then, $16000000$ sec x $(3/2)$, then we see that 24 million seconds $= 9.26$ months $= 9.3$ months.
If $2 \times [M^1 \times 2 \times 1] = 2 \times [1^1 \times 2 \times 1] = 40$ numbers and if we treated as weeks days, then, “Average human baby pregnancy time vs. other mammals. In general, the larger the animal, the longer the gestation period. In general, the larger the animal, the longer the life.

Human body* 266 days (40 weeks = 280 days)*

40 week days = 9.33 months, this value is tallied to 9.3 months followed by the equation $(3/2) \times M^6 \times 4 \times 4 = 1^6 \times 4 \times 4 = 9.26$ months or 277.8 days. Or $[M^2 \times 2 \times 2] / (3/2) = 266.66$ days. Therefore, Couple System and obtained relative numbers are most important in the case of birth cells.

We can give other examples from another animal also.

Average Gestation Period Of Different Animals [8]

<table>
<thead>
<tr>
<th>Animal</th>
<th>Period (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>African elephant</td>
<td>22</td>
</tr>
<tr>
<td>Giraffe</td>
<td>15.25</td>
</tr>
<tr>
<td>Humpback whale</td>
<td>10 to 12</td>
</tr>
<tr>
<td>Bison (buffalo)</td>
<td>9.5</td>
</tr>
<tr>
<td>Human</td>
<td>9</td>
</tr>
<tr>
<td>Hippopotamus</td>
<td>8</td>
</tr>
<tr>
<td>Grizzly bear</td>
<td>7</td>
</tr>
<tr>
<td>Baboon</td>
<td>5 to 6</td>
</tr>
<tr>
<td>Giant panda</td>
<td>4 to 5</td>
</tr>
<tr>
<td>Jaguar</td>
<td>3.5</td>
</tr>
<tr>
<td>Dog</td>
<td>2</td>
</tr>
<tr>
<td>Cat</td>
<td>2</td>
</tr>
<tr>
<td>Rabbit</td>
<td>1.33</td>
</tr>
<tr>
<td>Hamster</td>
<td>0.5 to 1</td>
</tr>
<tr>
<td>Lion</td>
<td>108 days</td>
</tr>
<tr>
<td>Ant</td>
<td>8 to 12 weeks</td>
</tr>
<tr>
<td>Camel</td>
<td>400 days</td>
</tr>
<tr>
<td>Rhino, Gray</td>
<td>485 days</td>
</tr>
<tr>
<td>Elephant, Asian</td>
<td>610 days</td>
</tr>
</tbody>
</table>

Likewise, we can estimate the period of other animals.

Breaking of couple system (for example cancer cell):

What is cancer?

Cancer is the name given to a collection of related diseases. In all types of cancer, some of the body’s cells begin to divide without stopping and spread into surrounding tissues. Cancer can start almost anywhere in the human body, which is buildup of trillions of cells. Human cells grow and divide to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place. When cancer develops, this orderly process breaks down. As cells become more and more abnormal, old or damaged cells survive when they should die, and new cells form when they are not needed. These extra cells can divide without stopping and may form growths called tumors.

Many cancers form solid tumors, which are masses of tissue of the blood, such as leukemias, generally do not form solid tumors. Cancerous tumors are malignant, which means they can spread into or invade nearby tissues. Also as these tumors grow, some cancer cells can break off and travel to distant places in the body through the blood or the lymph system and form new tumors far from the original tumor. Unlike malignant tumors, benign tumors
do not spread into or invade nearby tissues. Benign tumors can sometimes be quite large, however. When removed, they usually don’t grow back, whereas malignant tumors sometimes do. Unlike most benign tumors elsewhere in the body, benign brain tumors can be life-threatening.

**Differences between Cancer Cells and Normal Cells**

Cancer cells differ from cells in many ways that allow them to grow out of control and become invasive. The difference is that cancer cells are less specialized than normal cells. That is, whereas cells mature into very distinct cell types with specific functions, cancer cells do not. This is one reason that, cancer cells continue to divide without stopping [9]. So, without the coupling of two cells, it is never possible to give birth to a new generation. When the virus attracts in a body, then the system of a couple feels disturbed, and as a result, it cannot functioned smoothly. The living body feels trouble called diseases. Cancer is one example in this case. These cells do not follow the couple system in the long run; it breaks haphazardly and destroys other connected cells. A figure is given here, for example.

In these figures (13), we see that individual cancer cell have no clear hand to proceed in front, but the cell attack to cell and then progress in couple system by damaging each cell. According to reference [11], a figure is given here that how cancer cells dangerous for the body.
Therefore to fit a body, this is very important that the coupling reaction needs to keep steady by doing exercise. Regular exercise has roll to run coupling cells in every corner of a body and needs balanced food to fit. When the strength of the virus > the strength of coupling, then the virus acts on the body. The same number of cell attacks by the same number of the virus; this is one of the main properties of all cells reaction in the living body. If it is possible to stop growing cancer cells, no entry of cancer cells to the couple system by hook or kook; it is required by adding such medicine or rearranging cells by coupling system in this field to stop the activity of cancer cells. Here is the touch of couple system that how it is important in cell properties.

*The probable cause of breaking of cells:*

![Diagram of cancer progression](image1)

![Diagram of relative number](image2)

*The life or decay of corona virus [80]*
The coupling system of life of corona virus.
We have the equation,
\[ M^0 \times 1 \times 1 = 1^0 \times 1 \times 1 = 1 \text{ number (} M^0 = 1^0 = 1, \text{ when } M = 1) \text{ and 1 number = 1 hour.} \]

For a titers of viable virus (Aerosols)
1) \[ [M^0 \times 1 \times 1] = 1^0 \times 1 \times 1 = 1 \text{ hour.} \]
2) \[ \frac{1}{2} [M^0 \times 1 \times 1] = 0.5 \text{ hour.} \]
3) \[ 2 [M^0 \times 1 \times 1] = 2 \text{ hours.} \]
4) \[ 3 [M^0 \times 1 \times 1] = 3 \text{ hours} \]
5) \[ 4 [M^0 \times 1 \times 1] = 4 \text{ hours} \]

For Copper, Cardboard, Stainless steel, Plastic: (\(M^0 = 10 = 1, \text{ when } M = 1\))
a) \[ 2 [M^0 \times 2 \times 1] = 2 [2] = 4 \text{ hours on copper.} \]
b) \[ 3 [M^0 \times 2 \times 1] = 3 [2] = 6 \text{ hours on cardboard.} \]
c) \[ 4 [M^0 \times 2 \times 1] = 4 [2] = 8 \times 6 \times 12 = 72 \text{ hours on stainless steel and plastic.} \]
d) \[ 5 [M^0 \times 2 \times 1] = 5 [2] = 10 \text{ hours} \]
e) \[ 6 [M^0 \times 2 \times 1] = 6 [2] = 12 \times (12 \times 2) = 24, (24 \times 2) = 48, (48 \times 2) = 96 \]
f) \[ 6 (6 [M^0 \times 2 \times 1]) = 6 (6 [2]) = 72 \times (72 \times 2) = 144 \text{ hours.} \]

Virus spread throughout the word. An example is stated here [81]


How fast could COVID-19 spread in Australia? And how many people could potentially be infected?
We can’t of course know for sure, but we have enough data to make some rough forecasts; and being forewarned is to be forearmed. So we’ve developed an interactive website that gives a ten-day forecast, by country, on likely numbers of COVID-19 cases. We may compare the above figure of spreading of corona virus in 10 days in Australia with the figure of series of Pi [1, 2] as given here:
From the above comparison of the figures - 19, 20, 21 we may get idea that corona virus spread by obeying the couple system. Corona virus behave like host cells and ‘spike’ protein. Its hand is unstable. Due to this reason cell attacks body seriously. It is possible to destroy a corona virus cells by destroying the couple protein. All cells are interlinked to a couple systems. Couple breaks means cell will weak & cannot mixed with each other, at that time medicine can destroy the virus cells forever. Or we may add another protein cell to corona to change its harmful properties through a coupling process. If it is possible to fit cell by micro-cell-fitting- equipment, then no need medicine. Few medicines has side effect, but to rearranging the cell, body will safe from disease.

II. Brain and other Cells

Neurons are responsible for the transport and uptake of neurotransmitters - chemicals that relay information between brain cells. Depending on its location, a neuron can perform the job of a sensory neuron, a motor neuron, or an interneuron, sending and receiving specific neurotransmitters. In the adult brain, neural circuits are already developed, and neurons must find a way to fit in. As a new neuron settles in, it starts to look like surrounding cells. It develops an axon and dendrites and begins to communicate with its neighbors. The following figure [20] related to the couple system.

a) Death of brain cells

The lives of some neurons can take abnormal turns. Some diseases of the brain are the result of the unnatural deaths of neurons. Due to Parkinson’s disease, Huntington’s disease, Alzheimer’s disease, Blows to the brain, Spinal cord injury and, other cause cells damaged. When virus attacks or for other causes, cells destroyed. Here we see neuron, oligodendrocyte, astrocyte in the form of a couple systems. This system breaks when the attack by diseased. The reaction of coupling disturbs means cells damaged. We can realize these facts to see the following figure [22].

Fig. 22: Stem cells differentiate to produce different types of nerve cells
b) **Death of brain cells**

The lives of some neurons can take abnormal turns. Some diseases of the brain are the result of the unnatural deaths of neurons. Due to Parkinson’s disease, Huntington’s disease, Alzheimer’s disease, Blows to the brain, Spinal cord injury and, other cause cells damaged. When virus attacks or for other causes, cells destroyed. Here we see neuron, oligodendrocyte, astrocyte in the form of a couple systems. This system breaks when the attack by diseased. The reaction of coupling disturbs means cells damaged. We can realize these facts to see the following figure [23].

![Image of A Diseased Neuron and A dying Neuron](image.png)

*Fig. 23*

So, we need to think in what way a couple to be fit to work cells. The proper medicine has the power to clear diseases and can run a couple in proper ways. We need to know the number of cells worked in a body. It is possible to find many numbers from a couple systems. Therefore, the application of finding of a number of cells is vital one. The following figure of Ependymal cells [14] of the brain functioned as a couple system. If one of the cells breaks to complete its function, then it is to be assumed that memory cells will lose their property. As a result, the number of cells differs from the original format.

So, it needs to function in a body in a properly adding medicine or by sending a messenger to the brain to keep fit. The coupling can multiply by numbers as to produce many cells. Many coupling is possible at a time to yielding a cell’s birth also.

![Image of Ependymal cells](image.png)

*Fig. 24*

Functions of Couple System in the Brain Cells, Fig. 24 and 25.
Another example of the brain cells.

**Fig. 25:** Brain cells are obeying couple system

*Calculation of the number of cells*

1. \( M^3 \times 2 = (3 \times 2) = 6000 = 6 \times 10^3 = 6 \text{ thousand} \times 2 = 12000, \) (11000 Neurons in Pond Snail brain) [9]. Now ratio of \( M^3 \times 3 \times 2 = 1^3 \times 3 \times 2 = 6000 \) number and \( M^2 \times 2 \times 2 = 1^2 \times 2 \times 2 = 40 \) numbers = 6000/40 = 150. But 6000 / (3x2) = 1000 & 1000x100 = 100000, the number of hairs that someone has on their head can vary by individual. However, the average person has about 100,000 hairs on their head at one time [15]. Again, 150 x1000 = 150000 number of blonde hair [15].

2. \( M^4 \times 3 \times 3 = 1^4 \times 3 \times 3 = 90000 = 9 \times 10^4 = 0.09 \text{ million} = 90 \text{ thousand of red hair} [15].

Ratio of \( M^4 \times 3 \times 3 = 1^4 \times 3 \times 3 = 90000 \) and \( M^3 \times 3 \times 2 = 1^3 \times 3 \times 2 = 6000 \) & 90000/6000 = 15.

3. \( M^5 \times 4 \times 3 = 1^5 \times 4 \times 3 = 1200000 = 1.2 \times 10^6 = 1.2 \text{ million.} \) Now, 1.2 million / 90000 = 13.33.

*Application of 1.2x10^6 -----*

**Numbers from Couple System**

<table>
<thead>
<tr>
<th>Cells</th>
<th>[References]</th>
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</thead>
<tbody>
<tr>
<td>1) 1.2x10^6 = 1.2 million</td>
<td>A healthy adult male can release between 40 million and 1.2 billion sperm cells in a single ejaculation. [16]</td>
</tr>
<tr>
<td>2) 1.2 x 10^6 = 1.2 million</td>
<td>The number of fibers in human optic nerve = 1,200,000 [17].</td>
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<tr>
<td>3) 1.2x10^6 / 3 = 400000</td>
<td>The purple structures inside the ovary are immature egg cells, or oocytes. All of the 400,000 egg cells a woman will ever produce are already present in her ovaries when she is born, although the eggs are in an undeveloped form [18] and 400000 / 10 = 40000. The human gut alone contains on average: 40,000 bacterial species [25d], 40000 / 2 = 20000, According to Asher Mullard, “Between them [the bacteria in our bodies], they harbor millions of genes, compared with the paltry 20,000 estimated in the human genome. [19]</td>
</tr>
<tr>
<td>4) 1.2 million / 10 = 120000</td>
<td>Number of fibers in cat optic nerve = 119,000[20].</td>
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<tr>
<td>5) 1.2 million / 16 = 75000</td>
<td>Number of fibers in albino rat optic nerve = 74,800 [20].</td>
</tr>
<tr>
<td>6) 75000 / 10 = 7500</td>
<td>Number of neurons in nucleus of the hypoglossal nerve 7500 / (3/2 = 1.5) = 5000… 4,500-7,500[20].</td>
</tr>
<tr>
<td>7) 7500 / 2 = 3750</td>
<td>Number of hair cells in cochlea = 3,500 inner hair cells [21].</td>
</tr>
<tr>
<td>8) 1.2 million/100 = 12000</td>
<td>Number of hair cells in cochlea 12,000 outer hair cells [21].</td>
</tr>
<tr>
<td>9) 1200000 / (3/2)= 800000</td>
<td>Number of retinal ganglion cells = 800 thousand to 1 million.</td>
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<tr>
<td>10) 1200000 / (3/2) = 97979.58 ( \approx ) 1 million. Again, 1200000x( (3/2) = 14.6969 \approx 14.7 \text{ million. There are about 0.7 to 1.5 million retinal ganglion cells in the human retina} [22].</td>
<td></td>
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<tr>
<td>11) 800000 / 10 = 800000</td>
<td>800000 / ( \sqrt{2} = 565685.42 ) Number of neurons in the human LGN 565,835[23].</td>
</tr>
</tbody>
</table>
It is not possible to know all cells of all animals in our Earth; these are the examples to support the number yielded from a couple system, and it is related to different cells. 

\[ M^6 \times 4 \times 4 = 1^6 \times 4^4 = 16000000 = 1.6 \times 10^7 = 16 \text{ million}. \]

**Ratio of 16 million/1.2 million = 13.33:1**, for example, Currently the largest artificial neural networks, built on supercomputers, have the size of a frog brain (about 16 million neurons) [20].

\[ M^6 \times 5 \times 4 = 1^6 \times 5 \times 4 = 200000000 = 2 \times 10^8 = 0.2 \text{ billion} = 200 \text{ million} / 10 = 20 \text{ million}, \]

(Neurons in the brain of rat, 15000000 - 21000000) [24]. **Ratio = 200 million/16 million = 12.5:1**

\[ M^8 \times 5 \times 5 = 1^8 \times 5 \times 5 = 2500000000 = 2.5 \times 10^9 = 2.5 \text{ billion} / 10 = 250 \text{ million} (\text{Number of fibers in corpus callosum} = 250,000,000) \] [25]. **Ratio = 2.5 billion/0.2 billion = 12.5:1**

\[ M^6 \times 6 \times 5 = 1^6 \times 6 \times 5 = 3000000000 = 3 \times 10^10 = 30 \text{ billion}, \]

**Ratio = 30 billion/2.5 billion = 12:1**. 

Here we see, when \( M^1 \times 2 \times 1 \) then, negative portions is \( M^1 \times 1 \times 0 \), we can arrange this figure as:

1) \((M^2 \times 2 \times 1) \times (M^1 \times 1 \times 0) = M^3 \times M^1 \times 2 \times 1 \times 1 \times 0 = 0 \) Now, \( 2 \times 0 = 2 \)

2) \((M^1 \times 2 \times 1) / (M^1 \times 1 \times 0) = 10 \times 2 / 0 = 20 \) Now, \( 2 \times 0 = 2 \)

3) \((M^1 \times 2 \times 1) + (M^1 \times 1 \times 0) = 10 \times 2 + 0 = 20 \) Now, \( 2 \times 0 = 2 \)

4) \((M^1 \times 2 \times 1) - (M^1 \times 1 \times 0) = 10 \times 2 - 0 = 20 \) Now, \( 2 \times 0 = 2 \)

5) \( M^2 \times 2 \times 2 \times M^2 \times 0 \times 0 = 0 \) Now, \( 4 \times 0 = 4 \)

6) \((M^2 \times 2 \times 2) / (M^2 \times 0 \times 0) = 100 \times 4 / 0 = 400 \) Now, \( 4 \times 0 = 4 \)

7) \((M^2 \times 2 \times 2) + (M^2 \times 0 \times 0) = 100 \times 4 + 0 = 400 \) Now, \( 4 \times 0 = 4 \)

\((M^2 \times 2 \times 2) - (M^2 \times 0 \times 0) = 100 \times 4 - 0 = 400 \times \sqrt{2} = 5656.8, (\text{Neurons in the brain of Jellyfish is 5600}) \] [27]. Again, \( 400 \times 4 = 4000 \). The ANC is found by multiplying the WBC count by the percent of neutrophils in the blood. For instance, if the WBC count is 8,000 and 50% of the WBCs are neutrophils, the ANC is 4,000 (8,000 \times 0.50 = 4,000).

The most important infection-fighting WBC is the neutrophil (NEW-truh-fil). The number doctors look at is called your absolute neutrophil count (ANC). A healthy person has an ANC between 2,500 and 6,000 [28].

Again, \((M^5 \times 2 \times 2) - (M^5 \times 0 \times 0) = 100 \times 4 - 0 = 400 \times 4 = 1600 \) is for acting as “Within germinal centers, B cells proliferate and mutate the genetic region coding for their surface antibody (also known as immunoglobulin). The process is called somatic hypermutation and is responsible for introducing spontaneous mutations with a frequency of about 1 in every 1600 cell division (a relatively high frequency considering the low mutation frequency of other cells of the body is in 10^6 cell divisions) . . .” [29].

In this case, for 1600 cell division, when acts with \( 10^6 \) cells, then we get \( 1.6\times10^6 \) cells, and it is equal to \((M^6 \times 4 \times 4) = 1.6\times10^6, \) and 1000 times of this value is \( 1.6\times10^9 \) or 1.6 billion. Again, \((M^6 \times 4 \times 4) = 1.6\times10^6 / (3/2) = 1.066x10^6 \) or about 1 million. The **total number of Sodium pumps for a small neuron = 1 million** [30]. Again, \( \sqrt{1.066x10^6} \) number = 1032.47number and 800 x \( \sqrt{1.5} = 979.79 \) number. The scientists estimated that “Researchers have learned a lot about this worm — enough for several Nobel Prizes — and they know that there are exactly 1,031 cells in the adult male and 959 in the adult hermaphrodite (there is no female C. Elegans)” [31].

\[ M^5 \times 3 \times 2 \times M^3 \times 0 \times -1 = M^3 \times M^3 \times 3 \times 2 \times 0 \times -1 = 0 \]

8) \((M^3 \times 3 \times 2) / (M^3 \times 0 \times -1) = 1000 \times 6 / 0 = 6000 \)

9) \((M^3 \times 3 \times 2) + (M^3 \times 0 \times -1) = 1000 \times 6 + 0 = 6000 \)

10) \((M^3 \times 3 \times 2) - (M^3 \times 0 \times -1) = 1000 \times 6 - 0 = 6000 \)

11) \((M^4 \times 3 \times 3) \times (M^4 \times -1 \times -1) = 90000 \times 0.0001 = 9, \) but 90000 The nervous system: more than 90,000 miles of sensations [32] and according to University of Rochester Medical Center (UMRC), these are the normal range of WBCs per microliter of blood (mcL) [33]

**Age range** **WBC count per mcL of blood.**

- **Newborns** 9000 to 30000, let 9000 x (3/2)^2 x 3/2 = 3.375 = 30375 = 30000
- **Children under 2** 6200 to 17000, let 9000 / \( \sqrt{2} = 6363.9 \approx 6200 \) & 9000 x 2 = 18000≈ 17000

Children over 2 and adults 5000 to 10000, let 18000 / 4 = 4500≈ 5000 & 9000 x \( \sqrt{3/2} = 11022.7 \approx 10000. \) Or 9000 is near value of 10000.

12) \((M^4 \times 3 \times 3) / (M^4 \times -1 \times -1) = 90000 / 0.0001 = 9x10^8 = 0.9 \text{ billion} \times 100 = 90000, \) application of 90 billion:
a) \( 9 \times 10^6 \times 10 = 9 \times 10^9 \times 3 = 2.7 \times 10^{10} \) = (difference of \( 5.00 \times 10^{10} \) & \( 3.20 \times 10^{10} \) is \( 2.7 \times 10^{10} \) cell of mean cell number of Adipocytes and standard deviation of it, An average human adult has 30 billion fat cells with a weight of 30 lbs or 13.5 kg [34].

b) \( 9 \times 10^6 / 6 = 1.5 \times 10^8 \approx \) Femoral cartilage cells (1.49 \( \times 10^6 \) cells) [35].

c) \( 9 \times 10^6 / 7 = 1.5 \times 10^8 \approx \) 1.285 \( \times 10^6 \) \( \approx (\) Humeral head cartilage cells) [35].

d) \( 9 \times 10^6 / 11 = 8.18 \times 10^5 \approx \) 8.06 \( \times 10^5 \) = (Talus cartilage cells) [35].

e) \( 4 \times 10^6 \) cells / 2 = \( 2 \times 10^6 \) = \( 2 \times 10^6 \) cells (Heart muscle cells) [36].

12) \((M^x 3 \times 3) + (M^x -x \times -1) = 90000 + 0.0001 \approx 90000.0001\)

13) \((M^x 3 \times 3) - (M^x -x \times -1) = 90000 - 0.0001 = 89999.9999 \approx 90000 \times 10 = 9 million,

14) \((M^x 4 \times 3) + (M^x -x \times -2) = 1200000 + 0.00002 = 24

15) \((M^x 4 \times 3) / (M^x -x \times -2) = 1200000 / 0.00002 = 6 \times 10^6 = 6 billion \times 10 = 60 billion \approx 6.84 billion glia cells in brain [37].

Discussion: Coefficient of \( M^5 \) & \( M^5 \) is (4 x 3) / (-1 x -2) = 12/2 = 6, Number of cortical layer = 6. The percentage of oxygen consumption by white matter = 6 and by gray matter = 94% [38].

In general, neuroglial cells are smaller than neurons; there are about 86 billion neurons and 85 billion "nonneuronal" (glial) cells in the human male brain. While that of the cerebellum is only 0.23 (16.04 billion glia; 69.03 billion neurons). The ratio in the cerebral cortex gray matter is 1.48, and the combined gray and white matter is 3.76. The ratio of the basal ganglia, diencephalon, and brainstem combined is 11.35 [39].

16) We see that, 60 billion \( x/2 = 84.852 \) billion \( \approx 85 \) billion glial cells. Therefore, the equation \((M^x 4 \times 3) / (M^x -x \times -2)\) is tallied to glia cells in human brain. Again, in the case of cerebral cortex gray matter is 16.04 billion glia and 69.03 billion neurons. Again, 84.852 billion = 89.28 billion \approx 89.03 billion neurons. When that equation takes place in the form of \((M^x 4 \times 3) x (M^x -x \times -2)\), then we get = 1200000 \times 0.00002 = 24 number / (2/3) = 16. So, when 69 billion neuron cells will present in brain, then, rest of the glia will 16 billion. Now, 69 billion / 16 billion = 0.23 (present cerebellum in brain). If glia cell decreased as 16 billion cells \( \times (2/3) \), we get 13.06 billion and then, 13.06 billion / 69 billion = 0.189 or 0.19, this factor save from cerebellum attack.

If we arrange, (4 x 3) & (-1 x -2) as (4 + 3) = 7 and 1 / (-1 x -2) = \( \frac{1}{2} \) = 0.5, now, 7 + 0.5 = 7.5/2 = 3.75 this number brings the ratio of combined gray and white matter in the brain (3.76). Again, (4 - 3) = 1 and 1 / (-1 x -2) = \( \frac{1}{2} \) = 0.5, now, 1 + 0.5 = 1.5 which is almost same value of 1.48, the ratio of cerebral cortex gray matter. These facts prove that the cells obey the couple system in all respect.

17) \((M^x 4 \times 3) + (M^x -x \times -2) = 1200000 + 0.00002 = 1200000 = 1.2 million \times 2 = 2.4 million. There are about 2.4 million to 3 million ganglion cells in the human visual system.

18) \((M^x 4 \times 3) - (M^x -x \times -2) = 1200000 - 0.00002 = 1200000 = 1.2 million / (2/3) = 800000, \) (Number of retinal ganglion cells = 800 thousand to 1 million) [40].

19) \((M^x 4 \times 4) x (M^x -x \times -2) = 16000000 \times 0.000004 = 64

20) \((M^x 4 \times 4) / (M^x -x \times -2) = 16000000 / 0.000004 = 400 \times 10^6 = 400 billion \times 2 = 565.68 \) (In fact, the average male will produce roughly 525 billion sperm cells over a lifetime and shed at least one billion of them per month) [41]. So, 565.68 will be the maximum sperm cells over a life time.

21) \((M^x 4 \times 4) + (M^x -x \times -2) = 16000000 + 0.000004 = 1600000 = 16 million / 4 = 4000000, \) (Neurons in the brain of Mouse, 4000000) [9]. But 4000000/40 = 100000 or 10^4, the average loss of neocortical neurons = 100000 or 10^4 per day [42]. Again, 100000/100 = 1000 and 100000/10 = 10000 number, number of synapses for a "typical" neuron = 1000 to 10000 [43].

22) \((M^x 4 \times 4) - (M^x -x \times -2) = 16000000 - 0.000004 = 1600000 = 16 million, \) (Neurons in the brain of Frog, 1600000) [44].

23) \((M^x 5 \times 4) x (M^x -x \times -3) = 200000000 \times 0.0000006 = 120

24) \((M^x 5 \times 4) / (M^x -x \times -3) = 3.333333333 x 10^14 = 333 x 10^{12} = 333 trillion, 3.33 x 10^{14} / 9 = 3.737 \times 10^{13} or 37.04 trillion, Dr. Bianconi and her colleagues concluded that there were 3.72 \times 10^{13} cells in each of us. That is, 37.2 trillion [45].

25) \((M^x 5 \times 4) + (M^x -x \times -3) = 200000000 + 0.0000006 = 2x10^6 = 200 million, now \( \frac{1}{2} \) of this 100 million Number of neuron of Cockroch cells [46]. Again, 100 million x 5 = 500 million --- An octopus brain is formed by 500 million large neurons (while the human brain is made of roughly 100 billion smaller neurons), but the intelligence of this aquatic creature is comparable with that of the apes [47].

26) \((M^x 5 \times 4) - (M^x -x \times -3) = 200000000 - 0.0000006 = 2x10^6 = 200 million, Brown rat contain 200 million cells [48].
On the other hand, we can arrange the series as:

A) \[ M^1 \times 2 \times 1 - M^0 \times 1 \times 1 = 20 - 1 = 19 \quad \text{(Ratio} = (2x1)/(1x1) = 2:1) \]

\[ M^2 \times 2 \times 2 - M^1 \times 2 \times 1 = 400 - 20 = 380 \quad \text{(Neurons in the brain of Roundworm, 302)} \] \([49]\).

\[ \textbf{Caenorhabditis elegans} \text{ (roundworm)} \]

\[ M^3 \times 3 \times 2 - M^2 \times 2 \times 2 = 6000 - 400 = 5600 \quad \text{(Ratio} = (3x2)/(2x2) = 3/2 = 1.5:1) \]

B) \[ M^4 \times 3 \times 3 - M^3 \times 3 \times 2 = 90000 - 6000 = 84000 \quad \text{(Brain cells of ant is about 250000 \[50\])} \]

\[ \text{Brain cells of ant is about 250000} \quad \text{[50]} \]

\[ \text{(Ratio} = (3x3)/(3x2) = 3/2 = 1.5:1) \]

Again, a) \[ 8.4 \times 10^4 \times 3/2 = 1.26 \times 10^5 \quad \text{(Cell-associated viral loads for sorted memory CD4+ T cells)} \]

and it losses day after day from the memory, range from \[ 1 \times 10^5 \text{ to } 2 \times 10^5 \text{ cells} \] \([51]\). Therefore, we can increase the memory by adding at least \[ 1.26 \times 10^5 \text{ cells to memory through proper medicine. In almost primary stage of couple system this} \]

\[ M^4 \times 3 \times 3 - M^3 \times 3 \times 2 = 90000 - 6000 = 84000 \quad \text{Brain cells of ant is about 250000 [50]} \]

\[ \text{(Ratio} = (3x3)/(3x2) = 3/2 = 1.5:1) \]

The decrease in PV-specific CD8 T cells was also even more apparent when the percentage was translated into absolute numbers per spleen, as there was an overall reduction in the size of the spleens of the virus-infected mice at this point (NP38: PV-immune, \[ 5.8 \pm 2.5 \times 10^5 \]; PV + LCMV Armstrong, \[ 2.4 \pm 1.3 \times 10^5 \]; PV + LCMV-clone 13, \[ 1.5 \pm 0.4 \times 10^5 \]). We assume that these observed reductions in virus-specific T cells as monitored by intracellular IFN-\(\gamma\) production indicate a loss in T cell number instead of just function, because there is a loss in the total number of CD44highCD8+ cells \([53]\).

\textbf{Now value of}

i) \[ \text{For } 5.8 \pm 2.5 \times 10^5 \text{ cells,} \]

\[ 5.8 \pm 2.5 \times 10^5 = 8.3 \times 10^5 \quad \text{cells supporting the couple number, } 8.4 \times 10^4 \times 10 = 8.4 \times 10^5 \]

\[ 5.8 - 2.5 \times 10^5 = 3.3 \times 10^5 \quad \text{cells supporting the couple number, } 8.4 \times 10^5 / \sqrt{3/2} = 3.429 \times 10^5 \]

ii) \[ \text{For } 2.4 \pm 1.3 \times 10^5 \text{ cells,} \]

\[ 2.4 \pm 1.3 \times 10^5 = 3.7 \times 10^5 \quad \text{cells supporting the couple number, } 8.4 \times 10^4 \times 4 = 3.36 \times 10^5 \]

\[ 2.4 - 1.3 \times 10^5 = 1.1 \times 10^5 \quad \text{cells supporting the couple number, } 3.36 \times 10^5 / 3 = 1.12 \times 10^5 \]

iii) \[ \text{For } 1.5 \pm 0.4 \times 10^5 \text{ cells,} \]

\[ 1.5 \pm 0.4 \times 10^5 = 1.9 \times 10^5 \quad \text{cells supporting the couple number, } 8.4 \times 10^4 \times 2 = 1.68 \times 10^5 \]

\[ 1.5 - 0.4 \times 10^5 = 1.1 \times 10^5 \quad \text{cells supporting the couple number, } 3.36 \times 10^5 / 3 = 1.12 \times 10^5 \]

To calculate these values, we can assume that minimum of \[ 1.26 \times 10^5 \text{ cells be active in loss of memory. So, it} \]

requires filling the cell in a proper way by adding medicine, which can play in the coupled system to increase memory.
Cell-associated viral loads for sorted memory CD4+ T cells

C) \( M^0 \times 4 \times 3 - M^4 \times 3 \times 3 = 1200000 - 900000 = 1110000 = 1.1 \text{ million} \) (Neurons in the brain of Cockroach, 1 million) \([54]\). (Ratio = \((4x3)/(3x3) = 1.3333\))

D) \( M^0 \times 4 \times 4 - M^4 \times 4 \times 3 = 16000000 - 1200000 = 14.8 \times 10^6 = 14.8 \text{ million} \) (Ratio = \((4x4)/(4x3) = 1.7777\). but \(14.8 \times 10^6 / \sqrt{3/2} = 12.084 \times 10^6\). We see that \(1/2\) of 12 million or 6 million cone cells and 10 times of 12 million or 120 million rod cells are in human retina \([55]\). \( M^0 \times 5 \times 4 - M^4 \times 4 \times 4 = 200000000 - 16000000 = 1.84 \times 10^6 = 184 \text{ million} \times \sqrt{2} = 260.2 \text{ million}. \) (Neurons in the brain of Common treeshrew is 261 \times 10^6 \text{ cells} \([56]\).

E) \( M^0 \times 5 \times 5 - M^5 \times 5 \times 4 = 2500000000 - 20000000 = 2.3 \times 10^9 = 2.3 \text{ billion} \times 10 = 23 \text{ billion} \) and 23 billion \( / \sqrt{3/2} = 18.7794 \text{ billion} \approx 19 \text{ billion}. \) [57]. The average number of neocortical neurons was 19 billion in female brains and 23 billion in male brains. In terms of cells for male brain, 23 billion/2 = 11.5 billion, African Elephant, 11000000000 \([58]\). (Ratio = \((5x5)/(5x4) = 1.25\).

F) \( M^0 \times 6 \times 5 - M^5 \times 5 \times 5 = 30000000000 - 250000000 = 27.5 \times 10^8 = 27.5 \text{ billion} \) (Ratio = \((6x5)/(5x5) = 1.2\). Now 27.5 \times 10^8 / \sqrt{3/2} = 22.45 \times 10^8 \text{ cells in human body.} \) The average number of neocortical neurons was 19 billion in female brains and 23 billion in male brains. \([59]\)

G) \( M^0 \times 6 \times 6 - M^{10} \times 6 \times 5 = 360000000000 - 300000000000 = 330 \times 10^9 = 330 \text{ billion} / \sqrt{3/2} = 269.44 \text{ billion}, \) (Neurons in the brain of Elephant is 267 billion) \([60]\). (Ratio = \((6x6)/(6x5) = 1.2\)

H) \( M^0 \times 7 \times 6 - M^{11} \times 6 \times 6 = 4200000000000 - 3600000000000 = 3840 \times 10^9 = 3840 \text{ billion} \) Couple shifting type formation: (Ratio = \((7x6)/(6x6) = 1.166\)

a) \( M^2 \times 2 \times 2 - M^3 \times 2 \times 1 = 400 - 1 = 399 \)
b) \( M^3 \times 3 \times 2 - M^4 \times 2 \times 1 = 6000 - 20 = 5980 \)
c) \( M^4 \times 3 \times 3 - M^5 \times 2 \times 2 = 90000 - 400 = 89600 \)
d) \( M^5 \times 4 \times 3 - M^6 \times 3 \times 2 = 1200000 - 6000 = 1194000 = 1.194 \text{ million} \)
e) \( M^6 \times 4 \times 4 - M^7 \times 3 \times 3 = 160000000 - 90000 = 15910000 = 15.91 \text{ million} \)
f) \( M^7 \times 5 \times 4 - M^8 \times 4 \times 3 = 2000000000 - 120000 = 1988000000 = 198.8 \text{ million} \times 2 = 3.976 \times 10^8, \) (Neuron of Octopus - 300,000,000/500,000,000) \([61]\). \( M^8 \times 5 \times 5 - M^9 \times 4 \times 4 = 25000000000 - 16000000 = 24840000000 - 2.484 \text{ billion} \times 4 = 9.9 \times 10^9 \approx 10 \text{ billion}, \) Neuron of False killer whale, 1050000000 and 2.484 billion \times 2 = 4.968 billion, Neuron cells of Greater Kudu \([62]\).

It was estimated that long-finned pilot whales have an average of \(2.3 \times 10^8 \) neurons and \(8.3 \times 10^9 \) glial cells in the auditory cortex, and \(2.3 \times 10^9 \) neurons and \(7.6 \times 10^9 \) glial cells in the visual cortex. \([63]\).
g) $M^9 \times 6 \times 5 - M^7 \times 5 \times 4 = 300000000000 - 200000000 = 29.8 \text{ billion}\times \sqrt{1.5} = 36.13 \text{ billion}$, Neurons of Long-finned pilot whale, 372000000000.[64].

h) $M^{10} \times 6 \times 6 - M^8 \times 5 \times 5 = 360000000000 - 2500000000 = 357.5 \text{ billion}$. Here we see that 40 times of $2.5 \times 10^9$ is $10^{11}$ or 100 billion, average number of neurons in the brain is 100 billion.[65].

i) $M^{11} \times 7 \times 6 - M^9 \times 6 \times 5 = 420000000000 - 30000000000 = 4.17 \times 10^{12} = 41.7 \text{ billion}$. Here we see that 40 times of $2.5 \times 10^9$ is $10^{11}$ or 100 billion, average number of neurons in the brain is 100 billion.[66]. Another way of looking at it is that this is 100,000,000,000,000 or 100 trillion atoms. Interestingly, the number of cells in the human body is estimated to be about the same as the number of atoms in a human cell. We can determine more accurate value as $49 \times 10^{12} \times 2 = 98 \text{ trillion} = 100 \text{ trillion}$.

39.7144 trillion & $\sqrt{3/2}$ trillion $= 34.3946$ trillion, now, average is $(39.7144 + 34.3946)$ trillion. For higher derivatives do not apply to living bodies, cells probably will not be permitted to build their bodies. The individual body has its limited power to form cells. Again, from g): $M^9 \times 6 \times 5 - M^7 \times 5 \times 4 = 300000000000 - 200000000 = 29.8 \text{ billion}= 30 \text{ billion}\times \sqrt{1.5} = 36.74 \text{ billion}$, Neurons of Long-finned pilot whale, 372000000000.[64].

For a long time, scientific estimates of the number of cells in the human body ranged between $10^{12}$ and $10^{16}$[73].

A odd - odd function:

$M^{11} \times 7 \times 6 - M^{10} \times 6 \times 6 = 4.9 \times 10^{13} - 3.6 \times 10^{11} = 4.86 \times 10^{13} \text{ & ratio} = (7 \times 6) / (6 \times 5) = 1.4$

Even – even function:

$M^{12} \times 7 \times 7 - M^{10} \times 6 \times 6 = 4.9 \times 10^{13} - 4.2 \times 10^{12} = 4.86 \times 10^{13} \text{ & ratio} = (7 \times 7) / (6 \times 6) = 1.36$

Odd - odd function:

$M^{13} \times 8 \times 7 - M^{10} \times 7 \times 6 = 5.6 \times 10^{14} - 4.2 \times 10^{12} = 5.58 \times 10^{14} \text{ & ratio} = (8 \times 7) / (6 \times 5) = 1.33$

$M^{14} \times 8 \times 8 - M^{13} \times 8 \times 7 = 6.4 \times 10^{15} - 5.6 \times 10^{14} = 5.84 \times 10^{15} \text{ & ratio} = (8 \times 8) / (8 \times 7) = 1.14$

$M^{15} \times 9 \times 8 - M^{14} \times 9 \times 7 = 7.2 \times 10^{16} - 7.2 \times 10^{16} = 7.14 \times 10^{16} \text{ & ratio} = (9 \times 8) / (8 \times 7) = 1.28$

For higher derivatives do not apply to living bodies, cells probably will not be permitted to build their bodies. The individual body has its limited power to form cells. Again, from g): $M^9 \times 6 \times 5 - M^7 \times 5 \times 4 = 300000000000 - 200000000 = 29.8 \text{ billion}= 30 \text{ billion}\times \sqrt{1.5} = 36.74 \text{ billion}$, Neurons of Long-finned pilot whale, 372000000000.[64]. Again, 30 billion x 3 = 90 billion, Number of neurons in the Human Nervous System ~ 90 billion Number of glial cells[72].
How couple equation acts on the body, few examples are there: [74]

<table>
<thead>
<tr>
<th>Name</th>
<th>Neurons in the brain/whole nervous system</th>
<th>Synapses</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sponge</strong></td>
<td>0</td>
<td>( M^{2 + 2(N-2)} .Nr.(N - 2)r \rightarrow M : 0 x r.(0 - 2)r = 0, \text{when } N = 0 )</td>
<td>Despite no nerve system, it exhibits a coordinated feeding and behaviors. [4]</td>
</tr>
<tr>
<td><strong>Trichoplax</strong></td>
<td>0</td>
<td>( M^{2 + 2(N-2)} .Nr.(N - 2)r \rightarrow M : 0 x r.(0 - 2)r = 0, \text{when } N = 0 )</td>
<td></td>
</tr>
<tr>
<td><strong>Asplanchna brightwellii</strong> (rotifer)</td>
<td>about 200</td>
<td>( M^{2 + 2(N-2)} .Nr.Nr \rightarrow M^2 : 2r.2r, \text{when } N = 2, N &gt; 1, \text{if } M = r = 1, M^2.2r.2r = 400 ) &amp; 400/2 = 200.</td>
<td>Brain only</td>
</tr>
<tr>
<td><strong>Ciona intestinalis larva</strong> (sea squirt)</td>
<td>231</td>
<td>( M^{2 + 2(N-2)} .Nr.Nr \rightarrow M^4 : 3r.3r, \text{when } N = 3. \text{If } M = r = 1, \text{then } M^4.3r.3r = 9000 ) near value of 8617.</td>
<td>is the only organism to have its whole connectome (neuronal “wiring diagram”) completed. [9] [10] [11]</td>
</tr>
<tr>
<td><strong>Caenorhabditis elegans</strong> (roundworm)</td>
<td>302</td>
<td>( M^1 + 2(N-2) \text{Nr.}(N - 1)r \rightarrow M^3 : 3r.2r, \text{when } N = 3, \text{and } M^4 + 2(N-2) \text{Nr.}Nr \rightarrow M^4 : 3r.3r, \text{when } N = 3, \text{if } M = r = 1, \text{then average number} = (M^3.3r.2r + M^4.3r.3r) / 2 = (6000 + 9000) / 2 = 7500.</td>
<td></td>
</tr>
<tr>
<td><strong>Jellyfish</strong></td>
<td>5,600</td>
<td>( 2(M^2 \times 2 \times 2) - (M^2 \times 0 \times 0) = 100 \times 4 - 0 = 400 \times \sqrt{2} = 5656.8 )</td>
<td>Hydra vulgaris (H. attenuate)</td>
</tr>
<tr>
<td><strong>Megaphragma mymaripenne</strong></td>
<td>7,400</td>
<td>( 4M^3 : 3r.3r = 9000 \times 1.5 = 7348.5 = 7350, \text{When } M = r = 1 )</td>
<td>adult Tripedalia cystophora (8 mm diameter) – does not include 1000 neurons in each of the four rhopalia</td>
</tr>
<tr>
<td><strong>Box jellyfish</strong></td>
<td>8,700–17,500</td>
<td>( 4M^3 : 3r.3r = 9000 ) and ( 4(2M^3 .3r.3r) = 18000 ) ( \approx 17,500. )</td>
<td></td>
</tr>
<tr>
<td><strong>Medicinal leech</strong></td>
<td>10,000</td>
<td>( [1 + 2(N-2)] .Nr.(N - 1)r \rightarrow M^3 : 3r.2r, \text{when } N = 3, \text{if } M = r = 1, \text{then } M^3 .3r.2r = 6000. ) but coefficient of ( M ) is ( 3r.2r ). If ( M .3r.2r / 3r.2r = 1000. )</td>
<td></td>
</tr>
</tbody>
</table>
## Wonder Findings of Number of Cells in a Body Including Sexual Cells, Remedy of Corona Virus, Increase of Memory, and Other Cells by Couple System

<table>
<thead>
<tr>
<th>Name</th>
<th>Neurons in the brain/whole nervous system</th>
<th>Synapses</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pond snail</td>
<td>11,000</td>
<td>$\frac{3}{2} \times 3 \times 2 = 13 \times 3 \times 2 = 6000 = 6 \times 10^3$</td>
<td>$6000 \times 2 = 12000 \approx 11000$.</td>
</tr>
<tr>
<td>Sea slug</td>
<td>18,000</td>
<td>$\frac{4}{2} \times (M .3r.3r) = 18000$</td>
<td></td>
</tr>
<tr>
<td>Amphioxus</td>
<td>20,000</td>
<td>$\left\lfloor \frac{1 + 2(N - 2)}{3} \right\rfloor M \times 3 \times 2 = 90000$ and $3r.3r = 90000$</td>
<td>central nervous system only</td>
</tr>
<tr>
<td>Larval zebrafish</td>
<td>100,000</td>
<td>$\left\lfloor \frac{1 + 2(N - 2)}{3} \right\rfloor M \times 3 \times 2 = 90000$</td>
<td>$3r.3r = 90000$ and $\frac{M \times 3 \times 2}{3r.3r} = 1000$. If we multiply $1000 \times 20 = 20000$.</td>
</tr>
<tr>
<td>Lobster</td>
<td>100,000</td>
<td>$\left\lfloor \frac{1 + 2(N - 2)}{3} \right\rfloor M \times 3 \times 2 = 90000$</td>
<td>$3r.3r = 90000$ and $\frac{M \times 3 \times 2}{3r.3r} = 1000$. If we multiply $1000 \times 100 = 100000$.</td>
</tr>
<tr>
<td>Fruit fly</td>
<td>250,000</td>
<td>$\left\lfloor \frac{1 + 2(N - 2)}{3} \right\rfloor M \times 3 \times 2 = 90000$</td>
<td>$3r.3r = 90000$ and $\frac{M \times 3 \times 2}{3r.3r} = 1000$. If we multiply $1000 \times 100 = 100000$.</td>
</tr>
<tr>
<td>Ant</td>
<td>250,000</td>
<td>$\left\lfloor \frac{1 + 2(N - 2)}{3} \right\rfloor M \times 3 \times 2 = 90000$</td>
<td>$3r.3r = 90000$ and $\frac{M \times 3 \times 2}{3r.3r} = 1000$. If we multiply $1000 \times 100 = 100000$.</td>
</tr>
<tr>
<td>Honey bee</td>
<td>960,000</td>
<td>$\frac{4}{3} \times 3 \times 3 = 90000 - 6000 = 84000 = 8.4 \times 10^4 \times 3 = 252000 \approx 250000$.</td>
<td>$\frac{M \times 3 \times 2}{3} = 90000$. If we multiply $1000 \times 100 = 100000$.</td>
</tr>
<tr>
<td>Cockroach</td>
<td>1,000,000</td>
<td>$\left\lfloor \frac{1 + 2(N - 2)}{3} \right\rfloor M \times 3 \times 2 = 90000$</td>
<td>$3r.3r = 90000$ and $\frac{M \times 3 \times 2}{3r.3r} = 1000$. If we multiply $1000 \times 100 = 100000$.</td>
</tr>
<tr>
<td>Guppy</td>
<td>4,300,000</td>
<td>$\left\lfloor \frac{1 + 2(N - 2)}{3} \right\rfloor M \times 3 \times 2 = 90000$</td>
<td>$3r.3r = 90000$ and $\frac{M \times 3 \times 2}{3r.3r} = 1000$. If we multiply $1000 \times 100 = 100000$.</td>
</tr>
<tr>
<td>Adult zebrafish</td>
<td>$-10,000,000$</td>
<td>$\left\lfloor \frac{1 + 2(N - 2)}{3} \right\rfloor M \times 3 \times 2 = 90000$</td>
<td>$3r.3r = 90000$ and $\frac{M \times 3 \times 2}{3r.3r} = 1000$. If we multiply $1000 \times 100 = 100000$.</td>
</tr>
</tbody>
</table>

- $M, r, N$: Variables representing numbers of cells.
- $3r.3r$: Product of $3r$ and $3r$.
- $\frac{M \times 3 \times 2}{3r.3r}$: Ratio of total cells to sexual cells.
- $\times 10^3$: Multiplication by $10^3$.
- $\approx$: Approximate value.
- In 2020 a research group announced the most sophisticated connectome [23].
- Varies per species.
- Cells (neurons + other).
<table>
<thead>
<tr>
<th>Name</th>
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<th>Synapses</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frog</td>
<td>16,000,000</td>
<td>(6 \times 4 \times 4 = 1 \times 4 \times 4 = 1600000)</td>
<td></td>
</tr>
<tr>
<td>Naked mole-rat</td>
<td>26,880,000</td>
<td>([IM \times 4 \times 4 = 1 \times 4 \times 4] \times 2 = 32000000)</td>
<td>(\sqrt{1.5} = 26127890.59)</td>
</tr>
<tr>
<td>Smoky shrew</td>
<td>36,000,000</td>
<td>(4 \times 3 \times 3 \times 4 = 4 \times 900000)</td>
<td>(4 = 3600000) and ([3600000 \times 100 = 36000000)</td>
</tr>
<tr>
<td>Short-tailed shrew</td>
<td>52,000,000</td>
<td>([IM \times 4 \times 4 = 1 \times 4 \times 4]) \times 4 = 64000000)</td>
<td>(\sqrt{1.5} = 52.2255781.18)</td>
</tr>
<tr>
<td>Hottentot golden mole</td>
<td>65,000,000</td>
<td>([IM \times 4 \times 4 = 1 \times 4 \times 4]) \times 4 = 64000000)</td>
<td></td>
</tr>
<tr>
<td>House mouse</td>
<td>71,000,000</td>
<td>([IM \times 4 \times 4 = 1 \times 4 \times 4]) \times 4 = 64000000 \times 10)</td>
<td>(-1 \times 10^{12}) ([IM \times 4 \times 4 = 1 \times 4 \times 4]) \times 4 = 64000000 \times 10)</td>
</tr>
<tr>
<td>Nile crocodile</td>
<td>80,500,000</td>
<td>([IM \times 4 \times 4 = 1 \times 4 \times 4]) \times 4 = 64000000 \times 5)</td>
<td>(80000000) ([IM \times 4 \times 4 = 1 \times 4 \times 4]) \times 7 = 49000000 \times 4 = 196000000)</td>
</tr>
<tr>
<td>Golden hamster</td>
<td>90,000,000</td>
<td>(4 \times 3 \times 3 \times 3 \times 4 = 90000000)</td>
<td>(9000 \times 10000 = 90000000)</td>
</tr>
<tr>
<td>Ansell's mole-rat</td>
<td>103,000,000</td>
<td>([IM \times 4 \times 4 = 1 \times 4 \times 4]) \times 10 = 160000000)</td>
<td>(\sqrt{2} = 113137085)</td>
</tr>
<tr>
<td>Mashona mole-rat</td>
<td>113,000,000</td>
<td>([IM \times 4 \times 4 = 1 \times 4 \times 4]) \times 10 = 160000000)</td>
<td>(\sqrt{2} = 113137085)</td>
</tr>
<tr>
<td>Hairy-tailed mole</td>
<td>124,000,000</td>
<td>(7 \times 5 \times 4 = 5 \times 4 \times 3 = 200000000 -)</td>
<td>(1200000 = 198800000) (= 198.8 \text{ million} =)</td>
</tr>
</tbody>
</table>
Herculano-Houzel and her colleagues used this technique to analyze the brains of four deceased men and published their results in 2009: they consistently found whole human brain glia to neuron ratio of almost 1:1. Specifically, they found that the human brain contains about 170.68 billion cells, 86.1 billion of which are neurons, and 84.6 billion of which are glial cells. Their study also suggests that the ratio of glia to neurons differs dramatically from one general brain region to the next. 60.84 billion cells in the cerebral cortex are glia, while only 16.34 billion cells are neurons, giving this region glia to neuron ratio of about 3.76 to 1. It’s the inverse in the cerebellum, an evolutionarily ancient part of the brain that sits astride the brain stem.

According to Herculano-Houzel's study, the cerebellum contains 69.03 billion neurons and only 16.04 glial cells, which means there are about 4.3 neurons for every glia in this region.[75]. If we apply the relative number of couple system & classify, we can get different numbers of the brain and shown in Fig 27- Brain here.
Adapted from “Equal Numbers of Neuronal and Nonneuronal Cells Make the Human Brain an Isometrically Scaled-Up Primate Brain”

What is gray matter in the brain?

According to reference [76], the grey matter is mainly composed of neuronal cell bodies and unmyelinated axons. Axons are carrying signals between those bodies. The grey matter serves to process information in the brain. Structures within the grey matter process signals generated in the sensory organs or other areas of the grey matter. These signals reach the grey matter through myelinated axons that make up the bulk of the white matter in the cerebrum, cerebellum, and spine. Also found in the grey matter are the glial cells (astroglia and oligodendrocytes) and capillaries. The glial cells transport nutrients and energy to the neurons and may even influence how well the neurons function and communicate.

What is the white matter in the brain?

White matter, on the other hand, is mainly composed of long-range myelinated axons (that transmit signals to the grey matter) and very few neuronal cell bodies. Myelin forms a protective coating around these axons, insulating them and improving their transmission of neuronal signals. White matter is found buried in the inner layer of the brain’s cortex, while the grey matter is mainly located on the surface of the brain. The spinal cord is arranged in the oppositely way, with grey matter found deep inside its core and the insulating white matter wrapped around the outside. Some grey matter is also found deep inside the cerebellum in the basal ganglia, thalamus, and hypothalamus and white matter is also found in the optic nerves and the brainstem. How Gray and White Matter functioned through a couple system, that is given here. Glia and neuron cells are present in the gray matter. Brain and mind are related, and this relationship works to do think which stored in memory. It transmits timely from a store and hits mind that what happened in the past. In the present situation, the mind has determinable property. But for the future, which we think for tomorrow, all-time it may not be applicable due to variation of the present situation. A couple system is a system of dual property between two objects (A) & (B) related to the third party (R). This third party ordered to do function. Suppose, he loves a girl, love comes out from the mind; here mind is a third property which calls each other to meet in the form of the direction of love. A figure is given here, for an example:

Fig. 27: Coupling mechanism of Brain Cells
Couple system is related to Mind and Brain

[Reference – 77] [From the view of Couple System]

A taken from Reference and Figure – 30 is the reverse of Figure – 29 and obeying the figure of Couple System. In figure – 28, we see that A & B is related to R. This R may term as RELATIVE NUMBER between A & B. This relation will continue when the series extend from A & B to C & D to keep relation R. Let, A & B placed in A zone and B zone respectively, and C & D will produce another zone as C zone and D zone. If C & D another two number makes relation to A & B together, then the zone may be represented by a round symbol as , and we can write it in the form of:

No. 1, Formation:

The Reaction of a couple:

If A & B takes place in the form of A+ & B+ in the positive zone and C & D takes place in the negative zone in the form of D- & C+ related to A+ & B+ in the positive zone, then if dressed it in the form of:

\[ R \rightarrow (D- \cdot A + C+ B+) \]
we can write, D- = - D & C+ = +C

\[ R \rightarrow (C \cdot A + C \cdot A) \]

\[ R \rightarrow 2CA \]

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The Reaction of a couple:

If A & B takes place in the form of \( A^+ & B^+ \) in the positive zone and C & D takes place in the negative zone in the form of \( D^- & C^+ \) related to \( A^+ & B^+ \) in the positive zone, then if dressed it in the form of:

\[
R \rightarrow (D^- . A^+ + C^+ . B^+), \text{ we can write, } \frac{D^-}{C^+} = \frac{-D}{+C} \tag{1}
\]

\[
R \rightarrow (C . A + C . A) \tag{2}
\]

\[
R \rightarrow 2 CA \tag{3}
\]

Description:

1) D is related to A (Fig-31, L.H.S.), and C to B (Fig -32, R.H.S.). But the value of A and C will be same as the value of B and D. D- in L.H.S. means that it is the end of the reaction of the problem to form relative number ( R ) acting with C+ in R.H.S.

2) The original value of D is C; therefore, D reacts with A and forms CA. Similarly, C is the value of D, but the real value of B is A. So, C reacts with B and forms CA.

3) The total couple reaction with respect to R is 2CA.

When the value of A, B, C, D…., individual, then the result of 2 CA will bring another number [1].

**Effect of Gray Matter by Couple System: (Figure – 33)**

\[
R \rightarrow 8.68 \text{ billion glia cells} \times 69.03 \text{ billion neuron cells in brain} + M_1 \times 69.03 \text{ billion neuron cells}
\]

Or, \( R \rightarrow 599.1803 \text{ billion neuron cells}^2 + M_1 \times 69.03 \text{ billion neuron cells} \)

Or, \( M_1 \times 69.03 \text{ billion neuron cells} + 599.1803 \text{ billion neuron cells}^2 = 2 \text{ billion cells}^2 \text{ need for couple (Assuming that at least 2 billion cells}^2 \text{ need for function where, } R = 1 \text{ brain} + 1 \text{ mind} = 2) \)

Or, \( M_1 \times 69.03 \text{ billion neuron cells} = 2 \text{ billion neuron cells}^2 \text{ - 599.1803 billion neuron cells}^2 = -597.1803 \text{ billion}^2 \)

Or, \( M_1 = -599.1803 \text{ billion neuron cells}^2 \div 69.03 \text{ billion neuron cells} = -8.65102 \text{ billion neuron cells will function mind.} \)

This value 8.65 billion is almost 1/10th of 86.1 billion neuron cells out of total of 170.68 billion cells in the brain [75]. - negative sign indicating that mind on functioned to do give the order to work, which is mind-minus. When any view/ think/ ..., enter into brain, then we may call it mind-plus. These two compartments (+Memory & -Memory) are very active for the living body. We keep it in mind and stored in memory.

**Effect of white matter from the view of a couple systems. (Figure – 33)**

\[
R \rightarrow 19.88 \text{ billion glia cells} \times 69.03 \text{ billion neuron cells in brain} + M_1 \times 69.03 \text{ billion neuron cells}
\]

Or, \( R \rightarrow 1371.779 \text{ billion neuron cells}^2 + M_1 \times 69.03 \text{ billion neuron cells} \)

Or, \( M_1 \times 69.03 \text{ billion neuron cells} + 1371.779 \text{ billion neuron cells}^2 = 2 \text{ billion cells}^2 \text{ need for couple} \)

Or, \( M_1 \times 69.03 \text{ billion neuron cells} = 2 \text{ billion neuron cells}^2 - 1371.779 \text{ billion neuron cells}^2 = -1369.779 \text{ billion}^2 \)

Or, \( M_1 = -1369.779 \text{ billion neuron cells}^2 \div 69.03 \text{ billion neuron cells} = -198.432 \text{ billion neuron cells will function mind.} \)
For the white region, (Figure – 34, R.H.S.)

Effect of mind = 198.432 billion neuron cells x 1.29 billion cells / (8.68 billion glia + 6.18 billion neuron) = - 17.225 billion cells which is almost 1/10th of 170.68 billion cells of human brain.

This value 8.61 billion is almost 1/10th of 86.1 billion neuron cells out of total of 170.68 billion cells in the brain [74]. The negative sign indicating that mind on functioned to do give the order to work.

How many percentage cells work for mind?

For figure – Brain, Gray Matter: (Fig.- 34, L.H.S.)

Work function of the mind = 8.65102 billion cells x 100 / 170.68 billion cells = 5.068% = 5%. Only 5% of cells function for mind in the brain. According to reference [78], Vijay Kumar, who Realized God In 1993 - the connecting link between human form - cosmic mind. He said, “Albert Einstein used his brain only 4% while the rest of humanity, the average human being used between 1-2%, the balance portion always lying dormant. The human brain primarily acts as a receiving and transmitting station. No human being ever had an independent mind. The mind of the entire cosmos is one; it had only two compartments... reservoir of mind plus and reservoir of mind-minus”.

For Figure – Brain, White Matter: (Fig.- 34, R.H.S.)

In the white region, this effect will, 17.225 billion cells x 100 / 170.68 billion cells = 10.09% = 10%. According to reference [79], nearly 90 percent of the brain is composed of glial cells, not neurons. Andrew Koob argues that these overlooked cells just might be the source of the imagination. Astrocytes are also the adult stem cell in the brain and control blood flow to regions of brain activity. Because of all these important properties, and since the cortex is believed responsible for higher thought, scientists have started to realize that astrocytes must contribute to thought. Calcium waves in the cortex are leading scientists to infer that this style of communication may be conducive to the processing of certain thoughts. This idea stems from dreams, sensory deprivation. Without input from our senses through neurons, how is it that we have such vivid thoughts? How is it that when we are deep in thought, we seemingly shut off everything in the environment around us? In this theory, neurons are tied to our muscular action and external senses. We know astrocytes monitor neurons for this information. Similarly, they can induce neurons to fire. Therefore, astrocytes modulate neuron behavior. This could mean that calcium waves in astrocytes are our thinking mind. Neuronal activity without astrocyte processing is a simple reflex; anything more complicated might require astrocyte processing.

From the above writings, we can assume that, for ordinary people used less than 4% comparing Einstein’s brain. Though that will vary from time to time depending on age, brain weight, power of thinking, keeping capacity of the memory of the brain. Altogether, in all cases, mind will touch the field of the body. The brain has capacity 100% to do work, anyone can cover this field. But this is very tub to do. Because we are losing memory, we do not keep the mind in attention in the same direction concerning time. Due to these reasons, a lot of things went out of the
memory which plays with the mind. The function of memory comes from the brain and remarkable memory stored in it; when the mind wishes to search and order to the brain, then it comes out, and we describe on that past facts. Again, we lost many memories every day. Suppose the most common matter, the sun is rising, we are observing this, but we do not keep in mind. That is the uses of everyday things sometimes ignore the mind. But in the case of the sexual field, most of the persons (Men & Women) want to meet together to get their mental and sexual satisfactions. These two opposite nature of the body always wants to attract each other. Due to our social construction, we can’t meet an unknown men or women to do sex work. For example, when a beautiful lady passing you, your mind attracts her by eyes and action to be start in your mind that, Oh! Shall I marry her? May I use it? So-so thinks stored in your brain as memory. It is very difficult to explain perfectly about the mind and its behavior connected to the brain. If I feel pain in my body, immediately brain will attain in that place and my mind does not think other on that moment, I shall not think on universe, planets, other picture, wife, person etc. how I get pain or how I can relieve from it, that will be main feature at that time. Therefore, our mind will work maximum 5 billion cells with effect of 10 billion cells of brain as shown in the Figure: Brain, Gray Matter and Brain, White Matter.

IV. Conclusion

Lot of examples is there, the relative number obtained from couple system naturally important. We can apply it in other fields. Body cell is most important, body fit means mentally fit. So need such discover equipment by which cell in a body will stay in normal position. Math is manmade properties; its application to nature helps us to increase the knowledge many ways.

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Ontogenetic Development of *Hedysarum Alpinum* L. in Prebaikalia

By Elena G. Khudonogova, Alena A. Mikhlyaeva & Svetlana V. Polovinkina

*Irkutsk State Agrarian University*

**Abstract** - *Hedysarum alpinum* L. is an important medicinal plant, which also has ornamental value and is used as a non-conventional feed resource. The medicinal value of this plant species is attributable to the content of the glycoside mangiferin in the aerial parts of a plant, which demonstrates immunostimulating properties. Harvesting of *H. alpinum* as a herbal raw material leads to a fast depletion of natural coenopopulation areas. When cultivated in Prebaikalia, the plant passes through all ontogenetic stages, entering the reproductive phase at year 2-3 of growth. The mid-reproductive stage, which is the most productive for feeding purposes, lasts 6 to 10 years. For introduced species, the life span of a plant growing in the same place ranges between 12 and 20 years. *H. alpinum* is a promising medicinal and fodder plant, which tolerates dry summer periods, is winter-hardy, and has high shoot biomass production.

**Keywords:** hedysarum alpinum, fodder plant, medicinal, ornamental, ontogenesis, introduction.

**GJSFR-C Classification:** FOR Code: 060499
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Elena G. Khudonogova, Alena A. Mikhlyaeva & Svetlana V. Polovinkina

Abstract - *Hedysarum alpinum* L. is an important medicinal plant, which also has ornamental value and is used as a non-conventional feed resource. The medicinal value of this plant species is attributable to the content of the glycoside mangiferin in the aerial parts of a plant, which demonstrates immunostimulating properties. Harvesting of *H. alpinum* as a herbal raw material leads to a fast depletion of natural coenopopulation areas. When cultivated in Prebaikalia, the plant passes through all ontogenetic stages, entering the reproductive phase at year 2-3 of growth. The mid-reproductive stage, which is the most productive for feeding purposes, lasts 6 to 10 years. For introduced species, the life span of a plant growing in the same place ranges between 12 and 20 years. *H. alpinum* is a promising medicinal and fodder plant, which tolerates dry summer periods, is winter-hardy, and has high shoot biomass production.

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I. Introduction

Forb production in an especially relevant issue in Prebaikalia with its developed livestock farming. In recent years, along with the bringing of wild plants into cultivation and development of new varieties of fodder crops, special attention has been paid to non-conventional plants, in particular *Hedysarum alpinum* L. (common name: alpine sweetvetch), which is a valuable fodder, medicinal and ornamental plant. In the study territory *H. alpinum* is has not been used as a fodder or ornamental plant, both due to conventional reasons and insufficient knowledge of this species.

*H. alpinum* is a perennial herbaceous plant of the family Fabaceae, growing up to 40-120 cm in height. Stem: upright, bare. Rhizome: thick, long, branched. Leaves: unpaired, 5-9 (10) pairs, oblong-ovate or elongated-elliptical, appressed-pilose on the top. Inflorescence: long thick multi-flowered raceme with 20-30 flowers. Flowers: up to 15 mm long, papilionaceous, on short pedicels with linear bracts, corollas pink, lilac or purple, rarely white, turn purple when dried. Bracts are usually shorter than pedicels. Calyx: about 4 mm long, bell-shaped, irregular, wide-triangular, the smallest calyces are 2-4 times shorter than the calyx tube. Corollas: 10-14 mm long, the keel is slightly longer than wings and the banner, or is nearly the size of the latter.

Fruit: bean (8-10 mm long), bean segments: 1-4 (2-5), round-oval, with narrow margins, easily breaking off. Each segment contains one seed enclosed in a hard shell. Bloom period: June-July, ripening: from August to early September [1].

Herbal raw materials are harvested for medicinal use in the blooming period (herb) and during withering of the aerial parts of plants, usually in September (roots). *H. alpinum* herb contains mangiferin, which demonstrates antiviral activity against herpes simplex, chickenpox, and cytomegaloviruses. Mangiferin is also known as an immunostimulant due to its stimulating action on cellular and humoral immunity and stimulation of the production of gamma-interferon in blood cells; it is used as an active ingredient in the newly developed "Alpizarin" drug. The aerial part contains quercetin, quercitrin, avicularin. Leaves contain flavonoids: hyperoside, mangiferin, isomangiferin, polyustachosate and hediziaride, traces of alkaloids, ascorbic acid (up to 137.5 mg%); herb contains triterpene saponins. The underground parts of a plant contain 30-40% of polysaccharides, which hydrolyze down to galactose, xylose, galacturonic acid, and low amounts of rhamnose. The sweetvetch root decoction is used in traditional medicine as an expectorant for treatment of coughing, bronchitis and pulmonary tuberculosis, as well as a sedative for treatment of nervous disorders, insomnia, epilepsy, heartache, and atherosclerosis [2,3,4,5,6].

In the territory of the Russian Federation *H. alpinum* its habitat area spreads from the south of the Kola Peninsula to the Urals and Siberia. It grows in the forest and forest-steppe zone along river and stream valleys. It grows riotously in shrub-and-grass meadows on well-drained, elevated sections of the central parts of floodplains [1].

It grows in the southern regions of Irkutsk Oblast, in the Sayan Mountains, in the Lake Baikal area, in Buryatia, in the Yablonovy Mountains and in Dauria, where it occupies moist forest meadows, shrubs, and river valleys.

No large beds of *H. alpinum* were found in Prebaikalia. Harvesting of *H. alpinum* as a herbal raw material leads to a fast depletion of natural coenopopulation areas.

To preserve this species, introduction studies must be carried out based on the investigation of...
ontogenesis of a species under conditions of the study area.

The aim of the present study was to investigate the ontogenetic development of *H. alpinum* introduced in the forest-steppe zone of the southwestern Prebaikalia. Research objectives: based on morphological characteristics, identify ontogenetic states of the species, trace the progress of *H. alpinum* ontogenesis in the Western Cisbaikalia.

II. MATERIALS AND METHODS

**Study object:** *H. alpinum*. Investigation of the ontogenetic state of the species was carried out in the training and experimental field of Irkutsk State Agrarian University. Scarified *H. alpinum* seeds were planted in May and September.

**Planting method:** wide-row planting. The soil in the Experimental field: grey wood soil.

The age-state structure of coenopopulations of useful plants was determined using a standard method for studying the age-state structure of populations making up the plant association, and in compliance with principles and methods for studying the age structure of coenopopulations developed by Yu.A. Zlobin [7] and Ye.L. Nukhimovsky [8]. Description of ontogenesis of *H. alpinum* growing in Prebaikalia also took into account the data on species biology obtained in the studies by L.I. Fomina carried out in Chita Oblast [9], by N.S. Zinner in the forest-steppe zone of Siberia [10], by N. Portnyagina et al. in the Komi Republic [11,12,13], and by N.A. Karnaukhova, D.V. Sandanov, I.Yu. Selyutina in Buyatia [14].

III. RESULTS AND DISCUSSION

Cisbaikalia (or Pribaikalia) is a vast region in the south of Eastern Siberia located in the center of Asia with an area of over 800 thousand sq.km. Cisbaikalia is considered to include the entire area adjacent to Lake Baikal, while the western territories, in a similar way to Transbaikalia (meaning "beyond" (trans-) Lake Baikal), is called Prebaikalia (i.e. "before" Lake Baikal). In the system of physical and geographical zoning of Siberia, most of the Cisbaikalia territory is occupied by two physical and geographical areas: Central Siberia and the mountains of Southern Siberia. According to the landscape zoning of the south of Eastern Siberia, the Cisbaikalia territories occupied by taiga belong to three physical and geographical regions of North Asia: Central Siberian, South Siberian, and Baikal-Dzhugdzhur regions.

The climate of Prebaikalia is determined by the geographical latitude and the position of Lake Baikal almost in the center of Asia, which result in a harsh continental climate. The average January temperature in Cisbaikalia can go down to -20°C. The average air temperature in July reaches +15°C in the mountainous regions along the shores of Lake Baikal, and +18°C in the central regions of Cisbaikalia. Climate of Prebaikalia is determined by the geographical latitude and the position of Lake Baikal almost in the center of Asia, which result in a harsh continental climate. The average January temperature in Cisbaikalia can go down to -20°C. The average air temperature in July reaches +15°C in the mountainous regions along the shores of Lake Baikal, and +18°C in the central regions of Cisbaikalia. The sum of air temperatures above 10°C is 1550-1670 °C, and the frost-free period lasts about 100 days. Maximum air fluctuations are observed in early spring and late autumn. Annual precipitation ranges 380-480 mm [15].

In the entire Prebaikalia, the most prevalent type of soils is grey wood soils [16], while in island steppes chestnut soils, and less often chernozem and meadow chernozem can be found. There are also saline-alkali soils, and black alkali soils.

Our study results show that *H. alpinum* introduced in the region goes through the following ontogenetic stages (Fig. 1):

1. **Seedling stage:** a 1-1.5 high shoot is formed, with 1-3 simple broadly ovate or rarely round leaves and a 1-3 cm long taproot, with clearly visible round-shaped, yellowish cotyledons [17].

2. **Juvenile stage:** monopodial shoot growth continues, cotyledons whither. At this stage, two subgroups can be distinguished: subgroup 1 of juvenile plants (shoot height increases to 1.6-2 cm, the number of simple leaves increases to 3-6, the primary root begins to branch off to secondary roots), and subgroup 2 of juvenile plants (shoot height of 1.7-2.3 cm, with 1-3 ternate leaves appearing on a shoot in addition to simple leaves). In the basal portion of a shoot, 1-3 cataphyll leaves are formed, the 3-5 cm-long primary root begins to branch off to secondary roots, the root is thin, with a diameter of 1.0 to 1.6 mm. In the region of cotyledonary nodes, two primary buds are formed (1 mm wide, 2-2.5 mm high), in the axes of which an additional small third bud is formed, and in turn, in the axes of the third bud is the fourth bud. At this, all buds are formed in a ladder-like manner: with the base of each bud slightly above the other. All buds are different in terms of the stage of formation, they are mostly poorly formed, but in the next year of life they give fast-growing shoots with sympodial branching. The juvenile stage duration varies from 1-3 months to a year. Some plants enter the immature stage already in the first year of growth, while others enter the immature stage only the next year.

3. **Immature stage.** Individuals that entered the immature stage during the first vegetative phase remain single-shooted, the shoot (2.5-3 cm high) continues to grow monopodially, sometimes with...
Plants stop flowering. The subsenile stage is characterized by incomplete caudex particulation. This stage lasts about 2 years.

7. Senile stage: at this stage individuals are in a depressed state, the number of vegetative of shoots is 1-2, necrotic caudex particulation is clearly visible, with the caudex completely destroyed in the center. Plant individuals remain at this stage for 1-2 years.

In Prebaikalia H. alpinum goes through all age periods of development: from immature and virginile to reproductive and senile.

The lifespan of H. alpinum introduced in the southwest Prebaikalia is from 12 to 20 years, at this, seedlings germinate in 10-15 days, the juvenile stage lasts from 1-3 months to 1 year. Some individuals enter the immature stage already at their first year of life, while others enter the immature stage only next year. The virginile stage lasts for about one year. Plants usually enter the reproductive stage in the third year of their growth; and winter-sown individuals may flower during the second year, but only very few of them. The duration of the reproductive stage is from 9 to 14 years. The subsenile stage is characterized by an incomplete caudex particulation and lasts for 2 years. A plant quickly enters a senile stage, which is characterized by a complete caudex particulation and the subsequent death after overwintering.

Alpine sweetvetch goes through a full development cycle: it grows, blooms, bears fruit, forms viable seeds (3.02 ± 0.1 mm long and 1.97 ± 0.09 mm wide). The weight of 1000 pieces of seeds ranges from 4.24 to 6.17 g. Seed germination rate is 40–68%.

Mechanical scarification, light, and removal of seed pericarp improve germination rates and increase the germination energy to 58–88%.

In the first year after sowing, the plant develops slowly, forming one brittle shoot, so in this period it is nearly unable to compete with weeds. Like many other legumes, the sweetvetch forms a symbiotic relationship with nitrogen-fixing bacteria. Many researchers consider the slow growth of H. alpinum in the first year of life to be due to an insufficient number of nitrogen-fixing bacteria; therefore, when seeds are planted in the soil where this species did not grow before, it is recommended to sprinkle the seeds with soil taken from under adult individuals of the species. In the next years, H. alpinum perfectly adapts to the climatic conditions of Prebaikalia, being a frost- and drought-tolerant species, competitively resistant to weeds. Plants have a relatively long life span and can grow in the same place for 10 years or longer.

The 7-year study of biomorphology (Table 1), demonstrated that the productivity was the highest in middle-age reproductive stage plants, which had the highest shoot growth gain (up to 16.5 ± 1.2), shoot height (up to 130.2 ± 4.0 m), number of leaves per shoot (up to 16.0), plant mass per plant (up to 120.2 ±
1.9 g). The productivity of plants at the mid-reproductive stage was affected by the plant age, geographical location of the region, climatic conditions of the study area, environmental [18,19] and other factors. Thus, for example, several authors observed the highest biomass in 4-year-old reproductive plants in the Chita region (up to 13.7 g) [9], and in 8-year-old plants cultivated in the middle-taiga subzone of the Komi Republic (from 91 to 114 g) [11,12,13] and in Tomsk Oblast (up to 174.2 g) [20]. In Prebaikalia, the most productive are 7-year-old plants (up to 120 g).

*Fig. 1: Ontogenetic development of Hedysarum alpinum L. growing in Prebaikalia: 1. Seedling; 2. Juvenile plant of subgroup 1; 3. Juvenile plant of subgroup 2; 4. Immature plant, 5. Virginile plant; 6. Early-reproductive stage plant; 7. Middle-reproductive stage plant; 8. Late-reproductive stage plant; 9. Subsenile plant; 10. Senile plant*

### Table 1: Biomorphology of Hedysarum alpinum L. growing in the conditions of Prebaikalia

<table>
<thead>
<tr>
<th>Year of life</th>
<th>Number of shoots</th>
<th>Shoot height (cm)</th>
<th>Number of leaves per shoot</th>
<th>Weight of seeds per one plant (g)</th>
<th>Phytomass per plant (g) (air-dry)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3.1±0.5</td>
<td>69.4±4.8</td>
<td>12.0±1.1</td>
<td>-</td>
<td>30.2±2.7</td>
</tr>
<tr>
<td>4</td>
<td>4.5±0.8</td>
<td>88.6±6.5</td>
<td>13.5±0.7</td>
<td>9.0±0.6</td>
<td>42.5±1.9</td>
</tr>
<tr>
<td>5</td>
<td>8.3±0.3</td>
<td>112.5±5.5</td>
<td>13.6±1.0</td>
<td>17.2±1.0</td>
<td>86.5±3.6</td>
</tr>
<tr>
<td>6</td>
<td>13.7±0.9</td>
<td>120.0±3.6</td>
<td>16.0±1.5</td>
<td>25.8±2.9</td>
<td>117.0±1.5</td>
</tr>
<tr>
<td>7</td>
<td>16.5±1.2</td>
<td>130.2±4.0</td>
<td>15.3±1.9</td>
<td>38.5±3.0</td>
<td>120.2±1.9</td>
</tr>
</tbody>
</table>

**IV. Conclusion**

When cultivated in Prebaikalia, the plant passes through all ontogenetic stages, entering the reproductive phase at year 2-3 of growth. The duration of the most productive for feeding purposes mid-reproductive stage is 6-10 years. For introduced species, the life span of a plant growing in the same place ranges between 12 and 20 years. *H. alpinum* is a promising medicinal and fodder plant, which tolerates dry summer periods, is winter-hardy, and has high shoot biomass production (up to 120.2 g per plant on year 7 of growth).

**References Références Referencias**


The Significance of Length of Proboscis, the Tubular Feeding Organ in Butterflies of Family: Hesperiidae

By Vitthalrao B. Khyade & Sakdeo Babita Marutirao

Abstract- Butterflies have adapted themselves to different modes of ingestion of food. The feeding in butterflies is analogous to inserting a straw into a drink to withdraw fluid. Modifications in the parts around the mouth in butterflies appear to be the most significant feature for their life. Most of the butterflies use to feed on floral nectar. Butterflies therefore may have a role as efficient pollinators for respective host plants. Development of long proboscis as modified mouth parts in butterflies is to be regarded as an example of a coevolutionary line in the animal kingdom. The Hesperiidae butterflies of Mayureshwar Wildlife sanctuary shown variations in their length of proboscis. The hesperiidae butterflies with longer proboscis visit plant species having flowers with long or deep-tube. Hesperiidae butterfly proboscis help to take up nectar food from the flowers with long or deep as well as short tube of the corolla. The hesperiidae butterflies with extremely long proboscis in present attempt were observed to obtain the nectar from their preferred host plants.

Keywords: hesperiidae; mayureshwar; siphoning; corolla tube; proboscis.

GJSFR-C Classification: FOR Code: 279999

Strictly as per the compliance and regulations of:
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Abstract - Butterflies have adapted themselves to different modes of ingestion of food. The feeding in butterflies is analogous to inserting a straw into a drink to withdraw fluid. Modifications in the parts around the mouth in butterflies appear to be the most significant feature for their life. Most of the butterflies use to feed on floral nectar. Butterflies therefore may have a role as efficient pollinators for respective host plants. Development of long proboscis as modified mouthparts in butterflies is to be regarded as an example of a co-evolutionary line in the animal kingdom. The Hesperiidae butterflies of Mayureshwar Wildlife sanctuary shown variations in their length of proboscis. The hesperiidae butterflies with longer proboscis visit plant species having flowers with long or deep-tube. Hesperiidae butterfly proboscis help to take up nectar food from the flowers with long or deep as well as short tube of the corolla. The hesperiidae butterflies with extremely long proboscis in present attempt were observed to obtain the nectar from their preferred host plants. The Calathea species are well known as nectar host plants for the Hesperiidae butterflies of Mayureshwar Wildlife sanctuary. Species of skipper butterflies (family: Hesperiidae) with extremely long-proboscis, generally did not visit flowers with short nectar spurs. Both Lantana camera (L) (Family: Verbenaceae) and Stachytarpheta frantzii (L) (Family: Verbenaceae) attract many different flower-visiting insects. The flowers of Lantana camera (L) (Family: Verbenaceae) and Stachytarpheta frantzii (L) (Family: Verbenaceae) are easily accessible. The skipper butterflies (family: Hesperiidae) with long-proboscis are crowded out to deep-tubed flowers. Species of butterflies are well known as nectar host plants for the Hesperiidae butterflies of Mayureshwar Wildlife sanctuary. The Calathea species are well known as nectar host plants for the Hesperiidae butterflies of Mayureshwar Wildlife sanctuary. Species of skipper butterflies (family: Hesperiidae) with extremely long-proboscis, generally did not visit flowers with short nectar spurs. Both Lantana camera (L) (Family: Verbenaceae) and Stachytarpheta frantzii (L) (Family: Verbenaceae) attract many different flower-visiting insects. The flowers of Lantana camera (L) (Family: Verbenaceae) and Stachytarpheta frantzii (L) (Family: Verbenaceae) are easily accessible. The skipper butterflies (family: Hesperiidae) with long-proboscis are crowded out to deep-tubed flowers. This pressure is generated in the stipes which is associated with each galea. Coiling results from the elasticity of the cuticle of galea together with the activity of the intrinsic muscles. The uncoiled-proboscis thrusts out into the nectarines of the flower. Due to the sucking action of cibarium muscles and pharyngeal muscles, the nectar is sucked up.

Earlier researchers, including Darwin have pondered over the evolutionary processes of long proboscis of flower-visiting butterflies (Charles Darwin, 1862; Johnson, 1997; Johnson and Anderson, 2010; Muchhala and Thomson, 2009; Nilsson, 1988, 1998; Pauw et al., 2009; Rodríguez-Girone’s and Llandres, 2008; Rodríguez-Girone’s and Santamaría, 2007; Wasserthal, 1997, 1998; Whit tall and Hodges, 2007). The evolution of proboscis in butterflies is supposed to be related with evolution of nectar spurs in angiosperm plant species. (Darwin 1862; Nilsson 1998). Earlier studies by Krenn (2010); Courtney et al. (1982); Wiklund et al. (1979) and Wiklund (1981), mentioned doubtfulness regarding some of the butterflies as efficient pollinators. There is a rare reports on “Mutual relation for co-evolution between species of butterflies and the species of preferred nectar host plants” (Gilbert, 1972, 1975; Grant and Grant, 1965; Levin and Berube, 1972). According to some researchers like Stefanescu and Traveset (2009) and others, butterflies are the flower visitors of “Opportunistic Category” and they are using the available natural resources in the form of plant flower – nectar as they become available during the season (Shreeve, 1992; Stefanescu and Traveset, 2009; Tudor et al., 2004). The influence of length of butterfly proboscis for visiting common plant or a special plant is supposed to remain contradictory. Here, in the present attempt tried it’s best to study the Hesperiidae butterflies.
II. Materials and Methods

a) Area of Study; Plant Species and Butterfly Species For the Study

Area of study for the present attempt was “Mayureshwar Wildlife Sanctuary” belonging to Deccan Plateau. It is a part of Supe village (Tal. Baramati Dist. Pune Maharashtra India) (Co-ordinates: 18° 20’ 6" N 74° 22’ 15" E) (Fig. 1 and 2). The higher density of host plants for hesperiidae butterflies in this region include Lantana camara (L) (Verbenaceae); Stachytarpheta frantzii (L) (Verbenaceae); Calathea lutea (L) (Marantaceae) and Calathea crocalifera (L) (Marantaceae). Therefore, these flowering plant species were selected by the present attempt, recording hesperiidae butterflies visitation. The study was carried during September, October, 2017 and January, February, 2018.
Fig. 1: Mayureshwar Wildlife Sanctuary Site.
The species of plants: *Lantana camera* (L) (Verbenaceae); *Stachytarpheta frantzii* (L) (Verbenaceae); *Calatheca lutea* (L) (Marantaceae) and *Calathea crotalifera* (L) (Marantaceae) were observed in a flowering condition in the study area during the whole tenure of the study attempt. These plant species were in the seminatural garden of Mayureshwar Wildlife Sanctuary of Supe, which borders on natural forest habitats. One more feature of these plant species were growing in close proximity and within reach of the butterfly species foraging in this area. Mayureshwar Wildlife Sanctuary of Supe, the study area, avails the rich supply of nectar throughout the year. This system making the study area highly attractive for varied number and variety of butterflies. The butterflies use the system for colonizing the surrounding natural forest and semi-natural habitats (the habitats modified by human influence but retaining many natural features) (Viththalrao B Khyade and Sharad G Jagtap, 2017). The four studied flowering plant species *Lantana camera* (L) (Verbenaceae); *Stachytarpheta frantzii* (L) (Verbenaceae); *Calatheca lutea* (L) (Marantaceae) and *Calathea crotalifera* (L) (Marantaceae) make different demands on their butterfly visitors. Warren *et al.* (2009) reported that, the observation of butterflies visiting these flowers allows for conclusions on the flower morphology preferences, i.e., corolla length, of butterflies with varying proboscis lengths.

Collection of skipper butterflies was carried out soon after their landing on flowers and subsequently uncoiled the proboscis. Collection of butterflies was carried with the hand nets. The collected skipper butterfly specimens were stored in seventy percent ethanol.

**b) Measurement of Length of the Proboscis of Skipper Butterflies**

The proboscis length of skipper butterfly specimens (preserved in seventy percent ethanol) was measured. The proboscis of each skipper butterfly specimen was separated from the head at its base. It was then uncoiled and fixed on a foam mat using insect pins. Micrographs of the proboscis were taken using a Nikon SMZ 1500 stereomicroscope (Nikon, Tokyo, Japan) equipped with an Optocam-I digital camera (Nikon, Tokyo, Japan). Micrographs were imported to Image J (Savitribai Phule Pune University, Pune India), and proboscis length was measured with the aid of the segmented line tool.
c) Floral Biology and Length of Corolla

The host plant flowers, for example, Lantana camera (L) (Family: Verbenaceae) are small and mostly yellow or orange in colour changing to red or scarlet with age. The lantana flowers form a slightly curved corolla tube. Lantana flowers are arranged in hemispheric inflorescences, measuring up to 3 cm wide, that can be used by butterflies as a landing platform (Woodson et al., 1973). The flowers of Stachytarpheta frantzii (L) (Family: Verbenaceae) are larger than that of Lantana camera (L). The colour of flowers of Stachytarpheta frantzii (L) (Family: Verbenaceae) is purple. The petals of flowers in Stachytarpheta frantzii (L) (Family: Verbenaceae) are and forming a slender cylindrical tube. It is semi-immersed in the rachis of spikes. The flowers are arranged in terminal inflorescences (Woodson et al. 1973). The flowers of Calathea crotalifera (L) (Family: Marantaceae) and Calathea lutea (L) (Family: Marantaceae) are with a yellow tube and hooded staminode, that holds the style under tension. These flowers exhibit unique feature for pollination mechanism (Bauder, et al. 2011). The triggering movement of the style deserves “Irreversible Nature”. Therefore, there is only one opportunity for the flower for pollination. The position of the style after releasement prevents any pollen from subsequently entering the stigma (Kennedy 2000). Since the movement of style is easily visible and flowers can be inspected after visitation, the present attempt was able to determine whether skippers released the trigger and thus potentially act as efficient pollinators. For the purpose of measuring the length of the corolla, flowers from the individual plant of the concerned group at different locations of the study area. Freshly collected flowers were used for the estimation length of corolla. With the help of dissecting needles, the curved corolla, each flower was made straight. Digital caliper was used for measurement of the length of the corolla. Tip of petal and the point of origin of ovary were considered for the length of corolla of individual flower.

d) Record (Video) of Visit of Skipper Butterflies to the flowers

Sony HDR-XR550VE Handycam (Sony Corporation, Tokyo, Japan) was used for recording the foraging activity of the butterflies in their natural environment. One recording period was approximately 64 minutes due to the camera’s memory capacity, and recording was carried out twice a day at each experimental site. Video was recorded at 15 frames per second at a spatial resolution of 320 × 240 pixels. The camera was located approximately 120 cm away at an upward angle from the flowers.

e) Statistical Analysis of the data

Three repetition of whole attempt was followed for consistency in the results. The collected data was subjected for statistical analysis. The statistical package IBM SPSS Statistics 21.0 (IBM Corporation, New York, USA) was utilized for calculation. The Kruskal–Wallis ANOVA was used for analysis. Mann - Whitney U tests (Bonferroni-corrected significance level: p = 0.008) were used for the post hoc tests. The Sigma Plot 12.5 (Systat Software Incorporated, San Jose, California, USA), Corel DRAW X6 (Corel Corporation, Munich, Germany) and Adobe Photoshop CS4 Extended 11.0.2 (Adobe Systems Incorporated, San Jose, California, USA) were used for Graphical illustrations.

III. Results and Discussion

The results on the significance of extremely long-proboscis in Hesperiidae butterflies at Mayureshwar Wildlife Sanctuary is summarized in the tables (1, 2 and 3) and Fig. (3, 4, 5 and 6). The total number of individuals skipper butterflies visited the flowers of Lantana camera (L) (Family: Verbenaceae); Stachytarpheta frantzii (L)(Family: Verbenaceae); Calathea crotalifera (L) (Family: Marantaceae) and Calathea lutea (L) (Family: Marantaceae) was found measured 228. They belong to 43 species and 30 genera (Table - 1). All the species of plants were found differed significantly in corolla length (X^2 (3) = 121. 5; p < 0.0001) were subjected for statistical analysis. The statistical package IBM SPSS Statistics 21.0 (IBM Corporation, New York, USA) was utilized for calculation. The Kruskal–Wallis ANOVA was used for analysis. Mann - Whitney U tests (Bonferroni-corrected significance level: p = 0.008) were used for the post hoc tests. The Sigma Plot 12.5 (Systat Software Incorporated, San Jose, California, USA), Corel DRAW X6 (Corel Corporation, Munich, Germany) and Adobe Photoshop CS4 Extended 11.0.2 (Adobe Systems Incorporated, San Jose, California, USA) were used for Graphical illustrations.
had the skipper butterflies visitors with significantly shorter proboscis. This is in comparison with the skipper butterflies visitors of the other three nectar host plant species in the study [Stachytarpheta frantzii (L) (Family: Verbenaceae); Calathea crotalifera (L) (Family: Marantaceae) and Calathea lutea (L) (Family: Marantaceae)]. The skipper butterflies visitors of Stachytarpheta frantzii (L) (Family: Verbenaceae) in the present attempt were also observed significantly different from other flower visitors with reference to length of their proboscis (Table 3). The skipper butterflies visitors of Stachytarpheta frantzii (L) (Family: Verbenaceae) had longer proboscis in comparison with skipper butterflies of Lantana camera (L) (Family: Verbenaceae). Furthermore, the skipper butterflies visitors of the flower visitors of Calathea crotalifera (L) (Family: Marantaceae) and Calathea lutea (L) (Family: Marantaceae) in the present attempt are reported with significantly longer proboscis than that of skipper butterflies visitors of visitors of Lantana camera (L) (Family: Verbenaceae) and Stachytarpheta frantzii (L) (Family: Verbenaceae) (Table 3). The length of corolla of Calathea crotalifera (L) (Family: Marantaceae) and Calathea lutea (L) (Family: Marantaceae) in the present attempt were also observed significantly different from other flower visitors with reference to length of their proboscis (Table 3). The length of corolla of Calathea crotalifera (L) (Family: Marantaceae) and Calathea lutea (L) (Family: Marantaceae) in the present attempt were also observed significantly different from other flower visitors with reference to length of their proboscis (Table 3).

The skipper butterflies (family: Hesperiidae) are with extremely long proboscis, measures longer than 30 mm. Such butterflies visit flowers with deep nectar spur. The skippers butterflies (family: Hesperiidae) with shorter proboscis use to visit flowers with shorter nectar spurs. The data of present attempt indicate that, skipper butterflies (family: Hesperiidae) with extremely long proboscis refrained from visiting short-tubed flowers, since the number of interactions with flowers of different nectar host plant species did not increase with increasing proboscis length. Moreover, the pattern of interaction is compartmentalized and indicating that skipper butterflies (family: Hesperiidae) with shorter proboscis are separated from skippers with longer proboscis with reference to preference of flowers. Each of skipper butterflies (family: Hesperiidae) with shorter proboscis was using different sets of flowering plants as their source of nectar. The video recordings of visits of thirteen skipper butterflies (family: Hesperiidae) on un-triggered flowers of Calathea crotalifera (L) (Family: Marantaceae) reported that 92.4 % of the visited flowers, remained un-triggered after the skipper left the flower. During a single flower visit, the skipper butterfly (family: Hesperiidae) released the trigger mechanism with a leg through water droplet onto the style of flower.

The resources of food material is the force of driving to establish the coexistence among living beings (Hespenheide, 1973; Inouye, 1980; Ranta and Lundberg, 1980 and Schoener, 1974). It is often method of estimation of correlation through the use of morphological characters. These morphological characters include: size differences between animals or differences in mouthparts in relation to the size of food particles. The butterflies and the moths deserve significant feature of development of siphoning type of mouth parts. The mandibles and labium in butterflies and moths are very much reduced. The labrum is nearly a narrow transverse band, very long and deeply grooved medially. When applied together, the two galea use to enclose fine food channel and it forms a prominent proboscis. It is the main

**Table 1:** The length of proboscis of Hesperiidae Butterflies Visited the Flowers of Selected Plant Species at Mayureshwar Wildlife Sanctuary of Baramati Tehsil of Pune (India).

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Hesperiidae Butterfly Species</th>
<th>N</th>
<th>Proboscis Length (mm)</th>
<th>Flower Visited By Hesperiidae Butterfly</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Eudaminae Astraptes alardus latia (Evans, 1952).</td>
<td>3</td>
<td>23.735 (± 2.436)</td>
<td>Calathea lutea (L) (Family:Marantaceae).</td>
</tr>
<tr>
<td>5.</td>
<td>Eudaminae Bungalotis quadratum quadratum (Sepp, 1845)</td>
<td>3</td>
<td>28.129 (± 2.547)</td>
<td>Calathea lutea (L) (Family: Verbenaceae).</td>
</tr>
<tr>
<td>No.</td>
<td>Genus and Species</td>
<td>Sample Size</td>
<td>Mean Length (± Standard Error)</td>
<td>Family and Subfamily</td>
</tr>
<tr>
<td>-----</td>
<td>-------------------</td>
<td>-------------</td>
<td>--------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>8.</td>
<td>Eudaminae Typhedan us undulates (Hewitson, 1867).</td>
<td>3</td>
<td>12.524 (± 1.043)</td>
<td>Lantana camera (L) (Family: Verbenaceae)</td>
</tr>
<tr>
<td>13.</td>
<td>Eudaminae Saliana sevens (Mabille, 1895).</td>
<td>3</td>
<td>52.319 (± 3.786)</td>
<td>Calathea crotalifera (L) (Family: Marantaceae)</td>
</tr>
<tr>
<td>23.</td>
<td>Moncini Moris micythus (Godman, 1900).</td>
<td>3</td>
<td>19.796 (± 1.392)</td>
<td>Stachytarpheta franzii (L) (Family: Verbenaceae) (N=3). Lantana camera (L) (Family: Verbenaceae) (N=3).</td>
</tr>
<tr>
<td>30.</td>
<td>Pyrginae Pyrrhopygini Mysoria ambigua (Mabille and Boulet, 1908)</td>
<td>7</td>
<td>15.453 (± 2.423)</td>
<td>Stachytarpheta franzii (L) (Family: Verbenaceae).</td>
</tr>
</tbody>
</table>
### Table 2: Pair-wise post hoc tests (Mann–Whitney U tests, \(p < 0.008\); Bonferroni corrected).

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Host Plant and Corolla length of flower (mm)</th>
<th>Lantana camera (L)(Family: Verbenaceae)</th>
<th>Stachytarpheta frantzii (L)(Family: Verbenaceae)</th>
<th>Calathea crotalifera (L)(Family: Marantaceae)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lantana camera (L) (10.3; 8.5–11.7)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Stachytarpheta frantzii (L) (15.8; 14.7–18.2)</td>
<td>(p &lt; 0.0001^*)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Calathea crotalifera (L) (25.3; 22.3–28.4)</td>
<td>(p &lt; 0.0001^*)</td>
<td>(p &lt; 0.0001^*)</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Calathea lutea (L) (31.3; 26.6–36.3)</td>
<td>(p &lt; 0.0001^*)</td>
<td>(p &lt; 0.0001^*)</td>
<td>(p = 0.85)</td>
</tr>
</tbody>
</table>

Median; Minimal and Maximal Coroll Length of Each Nectar Host Plant are given in bracket. The “Pairwise post hoc tests” showed that all nectar host plants differ significantly in corolla length.

### Table 3: Pairwise post hoc tests (Mann–Whitney U tests, \(p < 0.008\); Bonferroni-corrected).

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Host Plant and Corolla length of flower (mm)</th>
<th>Lantana camera (L)(Family: Verbenaceae)</th>
<th>Stachytarpheta frantzii (L)(Family: Verbenaceae)</th>
<th>Calathea crotalifera (L)(Family: Marantaceae)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lantana camera (L) (15.5; 10.8–49.4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Stachytarpheta frantzii (L) (17.7; 13.1–52.8)</td>
<td>(p &lt; 0.0001^*)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Calathea crotalifera (L) (42.2; 27.5–52.6)</td>
<td>(p &lt; 0.0001^*)</td>
<td>(p &lt; 0.0001^*)</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Calathea lutea (L) (43.0; 23.6–52.7)</td>
<td>(p &lt; 0.0001^*)</td>
<td>(p &lt; 0.0001^*)</td>
<td>(p = 0.85)</td>
</tr>
</tbody>
</table>

Median; Minimal and Maximal Coroll Length of Each Nectar Host Plant are given in bracket.
siphoning tube. At the time of feeding, the proboscis remain uncoiled and inserted in the flower. It is hypothesized that, the length of proboscis vary according to the length of corolla tube of the flowers selected by the butterflies for feeding. The skipper butterflies (family: Hesperiidae) with extremely long-proboscs should specialize in visiting flowers that correspond to the length of their proboscis of mouth parts. The skipper butterflies (family: Hesperiidae) with extremely long-proboscs may avoid the flowers with short corolla tube. Many researchers (Corbet, 2000; Nilsson, 1988; Nilsson et al., 1985) consider the butterflies as "Generalist Flower Visitors". The attempt of the butterflies is to visit the maximum number of flowers for the nectar. They use to visit the flowers of the number of plant species available for them. This is possible due to the presence of extremely long proboscis in the mouth parts of the butterflies (Agosta and Janzen, 2005).

Conclusively enough, species of skipper butterflies (family: Hesperiidae) with long proboscis could potentially utilize short flowers in addition to long flowers. It would be expected that, the number of flowering species visited by skipper butterflies (family: Hesperiidae) would be greater than that of species skipper butterflies (family: Hesperiidae) with short proboscis. The data in present attempt support the hypothesis. The skipper butterflies (family: Hesperiidae) with extremely long-proboscs, generally did not visit flowers with short nectar spurs. Both Lantana camara (L) (Family: Verbenaceae) and Stachytarpheta frantzii (L) (Family: Verbenaceae) attract many different flower-visiting insects. This is because, the flowers of Lantana camara (L) (Family: Verbenaceae) and Stachytarpheta frantzii (L) (Family: Verbenaceae) are easily accessible. These flowers are continuously exploited by a great variety of butterfly species possessing rather short proboscis. The skipper butterflies (family: Hesperiidae) with long-proboscs are crowded out to deep-tubed flowers. Here, in these flowers, the skipper butterflies can benefit from a more exclusive access to nectar.

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Viththalrao B. Khyade and Sakdeo Babita Marutirao extend their sincere thanks to Administrative Staff at the "Mayureshwar Wildlife Sanctuary" of Baramati Tehsil District Pune (India) for constant guidance and providing valuable information regarding the research project. The guidance from the team of well esteemed “Global Journal of Science Frontier Research: Biological Science” exert a grand salutary influence.

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**Fig. 3:** Corolla Tube of Individual Flower of *Lantana camera* (L) (Family: Verbenaceae) Mayureshwar Wildlife Sanctuary [Supe Tal. Baramati Dist. Pune Maharashtra India (Co-ordinates: 18° 20’ 6” N 74° 22’ 15” E)].
Fig. 4: Corolla Tube of Individual Flower of *Stachytarpheta frantzii* (L) (Family: Verbenaceae) Mayureshwar Wildlife Sanctuary [Supe Tal. Baramati Dist. Pune Maharashtra India (Co-ordinates: 18° 20' 6" N 74° 22' 15" E)].
Calathea crotalifera (L) (Family: Marantaceae)

Fig. 5: Corolla Tube of Individual Flower of *Calathea crotalifera* (L) (Family: Marantaceae) Mayureshwar Wildlife Sanctuary [Supe Tal. Baramati Dist. Pune Maharashtra India (Co-ordinates: 18° 20' 6" N 74° 22' 15" E)].
Fig. 6. Corolla Tube of Individual Flower of *Calathea lutea* (L) (Family: Marantaceae) Mayureshwar Wildlife Sanctuary [Supe Tal. Baramati Dist. Pune Maharashtra India (Co-ordinates: 18° 20’ 6” N 74° 22’ 15” E)].
Effect of Low Temperatures on Phenolic Compounds Flavonoid Content and Antioxidant Activity of Egyptian Olive Oils

By Yasser A. Selim, Rehab E. Tag & Zeinab E. Eid

Zagazig University

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Keywords: olive oil; phenolic compounds; flavonoids; antioxidant activity.

GJSFR-C Classification: FOR Code: 069999

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Effect of Low Temperatures on Phenolic Compounds Flavonoid Content and Antioxidant Activity of Egyptian Olive Oils

Yasser A. Selim, Rehab E. Tag & Zeinab E. Eid

Abstract: This work studied the low temperatures on the chemical properties of two types of local Egyptian olive oils, one of them from Marsa Matrouh in Western desert (OO1) and the other from Al-Arish in the eastern desert (OO2). The parameters for edible olive oil are established by European Union regulations and by the International Olive Council. This study presents the influence of gentle heating on the content of saturated and unsaturated fatty acids. Results showed that, heat treatment causes a slow decrease in the nutritional quality of the types of olive oils specially in that were heated at 80 °C for 15 min. The content of unsaturated fatty acids decreased significantly from 92.15% in OO1 before heating to 79.32% at 80 °C for 15 min, while no significant change was found at 60 °C of OO1. Dihomo-γ-linolenic acid [DGLA, 20:3 (ω−6)] detected in OO1 type after heating treatment where, which has anti-thrombotic effects. Also, these results indicate that the version OO1 have a higher anti-inflammatory and antimicrobial activities. Also the higher antioxidant activity which not affected by low temperatures prevent the oils from deterioration. Also, the chemical analysis showed that the nutritional quality of OO1 was higher than OO2 due to a higher content of flavonoids which do not affected by heat.

Keywords: olive oil; phenolic compounds; flavonoids; antioxidant activity.

I. Introduction

Vegetable oils are regarded as the healthier choice relative to animal fats in view of their unsaturated fatty acid and cholesterol-free content. Extra virgin olive oil (EVOO) is one of the most important ingredients of the Mediterranean diet. While originally limited to the Mediterranean regions, olive oil remains as source of external fat, Olea europaea L. Olive oil is the vegetable oil obtained from the fruit of the olive tree by mechanical extraction (IOOC 2008). The composition of olive oil is primarily triacylglycerols and secondarily free fatty acids, mono- and diacylglycerols, and an array of lipids such as hydrocarbons, sterols, aliphatic alcohols, tocopherols, and pigments. Some of these compounds contribute to the unique character of the oil (Dimitrios et al., 2006). The phenolic content was significantly highest in the unheated EVOO and decreased constantly by increasing thermal stress. The temperature very highly significantly influenced phenolic content, whereas the duration of heating showed a minor effect (Giuffrè et al., 2017). Several olive oil grades are commercially available. Its classification based on sensorial attributes and chemical parameters that give a general overview of its quality and authenticity. These characteristics, namely the free fatty acids (FFA) are regulated by several organizations, including the European Commission itself, the International Olive Oil Council (IOC). Olive oil possesses a highly distinctive taste and flavor due to specific volatile organic compounds, namely aliphatic and aromatic hydrocarbons, aliphatic and triterpenic alcohols, furan and thiophene derivatives (Kiritsakis et al., 1998). Oleic acid is the most abundant fatty acid in olive oil that is claimed to affect the increase the level of high-density lipoprotein (HDL) and to reduce levels of low-density lipoprotein (LDL) in the blood plasma. For this reason, it is considered that oleic acid could prevent the occurrence of certain cardiovascular diseases which are still one of the major causes of death. The high concentration of phenolic compounds in olive oil contributes significantly toward its antimicrobial activity (Kecel & Robinson 2002; Markin et al., 2003; Pereira et al., 2006). Olive oil, in particular virgin olive oils with a high content in certain phenolic compounds, can inhibit the growth of pathogenic bacteria. Virgin olive oil (VOO) has nutritional and sensory characteristics that make it unique and a basic component of the Mediterranean diet. Its importance due to its richness in polyphenols which, act as natural antioxidants and may contribute to the prevention of several human diseases (Dimitrios et al., 2006). Olive oil (OO) constitutes the basis of the Mediterranean diet, and it seems that its biophenols, such as hydroxytyrosol (HT) may scavenge free radicals, attracting distinct attention due to their beneficial effects in many pathological conditions, such as cancer. To the best of our knowledge, this is the first study in which the functional properties of an olive oil total polyphenolic fraction (TPF) and pure hydroxytyrosol were examined in order to determine their antioxidant effects at a cellular level in endothelial cells and myoblasts (Maalej et al., 2006; Tagliaferro et al., 2015; Kalaiselvan et al., 2015). Direct evidence for the protective role of olive oil against cancer has been recently published (Anna et al., 2013). Testing methods are needed to address the real cooking effect on olive oil composition and under
different processing conditions, in comparison with other vegetable oils; such that, has good thermal resistance in general. High temperature and prolonged timed used on repeated frying, the oils progressively degraded by a complex series of chemical reactions including oxidation, hydrolysis, and polymerization. These reactions, however, are not equivalent for all the vegetable oils, and there is a particular concern regarding olive oil since its bioactive attributes might be lost during this process, despite being highly resistant to thermal oxidation. The most common frying methods are deep-frying, being the food immersed in hot oil, and pan-frying when the food is cooked in a pan with little amounts of oil (Andrikopoulos et al., 2002).

II. Materials and Methods

a) Chemicals

All solvents and chemicals were from Sigma-Aldrich (St. Louis, MO, USA). The international standards use different methods and parameters to evaluate the quality of the oils according to their use as food. Physicochemical parameters were considered about the requirements of standards.

b) Phytochemical Screening

Phytochemical screening for flavonoids, alkaloids, saponins and terpenoids were done following standard methods as described by Harborne (1998) and Sofowora (1993).

c) Experimental Design

One hundred-gram samples of OO1 and OO2 were placed in steel containers and heated to either 60 or 80 °C. These temperatures were chosen based on the fasting temperature of olive oil (60–80 °C) and of the temperature that can be reached during fasting cooking (100 °C). The samples were held at each temperature for 5, 10 and 15 min., giving a total of 12 trials. After heating, the oil was cooled to room temperature and analysed within two hours.

d) Gas Chromatography-Mass Spectrum Analysis (GC-MS) analysis

The chemical composition of your samples was performed using Trace GC1310-IQSamass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50 °C and then increased by 5°C /min to 230°C hold for 2 min. increased to the final temperature 290°C by 30°C /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 250, 260°C respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was three min. and diluted samples of 1 µl were injected automatically using Auto sampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

e) High-Performance Liquid Chromatography-Mass Spectrum

HPLC-MS technique is often used for separation, identification and quantitation of flavonoids and phenolic acids in plants. The HPLC-MS system (Agilent 1100) is composed of a quaternary pump, a photodiode array detector, a UV/Vis detector, and a single quadruple MS detector with ion source (ESI).

f) Phenolic contents

Phenolic contents were separated from within 60 min by employing a gradient mobile phase of water/acetonitrile/glacial acetic acid (980/20/5, v/v/v, pH 2.68) and acetonitrile/glacial acetic acid (1000/5, v/v) with flow rate at 3 mL/min and detection at 325 nm.

g) Flavonoids contents

Phenolic contents were separated from within 60 min by employing contents were separated from within 60 min by employing a gradient solvent system of 0.1% formic acid solution with flow rate at 1.0 mL/min, detection at 280 nm and identification by ESI -MS, were separated within 70 min.

h) Antioxidant Activity (DPPH assay) oil

The DPPH assay measures the radical scavenging activity of vegetable oil. It was conducted in UV/Vis Spectrometer model Lambda 2, Perkin Elmer (Waltham, MA, USA), using the method proposed by kalantzakis et al. (2006), modified as follows. Firstly, the oil was diluted with ethyl acetate (1:10, v/v). Secondly, 500 µL of diluted oil were added to 2 mL of a 10-4 M DPPH solution, previously prepared with ethyl acetate and, thirdly, the absorbance of the mixture was measured immediately at 515 nm (t0) and after 30 minutes of incubation (t30). The results were calculated with the following formula: % inhibition = [(T0 – T30)/T0] x 100 and they were expressed as % inhibition.

III. Results and Discussion

a) Phytochemical Screening

Phytochemical screening showed that the phenolic compounds were decreased for both OO1 and OO2, while the flavonoids contents do not affect by heating. These results indicated that, the olive oils after treatment with low temperatures have high antioxidant activity due to flavonoids compounds which do not affect by heat (Tables 1&2). The results showed that the presence of terpenoids, steroids and phenolic compounds in both which, their concentrations were decreased by heating at low temperatures. The contents of flavonoids not affect by heating.
Table 1: Phytochemical screening of OO1 at 60°C, 80°C

<table>
<thead>
<tr>
<th>Compounds</th>
<th>60°C</th>
<th></th>
<th></th>
<th>80°C</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min.</td>
<td>10 min.</td>
<td>15 min.</td>
<td>5 min.</td>
<td>10 min.</td>
<td>15 min.</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

Highly positive ‘+++’, Moderate ‘++’, Negative ‘-’

Table 2: Phytochemical screening of OO2 at 60°C, 80°C

<table>
<thead>
<tr>
<th>Compounds</th>
<th>60°C</th>
<th></th>
<th></th>
<th>80°C</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min.</td>
<td>10 min.</td>
<td>15 min.</td>
<td>5 min.</td>
<td>10 min.</td>
<td>15 min.</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

Highly positive ‘+++’, Moderate ‘++’, Negative ‘-’

b) The effect of heat treatment on the content of free fatty acids

Fatty Acids (FAs) are among the most important parameters to establish the edibility of vegetable oil; in particular, oleic acid content. The high saturated fatty acid (SFA) content determines vegetable oil solidification at low temperatures and increases the cholesterol content in the blood. The high unsaturated fatty acid (UFA) content determines an increase in oxidation ability of the vegetable oil due to the presence of double bonds. However, mono-unsaturated fatty acids (MU FAs) are lower the bad cholesterol in the blood and represented as essential fatty acids (EFAs) which, it must have to be taken with the diet. Saturated fatty acids namely, Palmitic acid (16:0), Heptadecanoic acid (C17:0) and Stearic acid(C18:0) were calculated as percentage content increased with increasing temperature. The minimum content (0.80%) and (1.20 %) were found in the OO1 and OO2 respectively, before thermal treatment and the maximum(11.95% and 16.99 % ) of both OO1 and OO2 were found at 80 °C for 15 min.(Table 3 & Table 4). Linoleic acid (18:2) showed a decreasing trend from 22.73% in the original OO1 to 20.73% and 16.32% after 15 min heating at 60°C and 80°C respectively (Table 2). On the other hand, there is a significant decrease in linolenic acid content in OO2 from 20.71% to 17.92% and 15.32% after 15 min heating at 60°C and 80°C respectively. These observations confirmed the fact that the fatty acid degradation rate increases with the number of double bonds in the molecule (Guillén and et Ruiz 2008). The results also confirmed that, different types of Egyptian olive oils OO1 and OO2 have higher content of active antioxidant compounds which prevent or decreases the unsaturated fatty acids against deterioration during gentle heating. Also, the results showed that after heating Dihomo-γ-linolenic acid (DGLA) was detected in OO1 type where, it is a 20-carbon ω−6 fatty acid. In physiological literature, it is given the name 20:3 (ω−6) contains three cis double bonds. DGLA is an extremely uncommon fatty acid, found only in trace amounts in animal products which have antithrombotic effects. Also, these results indicate that the version of olive oil OO1 has higher anti-inflammatory and antimicrobial activities (Jill and Alexander 2000).
Table 3: Fatty acids composition% of OO₂ at 60°C, 80°C

<table>
<thead>
<tr>
<th>Compounds</th>
<th>60°C</th>
<th>80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min.</td>
<td>10 min.</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>0.80±0.11</td>
<td>1.11±0.01</td>
</tr>
<tr>
<td>Heptadecanoic acid (C17:0)</td>
<td>4.52±0.10</td>
<td>4.32±0.11</td>
</tr>
<tr>
<td>Heptadecenoic acid (C17:1)</td>
<td>0.40±0.12</td>
<td>0.40±0.13</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>1.85±0.12</td>
<td>2.15±0.11</td>
</tr>
<tr>
<td>Oleic acid (C18:1)</td>
<td>52.53±0.10</td>
<td>52.13±0.11</td>
</tr>
<tr>
<td>Linoleic acid (C18:2)</td>
<td>15.55±0.10</td>
<td>15.15±0.10</td>
</tr>
<tr>
<td>Linolenic acid (C18:3)</td>
<td>22.73±0.10</td>
<td>21.13±0.10</td>
</tr>
<tr>
<td>Dihomo-γ-linolenic acid (C20:3)</td>
<td>-</td>
<td>0.23±0.10</td>
</tr>
<tr>
<td>Arachidonic acid (C20:4)</td>
<td>0.94±0.10</td>
<td>0.86±0.10</td>
</tr>
<tr>
<td>Total saturated fatty acids</td>
<td>7.17±0.11</td>
<td>7.58±0.11</td>
</tr>
<tr>
<td>Total unsaturated fatty acids</td>
<td>92.15±0.11</td>
<td>89.90±0.11</td>
</tr>
<tr>
<td>Ratio unsaturated/saturated fatty acids</td>
<td>12.85±0.11</td>
<td>11.86±0.11</td>
</tr>
</tbody>
</table>

Means ± DS, n = 3. Values are significantly different from each other (p < 0.05)

Table 4: Fatty acids composition% of OO₂ at 60°C, 80°C

<table>
<thead>
<tr>
<th>Compounds</th>
<th>60°C</th>
<th>80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min.</td>
<td>10 min.</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>1.20±0.10</td>
<td>1.91±0.00</td>
</tr>
<tr>
<td>Heptadecanoic acid (C17:0)</td>
<td>6.12±0.10</td>
<td>6.37±0.00</td>
</tr>
<tr>
<td>Heptadecenoic acid (C17:1)</td>
<td>0.33±0.11</td>
<td>0.31±0.13</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>2.15±0.22</td>
<td>2.23±0.00</td>
</tr>
<tr>
<td>Oleic acid (C18:1)</td>
<td>45.51±0.10</td>
<td>44.93±0.11</td>
</tr>
<tr>
<td>Linoleic acid (C18:2)</td>
<td>13.59±0.10</td>
<td>13.45±0.10</td>
</tr>
<tr>
<td>Linolenic acid (C18:3)</td>
<td>20.71±0.10</td>
<td>18.13±0.10</td>
</tr>
<tr>
<td>Arachidonic acid (C20:4)</td>
<td>0.73±0.10</td>
<td>0.72±0.10</td>
</tr>
<tr>
<td>Total saturated fatty acids</td>
<td>9.47±0.11</td>
<td>10.51±0.11</td>
</tr>
<tr>
<td>Total unsaturated fatty acids</td>
<td>80.87±0.11</td>
<td>77.54±0.11</td>
</tr>
<tr>
<td>Ratio unsaturated/saturated fatty acids</td>
<td>8.59±0.11</td>
<td>7.37±0.10</td>
</tr>
</tbody>
</table>

Means ± DS, n = 3. Values are significantly different from each other (p < 0.05)
c) Phenolic compounds content

Olive oil contains many biologically active components, which exert antioxidant activity, differently from other edible vegetable oils. This implies that olive oil contains many minor bioactive compounds such as phenols whose content was found to decrease with heating. The phenolic content was significantly highest in the unheated of both olive oils OO1 and OO2 and decreased constantly with the increasing temperature. After 15 min. of heating the phenolic content of OO1 decreased from 92.91 to 63.77, and 31.24%, respectively at 60 and 80 °C (Table 5). On the other, the phenolic content of OO2, decreased from 89.17 to 34.23, and 32.4 %, respectively at 60 and 80 °C (Table 6).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>60°C</th>
<th>80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min.</td>
<td>10 min.</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>25.21</td>
<td>16.42</td>
</tr>
<tr>
<td>Ellagic</td>
<td>18.44</td>
<td>8.40</td>
</tr>
<tr>
<td>Quercetin</td>
<td>15.82</td>
<td>12.82</td>
</tr>
<tr>
<td>Total</td>
<td>92.91</td>
<td>65.61</td>
</tr>
</tbody>
</table>

Table 5: HPLC of Phenolic compounds of OO1 at 60°C, 80°C

<table>
<thead>
<tr>
<th>Compounds</th>
<th>60°C</th>
<th>80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min.</td>
<td>10 min.</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>24.65</td>
<td>19.15</td>
</tr>
<tr>
<td>Catechol</td>
<td>28.20</td>
<td>25.32</td>
</tr>
<tr>
<td>Ellagic</td>
<td>14.66</td>
<td>12.61</td>
</tr>
<tr>
<td>Gallic</td>
<td>12.65</td>
<td>9.65</td>
</tr>
<tr>
<td>Quercetin</td>
<td>9.01</td>
<td>11.01</td>
</tr>
<tr>
<td>Total</td>
<td>89.17</td>
<td>77.74</td>
</tr>
</tbody>
</table>

Table 6: HPLC of Phenolic compounds of OO2 at 60°C, 80°C

d) Flavonoids content

Total flavonoid content for all the two types of local Egyptian olive oils OO1 & OO2 was evaluated and had been represented in (Table 7 & Table 8). Results revealed that, OO1 possessed the highest flavonoid content when it was heated at 60 °C for 15 min. The most important flavonoid is Apigenin (22.35) compared to other version OO2. The total flavonoid content decreased in the following order for all the other variants: OO2 at (80 °C) < OO2 at (60 °C) < OO2 at (60 °C).

Table 7: HPLC of Flavonoid compounds of OO1 at 60°C, 80°C

<table>
<thead>
<tr>
<th>Compounds</th>
<th>60°C</th>
<th>80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min.</td>
<td>10 min.</td>
</tr>
<tr>
<td>Myrcetin</td>
<td>22.32</td>
<td>21.12</td>
</tr>
<tr>
<td>Apigenin</td>
<td>11.05</td>
<td>18.15</td>
</tr>
<tr>
<td>Oleuropein</td>
<td>20.3</td>
<td>18.32</td>
</tr>
</tbody>
</table>

Table 8: HPLC of Flavonoid compounds of OO2 at 60°C, 80°C

e) Antioxidant Activity

Fast food is one of the most popular culinary methods globally. Organoleptic and sensorial properties of fried food products, such as juicy taste, nice flavor, crispy texture and brownish color are largely desired and relished by consumers. Deterioration of natural antioxidant such as phenolic compounds was observed when virgin olive oil is heated (Evuen et al., 2013). In the present study, the antioxidant activity of original olive oils OO1 & OO2 or the ability of antioxidants to retain antioxidant activity after heat treatment was tested at two different temperatures, 60°C and 80°C. Heating causes changes in the physical and chemical characteristics of the oils leads to the degradation in the oil quality, with the formation of more saturated compounds such as hydro peroxides, monomers and high-molecular-weight.
Effect of Low Temperatures on Phenolic Compounds, Flavonoid Content and Antioxidant Activity of Egyptian Oils

Compounds along with less proportion of unsaturated fats. Lipid peroxidation may be initially prevented by antioxidants. In this study due to the higher content of flavonoids which, dose not affected by low temperatures especially, when were heated at 60 °C. After 15 min. of heating the antioxidant activity of OO1 was shown to possess greater antioxidant capacity (93.56) at concentration 1.2 mg/mL with IC50 (0.272±0.001) as compared with its version when was heated at 80 °C (81.64, IC50 0.451±0.006) (Table 9). Also, the highest antioxidant activity due to the presence of Apigenin which, represented as more potent cytotoxic activity against most of tumor cell lines because of their highest antioxidant activity and prevent the formation of free radicals (Selim et al., 2019). On the other hand, the antioxidant activity of the second version of olive oil OO2 was less than OO1 as (71.56, IC50 0.967±0.007) and (76.38, IC50 0.451±0.006) at 60 °C and 80 °C after 15 min. respectively (Table 10).

### Table 9: Average of % inhibition of DPPH anti-oxidant assay OO1

<table>
<thead>
<tr>
<th>Conc. (mg/mL)</th>
<th>60 °C</th>
<th>80 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>10 min</td>
</tr>
<tr>
<td>1.2</td>
<td>77.38</td>
<td>87.32</td>
</tr>
<tr>
<td>0.8</td>
<td>69.10</td>
<td>71.28</td>
</tr>
<tr>
<td>0.4</td>
<td>51.07</td>
<td>56.49</td>
</tr>
<tr>
<td>0.2</td>
<td>26.41</td>
<td>35.11</td>
</tr>
<tr>
<td>0.1</td>
<td>15.16</td>
<td>17.27</td>
</tr>
<tr>
<td>IC50 (mg/dl)</td>
<td>0.417</td>
<td>±0.003</td>
</tr>
</tbody>
</table>

### Table 10: Average of % inhibition of DPPH anti-oxidant assay OO2

<table>
<thead>
<tr>
<th>Conc. (mg/mL)</th>
<th>60 °C</th>
<th>80 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>10 min</td>
</tr>
<tr>
<td>1.2</td>
<td>65.34</td>
<td>75.64</td>
</tr>
<tr>
<td>0.8</td>
<td>43.11</td>
<td>63.93</td>
</tr>
<tr>
<td>0.4</td>
<td>28.67</td>
<td>49.33</td>
</tr>
<tr>
<td>0.2</td>
<td>11.29</td>
<td>21.17</td>
</tr>
<tr>
<td>0.1</td>
<td>4.03</td>
<td>10.28</td>
</tr>
<tr>
<td>IC50 (mg/dl)</td>
<td>0.844</td>
<td>±0.009</td>
</tr>
</tbody>
</table>

### IV. Conclusion

In conclusion, olive oil is safe to cook with heating at low temperatures and will not destroy the health benefits or turn olive oil unhealthy. You can feel confident using olive oil in all of your recipes especially when heating at 60°C. The antioxidant activity of both two versions of olive oils OO1 & OO2 were increased due to higher contents of phenolic and flavonoids compounds.

### References Références Referencias


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ASSOCIATE OF SCIENCE FRONTIER RESEARCH COUNCIL is the membership of Global Journals awarded to individuals that the Open Association of Research Society judges to have made a substantial contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Associate membership can later be promoted to Fellow Membership. Associates are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Associate Members.
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2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

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Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

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Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.
Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27” x 11””, left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word “Abstract” in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper’s subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.
It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

**Title**

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

**Author details**

The full postal address of any related author(s) must be specified.

**Abstract**

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

**Keywords**

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

**Numerical Methods**

Numerical methods used should be transparent and, where appropriate, supported by references.

**Abbreviations**

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

**Formulas and equations**

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

**Tables, Figures, and Figure Legends**

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.
Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Electronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can’t clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.
6. **Bookmarks are useful**: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. **Revise what you wrote**: When you write anything, always read it, summarize it, and then finalize it.

8. **Make every effort**: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. **Produce good diagrams of your own**: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. **Use proper verb tense**: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. **Pick a good study spot**: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. **Know what you know**: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. **Use good grammar**: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. **Arrangement of information**: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. **Never start at the last minute**: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. **Multitasking in research is not good**: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. **Never copy others’ work**: Never copy others’ work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. **Go to seminars**: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. **Refresh your mind after intervals**: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.
20. **Think technically:** Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. **Adding unnecessary information:** Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn’t be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. **Report concluded results:** Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. **Upon conclusion:** Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

**Informal Guidelines of Research Paper Writing**

**Key points to remember:**

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

**Final points:**

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

**The introduction:** This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

**The discussion section:**

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

**General style:**

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

**To make a paper clear:** Adhere to recommended page limits.
Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract:

This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.
The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

**Approach:**

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

**Procedures (methods and materials):**

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

**Materials:**

*Materials may be reported in part of a section or else they may be recognized along with your measures.*

**Methods:**

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that’s all.

**Approach:**

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

**What to keep away from:**

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.
Results:
The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:
- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:
- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:
As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:
If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:
The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

**Approach:**

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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