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RESEARCH ARTICLE

EFFECT OF CROSSING BETWEEN TWO GENETICALLY DISTANT STRAINS OF CALLOSOBRUCHUS MACULATUS ON THEIR BIODEMOGRAPHIC PARAMETERS

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Abstract

The effect of gene flow on fecundity was negligible: 57 eggs per female, with non-significant differences.However, over 90% of eggs are laid during the first four days of female life, with a peak on the second day. Egg fertility was increased by cross-breeding (reaching 60%). Larval survival rates are relatively unchanged (around 78%), while emergence rates (around 65%) are higher than controls. The sex ratio is in favor of females following crossbreeding. The total duration of the development cycle was not significantly affected for Fouta seeds, at around 30 days. For Barkedji seeds, on the other hand, there was a significant reduction in total cycle length between the controls (batch 3) and crossbreeding batch 1. Ultimately, this study has enabled us to understand, through crossbree ding tests, the evolutionary processes of biodemographic parameters. The latter are influenced by strain genetics, geographical origin and substrate type.

Introduction:-

Global cowpea production amounts to more than 5.7 million tonnes of dry seeds per year, on 7.5 million ha in 2008, 70% of which is produced in Africa (Tengo, 2011). Thus, to achieve food security, it is necessary to increase yields by limiting, for example, losses due to plant enemies, particularly insect pests and especially storage pests.

Callosobruchus maculatus is a cosmopolitan pest of cowpea stocks. It is found in all agro-ecological zones of Senegal, as in many other countries. Its genetic characteristics may differ from one zone to another. From a general point of view, knowledge of genetic diversity and structure is of paramount importance for the species we wish to study. Given that this genetic structure is shaped by multiple demographic events and evolutionary processes, we can postulate that intra and interpopulation gene flow makes a major contribution. According to Pritchard et al., 2000a and Flint-Garcia et al., 2005, genetic structure is particularly important for linkage disequilibrium studies and, by extension, association genetics.

In bruchid populations, genes can be exchanged through migratory flows resulting from the commercialization of cowpea seeds (trade), or through bartering in the search for new seeds or varieties. These gene exchanges tend to reduce the isolation of populations and create new groups with close phylogenetic relationships. They may also show strong genetic differentiation, perhaps linked to geographical distance or substrate (Faye et al., 2023).

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Genetic studies on the characterization of C. maculatus populations according to agro-ecological zones in Senegal have shown that populations from the Sylvopastoral Zone (SPZ) and those from the Senegal river Valley Zone (SRVZ) are the most genetically distant (Faye et al., 2023). So we asked ourselves whether genetic characteristics have any effect on the physiology of this insect?

This is why, in this article, we have determined the biodemographic parameters of populations resulting from crossbreeding. To do this, we carried out cross-breeding tests between populations from the agro-ecological zones of Senegal that are the most distant genetically, namely the Fouta and Barkedji populations, to determine these parameters. The new generation resulting from the crosses (F1) enables us to:

- Determine biodemographic parameters such as total cycle length, adult lifespan or adult longevity, oviposition-emergence time, adult emergence rate, sex ratio, larval survival rate, egg fertility rate and average number of eggs laid per female.

- Compare the results of the crossover tests with those obtained from controls (Fouta and Barkedji populations) to see the effect of gene flow on the biodemography of the cowpea bruchid, C. maculatus.

Material and Methods:

I. 1 Cowpea seed sampling

To carry out the experiments, cowpea seeds were collected from the localities of Barkedji and Fouta, belonging to the Sylvopastoral and Senegal River Valley agro-ecological zones respectively. The choice of these localities was based on the fact that their populations are the most genetically distant from each other, if we consider all of Senegal's agro-ecological zones (Faye et al., 2023). Seeds from these localities were taken from growers. The uninfested part was kept in the freezer for testing, and the infested part was put in aerated jars for mass rearing.

I. 2. Mass rearing of Callosobruchus maculatus strains

Cowpea samples from Barkedji and Fouta were kept in the laboratory in aerated jars until adults emerged. Mass rearing aims to maintain and multiply strains in order to have a sufficient number of individuals to pair or cross. Each strain was reared on cowpea from the same locality in well-ventilated glass jars with a volume of approximately 1 liter under ambient temperature and humidity conditions. Continuous monitoring of the rearing was carried out until a sufficiently large number of insects emerged.

I. 3. Insect identification by sex

Females of C. maculatus are characterized by a shorter, dark red or black prothorax with vague patterns of pale pubescence and convex sides of the protonum. Females are larger than males. They have a median line of white bristles. Males, on the other hand, have longer, more toothed antennae and often larger eyes.

The most distinctive feature is the coloration of the plate covering the pygidium (last abdominal segment). In females, the plate is enlarged and dark on both sides. Males have a reduced pygidium without any coloration (Beck and Blumer, 2014).

I. 4 Experimental protocol

I. 4. 1. Choice of individuals

Mass rearing enabled us to obtain a large number of insects. At the last emergence considered, the contents of the boxes were sieved to eliminate all adults that had emerged. Six (06) hours after sieving, the newly emerged insects were isolated. This procedure enabled us to obtain adults less than 12 hours old. These adults were used for the experiment. Those emerging from the jars and less than 12 hours old were isolated individually and immediately identified according to sex and locality. Once identified, these insects were used in the tests. This avoided mating between conspecifics.

I. 4. 2. Experimental conditions

Experiments were carried out under natural climatic conditions at the Laboratory of Entomology and Acarology, Faculty of Science and Technology atCheikh Anta Diop University, between August 15 and October 15. During this period, average temperatures ranged from 29 to 36°C and relative humidity from 70 to 75%. Sterilized cowpea seeds from the localities of Barkedji and Fouta were used as test substrates.Before use, the sterilized seeds were returned to room temperature for at least 24 hours to warm up.

I. 4. 3. Crossover tests

Two crossover tests and two control tests were carried out. Each crossover test comprises two batches, while the control tests are made up of a single batch per test. This means that we have a total of six (06) batches defined as follows with their code names:

- ✓ Batch 1: Barkedji seeds; Barkedji males X Fouta females (BSBMFFe).
- ✓ Batch 2: Barkedji seeds; Fouta males X Barkedji females (BSFMBFe).
- ✓ Batch 3: Barkedji seeds; Barkedji males X Barkedji females (BSBMBFe), Barkedji control batch.
- ✓ Batch 4: Fouta seeds; Fouta males X Barkedji females (FSFMBFe).
- ✓ Batch 5: Fouta seeds; Barkedji males X Fouta females (FSBMFFe).
- ✓ Batch 6: Fouta seeds; Fouta males X Fouta females (FSFMFFe), Fouta control batch.

B = Barkedji; Fe = Female; F = Fouta; S = Seed; M = Male.

In each batch, 10 pairs were used. Each pair was placed in a box containing 25 sterilized cowpea seeds. From these fertilized females come the different populations whose biodemographic parameters are studied here. This gives us a total of six populations (each batch constituting one population). These populations are of the first generation.

The cans used are made of plastic, with a volume of around 85 ml and an average diameter of 05 cm. The lids of these cans, which were hermetically sealed, were finely perforated for aeration.

I. 4. 4. Determination of biodemographic parameters of C. maculatus

Determination of the biodemographic parameters studied here began on the first day of infestation. Every day for 08 to 09 days, seeds infested by females were removed and replaced by new sterile seeds. Those removed were placed in another box bearing the same label or code name, and the eggs laid were counted using a magnifying glass. This operation revealed the daily egg-laying rate of each female. Once the eggs had been counted, the boxes that had already been properly referenced were put away on the bench.

Between the seventh (D7) and tenth (D10) days from the start of the experiments, sterile or infertile eggs and fertile eggs were counted using a magnifying glass or even the naked eye. Sterile eggs can be distinguished from fertile eggs by their translucent appearance on the seed. The boxes were then kept on the laboratory bench at room temperature until the adults emerged.

On first emergence, the date was noted and adult monitoring began. From this date onwards, and for each box, emerged adults were removed, identified according to sex. These adults were then counted. This monitoring of emergence lasted around two weeks. This procedure enabled us to determine the number of males and females, and consequently the total number of adults emerging from each box. Among the adults, a batch of 10 males and a batch of 10 females were formed, with up to 10 replicates per pair of the same type. A total of 100 males and 100 females were obtained from each initial test batch. Once the batches of 10 had been formed, the insects were tracked, and each day the number of deaths was counted down to the last. This enabled us to calculate the longevity of males and females.

The following parameters were determined as a result of this work:

- ✓ The average number of eggs laid per female (N) corresponds to the total number of eggs a female can lay during her lifetime. This number is obtained by summing all the eggs laid by all the females in a batch, for example (a total of ten females per batch) over the number of females, i.e. over 10.

$$N = \frac{\text{Numberofeggsfromallfemales}}{\text{Numberoffemales}}$$

- ✓ Fertility rate (FR): the percentage of fertile eggs in relation to the total number of eggs laid. It's the ratio between the number of fertile eggs multiplied by one hundred and the total number of eggs laid.

$$TF\ (\%) = \frac{\text{Numberoffertileeggsx100}}{\text{Totalnumberofeggslaid}}$$

- ✓ Larval survival rate (LSR): the percentage of emerged individuals in relation to the total number of fertile eggs.

$$LSR\ (\%) = \frac{\text{Numberofemergedindividualsx100}}{\text{Totalnumberoffertileeggs}}$$

- ✓ Emergence rate (TE): the percentage of individuals that have emerged in relation to the total number of eggs laid;

$$TE\ (\%) = \frac{\text{Numberofindividualsthathaveemergedx100}}{\text{Totalnumberofeggslaid}}$$

- ✓ The sex ratio (R) corresponds to the numerical ratio between males and females in the offspring.

$$R = \frac{\text{Numberofmales}}{\text{Numberoffemales}}$$

- ✓ Development time or oviposition-emergence time: this is the time between the emission of an egg on a seed and the emergence of the adult;
- ✓ Adult life span or adult longevity: the time interval between insect emergence and death;
- ✓ Total cycle length: this is the time interval between the emission of an egg and the death of the adult that emerges from it. It includes the development time and longevity of the adults.

I. 5. Statistical analysis

The Excel spreadsheet was used to list all the results obtained in a workbook and to calculate the value of each parameter described.Statistical analyses were carried out using R software version 4.3.1 at the 5% threshold (alpha = 0.05) with the Shapiro, Kruskal-Wallis and ANOVA tests. The Shapiro wilk test is a normality test with two hypotheses (H0 and H1):If the variable follows the normal distribution (H0), we performed the ANOVA test to determine the differences.On the other hand, if the variable does not follow the normal distribution (H1), we performed the Kruskal-Wallis test.

Results:

II.1 Study of egg-laying activity

II. 1. 1 Variation in the number of eggs laid per female C. maculatus

Figure 1 shows the average number of eggs laid per female according to batches or tests. Using Barkedji seeds as an oviposition substrate, we found that the average number of eggs laid per female is higher for batch 3 (BSBMBFe) made up of Barkedji control pairs (55.60 ± 19.144 eggs) and lower (45.40 ± 10.906 eggs on average per female) for the reverse pairs forming batch 2 (BSFMBFe).With regard to the egg-laying substrate made up of Fouta seeds, batch 6, formed by the Fouta control pairs, together with batch 5 (made up of the inverse pairs to batch 4), had the lowest average number of eggs per female (45 on average). Those in batch 6 have 45 ± 18.577 eggs and those in batch 5 have 45.10 ± 23.715 eggs on average per female. Batch 4 (FSFMBFe; pairs from the first cross) has the highest average number of eggs (57.50 ± 21.141 eggs per female on average).

The Kruskel Wallis test gave a p-value = 0.164, so the differences are not significant.

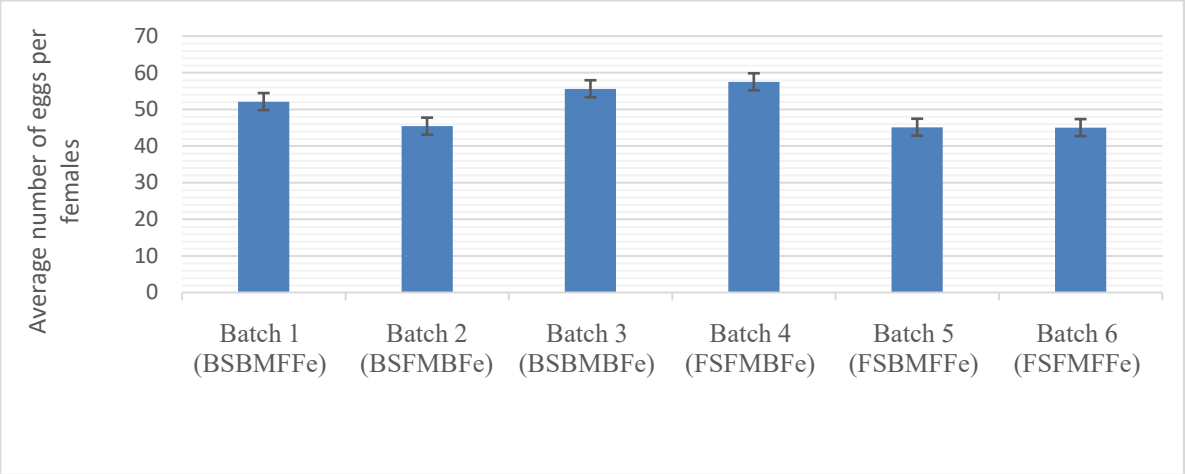

Figure 1: Average number of eggs laid per female by batch

II. 1. 2. Evolution of egg-laying activity

Egg-laying activity began on the first day of the females' lives, with over 20% of eggs laid. Daily egg laying peaked on day 2 for batches 1 and 5, which are crossbred batches, and for batches 3 and 6, which are control batches (40.307% for batch 1; a Barkedji crossbred batch, 33.453% for batch 3; a Barkedji control batch, 32.815% for batch 5; a Fouta crossbred batch and finally 38% for batch 6; which is the Fouta control batch...). However, for females in batch 2 (reverse crossbreed batch to batch 1) and batch 4 (reverse crossbreed batch to batch 5), egg-laying peaks on day 1 at 33.295% and 34.260% respectively.) Egg-laying activity declines as the age of the females increases. This decline is considerable after the peak, and becomes very low or non-existent after four days of female survival. The results obtained are shown in Figure 2.

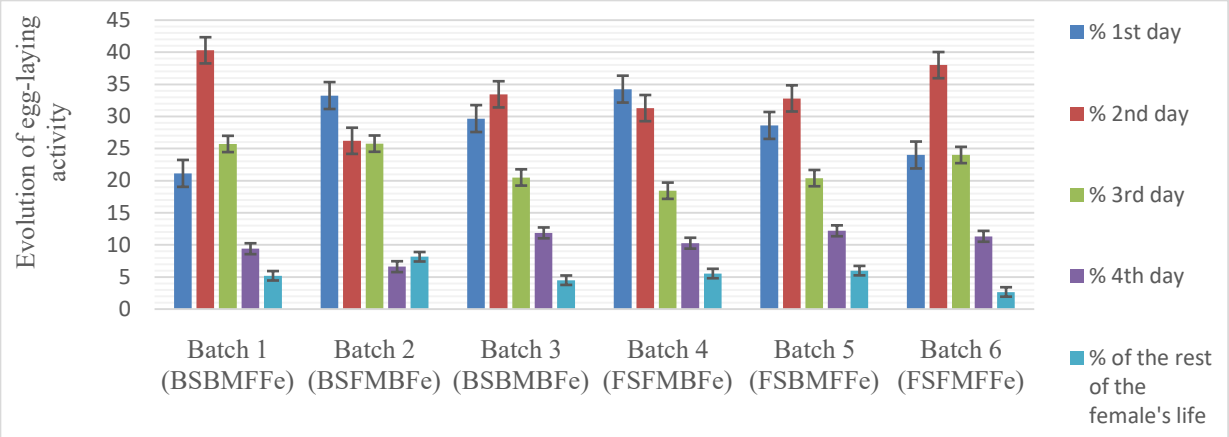

Figure 2: Evolution of egg-laying activity of female *Callosobruchus maculatus*

II. 2. Study of embryonic development

II. 2. 1. Egg fertility

The egg fertility rate seems to vary more with the type of pair than with the laying substrate. We found that whatever the type of pair considered, the fertility rate was over 60% (**Figure 3**). It was lowest with the control pairs from Barkedji (62.589%) and highest with the pairs from batch 5 (FSBMFFe), with 76.940% fertile eggs. Control pairs from Barkedji (batch 3: BSBMBFe) had a lower fertility rate than controls from Fouta (batch 6: FSFMFFe), with 72.666%. Depending on the food substrate, we found that for Barkedji seeds, the controls (batch 3) had the lowest fertility rate (62.589%) compared with the other Barkedji cross batches. On the other hand, for seeds from Fouta, pairs from lot 4 (FSFMBFe) had the lowest rate (70.260%), while those from lot 5 had the highest (76.940%). However, these differences are not significant based on the statistical tests performed.

The Shapiro Wilk test, with a non-significant p-value of 0.1238, allowed us to perform the ANOVA test which also gave a non-significant p-value (p-value = 0.148).

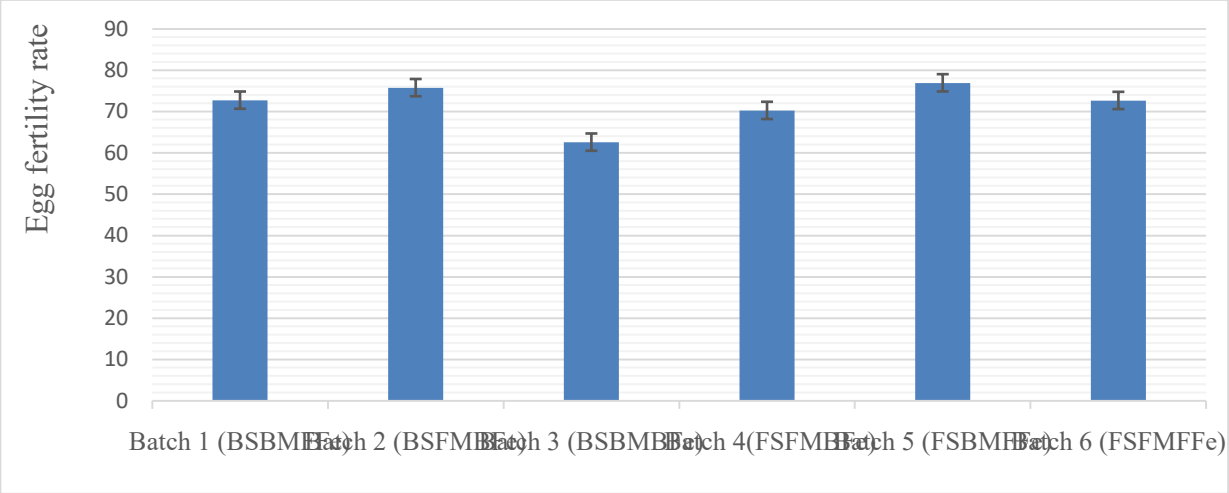

Figure 3: Fertility rate of C. maculatus eggs by batch

II. 2. 2. Larval survival

The results recorded in **Figure 4** show that, whatever the nature of the pairs or the batch considered, we have over 75% larval survival rates. However, the larval survival rates observed seem to depend on the type of food substrate used and sometimes on the nature of the pairs or batch considered.

Depending on the nature of the couples or the batches, we found that the average larval survival rate is 78% for the Barkédji batches while for the Fouta batches this average rate reaches 80%. Furthermore, we also found that the highest larval survival rate (84.403%) was obtained with the control couples of Fouta (batch 6). The larval survival

rate is much higher at the level of the controls and lower at the level of the couples of the first cross (couples of batch 1 with a rate of 78.627% and batch 4 with a rate of 75.495%). The couples of the reverse crosses (batch 2 and batch 5), have larval survival rates which are respectively 72.651% and 81.556%).The Kruskal-Wallis test shows no significant difference between these rates (p-value equal to 0.5428).

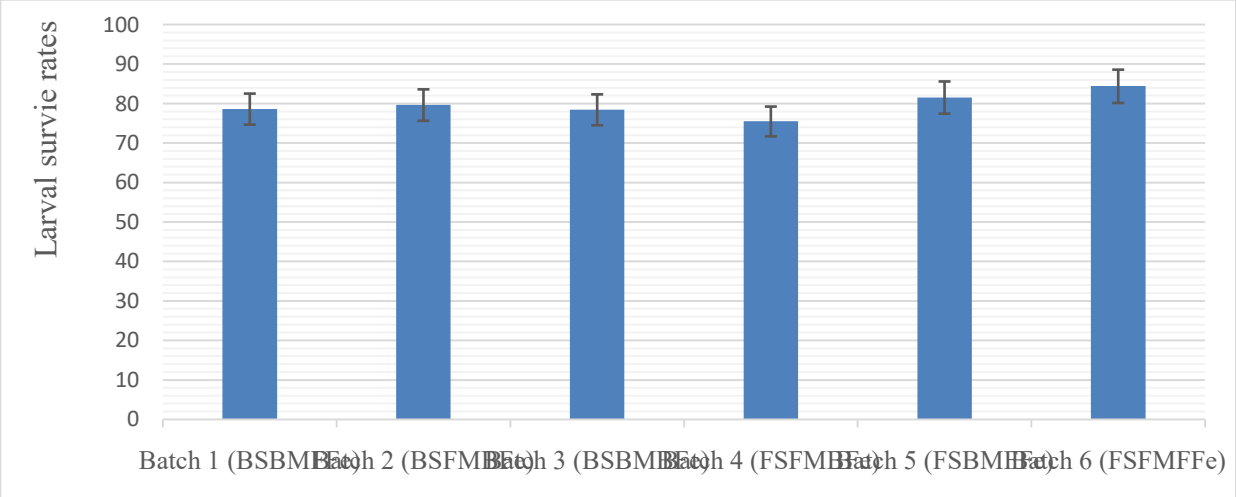

Figure 4: Larval survival rates by batch

II. 2. 3 Adult emergence

Variations in larval survival as a function of substrate are reflected in variations in adult emergence rates (**Figure 5**). In fact, emergence rates vary from batch to batch, depending on the nature of the pair and the spawning substrate used.

We found that the emergence rate of the Barkedji controls (49%) is lower than that of the Fouta controls (61%). Reverse crosses (batch 2 and batch 5) gave the best emergence rates, averaging 61% (with 60.352% and 62.749% respectively). The first crosses (batch 1 and batch 4) had emergence rates of 57.197% and 53.043% respectively.

Furthermore, whatever the batch considered, over 50% of larvae having penetrated the seeds gave rise to adults, with the exception of batch 3, made up of control pairs from Barkedji, which had 49.100%. It should also be noted that the emergence rate was higher when Fouta seeds were used as oviposition substrate.

The ANOVA test (p-value = 0.364) indicates that the differences observed between the calculated rates are not significant.

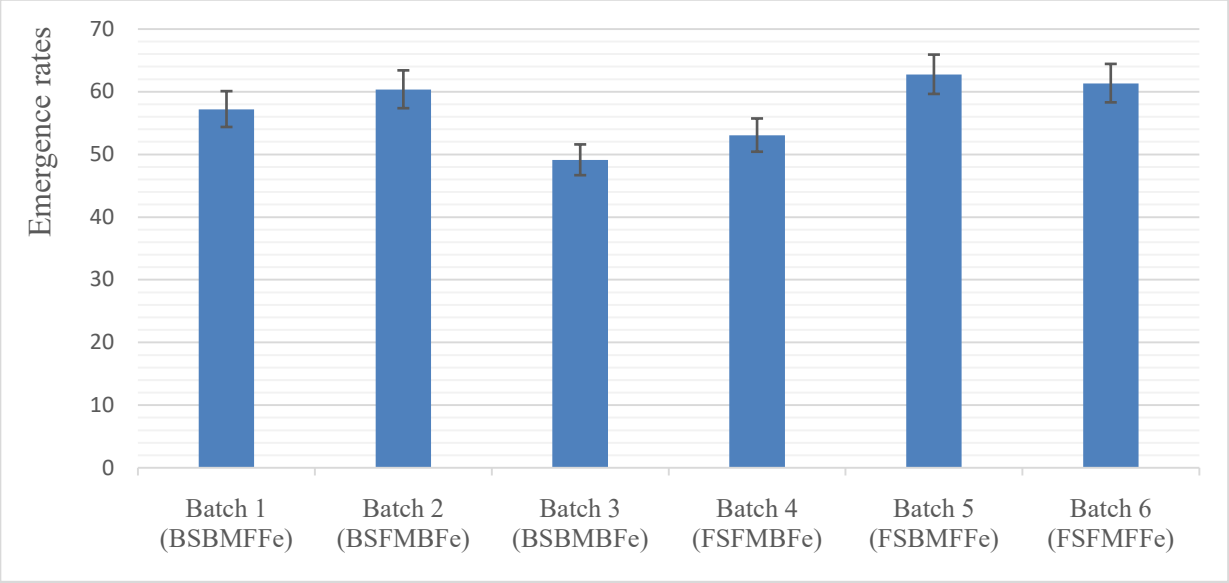

Figure 5: *C.* maculatus adult emergence rates by batch

II. 3. Sex ratio

The sex ratio, which is the ratio of male to female offspring (Table II), varied from batch to batch. Analysis of the sex ratio in the F1 (first generation) obtained shows that it favors males for batch 6 (1.109), batch 5 (01) and batch 3 (1.022). On the other hand, for batch 1 (0.986), batch 2 (0.889) and batch 4 (0.930), which are crossbreeding batchss, the sex ratio favors females. We also noted that the control batches had the highest sex ratio: 1.109 for the Fouta control batch and 1.022 for the Barkedji control batch, with males dominating females.

Table II: Variation in C. maculatus sex ratio (R) between batches

Batches and code names	Sex-ratio
Batch 1(BSBMFFe)	**0.986**
Batch 2(BSFMBFe)	**0.886**
Batch 3(BSBMBFe)	**1.022**
Batch 4(FSFMBFe)	**0.930**
Batch 5(FSBMFFe)	**01**
Batch 6(FSFMFFe)	**1.109**

II. 4 Study of the C. maculatus development cycle

II. 4. 1. Oviposition-emergence duration

Table III shows that the duration of embryonic development (oviposition-emergence duration) hardly varies according to the batches or the type of C. maculatus pairs used. The Kruskal Wallis test confirms this with a non-significant p-value of 0.3534, suggesting equality between the durations determined. The average duration was 21 days. It is longer in pairs from batch 3 (BSBMBFe) or the Barkedji control batch (21.576 ± 1.711 days) and shorter in pairs from batch 6 (FSFMFFe) or the Fouta control batch (20.382 ± 1.094 days). Inverse pairs from Barkedji and Fouta (batch 2 and batch 5) had the same duration, at around 21.133 days. Pairs from the first crosses (batch 1 and batch 4) have approximately the same duration (21 days on average), with a duration of 20.874 ± 0.905 days for batch 1 and 21.439 ± 1.146 days for batch 5 pairs.

Table III: Laying-emergence duration by batch

Batches and code names	*Laying-emergence duration in days*
Batch 1(BSBMFFe)	**20.872 ± 0.905 a**
Batch 2(BSFMBFe)	**21.133 ± 1.276 a**
Batch 3(BSBMBFe)	**21.576 ± 1.711 a**
Batch 4(FSFMBFe)	**21.050 ± 1.338 a**
Batch 5(FSBMFFe)	**21.439 ± 1.146 a**
Batch 6(FSFMFFe)	**20.382 ± 1.094 a**

The presence of the letter **‘'a’'** only indicates that there is no significant difference between the oviposition-emergence durations calculated.

II. 4. 2. Adult lifespan or longevity of adults

The results in **Table IV** show that the lifespan of C. maculatus adults varies according to the sex and nature of the pairs that produced them, or of the batches.

For each batch considered, we found that the lifespan of females was longer than that of males. For example, females in batch 1 (BSBMFFe) have a lifespan of 7.980 ± 1.388 days, which is higher than that of males, which is 6.890 ± 1.230 days. The same applies to all remaining batches.The control couples of Barkedji (lot 3: BSBMBFe) have a lower average lifespan (9.904 ± 0.733 days) than that of the controls of Foua (lot 6: FSFMFFe) which is 11.035 ± 1.527 days.But the difference observed is not significant.

Referring to the original substrates, we found that crossbred batches always have a lower average lifespan than control batches. This difference is significant in males between batch 5 and batches 4 and 6, which are derived from Fouta seeds. For females, the significant difference is between batch 3, a control from Barkedji, and batches 1 and 2,

which are crossbred batches from Barkedji.In terms of average lifespan, there was a significant difference between batch 3, the Barkedji control, and batches 1 and 2, which were Barkedji cross batches. On the other hand, the difference between the average longevity of adults from the Fouta substrate and batches 4, 5 and 6 was not significant.

Table IV: Variation in mean lifespan of *C. maculatus* by sex and batch

Batches and code names	*Average lifespan of males in days*	*Average lifespan of females in days*	*Average lifespan of adults in days*
Batch 1(BSBMFFe)	6.890± 1.230 **a**	7.980 ±1.388 **a**	7.426 ± 1.252 **b**
Batch 2(BSFMBFe)	7.431 ± 1.162 **a**	9.006 ± 2.344 **a.c**	8.210 ± 1.581 **b.c**
Batch 3(BSBMBFe)	7.845 ± 0.483 **a**	12.000 ± 1.327 **b**	9.904 ± 0.733 **a.c**
Batch 4(FSFMBFe)	8.484 ± 1.178 **b**	11.644 ± 2.354 **b**	9.863 ± 1.227 **a.c**
Batch 5(FSBMFFe)	7.800 ± 1.926 **a**	10.889 ± 2.548 **b.c**	9.344 ± 2.077 **a.b.c**
Batch 6(FSFMFFe)	9.758 ± 1.846 **b**	12.313 ± 1.930 **b**	11.035 ± 1.527 **a**

Batches with the same letter (**a, b or c**) are not significantly different. However, batches with different letters are significantly different.

II. 4. 3. Total cycle length of C. maculatus

Total cycle length also varied between batches or pairs, and according to the substrate used (**Table V**).

Whatever the food substrate used, we found that the control pairs (batch 3 and batch 6) always had the highest total cycle time. For example, for Barkedji seeds, batch 3 or Barkedji control pairs have a total cycle length of 31.480 ± 1.863 days, which is higher than that of batch 1 and batch 2 pairs, which have total cycle lengths of 28.298 ± 1.017 days and 29.352 ± 2.110 days respectively. But this difference is only significant between batch 1 and batch 3.

For Fouta seeds, too, pairs from batch 6 or Fouta controls had the highest total cycle length at 31.427 ± 2.357 days. However, there were no significant differences between these batches using Fouta seeds as substrate.

An initial comparison between batches shows that only the following pairs of batches have significant differences: the pair batch 1 - batch 3 (p-value = 0.015), the pair batch 1 - batch 4 (p-value = 0.043) and finally the pair batch 1 - batch 6 (p-value = 0.017). The Barkedji and Fouta controls show no significant difference in duration.

Table V: Variation in total development time of *C. maculatus* by batch

Batches and code names	*Total cycle duration in days*
Batch 1(BSBMFFe)	28.298 ± 1.0168 **b**
Batch 2(BSFMBFe)	29.352 ± 2.110 **b. a**
Batch 3(BSBMBFe)	31.480 ± 1.864 **a**
Batch 4(FSFMBFe)	31.113 ± 1.967 **a**
Batch 5(FSBMFFe)	30.783 ± 2.599 **b. a**
Batch 6(FSFMFFe)	31.427 ± 2.357 **a**

Batches with the same letter **a, b or c** do not differ significantly from one another. However, batches with different letters have significantly different cycle times.

Discussion:

The idea of crossing two strains that are genetically distant may be interesting insofar as it may enable us to see the effect of gene flow on the biodemographic parameters of the C. maculatus insect. The demographic parameter must not be forgotten in the quest for effective control methods. The effectiveness of any control method, whatever its nature, lies in its ability to control insect populations, for example, where they are wreaking havoc. These populations are often of variable size. Population size may therefore depend more on genetic parameters than on climatic and geographical ones. However, few studies on the effect of insect genetics on biodemography have emerged. Nevertheless, Diome (2014) believes that there may be a difference in development cycles between two genetically different populations of Tribolium castaneum.The results obtained in this study, following the crossing experiments carried out, will allow us to learn more.

A study of the egg-laying activity of C. maculatus shows that the average number of eggs laid by females varies according to the nature of the pairs formed and therefore according to the batches formed.This average number of eggs is higher for batch 4 (FSFMBFe) than for the other batches, with 57.50 eggs.The effect of crossbreeding on female fecundity is therefore negligible, since the pairs resulting from crossbreeding do not show a significant difference in egg-laying. However, the results found are comparable to those obtained by Doumma et al., 2011, who obtained an average number of 60 eggs in 5 days when studying varietal resistance. This fecundity appears to be low compared with the results obtained in the laboratory by Sanon (1995), who showed that between May and June, fecundity per Callosobruchus maculatus female varied between 76 and 95 eggs. Our results can be explained by the temperature and humidity conditions prevailing between July and October (Temperatures between 29 and 36°C and relative humidity between 70 and 75%), when we carried out our experiments. These conditions tend to make the veiled form appear less fertile. This can lead to a drop in female fecundity.

Results on the evolution of average daily fecundity show that for all batches, most eggs were laid during the first four days of infestation, as has been observed in many bruchidae. According to Kellouche (2005), the majority of eggs are laid during the first three days of bruchid life. On average, over 90% of eggs were laid by females after four days, with a peak on the second day, except for batch 2 (BSFMBFe) and batch 4 (FSFMBFe), whose peaks occurred on the first day. During these first four days, fertile females still have the energy to lay eggs. Egg-laying activity is therefore a function of age, and is highest at younger ages. These results corroborate those observed by Nyamador (2009) in C. subinnotatus, who showed that the female of this species lays most of her eggs (80.83%) in 6 days, with a peak on day 3, during an average lifespan of 11.36 ± 1.85 days.

However, whatever the batch considered, the egg fertility rate was high, at over 60%. These rates are close to those obtained by Kellouche et al., 2004. However, the batches resulting from crossbreeding (batch 1, batch 2, batch 4 and batch 5) show the best egg fertility rates (70% on average). Egg fertility is therefore influenced by the crosses made between the two selected strains of C. maculatus, namely the Barkedji strain and the Fouta strain, which are genetically distinct. The egg fertility rate increases if genetically distant strains of C. maculatus are crossed. However, the differences observed were not significant, so the influence of crossbreeding on egg fertility was negligible.

The larval survival rate varies rather more depending on the type of food substrate. This rate is higher with the Fouta substrate.These rates, depending on the Fouta substrate, seem to be induced by the origin of the females.Therefore, the females of batch 5; a Fouta cross batch (FSBMFFe) and those of batch 6; Fouta control batch (FSFMFFe), having the same origin as the seeds, allowed to have the best rates.Indeed, we observed that the larval survival rate is low, compared to those of batches 5 and 6, if the seeds and the females do not have the same origin. This fact is not confirmed by thelarval survival rates obtained from the different batches whose food substrate is Barkedji. For these batches of Barkedji, whatever the origin of the females and seeds, the survival rate is always approximately equal to 78%. The relatively high larval survival rates obtained may be due to the fact that the females were able to achieve good distribution of eggs on the seeds, which can allow for a low larval density in the seeds and thus availability of the food substrate. Indeed, according to the work of Zannou (2000), Booker (1967), Howe and Curie (1964), when the intra-granary larval density increases, the larval mortality rate also increases; but the emergence rate decreases.

The emergence rate of adults from the reverse crosses (batches 2 and 5) is higher than that of the controls (batches 3 and 6) and those of the first crosses (batch 1: BSBMFFe and batch 4: FSFMBFe) (Figure 5). For these reverse batches, the females have the same origins as the seeds on which they laid eggs. The origin of the seeds could be a factor influencing the emergence of adults. The nature of the seed also influences emergence because the highest emergence rates are obtained with Fouta seeds. Nevertheless, it could be said that high fertility rates generate equally high emergence rates.Indeed, a limited number of eggs on the seed thus avoids an increase in larval density inside the seed and therefore helps to avoid larval competition.This increases the adult emergence rate. According to Nyamador (2009), high larval survival and emergence rates are favored by the climatic conditions for larval development and the availability of the oviposition substrate. These high emergence rates could also be favored by climatic conditions that influence larval development.

The sex ratio also varies depending on the batches. It is in favor of males for the control couples of Barkedji and even Fouta. However, for the cross-breeding batches (batch 1, batch 2, batch 4 and batch 5), the sex ratio is in favor of females. We can therefore say that the cross has impacted the sex ratio of the insects. These results are consistent with those obtained by Moumouni et al., 2013 on the Gaya and Ayérou strains. The abundance of females in these

offspring resulting from the crosses could have an impact on the biodemographic parameters of the latter and the generations of Callosobruchus that will be born from them. The study of the biodemographic parameters of the first generation (the F1) could provide a clearer picture.

The duration of spawning-emergence varies slightly between groups (less than two days difference). This duration is approximately equal to 21 days in all pairs constituting the groups or populations considered. Crossbreeding has no effect on the embryonic development of Callosobruchus maculatus.

However, significant variations in the total duration of the development cycle of C. maculatus are noted between these batches. Indeed, the total duration of the cycle is relatively longer for batch 3, batch 4 (Barkedji cross batch) and batch 6 (Fouta control batch) and lower for batch 1 (31 versus 28 days). These differences are sometimes significant (Table V). They are significant between batch 1 (a Barkedji cross batch: BSBMFFe) and batch 3 (Barkedji control: BSBMBFe) and also between batch 1 and batch 5; a Fouta cross batch (FSBMFFe). This difference in the total duration of the observed development cycle is induced by a difference in the longevity of adults observed in the different batches. Indeed, this variable duration is due to the fact that the females presented a difference in longevity. The males have relatively the same lifespan regardless of the cross they come from. This variable longevity of females could be due to the genetic variability existing between the strains since the females were placed in the same conditions.

We also noted that crossbreeding appears to have a negative effect on adult longevity. Compared to the lifespan of control adults, the adults from the crossbreeds always have the shortest lifespan when considering the substrates separately. The relatively high lifespan of females is explained by the fact that females, being isolated, escape mating. Mating requires a lot of energy and is highly risky for the female. According to Perrier (2009), males cause enormous damage to the females' genital tracts with their spiny copulatory organ during mating.This means that in the female, by escaping this physical aggression from males, would increase her chances of living even longer, hence the higher longevity of females compared to that of males. According to Tatar et al.,1993, mating significantly affects the lifespan of a female. Hence the concept of "cost of reproduction" used by Williams (1966) to link the effort of reproduction to the other functions of the insect. The lifespan of males is shorter because they, being isolated, are agitated and active almost all the time. This exhausts them after a few days and ends up dying a little earlier.

The lifespan of the controls (batch 3: BSBMBFe and batch 6: FSFMFFe) being always higher, shows that the crosses (batch 1: BSBMFFe, batch 2: BSFMBFe, batch 4: FSFMBFe and batch 5: FSBMFFe) do not have a positive influence on the survival of adults. However, we noted an average lifespan which is approximately 09 days in all batches. These results are different from those observed by Sanon (1997), who obtained an average lifespan of 6.4 days under natural conditions in Burkina Faso and by fatimeh (2009) who observed a lifespan of 5.87 days under conditions of 25 ± 1 °C temperature, 20 ± 5% relative humidity and 16:8 (L:D) h photoperiod.Moumouni et al., 2013 and Doumma (2012) also demonstrated that the longevity of Callosobruchus maculatus hardly exceeds 6 days whatever the climatic conditions of Niger. But these results corroborate those observed by Nyamador (2009) in C. subinnotatus who showed that the female of this species deposits most of her eggs (80.83%) in 6 days with a peak on the 3rd day during an average lifespan of 11.36 ± 1.85 days.

Crossbreeding has no effect on the total development cycle (or total cycle duration) of the Fouta populations. We found that regardless of the nature of the couples, the Fouta substrate does not allow any significant difference between the calculated cycles. On the other hand, for the Barkedji substrate, we noted a significant difference between the total development cycles or total cycle durations of the couples in batch 1 and those in batch 2 (which are Barkedji crossbreeding batches) and batch 3 (Barkedji control), but the cycles of the couples in batch 2 (crossbreeding couples) and batch 3 (control couples) have no significant difference (Table V).These results can be explained by a probable influence of the origin of the females in relation to the origin of the seeds. Indeed, in batch 2 and batch 3, the females laid eggs on the substrate from which they were born. On the other hand, in batch 1, the females were born on the Fouta substrate, that is to say, on Fouta seeds, but laid eggs on Barkedji seeds. This seems to be confirmed by (Ndiaye, 1991), who, for some authors, the choice of egg-laying in insects is determined by hereditary factors. In addition to these factors, Sembene and Delobel (2004) believe that the choice of Caryedon serratus females obeys Hopkins' principle, according to which insects that have pupated inside the pods of a host plant show a greater preference for this host plant. Batch 1, the Barkedji cross batch, has a total cycle duration that is comparable to that of the Tchintabaraden strain calculated by Moumouni et al., 2013.

Conclusion:

The results obtained during this crossbreeding study show that Callosobruchus maculatus populations have significant reproductive potential. This potential can be enhanced by mixing different strains, thus increasing the insect's destructive effect. These results are a good indicator for research, and especially for finding better control methods to eradicate this destructive insect. Indeed, solid knowledge of egg-laying activity, female fertility, the longevity of male and female adults, the overall development cycle, etc., can help develop an effective control method that is accessible to all and without adverse consequences for the environment and other populations. The crossbreedings achieved improved larval survival rates, fertility, and adult emergence, as well as a sex ratio favoring females and a greater adult longevity. However, could the mixing of different strains not be the source of the development of resistance and adaptation mechanisms in the insect C. maculatus?

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