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MODELLING OF THE RESPIRATION OF THE TUNISIAN BEE *APIS MELLIFERA INTERMISSA* (BUTTEL REEPEN, 1906) (HYMENOPTERA: APIDAE)

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A B S T R A C T

The modelling of the respiration of the individual Tunisian bee was studied for two variants, the short one P_0Q with two haplotypes A1 and A8 and the medium-sized one P_0QQ with two haplotypes A4 and A9. This work studied the evolution of the oxygen consumption of 250 bees of each haplotype under different temperatures of 0, 10, 15, 20, 25, 30 and 35°C during 10 minutes. This study shows that the triggering of the thermogenesis process depends on temperature (T) and variant. The medium-sized variant P_0QQ consumes more oxygen than the short one in low temperatures of 0°and 10°C. It was proved that for the two variants there was a difference of 5°C in the temperatures for the triggering of thermogenesis. After this activation, the physiological reaction of the individual bee is inversely proportional to temperature. Two mathematical equations have been established for the modelling of the respiration of *Apis mellifera intermissa* in its stand- alone state. The theoretical work confirms the experimental work with percentage of 92 (±5) % according to the temperatures being tested.

Keywords: Tunisian bee, variant, respiration, modelling.

INTRODUCTION

The bee has been used for a long time as a biological model reference for physiological (Ken *et al*., 2012) and behavioural studies (Erdogan *et al*., 2009) in insects. The metabolism at rest of a bee is equal to the basal metabolism of small mammals (Lighton and Lovegrove, 1990). This metabolism has been studied by many researchers including Crailsheim *et al*. (1999) and Stabentheiner *et al*. (2003). The impact of the ecological conditions on the breathing of insects has been the subject of various investigations. (Moffat, 2001; Woods *et al*., 2005). The consumption of oxygen of *Apis mellifera intermissa,* in the isolated state, increases according to the lowering of the exterior temperature (Chouchaine *et al*., 2014). The largest amount of muscle in the body of a bee is found in the thorax (Nachtigall *et al*., 1995). This muscle mass is characterised by the abundance of mitochondria (Suarez *et al*., 1999) which were one of the major sources of oxygen in the cell (Lenaz, 2001). According to Suarez *et al*. (2005), the glycolysis and the catabolism of glycogen are the two main sources of energy of the domestic bee, *Apis mellifera* in the Embden-Meyerhof pathway. The bee is one of the nine species of arthropods that has had the mitochondrial genome totally sequenced (Rustin *et al*., 1994). The size of the inter genetic sequence COI-COII depends on latitude and altitude. In cold regions the average number of sequence Q is higher (Garnery, 1992). The molecular study of COI-COII of the mitochondrial DNA of the Tunisian bee, *Apis mellifera intermissa,* has proved that four haplotypes exist A1, A4, A8 and A9.The mitochondrial area of the haplotypes A1 and A8 is of the type P_0Q belonging to the short variant. However, the haplotypes A4 and A9 are of the type P_0QQ and belonging to the medium-sized variant (Chouchaine *et al*., 2007). Haplotypes A1 and A8 are present in all bioclimatic levels in Tunisia, but A9 and A4 only exist in

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certain humid, sub-humid and semiarid levels with cool winters (Chouchaine, 2010). For an individual Tunisian bee at low temperatures, there is a difference in the amount of oxygen consumed between the two variants (Chouchaine, 2014). At the level of the colony itself, the eco-ethological study of the internal parameters of the Tunisian bee show that the haplotypes A4 and A9 have a tendency to fight against the cold (Chouchaine *et al*., 2015). They lower the temperature of the brood nest. They produce a limited brood, a small and compact cluster, with a long life expectancy and provisions are used sparingly (Chouchaine, 2010). In extreme conditions the egg-laying stops, the temperature at the centre of the cluster of these two haplotypes is 25(±3) °C, the temperature on the periphery is 9°C and the cluster forms around the queen. The outermost layer of the cluster must never have a temperature of less than 9°C. When there is a drop in outside temperatures, the centre of the cluster produces extra heat to re-establish adequate conditions on the periphery. The haplotypes A1 and A8 are very sensitive to low temperatures and cannot get through difficult winters. They have a tendency to increase the thermogenesis mechanism, therefore the mechanism of thermolysis increases to keep the temperature of the periphery of the cluster at a superior level to the triggering threshold of individual thermogenesis (Chouchaine *et al*., 2015).

The modelling of the respiration of the Tunisian bee *Apis mellifera* intermissa in the isolated state aims to consolidate physiologic and eco-ethologic studies realized by Chouchaine *et al*., 2014 and Chouchaine *et al*., 2015. This work enables the establishment of the factors which have an effect on respiration, as well as establishing and showing two mathematical equations relevant to the respiration of the Tunisian bee.

MATERIAL AND METHODS

Biological material: During experiment we used four haplotypes of bee. Our experiments were based on 1000 bees, 250 of each haplotype, coming from 100 colonies. In each colony we took out 25 to 40 worker bees, in order to avoid problems of mortality in the individual bee. Five marked sister bees and five unmarked sister bees, taken at random from the same nest are studied at each temperature. For each colony, bees of different ages were extracted from the framework cover and the flight entrance. Whatever their age, all the worker bees participate in maintaining the temperature of the brood nest (Petz *et al*., 2004).

Operating mode: In the laboratory at ambient temperature, the recuperated bees were placed in an enclosure containing honey. The dehydrogenation of the glyceraldehyde-3 phosphate and the dehydration of the dihydroxy-acetone-phosphate, that exist in honey are the two main sources of heat production for *Apis mellifera* (Bruneau, 2006). After the measurement of each temperature, the bees were placed in an enclosure. The physical contact between the bees triggers the liberation of two pheromones, azelaic acid and pimelic acid. These two pheromones reduce gas exchange and limits energetic exhaustion of the individual bees (Chauvin *et al*., 1985). Calculations of the intensity of their breathing were carried out throughout the day from 8am until 4pm (GMt+1) using a Jeulin micro-respirometer regulated, at a resolution of 2.5µl fitted with an absorber of CO_2 (hydroxide of potassium-KOH). The bees consumption of oxygen for each haplotype was measured at different temperatures of 0, 10, 15, 20, 25, 30 and 35°C. These temperatures were obtained using a bain-marie with a heating agitator, Bioblock Polystat type. With these temperatures, the intensity of the respiration was measured every minute for 10 minutes. The micro vibration of the thoracic muscle is at its maximum during 10 minutes (Oliver, 2008). The ambient temperature of the laboratory was 25°C.

Analysis of the data: During this study we had recourse to three software programs

- **R project:** The calculation of the statistics and the graphic presentation was achieved using the software R project; In fact four tests were set up.
  - The analysis of the variance ANOVA was used to compare the volume of oxygen consumed by the four haplotypes under the different temperatures at p= 5%.
  - The Principal Component Analysis (PCA) is achieved by comparing the global variability of the oxygen consumption of the four haplotypes (space of the individuals) according to time and temperature (space of variables).
  - The Discriminate Function Analysis (DFA) is achieved by comparing oxygen consumption according to the temperature (space of variables), for the four haplotypes (space of the individuals).

- The graphic presentation of 'Scatterplot3d' is used to visualise the differences in the spatial layout of the oxygen consumption of the haplotypes for temperatures of 0°C and 10°C.
- **Software T183Plus:** The processing of the experimental respiratory data according to temperature was carried out using this software. For mathematical reasons, all the experimental values relative to 0°C and 0 min were removed, to be replaced respectively by 1° C and 1 min all the way through this procedure. For the establishment of the mathematical equations relative to the breathing of the individual bee, we have used the approach of approximation of the functions of several variables (Patrick Ciarlet, 2005) and the study of the Gauss function (Patrick Ciarlet *et al.*, 2014). The progression of the oxygen consumption of the four haplotypes depends on three variables: time (t), temperature (T) and the type of haplotype (n). This approach of the functions of several variables (Patrick Ciarlet, 2005) consists of setting one of the variables at each stage of the modelling.
- **Excel software:** Excel was used to plot the curves experimentally and theoretically, of the respiration according to time and temperature, for the four haplotypes.

RESULTS

Physiologic response of the Tunisian bee: Independently of the type of haplotype, the development of the oxygen consumption of the *Apis mellifera intermissa* in its isolated state according to temperature, designs a bell- like curve with a peak of 15°C (fig. 1). According to the ANOVA test, there is a significant difference in the oxygen consumption of the four haplotypes under the different tested temperatures (*dl =999*, *F*=22.98, *p*=2.2e-16). Above 15°C, oxygen consumption is inversely proportional to temperature, but lower than 15°C, it is proportional to temperature. At 0°C, the bee goes into a coma; oxygen consumption becomes very low but does not cancel out (figure 1.).

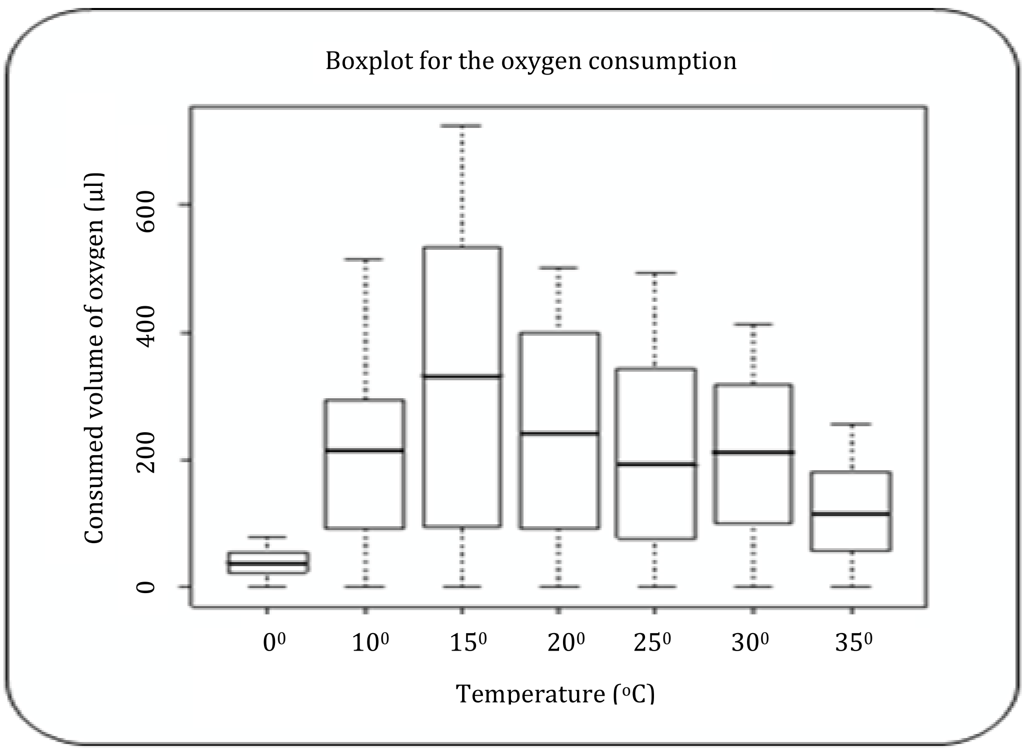

Figure 1. Progress of the oxygen consumption of an individual *Apis mellifera intermissa* according to temperature during 10 min.

The figure 2 illustrates the progression of oxygen consumption of the four haplotypes of the individual *Apis mellifera intermissa* A1, A4, A8 and A9 at different temperatures and in function of time. The oxygen consumption of the Tunisian bee increases progressively with time at different temperatures tested. It reaches its maximum at 15°C for all four haplotypes. In fact at this temperature and beyond it the ANOVA test shows no significant differences.

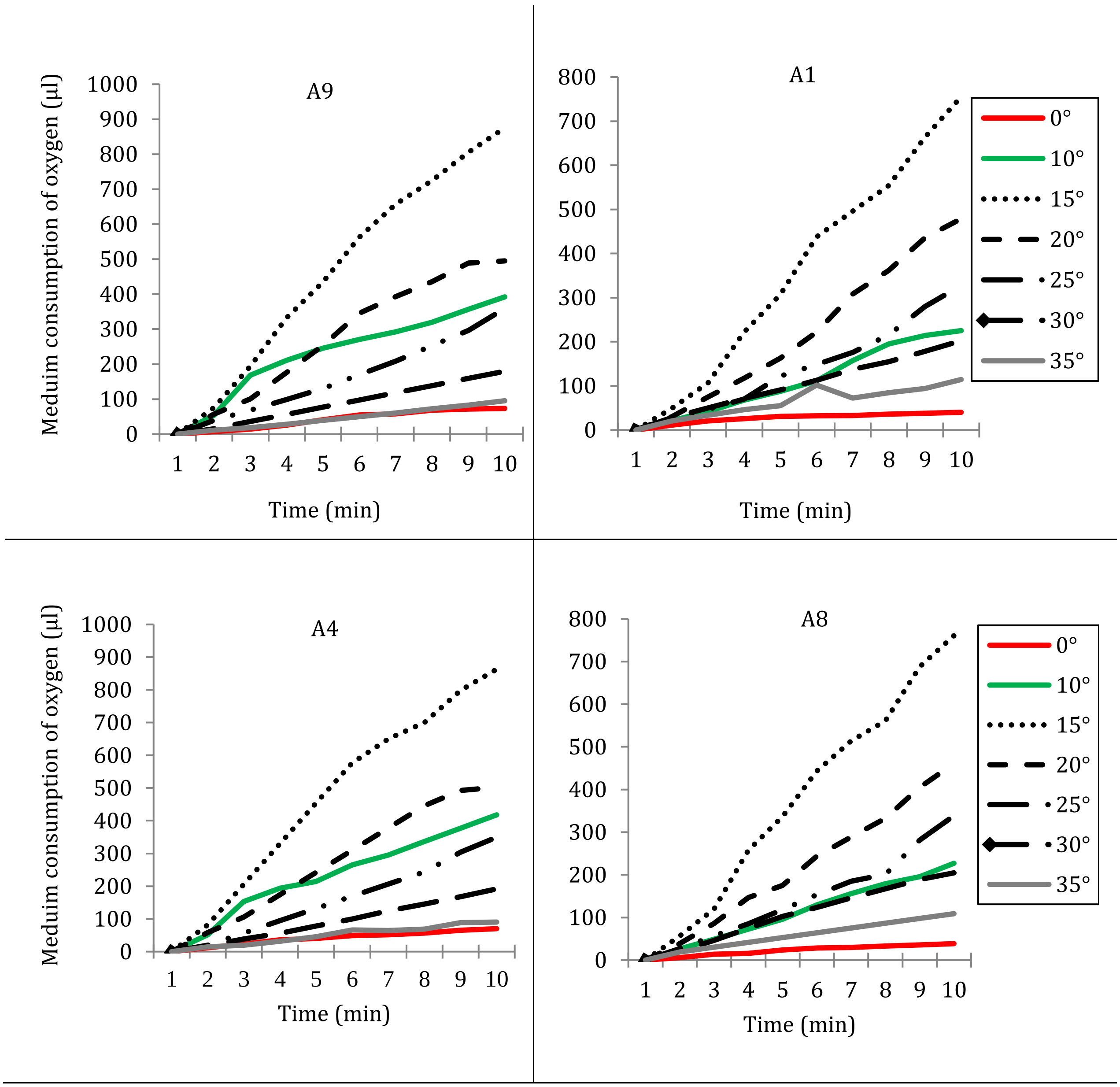

Figure 2. Progress of oxygen consumption of the four haplotypes of the individual *Apis mellifera intermissa* in function of time and temperature.

Genetic response of the Tunisian bee: The figure 3a shows the graphic representation of the principal component analysis (PCA) of the oxygen consumption for the four haplotypes during 10 min of each tested temperature. The first two lines of the conformation of the characteristic values of PCA represent 99.02% of the accumulated variances. The global variability of the respiration according to time and temperature of the four haplotypes is heterogeneous (fig. 3a). This variability is attributed to the genetic difference between the haplotypes after the thermic shock. In fact, figure 3 (a) revealed the existence of two haplotypes groups basing on the thermic responses which are (A1, A8) and (A4, A9). The figure 3b is the graphic representation of the Discriminant Function Analysis (DFA) of the oxygen consumption for the four haplotypes during 10 min of each tested temperature. The graduation of profile-line of DFA is 0, 5. Results relative to the first line of DFA (97.3% of the accumulated variances) show that haplotypes A4 and A9 consume more oxygen for the temperatures of 0, 10, 15 and 20°C than the haplotypes A1 and A8. The DFA globally confirms the variability between the haplotypes mentioned by PCA. This variability explains the existence of a genetic difference due to being exposed to a thermic shock. For the temperatures of 0 and 10°C, Scatterplot3D regroups the spatial development of oxygen consumption of the binary haplotypes. For these two temperatures, the oxygen consumption of haplotypes A4 and A9 is superior to that of A1 and A8. At 0°C, the angle (α) between the tracing (A1/A8) and the tracing (A4/A9) is small (fig 3c). At this temperature, the oxygen consumption of the four haplotypes is low. Statistical analysis showed a significant difference (*dl =999, F*=4.0147, *p*=0.012). At 10°C, the angle (β) is considerable (fig3d). According to the ANOVA test, there is a significant difference between the variants at this temperature (*dl =999, F*=5.0364, *p*=0.004).

Mathematical approach to the breathing of an individual bee

3.1 Relative to time (t): $V_{O2}(t)= a\ \mathbf{t} +b$: Figure 2 shows the process of oxygen consumption of the four individual haplotypes at different temperatures during 10 minutes. For each haplotype, we have established seven matrices each one relative to a temperature. Each matrix is formed of nine lines and a column and the values of this matrix are the volumes of oxygen consumed (V_{O2}).

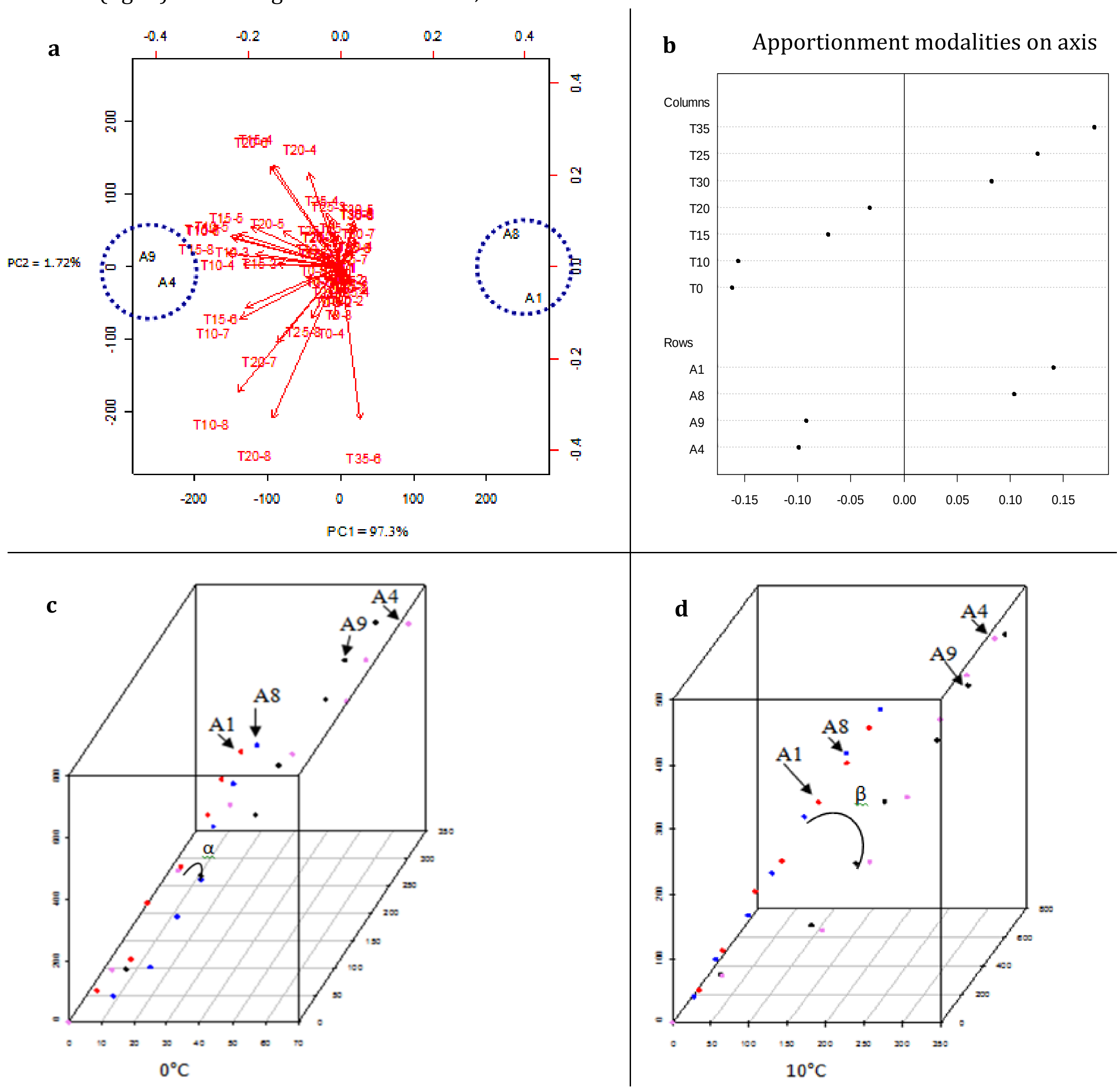

Figure 3. Global variability of the respiration according to time and temperature of the four haplotypes. a, PCA. b, DFA. c, Scatterplot3D at 0°C. d, Scatterplot3D at 10°C.

The data of the matrices relative to each haplotype was worked out from the data base of figure 2. For each haplotype, seven experimental curves were established. The respiration of the individual Tunisian bee follows a linear function in the form of V_{O2} (t)=at+b. Table 1 summarises the two ingredients a and b variable according to time (t) of the seven equations, relative to different temperatures. To do this, the amount of oxygen consumed by the two binary haplotypes is classified in table 1.

Table 1. Components a and b of the equations according to time (t).

A4 et A9	a.	b.	A1 et A8	a.	b.
1°C	6,42	11,43	1°C	2,52	16,34
10°C	40,72	-10,63	10°C	24,04	0,637
15°C	121,54	-44,61	15°C	89,54	-47,5
20°C	73,2	-33,67	20°C	58,5	-48,89
25°C	42,34	-27,74	25°C	35,4	-23,75
30°C	16,56	36,09	30°C	20,56	14,23
35°C	12,87	-14,53	35°C	15,04	27,04

***3.2 Relative to temperature (T)*:** The two components a and b of the formula V_{O2} (t) =a**t**+b already quoted in table 1 are also variable according to temperature (T). The two components a and b are each formed of two sub components, a_1, a_2, b_1 and b_2. The oxygen consumption of the four haplotypes according to the temperature (T) takes the form of a bell (fig. 1). The function V_{O2} (T, t) varies over the two intervals of temperature [1°C, 10°C] and [15°C, 35°C] and for each interval a linear mathematical equation is established.

3.2.1 T ∈[1, 10°C]: Two matrices are formed for the binary haplotypes. The two components a and b from table 1 are arranged according to the columns and the lines relative to the temperatures 1 and 10°C.

$$\mathrm{VO2(T,t)\ A4/A9} = \begin{bmatrix} 6.42 & 11.43 \\ 40.72 & -10.63 \end{bmatrix}$$

$$\mathrm{VO2(T,t)\ A1/A8} = \begin{bmatrix} 2.52 & 16.34 \\ 24.04 & 0.637 \end{bmatrix}$$

The table 2 summarises the two sub components a_1, a_2, b_1 et b_2 of the two matrices at [1°, 10°C]. Following on from this approach, the formula V_{O2} (t) = at+b becomes $V_{O2}t$ (T,t) =(a_1 T+ a_2) t + (b_1 T+ b_2).

Table 2. Sub-components a_1, a_2, b_1 et b_2 the equations according to temperature (T).

Haplotypes	A4 et A9				A1 et A8			
Sub components	a1	a2	b1	b2	a1	a2	b1	b2
[1, 10°C]	3.81	2.6	0.08	11.51	2.39	0.128	1.75	18.08

In this interval, the two empirical formulas of respiration (1) and (2) of the two binary haplotypes are the following:

- *Formula of respiration of the haplotypes A4 and A9*

$$\mathrm{VO2(T,t)} = (3{,}81\ \mathrm{T} + 2{,}6)\ \mathrm{t} - 0{,}08\mathrm{T} + 11{,}51, \text{with T : (°C) and t: (min)} \quad (1)$$

- *Formula of respiration of the haplotypes A1and A8*

$$\mathrm{VO2\ (T,t)} = (2{,}39\mathrm{T} + 0{,}128)\ \mathrm{t} - 1{,}75\mathrm{T} + 18{,}08, \text{with T : (°C) and t: (min)} \quad (2)$$

***3.2.2 T ∈[15°, 35°C]*:** Also in this interval, two matrices are formed for the binary haplotypes. The two components a and b of table 1 are distributed according to the columns and in accordance with the lines relative to the temperatures 15, 20, 25, 30 and 35°C.

$$V_{O2}(\mathrm{T,t})_{\ A4/A9} = \begin{pmatrix} 121.54 & -44.61 \\ 73.2 & -33.67 \\ 2.34 & -27.74 \\ 16.56 & 36.09 \\ 12.87 & -14.53 \end{pmatrix}$$

$$V_{O2}(\mathrm{T,t})_{\ A4/A9} = \begin{pmatrix} 89.54 & -47.5 \\ 58.5 & -48.89 \\ 35.4 & -23.75 \\ 20.56 & 14.23 \\ 15.04 & 27.04 \end{pmatrix}$$

Table 3 sums up the two sub components a_1, a_2, b_1 et b_2 of the two matrices at [15°,35°] tested by the software T183Plus. Following on from this approach, the formula becomes V_{O2} (T, t) = (a_1 e $(^{a_2 T})$ t + (b_1 T+ b_2).

Table 3. Sub components a_1, a_2, b_1 et b_2, of the equations according to temperature (T).

Haplotypes	A4 et A9				A1 et A8			
Sub components	a1	a2	b1	b2	a1	a2	b1	b2
[15, 35°C]	759.9	-0.12	2.6	-81.85	375.76	-0.092	4.24	-121.875

In this interval [15, 35°C], the two formulas of respiration (3) and (4) of the two binary haplotypes are:

- *Formula of respiration for haplotypes A4 and A9*

$$VO2(T,t) = 756{,}5\ e\ (-0{,}12\ T)t + 2{,}6\ T - 81{,}85, \text{with } T : (°C) \text{and } t: (min) \qquad (3)$$

- Formula of respiration for haplotypes A1 and A8

$$VO2(T,t) = 375{,}76\ e\ (-0{,}092\ T)t + 4{,}24T - 1221{,}875, \text{with } T : (°C) \text{and } t: (min) \qquad (4)$$

3.2.3 Overall formula

- ***T* ∈ *[1, 10°C]*:** Based on the statistical analysis of the ANOVA test, there is a difference between the variants (*dl =999*, *F*=12.343, *p*=0.0009) and (*dl =999*, *F*=15.711, *p*=0.0002) respectively for the temperatures of 0 and 10°C. As well as the factor of temperature (T), the overall formula of the respiration of the individual bee depends on the haplotype (n), given the two empirical functions (1) and (2).

$$VO2(T,t) = (3{,}81\ T + 2{,}6)t - 0{,}08T + 11{,}51, \text{with } T : (°C) \text{and } t: (min) \qquad (1)$$

$$VO2\ (T,t) = (2{,}39T + 0{,}128)t - 1{,}75T + 18{,}08, \text{with } T : (°C) \text{and } t: (min) \qquad (2)$$

Taking into account the factor (n) we then proceed to an equation of the following form: V_{O2} (T, t, n) = [g_1(n) T+g_2(n)] t-g_3(n) T+g_4(n), with T : (°c), t : (min) and n type of haplotype. The factor (n) takes two values1 and 2 because there are only two binary haplotypes. That is to say the function g_i (n) is a linear function of the form g_i (n) =a (n) +b.

Calculating a and b for the four functions g_i(n) ; $i \in [|1,4|]$

$$\text{a : Coefficient director : } a = \frac{gi(2) - gi(1)}{1 - 2}$$

$$\text{b : Original order : } b = gi\ (2) - a(1)$$

- Numeric program

$$g1(n) = 1.42n + 0.97 \qquad (5)$$
$$g2(n) = 2.472n - 2.344 \qquad (6)$$
$$g3(n) = -1.67n + 3.42 \qquad (7)$$
$$g4(n) = -6.57n + 24.65 \qquad (8)$$

Meanwhile the general formula of the respiration of the individual bee, *Apis mellifera intermissa* is the total of the functions (5), (6), (7) and (8).

$$VO2\ (T,t,n) = [g1(n)T + g2(n)]t - g3(n)T + g4(n)$$

$$VO2\ (T,t,n) = [(1.42\ n + 0.97)T + (2.472n - 2.344)]t - (-1.67n + 3.42)T + (-6.57n + 24.65) \qquad (9)$$

- ***T* ∈*[15°, 35°C]*:** According to the ANOVA test, in this interval, there is no difference between the variants. So the general formula of the respiration of the individual bee is the average of the two formulas (3) and (4) of the four haplotypes. The general equation of the respiration (10) is the following:

$$VO2\ (T,t) = 375\ t\ (e\ [-0{,}12\ T] + e\ [-0{,}092\ T]/2) + 3{,}42T - 101{,}86 \qquad (10)$$

DISCUSSION

- **T ∈[1, 10°C]:** In this interval, the medium sized variant consumes more oxygen than the short variant, going as far as double the amount. The equation (9) depends on two factors: the temperature (T) and the kind of haplotype (n). The theoretical work confirms the experimental work. The curve of the empirical equation passes through a maximum of points of the experimental representation with a margin of error mathematically insignificant (figure 4.).

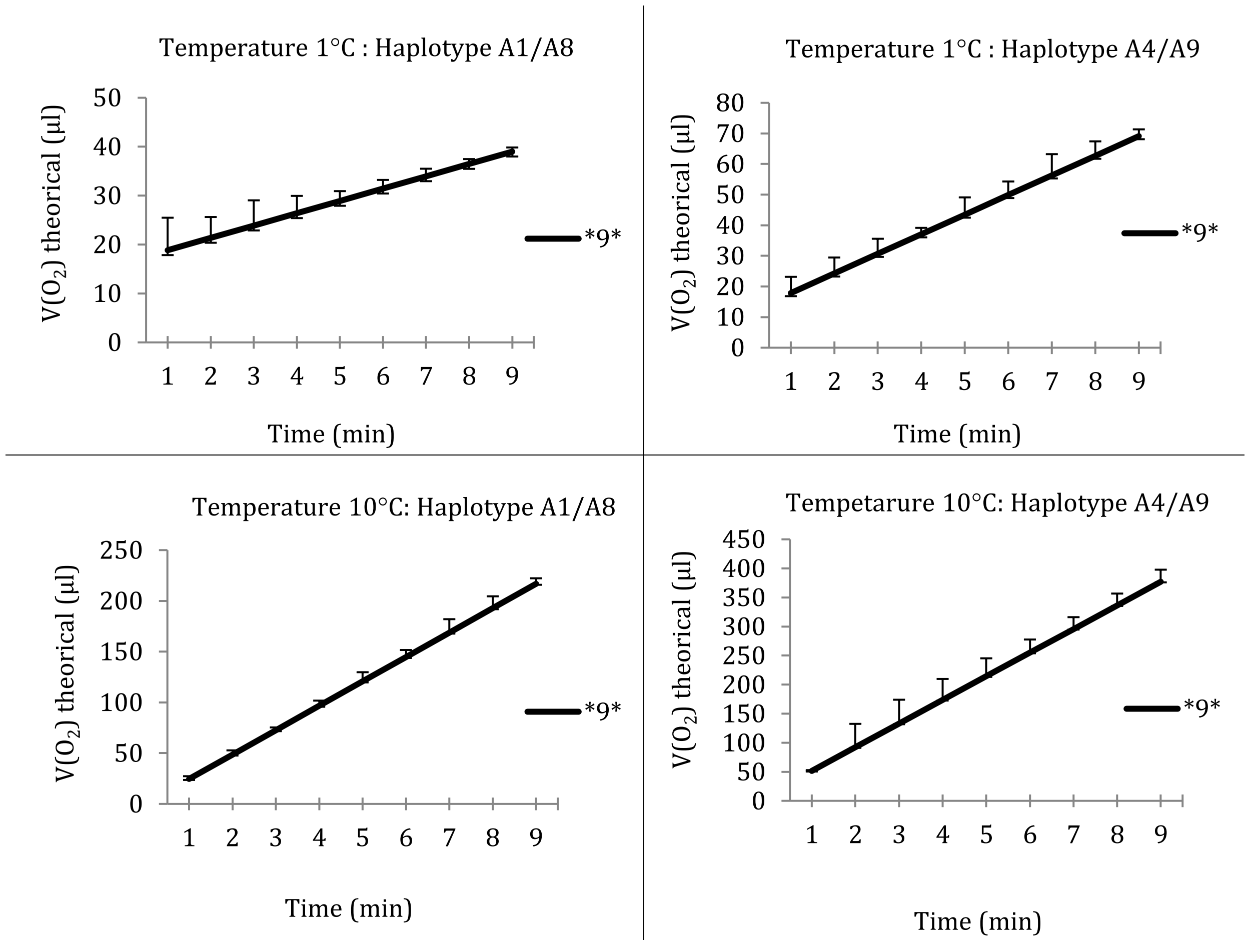

Figure 4. Equation (9). The theoretical evolution of the oxygen consumption of the individual *Apis mellifera intermissa* relative to time at [1°, 10°].

The individual bee is gifted with a real mechanism of thermogenesis to be able to fight against the cold (Rither, 1982). The difference of oxygen consumption in the individual bee is attributed to a biochemical difference (Oliver 2008). The physiologic response of haplotypes A4 and A9 starts at 10°C but does not reach the maximum state. At this moment the process of thermogenesis is triggered only for the medium sized variant (Chouchaine *et al.*, 2014). The temperature (T) has a vital role in the biology of bees (Ken *et al.*, 2012).The temperature at which the thermogenesis is triggered in the individual bee corresponds to the temperature on the outer edge of the cluster (Chouchaine *et al.*, 2015). Whatever the variant of haplotype, the oxygen consumption at 1°C is low. According to Heusner and Stussi. (2005), this weakness is explained by the inhibition of the thermogenic mechanism due to the cooling of the bee, which leads to the intercellular and intracellular liquid transforming into ice which provokes their partial or total paralysis.

- **T ∈[15°, 35°C]:** At this interval, on a statistics level there is no difference between the variants, including the haplotypes, for oxygen consumption. The equation depends uniquely on temperature (T); the theoretic work confirms the experimental work, as the margin of error between the experimental and the theoretical curve is mathematically insignificant (figure 5.). At 15°C, regardless of the type of haplotype, oxygen consumption is maximal. At this temperature, the thermogenic mechanism is triggered in the haplotypes A1 and A8, yet this process in haplotypes A4 and A9 is triggered at 10°C and reaches its maximum at 15°C (Chouchaine *et al.*, 2014). Beyond 15°C, oxygen consumption in the four haplotypes decreases according to the temperature (T). This decrease is explained by the fact that the individual bees adjust their thermic effort according to the intensity of the stimulus. The more one gets close to the thermal preferendum of the hive which is around 35± (2°C) the more the oxygen consumption diminishes (Heusner and Stussi, 2005).

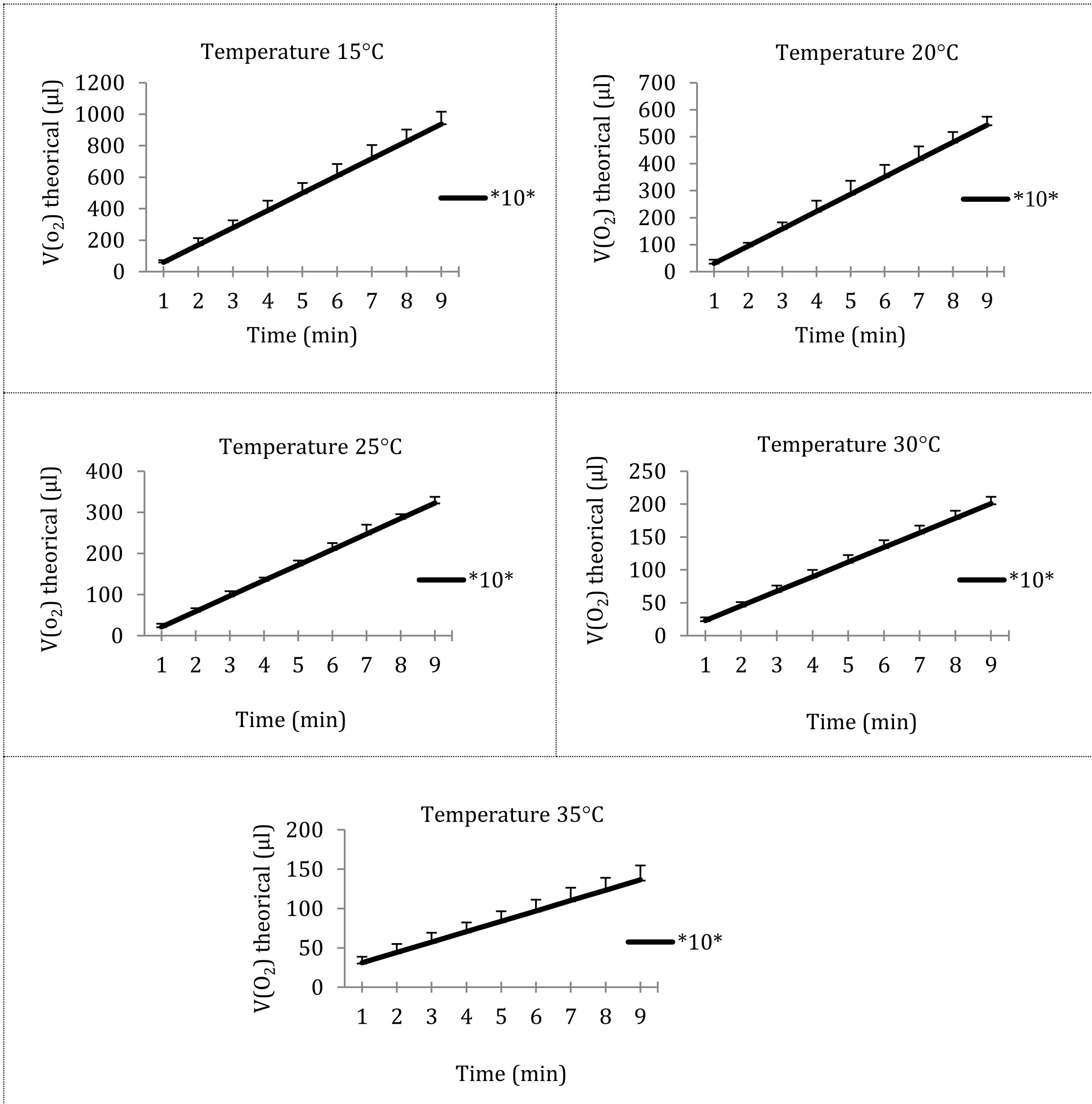

Figure 5. Equation (10).The theoretical evolution of the oxygen consumption of the individual *Apis mellifera intermissa* in function of time at [15°, 35°C].

CONCLUSION

The two mathematical approaches (9) and (10) of the respiration showed that the physiologic response of the Tunisian bee depends on the exterior temperature (T) and the type of variant (n). In the interval [0, 10°C], the medium sized variants P_0QQ: the haplotypes A_4 and A_9 consume more oxygen than the short variants P_0Q: the haplotypes A_1 and A_8. The process of thermogenesis is triggered in the medium size variant but blocked in the short variant. The modelling of the respiration of the individual bee has permitted the explanation of eco-ethological heterogeneousness studied by Chouchaine *et al.* (2015) between the two variants of the Tunisian bee. Our work has shown that the Tunisian bee has two different strategies.

At the level of the colony, the survival of the medium sized variant (P_0QQ) necessitates the protection of the queen. They adapt to the extreme cold by hibernating. The very outside covering of the cluster can never go to less than 9°C. The moment that there is a menacing situation, the centre of the cluster produces extra heat to re-establish the correct conditions on the periphery of the cluster. The temperature at the periphery is equivalent to the temperature at which thermogenesis is triggered in the individual bee.

For the short variant (P_0Q) the survival of the colony depends on the protection of the queen and the brood. P_0Q is much more sensitive to low temperatures, and therefore unable to live through extreme conditions in winter. This variant resists to cold by thermogenesis.

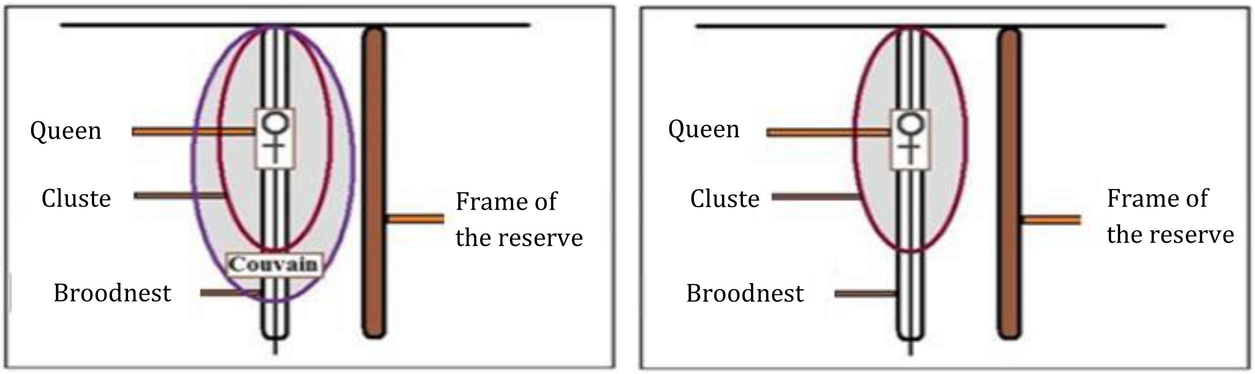

Figure 6. Diagrammatical form of the brood nest of the variants of the Tunisian bee during extreme climate conditions after the model Langstroth. a, short variant (Haplotype A1 and A8); b. medium sized variant (Haplotype A4 and A9).

With their two different strategies, the Tunisian bee presents a perfect adaption to the climatic conditions. The knowledge and understanding of the characteristics of the variants will certainly allow them to be better protected and preserved. The medium sized variant is menaced by extinction, as the apiculturists of the north are destroying its biological clock by inserting the broods of other colonies, thinking of the increase of the population. These apiculturists, because of lack of knowledge are not aware that these haplotypes go into hibernation.

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