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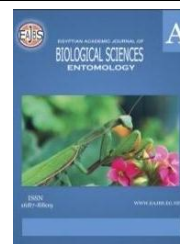
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Attempted Reproductive Isolation in Two Geotypes of *Calliptamus barbarus* (Orthoptera: Acrididae)

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ABSTRACT

This work aims to carried out a study in Algeria on the isolated reproduction between two different populations of *Calliptamus barbarus* one with three spots on their posterior femoral hind (3 S) living in wet region Jijel, the other with one spot (1 S) collected in the semiarid zone (Djelfa and Kasr El Boukhari). After sampling, the insects were placed in wooden rearing cages on the laboratory. The insects were fed on wheat seedlings mixed with barley bran. The reproductive isolation was positif, but the sexual act is not confirmed. Despite the apparent success in reproductive isolation, the obtained reproductive isolation data do not allow us to corroborate that the two geotypes of *C. barbarus* are distinct taxa or are experiencing a segregation process.

INTRODUCTION

Insects are the most formidable pests that harm agriculture. Among insects, Orthoptera are the most fearsome. The history of Orthoptera is very old. It dates back to more than 2400 years before Jesus Christ where in the Old Testament it is mentioned that locusts were one of the plagues of Egypt sent by God to punish the pharaoh and his people. The term Orthoptera derives from Orthos, which means "right" and pteron, "wing". It is a heterogeneous group of species known as grasshoppers. They constitute a vast order of insects, currently composed of more than 26,000 species, including fossil forms (Eades *et al.*, 2014). According to Copr (1982), there are 500 species of locusts out of 12000 described in the world as pests of agricultural or pastoral productions. Among these Orthoptera, the genus *Calliptamus* is one of the most dangerous for agriculture; it belongs to the family of Acrididae and subfamily Calliptaminae. It is included in a group of four closely related species whose identification often proves difficult, including the Italian Caloptene, *Calliptamus italicus* (L. 1758), the Provenzal Caloptene: *C. siciliae* (Monnerat C.,2007) and the Occitan Caloptene: *C. wattenwylanus* (Pantel, 1896). However, the most important of them having aroused the interest of scientists is undoubtedly *C. barbarus* also called "the Caloptene ochrace" or "prickly locust". It is a dangerous species causing a lot of damage in

Algeria especially during outbreaks. It is the most polymorphous species. It has a chromatic of the hind femora (femora red with three spots or orange with only one spot), and geographical polymorphism (Clemente *et al.*, 1987; Benzara, 2004; Larrosa, 2004; Rouibah *et al.*, 2016). In order to compare the two forms this locust has been the subject of several studies related to different aspects: morphometric (Clemente *et al.*, 1987; Benzara, 2004; Larrosa *et al.*, 2004). Geometric morphometric of wing shapes females (Rouibah *et al.*, 2018), ovarioles and biochemistry (Benzara, 2004), sexual behavior (Larrosa *et al.*, 2007), sound production (Larrosa *et al.*, 2008), or molecular study (Sofrane, 2015; Rouibah *et al.*, 2016).

In Algeria, *C. barbarus* was found for two areas: Littoral region for the form with three femoral spots (3S), and extremely semiarid and dry habitats for the form with only one femoral spot (1S).

Several authors have proposed reproductive isolation in order to be able to separate the two geotypes of *C. barbarus*. This is the case of Larrosa *et al.* (2007), who have already studied, in Spain, the intra and interspecific behavior of populations at 1T with those at 3T. These authors had reported that sexual behavior showed some differences between the two forms, each apart, in intra (between individuals of the same sex) and intersexual relationships (between individuals of different sex). These are: convulsive and reciprocating movements of the posterior femur, upward and downward movement of the latter, as well as forward and jump (Larrosa *et al.*, 2007).

This work aims to carry out a study in Algeria on the isolated reproduction between two different populations of *C. barbarus* one with three spots (3 S) living in a wet region, the other with one spot (1 S) collected in a semiarid zone. Additionally, this study will confirm or refute the hypothesis proposed by Benzara (2004) and Larrosa *et al.* (2008) on the start of speciation by geographical isolation observed in the two geotypes of this species.

MATERIALS AND METHODS

For the semi-arid population, we have chosen 2 sites. The first one is in Djelfa (300 km South East of Algiers) (34°40' N, 3°15' E). This is a steppe (Fig. 1) with a sparse vegetation grows, dominated by *Stipa tenacissima*, *Artemisia herba-alba*, *Artemisia campestris* and *Lygeum spartum*. Its altitude is 1138 m. The other site is located in Kasr El-Boukhari (150km South of Algiers) (2° 67' E, 35° 86' N). It is also a steppe installed on a predominantly sandy soil, on which grassy vegetation also grows (Fig. 2). For the coastal region, the samples were taken in the Texenna region near Jijel (350 km East of Algiers) (36° 69' N, 5° 77' E). This station is an open scrubland located at an altitude of 487 m on which grows vegetation dominated by the Cyst: *Cystus monspeliensis* (Fig. 3).

This work was first carried out during the months of July to November 2013. The samples were collected from Djelfa for the 1S form (7 females and 6 males) and from Texenna for the 3 S form (9 females and 6 males). A second reproductive isolation was carried out in July and August 2014. The behavior of 30 male and female individuals was studied by bringing back for this second experiment samples collected at Kasr El Boukhari (1S) and Texenna (3S). The isolation was done reciprocally for the 2 sexes belonging to the 2 populations.

There are many methods for sampling insects depending on their habitat (Franck, 2008). According to Savard (1991), Field intervention can be implemented in three ways: by taking a sample of individuals from a population, selected according to pre-established criteria chosen by the collector (reasoned choice); by taking a total sample of the population (enumeration or census) and by randomly taking a representative fraction of the population (sampling) according to a specific sampling plan. On the other hand, according to Franck

(2008), the classic methods (hunting by sight, mowing, quadrat method or shaking branches) must allow the collection of insects in good and clean condition.

In this study, we have chosen the reasoned choice approach using the mowing net. It consists of randomly collecting a sample of locusts. The surveys are carried out early in the morning for one and a half to two hours. When spotting locusts, proceed very gently so as not to scare the insect. During sampling, the sweeping net must be handled vigorously to sweep the vegetation with rapid, lateral movements. These energetic gestures take individuals by surprise.

After sampling, the insects collected were brought directly to the laboratory, when the fresh specimens were isolated individually in plastic bags. On these bags, labels bearing the date of collection, the name of the station and the sex of the species are stuck. Note that the isolation test was carried out in the zoology laboratory of the University of Jijel. In the laboratory, the insects were placed in wooden rearing cages (Fig. 4). The dimensions are 40 cm long by 40 cm wide and 50 cm high. As cover material, weak-link netting was used.

The experimental conditions are those of the laboratory, that is to say an ambient temperature and humidity. The insects were fed with seedlings of wheat with barley bran. Finally, note that to allow females to lay their eggs, the bottom of the cage has been filled with soil up to a height of 12 cm.



Fig. 1. Station of Djelfa



Fig. 2. Station of Kasr El-Boukhari



Fig. 3. Station of Texenna



Fig. 4. Rearing cage used during the reproductive isolation of the two bio forms of *C. barbarous*

RESULTS

Whether it was during the first or the second test produced in 2013 or 2014, the reproductive isolation was conclusive. Indeed, the results showed that only two couples out of all the individuals tested in the two populations succeeded in forming, as shown in Figures 5 and 6. On the other hand, the sexual act is not confirmed. The two couples may have formed by chance. In addition, the upward and downward movements of the posterior femur have also been observed on several occasions. It should also be noted that no female of the two geotypes was seen laying eggs or traces of eggs were recorded during the entire period of this experiment. These results effectively show that reproductive isolation was conclusive. This also perhaps interprets the upward and downward uplift of the posterior femur as a sign of recognition of each individual of the opposite sex towards the other by virtue of the femoral spots.



Fig. 5. Coupling between a 3S female and a 1S male



Fig.6. Coupling between a female at 1 S and a male at 3S

DISCUSSION

To study any species, especially those with a large polymorphism and distribution, as is the case with *C. barbarus*, it is necessary to take into account biometrics, ethology, ecology (Clemente *et al.*, 1987), sexual behavior (Larrosa *et al.*, 2007), sound production (Larosa *et al.*, 2008), or molecular study (Sofrane, 2015; Rouibah *et al.*, 2016).

With regard to reproductive isolation, it should be noted that the opposite pairs of the two geotypes did not form despite being brought into contact for a very long time. The exception is made for two couples who, apparently, were formed accidentally, without knowing whether or not mating took place. The test is therefore considered to be conclusive. This result confirms that of Selmi (1991) realized in Algeria. The latter did not obtain coupling between individuals from Kasr El Boukhari at 1S with those from Tidjellabine at 3S. This same author had attempted a cross, in captivity, between individuals 3S from Cognac in France with individuals 1S from Dir Yamma in Algeria, but to no avail. In contrast, mating

between the same individuals at 3S from Cognac (France) with those at 3S from Tadjellabine (Algeria) gave a generation with 3S (Selmi, 1991).

Those data show that the semi-arid 1S populations are reproductively isolated from those of the subhumid and the humid. It should be mentioned here that according to Clemente *et al.* (1987) femoral spots are a sign of partner recognition. They serve as a sexual attraction for the two partners; hence the convulsive movements of the posterior femur observed during their study in captivity. Likewise, the red of the wings is also very attractive, the males throw themselves on the females which pass in flight near them (Benzara, 2004). The latter author also reported that the size of the epiphalle, which is slightly different between the two geotypes, can lead to poor coadaptation at the time of mating, which can constitute a barrier to reproductive isolation.

By studying their morphometry, Clemente *et al.* (1987) noticed a clear difference between the 2 geotypes. According to them, the 2 groups of different sizes are related to the color of the internal surface of the posterior femur, so it is possible to observe the 2 geotypes together. According to (Dreux (1972), polymorphism materialized by size differences can generate mechanisms of geographical and ecological isolation of species. Femoral spots can change by decreasing or increasing in size, or merging; this is an adaptation to different ecological conditions (Jago, 1963)

CONCLUSION

Despite the apparent slight success in reproductive isolation between the 2 forms, despite the differences, considered significant enough for their recognition as distinct species: morphometry, Geometric morphometrics, biochemistry, sexual behavior, sound production or molecular study, the obtained reproductive isolation data do not allow us to corroborate that the 2 geotypes of *C. barbarus* are at separate taxa or are experiencing a segregation process.

We propose subspecies status for the 2 geotypes by designating the living form on the littoral by *C. barbarus* hygrophilus (preference for wet bioclimates) and the living form in the semiarid region by the name of *C. barbarus* xerophilus (trend for dry areas).

Declarations:

Ethics Approval: Not applicable.

Authors Contributions: I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

Competing Interests: The author declares no conflict of interest of any kind.

Availability of Data and Materials: All datasets analysed and described during the present study are available.

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