Biodemographic and live cycle development comparison of three genetic populations of *Tribolium castaneum* Herbst (Coleoptera, Tenebrionidae).

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ABSTRACT
Tribolium castaneum is a cosmopolitan parasite of dry agricultural products. It is harmful and must be the subject of a rational struggle to protect grains during the storage and preservation.
Objective: The objective of this study is to evaluate cycles of development of the populations genetically differentiated.
Methodology and results: Grains of millet were placed in eight Petri dishes and infested by three (3) couples of *T. castaneum* aged between three to ten days for 24 hours. A follow up of the eggs from the laying up to the emergence of the adults was done in order to determine the duration of the different stages. The results showed that the *T. castaneum* cycle of development differ between those non-differentiated ones. The average life development of the F1 of *T. castaneum* is 28.32±1.70 days in Djilas, 32.30±3.69 days in Karang and 31.07±2.23 days in Diaroume. The average life development of the F2 of *T. castaneum* in Djilas is 29.30±2.71 day's 35.58±5.24 days in Karang and 33.36±4.21 days in Diaroume. Between populations genetically differentiated (Djilas and Karang on the one hand and between Djilas and Diaroume on the other hand) the gap of the cycle of development is significant. On the other, between Karang and Diaroume, two populations genetically no differentiated, the difference (gap) of the cycle of development is not significant. The sex ratio is in favour of females in the three localities and in the two generations.
Conclusion and application of results: The producers should not mix their own grain to those from markets or other localities to avoid increasing the genetic diversity or introduce short-cycle populations in their storage areas. Indeed, populations of *T. castaneum* from Karang and Diaroume are genetically identical and have the same development cycle. The cycle of the population of *T. castaneum* of Djilas (less than 30 days) is shorter than the other two populations of Karang and Diaroume (over 30 days).
Key words: *Tribolium castaneum*, genetic differentiation, cycle of development.

INTRODUCTION

Insects can cause devastating losses by reducing the quality and the quantity of stocked products (Bounechada, 2011). In Sénégal, the specimens encountered in the stocks of fonio, maize and millet belong to eight genus following the order of the Coleoptera beetle (Sitophilus, Prostephanus, Tribolium, Rhyzopertha, Cryptolestes, Trogoderma, Oryzaephilus, Carpophilus) dispatched into six families (Curculionidae,Cucujidae,Bostrichidae, dermestidae, Nitidulidae and Tenebrionidae) (Ndao, 2011). *Tribolium castaneum* is one of the most common devastating tropical beetles of stocked products and main devastator of the cereal (Cotton, 1963 *in* Prabha Kumari, 2011). Because of its adaptability, *T. castaneum* ranks among the ones to be ostracized all the European countries at all costs (Erwin *et al*, 1993). Karunakaran *et al.* (2004) reported that the infestation caused by *T. castaneum* in stock and non-protected wheat reduces almost completely the germination from 9 per cent to 39 per cent after nine months of stocking in Canada and the grains present some damaged nucleus. The grain then emits a foul smelling secretion, which makes the products unsuitable for consumption. The adults and larvae live on the grain and flow. In case of infestation active larvae disappear into the grain and eat there remsted from the light (Mason, 2003). They produce lumpy excrement that is similar to dust. Several parameters may have an effect upon the fertility or about the duration of development for *T. castaneum.* According to Guèye *et al.* (2012), the

cycle of development of *T. castaneum* carry out their larval development in the maize. Different concentrations (1; 0.5 and 0.25) from millet extract have a significant effect in the fertility of *T. castaneum* but do not bring about a difference upon the duration of larval or nymphal development (Ahmed *et al*., 2009). Temperature acts also upon the duration of the cycle of development of the insect. Indeed, according to Kafka (2009), a temperature of 32°C accelerate the duration of the embryonic development (three days) of *T. castaneum*, 25°C slows down the embryonic development (seven days) and for a temperature of 23°C and 18°C, the embryos do not develop. According to the same author, at a temperature of 23°C and 18°C, the hatched larvae do not develop too; the adults survive but need higher temperature during a week in order to produce a normal offspring again. The effect of other parameters such as genetic structuring about the cycle of development of *T. castaneum* has not been shown. After a study about the genetic characterization, it demonstrated a significant genetic differentiation between three populations of *T. castaneum* from Djilas, Karang and Diaroume (Diome *et al*., 2013). The study raises the question as to whether this genetic differentiation has a physiological base. We are trying to determine the different stages of the cycle of development of these three populations so that to compare them.

MATERIAL AND METHODS

Grain of millet as selected samples: To carry out these experiences, grains of millet were taken from the shops of Djilas (14°14'N, 16°38'W) from Karang (13°35'N, 16°42'W) and Diaroume (12°59N, 15°37'W). The choice of these localities can be explained by their demonstrated genetic differentiation. The grains from these localities were selected from some producers' shops. One part was kept in a freezer for 15 days to sterilize them and the other part was put into jars for a breeding ground.

Tribolium castaneum breeding: Couples were chosen among the first adults emerging from the jars in order obtain second generation which corresponds to

F1 of the experimentation, the laboratory temperature ranged from 25 to 31°C and humidity from 41 to 68%.

Experimental study of the cycle of development of *T. castaneum*: The experiment was performed on millet. There were eight tins numbered from one to eight for each population and corresponding eight repetitions. Ten grammes of grains was infested by three couples of *T. castaneum* aged between three days to ten days , to ensure that the laying has really started (females of *T. castaneum* start their laying at the age of three days (Camara, 2009). Three couples of T. castaneum (table 1) infested each tin Petri dish. The

date of infestation, which corresponds, to the first day of the experimentation is recorded.

Statistic analysis: The excel spreadsheet was used to processes the data of the different determined parameters: the duration egg-larvae, egg-pupa, egg-adult and the sex-ratio.

Egg-larvae duration: this is the time between the emission of an egg on a seed and the appearance of larvae from it.

Egg-nymph duration: this is the time between the emission of an egg on a seed and the formation of a nymph from it.

Egg-adult duration: this is the time between an egg on a seed and the emergence of the adult from it.

The sex-ratio: it gives the percentage of females compared to all the descendants. If the sex-ratio is superior to 50% then it is in favour of females in the opposite case it takes advantage to males.

The differences of average between the different populations taken by couple were compared by the smallest significant difference about the threshold of 5% (test Fisher). Fisher starts therefore with the formulation of a hypothesis H0, according to which the statistic (the average for example) of a random sample, draw from a boundless hypothetic population, is equal to a given value (Bourque *et al.*, 2009). Then, we test the difference between the parameter of the distribution of theoretical selection of sample and the statistic observed in the sample. When the compared values differ from more than one gap in advance the hypothesis is rejected (Blais, 1991).

RESULTS

Average duration of embryonic development of the first F1 and of the second generation (F2) of *Tribolium castaneum.* : The average duration of embryonic development of the F1 of *T. castaneum* in the store of Djilas is of 4.86±0.79 days; 4.54±0.66 days in the store of Karang and 5.33±0.89 days in the store of Diaroume. The standard deviations were inferior to 1 for the three localities concerned. This shows that the distribution of the data is stable in each of the localities. This average duration (life) for the F2 of *T. castaneum* in the store of Djilas is of 5.20±0.85 days; 4.26±0.68 days in the store of Karang and 4.46±0.94 days in the store of Diaroume. The standards deviation of the data is stable in each of the populations (table 2). The comparison of the average durations of embryonic development of the F1 of *T. castaneum* from the localities of Djilas and Karang reveal a value of P=0.09>0.05. The difference between the average duration of the embryonic development of the populations of *T. castaneum* stemming from the localities of Djilas and Karang is not significant at the threshold of risk alpha=0.05. Between the *T. castaneum* populations of Diaroume and of Karang, there is a value of P=0.0000115<0.05. The difference between the duration of embryonic development between the localities of Diaroume and of Karang is significant in F1 at the threshold of risk alpha=0.05. The average duration of embryonic development of the population of *T. castaneum* coming from Djilas and Diaroume in F1 reveals a value of P=0.048<0.05, the difference between of the average duration of larvae development of the localities of Djilas and Diaroume is significant in F1 at the threshold of risk alpha=0.05. The comparison between the average duration of embryonic development of the localities of Djilas and Diaroume in F2 reveal a value of P=0.00039, the difference is significant between these two populations in F2 at the threshold of risk alpha=0.05. Between the average durations of embryonic development of the populations of *T. castaneum* stemming from the localities of Karang and Diaroume, we have a value of P=0.000015<0.05. The difference between the duration of embryonic development of *T. castaneum* stemming from stares of Karang and Diaroume, we have a value of P=0.283. The difference between the duration of embryonic development of the populations of *T. castaneum* of Karang and Diaroume is not significant at the threshold of risk alpha=0.05 (table 3).

Table 1: Methodology of infestation of boxes by the species _T. castaneum._

Species	From	Generation	Number of couples	Number of repetitions
T. castaneum	Djilas	F1	3	8
	Karang		3	8
	Diaroumé		3	8
	Djilas	F2	3	8
	Karang		3	8
	Diaroumé		3	8

Table 2: Average Duration of embryonic development of the F1 and F2 of _T. castaneum_ in the three Communities

	Djilas	Karang	Diaroumé
		F1	
Average Duration (in days)	4,86	4,54	5,33
Standard deviation	0,79	0,66	0,89
		F2	
Average Duration (in days)	5,20	4,26	4,46
Standard deviation	0,85	0,68	0,94

Average duration egg-nymph of the two generations F1 and F2 of _T. castaneum_; The average duration of egg-nymph development of _T. castaneum_ in the store of Djilas is 22.58±1.92 days, 27.24±3.41 days in the store of Karang and 26.49±2.43 days in the store of Diaroume. The standard deviations are superior to 1 for the three populations which shows that the distribution of data is unsettled in each of the populations of _T. castaneum_ stemming from stores of these localities. The average duration of egg-nymph development of the F2 of _T. castaneum_ in the store of Djilas is 24.48±2.67 days, 27.48±3.64 days in the store of Karang and 28.47±5.16 days in the store of Diaroume. The standard deviations are superior to one for the three localities; the data's distribution is then unsettled in each of the store of these localities (table 4) The comparison of the average durations of development of the F1 between the populations of _T. castaneum_ stemming from Djilas and Karang from the laying to the appearance of nymphs reveals a probability value $P=5.90 \times 10^{-8}<0.05$. The difference between the average durations egg-nymph of _T. castaneum_ of Djilas and Karand is significant at the threshold of risk alpha=0.05. It has been the same for the average duration of the development of the F1 between the populations of _T. castaneum_ stemming from Djilas and Diaroume from the laying to the appearance of nymphs which reveals a probability value $P=3.48 \times 10^{-6}<0.05$. The difference between the average durations egg-nymph of _T. castaneum_ coming from the stores of the localities of Djilas and Diaroume is significant at the threshold of risk alpha=0.05. In return, the comparison of the average durations of development between the populations of the F1 of _T. castaneum_ from Karang and Diaroume from the laying to the appearance of nymphs reveals a probability value $P=0.294>0.05$. The difference between the average duration egg-nymph of the stores of the localities of Diaroume and Karang is not significant at the threshold of risk alpha=0.05. Between the average durations of development from the laying to the appearance of the nymph of the second generation (F2) of the populations of _T. castaneum_ stemming from Djilas and Karang, we have a probability value of $P=0.000971<0.05$. The difference between the average duration egg-nymph of _T. castaneum_ from the stores of these localities is significant at the threshold of risk of alpha=0.05. The comparison of the average durations of development of the populations of _T. castaneum_ stemming from Djilas and Diaroume from the laying to the appearance of nymph reveals a probability value $P=0.000152<0.05$. The difference between the average durations egg-nymph of the populations of _T. castaneum_ from the stores of the localities such as Djilas and Diaroume is significant at the threshold of risk alpha=0.05. It reveals also a probability value $P=0.346>0.05$ between the average durations of development of the egg until the appearance of the nymphs of the populations of _T. castaneum_ from stores of Karang and Diaroume. The difference between the average durations egg-nymph

of *T. castaneum* between the stores of the localities of Diaroume and Karang is not significant at the threshold

of risk alpha=0.05 (table 5).

Table 3: Comparison of average durations of embryonic development of the F1 and F2 of *T. castaneum*

Shops	Djilas	Karang
	F1	
Diaroume	P=0.048	P=0.000015
Karang	P=0.09	
	F2	
Diaroume	P=0.00039	P=0.283
Karang	P=0.0000115	

Table 4: Average durations egg-pupa of the F1 and F2 of *T. castaneum*

	Djilas	Karang	Diaroume
		F1	
Average Duration (in days)	22.58	27.24	26.49
Standard deviation	1.92	3.41	2.43
	F2		
Average Duration (in days)	24.48	27.47	28.47
Standard deviation	2.67	3.64	5.16

Table 5: Comparison of durations egg-pupa of the F1 and F2 of *T. castaneum*

Shops	Djilas	Karang
	F1	
Diaroume	P=3.48 E-06	P= 0,294
Karang	P=5.90 E-08	
	F2	
Diaroume	P=0.000152	P=0.346
Karang	P=0.000971	

Average durations egg-adult of the F1 and F2 of *T. castaneum*: The average duration of development of *T. castaneum* in the stores of Djilas is 28.32±1.70 days, 32.8±3.69 days in that of Karang and 31.07±2.23 days in the store of Diaroume. The standard deviations are superior to one as for as the three localities are concerned, which shows that the data distribution is unsettled in each store of these three localities. The average duration of development of the F2 of *T. castaneum* in the store of Djilas is 29.30±2.71 days, 35.58±5.24 days in that of Karang and 33.36±4.21 days in the store of Diaroume. The standard deviations are superior to one for these three localities; the data distribution is unsettled in each store in each store of these three localities (table 6). The comparison of the average durations of development of the F1 of *T.*

castaneum from Djilas and Karang shows a probability value $P=1.22 \times 10^{-5} < 0.05$, the difference between the cycles of development of *T. castaneum* stemming from the stores of the localities such as Djilas and Karang at the threshold of risk alpha 0.05. The comparison of the average durations of development of the F1 of the *T. castaneum* populations stemming from Djilas and Diaroume from the laying to the appearance of the adults reveals a probability value $P=1.42 \times 10^{-5} < 0.05$. The difference between the average durations egg-adult of the *T. castaneum* populations stemming from the stores of these two localities is significant at the threshold of risk alpha=0.05. It shows also that between the average durations of development from the laying to the appearance of the adult of *T. castaneum* coming from the stores of the localities of Diaroume and

Karang is not significant P=0.019>0.05 at the threshold alpha=0.05. The comparison of average durations of development of the F2 of *T. castaneum* populations stemming from Djilas and Karang shows a probability value P=5.499 10^{-8}<0.05. The difference between the cycles of development of *T. castaneum* from the stores of the localities of Djilas and Karang is significant at the threshold of risk alpha=0.05. It shows also for the F2 a probability value P=0.00023<0.05 between the *T. castaneum* populations from Djilas and Diaroume which

shows that the difference is significant between these populations at the threshold of risk alpha=0.05. The comparison of the average durations of development of the F2 of *T. castaneum* from the laying to the appearance of adults between Diaroume and Karang reveals a probability value P=0.064>0.05. The difference between the average duration of egg-adult of the stores of localities of Diaroume and Karang is not significant at the threshold of risk alpha=0.05 (table 7).

Table 6: Average durations egg-adult of the F1 of *T. castaneum*.

Shops	Djilas	Karang	Diaroume
F1			
Average Duration (in days)	28.32	32.8	31.07
Standard deviation	1.70	3.69	2.23
F2			
Average Duration (in days)	29.30	35.58	33.36
Standard deviation	2.71	5.24	4.21

Table 7: Comparison of durations of egg-adult of the F1 and F2 of *T. castaneum* populations.

Shops	Djilas	Karang
F1		
Diaroume	P=1.4229 E-05	P=0.019
Karang	P=1.221 E-05	
F2		
Diaroume	P=0.00023	P=0.346
Karang	P=5.499 E-8	

Differentiation between the three populations of *T. castaneum* of Djilas, Karang and Diaroume: The strongest values of the Fst have been obtained between the populations of *T. castaneum* of Djilas and

Diaroume (0.57143). These values are also significant. On the other hand, between Karang and Diaroume the genetic differentiation is negative and not significant (table 8).

Table 8: Genetic differentiation between the three populations of *T. castaneum*

	Djilas	Karang
Karang	0.46296 *	
Diaroume	0.57143 *	-0, 07280

The sex ratio of the F1 of *T. castaneum* according to the differentiated populations: The sex ratio of the F1 is in favour of females in the three populations. The females represent 63.15% of the emerged adults from the population of *T. castaneum* of Djilas, 64.28% of the adults of Karang and 56% of that of the adults of

Diaroume. The number of females emerged is superior to the number of males regarding the three populations of the F2 as well. The females represent 59.27% of the adults emerged from the population of *T. castaneum* of Djilas, 66.66% of the adults from that of Karang and 59.42% of the adult from that of Diaroume. The number

of emerged females is superior to the number of males in the three populations (table 9).

The mortality rate regarding the two generations F1 and F2 : It results that the larval mortality of the F1 is more important in the population of *T. castaneum* coming from Diaroume (28.57%) it is weak in the population of Karang (8.51%) and Djilas (9.52%). On the other hand, regarding the F2, the larval mortality is weak in the population of *T. castaneum* of Diaroume (5%) just as in the populations of Djilas 10% and Karang (12%). The nymph mortality is non-existent in Djilas and in Diaroume in the two generations. On the other hand, for Karang, we have a death rate of 2.3% in the F1 and 12% in the F2 (table 10).

Table 9: Emergence of adults and sex-ratio (R) of two generations F1 and F2 of *T. castaneum* in all three populations.

Populations	Number adult	Male	Female	% Female	Sex-ratio
		F1			
Djilas	19	7	12	63.15	0.58
Karang	42	14	27	64.28	0.51
Diaroume	25	11	14	56	0.78
		F2			
Djilas	27	10	16	59.27	0.62
Karang	24	8	16	66.66	0.5
Diaroume	69	28	41	59.42	0.68

Table 10: Percentage of larval mortality and indicating

Populations	N. larvae	N. nymphs	M. larvae	N. adults	M. Nymphs
			F1		
Djilas	21	19	9.52 %	19	0
Karang	47	43	8.51 %	42	2.3 %
Diaroume	35	25	28.57 %	25	0
			F2		
Djilas	30	27	10%	27	0
Karang	32	28	12.5 %	24	12%
Diaroume	73	69	5%	69	0

N.: Number, M.: Mortality

DISCUSSION

This study has demonstrated that the populations of *T. castaneum* from Djilas, Karang and Diaroume are different genetically. The bio-ecology results have shown that both the first generation F1 and the second generation F2, have a significant difference for the durations of embryonic development between the populations of *T. castaneum* from Djilas (4.86 days) and Diaroume (5.33 days) are concerned. According to Bonneton (2010), the embryogenesis of *T. castaneum* last three days at 30°C. This duration is inferior to what this study obtained in the populations from the localities of Djilas and Diaroume. Indeed, the temperature was not steady during the testing, which might accentuate the difference mentioned. However, we can think that the duration of embryonic development can differ significantly between the populations of *T. castaneum* genetically differentiated. Diome *et al.* (2013) have shown that the genetic differentiation is more accentuated between the populations of *T. castaneum* from Djilas and Diaroume. This differentiation could have then an impact on the duration of embryonic development of the insect. For the average duration egg-nymph, the study had a significant difference between the populations from Djilas and Karang on the one hand and between Djilas and Diaroume on the other hand, for the two generations F1 and F2 between Karang and Diaroume the difference is not significant. Indeed the genetic differentiation is not significant

between the populations of *T. castaneum* of these last two localities. We can therefore think that the more there is a genetic differentiation, the more there is a difference in the duration of the cycle of development. The results obtained with the duration egg-nymph are confirmed by the results obtained on the cycle of development of these different populations. The cycle of development of the *T. castaneum* populations of Djilas is shorter and does not exceed thirty days for each of the two generations (F1 and F2). This shows that we can have several generations in this population during the years if the conditions are comfortable in a crucial period of the year. We can meet five generations in a year (Bennett, 2003). According to the Canadian commission of the grains (CCG), the red *Tribolium* of the flow is among the insects, which infest the stored foodstuff, one of those, which the populations increase the most rapidly. The duration of the cycle of development has slightly increased within each locality from one generation to another, which could be due to a variation of certain climatic parameters during the testing like the temperature and the humidity relative. The temperature and the relative humidity of the air, the type of food (Guèye *et al.*, 2012) the rate of breakage (Seck *et al.*, 1992) have an impact on the duration of the cycle of development of *T. castaneum*. The development of *T. castaneum* stops in below of 22°C but it resists very well to the low of hygrometry's (Camara, 2009). The cycle of development is longer in the population of Diaroume 32.8±3.69 days in F1 and 35.58±5.24 days in F2. According to ISS Suisse, while all the factors are almost optimal, the duration of a generation can be from 27 to 35 days. The cycle in F2 of the *T. castaneum* population of Diaroume is near at hand to that found by Guèye *et al.* (2012) on the *T. castaneum* populations erected upon maize (36.3±1.42 days). The shortest cycle is noted in Djilas and its duration is close to that found by Guèye *et al.* (2012) and distant to that

found by Delobel and Tran (1993) who have found an average duration of development from egg to adult on the millet of 26 days at 28% for an HR of 70%. This shows that the cycle of development of *T. castaneum* can differ between subservient in a same cereal and that the genetic differentiation would play a very important role. In the three populations, the average duration of the cycle of development does not exceed 36 days as well for the first and for the second generation. Some are comparable at the average duration 35 days obtained by Seck *et al.* (1992) on the *T. castaneum* populations erected in the grains of millet at 0% of breakage. This could be explained by the capacity of the insect to be able to attack the intact grains. Indeed, it has been for a long time ranked among the secondary devastators but the Canadian commission of the grain (CCG) has ranked it now among the primary devastators. Between genetically non-differentiated populations (like that of Karang and Diaroume), it was noted that a significant difference in their development duration from the bud until the emergence of adults. The non-differentiated populations are located in two different agro-ecological areas, which show that the populations located in different agro-ecological areas can present a genetically homogeneous population genetically (Diome *et al.*, 2012) and the same cycle of development. However, the differentiated populations would present a different duration of the cycle of development. The study noted that, the number of females is higher comparing to that of males in the 3 localities and in the two generations (percentage of females superior to 50 per cent). It can be concluded that the females prevail and tend to have a paramount role in the infestation of stocked grains in the different stores of the localities; this could accentuate the infestation in the stocking spots. The larval death rate of *T. castaneum* being equal to 8.51 percent in Karang, 9.52 percent in Djilas and 28.57 percent in Diaroume.

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