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Karyotypic patterns in populations of *Wasmannia auropunctata* (Roger, 1863): Formicidae, Myrmicinae, from Southeast Bahia, Brazil

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Abstract:

Wasmannia auropunctata is a species with considerable economic and environmental importance, which can lead to considerable losses in the cocoa cultivation. It is also capable of causing many problems when it comes to conservation, as it is a highly competitive and dominant species in many environments, capable of successfully invading disturbed areas and displacing native species. Thus, it is crucial to know it from several points of view, including the cytogenetic. This study proposed to examine the karyotypic set of populations of *W. auropunctata* established in cocoa plantations in Southeast Bahia, Brazil. The sampling was conducted in areas of cocoa plantations, from 2011 to 2015, located in: Itacaré, Dário Meira, Florestal (district of Jequié), Ilhéus and Jequié. Around five to ten *W. auropunctata* nests per location were used for cytogenetic studies. Approximately ten female prepupae from each nest were dissected for cytogenetic preparation. The slides containing metaphases were obtained from the cerebral ganglia of female prepupae, and chromosomes were stained with Giemsa. The constant karyotype of 2n = 32 chromosomes for the populations studied was registered for *W. auropunctata*, and differences were verified in the karyotypic formulas for the localities examined, so that three different patterns were found: (A) 2K = 30M+2SM (Itacaré), (B / C) 2K = 24M+8SM (Dário Meira and Florestal) and (D / E) 2K = 20M+8SM+4ST (Ilhéus and Jequié). Therefore, with a variation of twenty to thirty metacentric chromosomes and from two to eight submetacentric chromosomes for the populations studied, in addition to the occurrence of four subtelocentric chromosomes in the populations of Ilhéus and Jequié. We point to the occurrence of pericentric inversions as the origin or cause of these variations.

Key-words: Cytogenetic; Chromosome; Little fire ant; Cocoa plantation.

Padrões cariotípicos em populações de *Wasmannia auropunctata* (Roger, 1863): Formicidae, Myrmicinae, do Sudeste da Bahia, Brasil

Wasmannia auropunctata é uma espécie com considerável importância econômica e ambiental, o que pode levar a perdas consideráveis no cultivo cacaueiro. Também é capaz de causar muitos problemas no que diz respeito à conservação, pois é uma espécie altamente competitiva e dominante em muitos ambientes, capaz de invadir com sucesso áreas perturbadas e deslocar espécies nativas. Assim, é fundamental conhecê-la sob vários pontos de vista, inclusive o citogenético. Este estudo se propôs a examinar o conjunto cariotípico de populações de

W. auropunctata estabelecidas em plantações cacaueiras no Sudeste da Bahia, Brasil. A amostragem foi realizada em áreas de plantações de cacau, no período de 2011 a 2015, localizadas em: Itacaré, Dário Meira, Florestal (distrito de Jequié), Ilhéus e Jequié. Cerca de cinco a dez ninhos de *W. auropunctata* por local foram utilizados para os estudos citogenéticos. Aproximadamente dez pré-pupas fêmeas de cada ninho foram dissecadas para a preparação citogenética. As metáfases foram obtidas de gânglios cerebrais de pré-pupas fêmeas, e os cromossomos foram corados com Giemsa. O cariótipo constante de 2n = 32 cromossomos para as populações estudadas foi registrado para *W. auropunctata*, e foram verificadas diferenças nas fórmulas cariotípicas para as localidades examinadas, de modo que foram encontrados três padrões diferentes: (A) 2K = 30M+2SM (Itacaré), (B/C) 2K = 24M+8SM (Dário Meira e Florestal) e (D/E) 2K = 20M+8SM+4ST (Ilhéus e Jequié). Portanto, com variação de vinte a trinta cromossomos metacêntricos e de dois a oito cromossomos submetacêntricos para as populações investigadas, além da ocorrência de quatro cromossomos subtelocêntricos nas populações de Ilhéus e Jequié. Apontamos a ocorrência de inversões pericêntricas como a origem ou causa dessas variações.

Palavras chave: Citogenética; Cromossomo; Pequena formiga de fogo; Plantação cacaueira.

Introduction

In the different biogeographic regions of the world, approximately 800 morphospecies of ants have their chromosome number known (LORITE; PALOMEQUE 2010; CARDOSO et al. 2018); however, information on their karyotype is still lacking (MARIANO et al. 2015). Even though cytogenetic investigations with ants started in the 1960s and led to the description of the karyotypes of a considerable number of species, which allowed the discussion of aspects of phylogeny and karyotype evolution in this group (IMAI et al., 1988; MARIANO et al., 1999; LORITE; PALOMEQUE, 2010).

Borges et al. (2004) considers cytogenetics as an additional tool in the studies of the mechanisms involved in evolutionary processes, which provides support for studies of phylogeny, speciation mechanisms and genetic variability. According to Brito et al (2005), cytogenetic data are powerful phylogenetic tools, being indispensable in evolutionary studies, which include chromosome number and length, position of heterochromatin, number of NORs (nucleolar organizer regions), position of GC base pairs and AT, among others.

However, according to Delabie e Mariano (2005), the amount of information on the structure of the chromosomes in the family Formicidae is low compared to the number of described species (approximately 12,000) (BOLTON, 2021). Studies are scarce for Neotropical ants, hence the need to expand cytogenetic investigations to a greater number of neotropical species (MARIANO, et al. 2001).

Within this context, cytogenetic studies with *Wasmannia auropunctata* (Roger, 1863), which is currently part of the Attini tribe (WARD et al., 2015), are incipient (SOUZA et al., 2011; AGUIAR et al., 2020). Cytogenetic studies with other Attine ants are more frequent, such as with ants of the genera *Acromyrmex*, *Apterostigma*, *Atta*, *Cyphomyrmex*, *Mycocepurus*, *Mycetophylax*, *Sericomyrmex* and *Trachymyrmex* (GOÑI et al., 1983; FADINI et al., 1996; MURAKAMI et al., 1998; BARROS et al., 2010; MARIANO et al., 2011; CARDOSO et al., 2014; BARROS et al., 2016; CARDOSO et al., 2018).

The "little fire ant" *W. auropunctata* is a species with considerable economic and environmental importance (CLARK et al., 1982; LUBIN, 1984; MEIER, 1985), which can lead to considerable losses in the cocoa crop (Souza et al. 1998). Furthermore, it is capable of causing many problems when it comes to conservation, as it is a highly competitive and dominant species in many environments (MAJER et al., 1994), capable of successfully invading disturbed areas (BRETON et al., 2003; O'DOWD et al., 2003; CHIFFLET et al., 2018) and displacing native species (CLARK et al., 1982; LUBIN, 1984; MEIER, 1994). Thus, it is crucial to know it from several points of view, including the cytogenetic.

Studies focusing on the genetic variability of *W. auropunctata* populations and their relationship with their invasive potential are much more frequent (see studies by FOURNIER, et al. 2005a; 2005b; FOUCAUD et al., 2006; 2007; MIKHEYEV; MUELLER, 2007; FOUCAUD et al., 2010; SILVA et al., 2018; CHIFFLET et al., 2018) than cytogenetic investigation with this species (SOUZA et al., 2011; AGUIAR et al., 2020).

Wasmannia auropunctata is notable due to the genetic structure of its populations and its reproductive mechanism (FOURNIER et al., 2005a; 2005b), with three different genetic systems: haplodiploidy, clonality and thelytoky (FOUCAUD et al., 2006).

auropunctata (FOUCAUD et al., 2007; 2010; CHIFFLET et al., 2018).The clonality, a phenomenon responsible for the decrease in genetic variability, is a key factor, together with anthropization, in the invasive success of *W*. Thus, this study proposed to examine the karyotypic set of populations of *W. auropunctata* established in cocoa plantations in southeastern Bahia, Brazil, using conventional cytogenetics tools so that the information generated makes it possible to establish a pattern of organization of their karyotypes within the studied localities.

The information generated can be used to propose the management of their populations within cocoa plantations, since they can make it possible to detect a more invasive population, taking environmental conservation measures.

Material and mathods

The sampling was conducted in areas of cocoa plantations established under the "cabruca" system, where part of the Atlantic Forest is thinned and cocoa trees are planted under the shade of large native trees (PIASENTIN; SAITO, 2014), from 2011 to 2015, located in municipalities in the region southeast of the state of Bahia, Brazil: (A) Itacaré, (B) Dário Meira, (C) Florestal (district of Jequié), (D) Ilhéus and (E) Jequié (Figure 1).

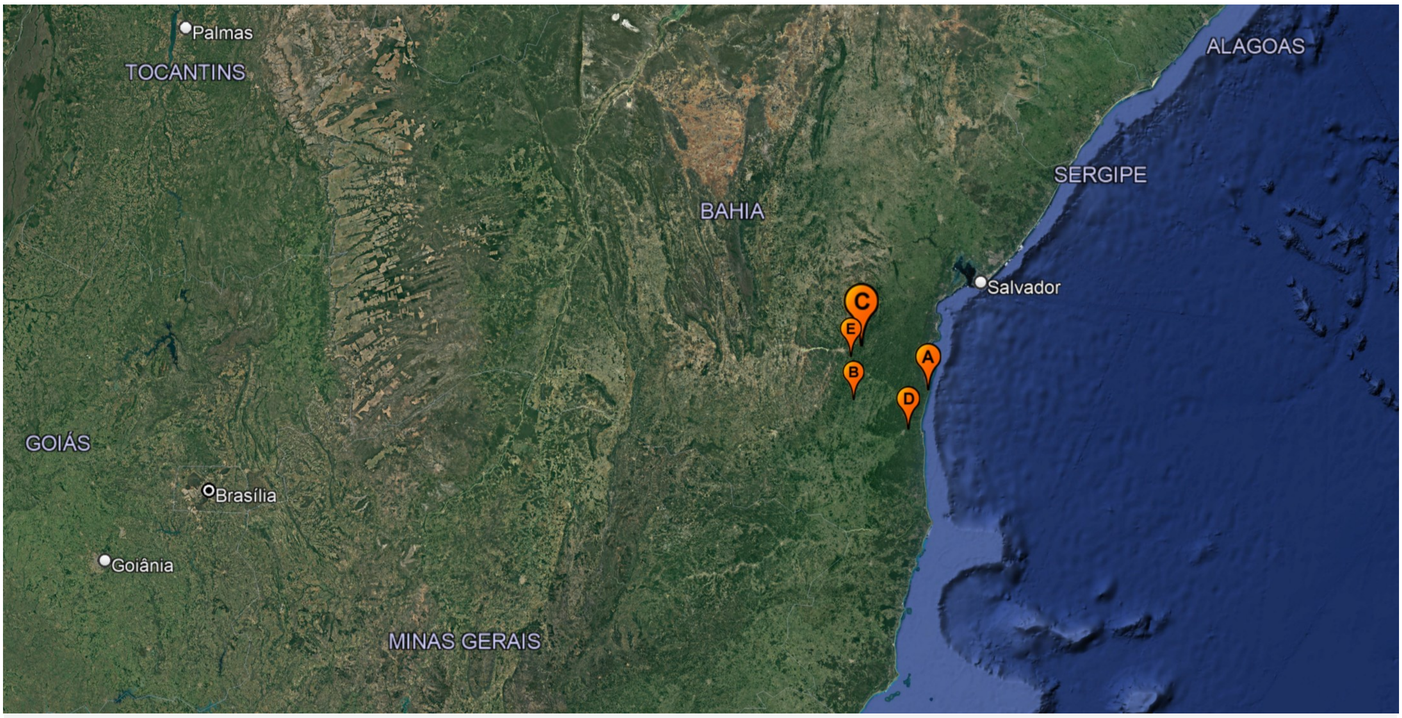

Figure 1. *Wasmannia auropunctata* populations from southeastern Bahia, Brazil. Municipalities: (A) Itacaré (Vila de Olinda), (B) Dário Meira (Fazenda Ribeirão do Cristal), (C) Florestal (Fazenda Floresta), (D) Ilhéus (Quadra G – CEPEC/CEPLAC) and (E) Jequié (Rio Preto do Criciúma).

Around five to ten *W. auropunctata* nests per location, containing differentiated castes and immature forms, were used for cytogenetic studies. The samples were labeled, packed in plastic bags and transported to the UESB Biology Laboratory, Jequié campus, where specimens and immature specimens were sorted.

Approximately ten female prepupae from each nest were dissected under a magnifying glass at the UESB Cytogenetics Laboratory, Jequié campus, for cytogenetic preparation. The slides containing metaphases were obtained from the cerebral ganglia of female prepupae, and chromosomes were stained with Giemsa, according to the protocol of Imai et al. (1988). Metaphase images were captured using a Q Color 3 video camera connected to an Olympus BX 60 microscope with an immersion objective (100x).

The chromosomal classification followed Levan et al. (1964), so that the proportion of the arms of the chromosomes was based on the division of the length of the largest arm by the smallest arm (r: arm ratio = L/S). Witnesses (dry-preserved imagos) from each colony collected were deposited in the myrmecological collection of the UESB Biology Laboratory, on the Jequié campus, Bahia, Brazil. The ants were identified by Dr. Jacques Hubert Charles Delabie, from the Myrmecology Laboratory at CEPLAC, in Ilhéus, Bahia.

Results

The constant karyotype of 2n = 32 chromosomes for the populations studied was registered for *W. auropunctata*, and differences were verified in the karyotypic formulas for the localities examined, so that three different patterns were found: (A) 2K = 30M+2SM (Itacaré), (B / C) 2K = 24M+8SM (Dário Meira and Florestal) and (D / E) 2K = 20M+8SM+4ST (Ilhéus and Jequié). Therefore, with a variation of twenty to thirty metacentric chromosomes and from two to eight submetacentric chromosomes for the populations studied, in addition to the occurrence of four subtelocentric chromosomes in the populations of Ilhéus and Jequié (Table 1; Figure 2).

Table 1. Karyotypic variation in relation to chromosomal morphology for *Wasmannia auropunctata* populations from southeastern Bahia, Brazil, where 2n = 32 chromosomes. M (metacentric), SM (submetacentric) and ST (subtelocentric); r: arm ratio = L (length of the largest arm)/S (length of the smallest arm).

Code	Municipality/ Populations	Geographical coodinates	Chromosomal number	Karyotypic formula	r: arm ratio = L/S
A	Itacaré	14°16'36"S 38° 59'56"O	2n = 32	2K = 30M+2SM	M = 1,0 - 1,5 SM = 2,9
B	Dário Meira	14°26’09"S 39° 54’25"O	2n = 32	2K = 24M+8SM	M = 1,0 - 1,7 SM = 1,8 - 2,0
C	Florestal	13°47’49"S 39° 50’48"O	2n = 32	2K = 24M+8SM	M = 1,1 - 1,6 SM = 2,3 - 2,6
D	Ilhéus	14°45'00"S 39° 13'00"O	2n = 32	2K = 20M+8SM+4ST	M = 1,0 - 1,3 SM = 2,0 - 2,7 ST = 3,4 - 6,0
E	Jequié	13°55’45"S 39° 57’57"O	2n = 32	2K = 20M+8SM+4ST	M = 1,1 - 1,7 SM = 1,8 - 2,5

Discussion

The chromosome number of *W. auropunctata* for all populations studied remained constant, 2n = 32 chromosomes, and variations were only found in the position of the centromeres, with most chromosomes being metacentric, and with their variations, such as submetacentric and subtelocentric; thus, we point to the occurrence of pericentric inversions as the origin or cause of these variations. Pericentric inversions are due to two breakpoints in different chromosome arms, including the centromere region (LORITE; PALOMEQUE, 2010).

Pericentric inversion is known to help maintain a relatively stable karyotype for a high chromosome number (IMAI et al., 1988), as is the case in the *W. auropunctata* populations studied. This type of inversion can present examples of these rearrangements, such as intra and interpopulation polymorphisms (LORITE; PALOMEQUE, 2010).

Comparing this work with the article by Souza et al. (2011), where 2K = 20M + 12A,for populations of Ilhéus and Una, both in southeastern Bahia, we found the same chromosome number, 2n = 32 chromosomes, and the same number of metacentric chromosomes (20M), when considering the populations of Ilhéus and Jequié. The difference observed was in relation to the smaller chromosomes, not having occurred acrocentric chromosomes, but instead, 8SM and 4ST.

This difference oberved in the populations mentioned above was probably due to the type of classification used, since the 12 acrocentric chromosomes, classified according to the terminology of Imai (1991), must be equivalent to the 8SM and 4ST, according to the classification of Levan et al. (1964). More recently, Aguiar et al. (2020) also found the same chromosome number and similar karyotype for populations of *W. auropunctata* from French Guiana, but with a difference in the karyotypic formula in relation to our study, due to the fact that it was a cytogenetic investigation with a

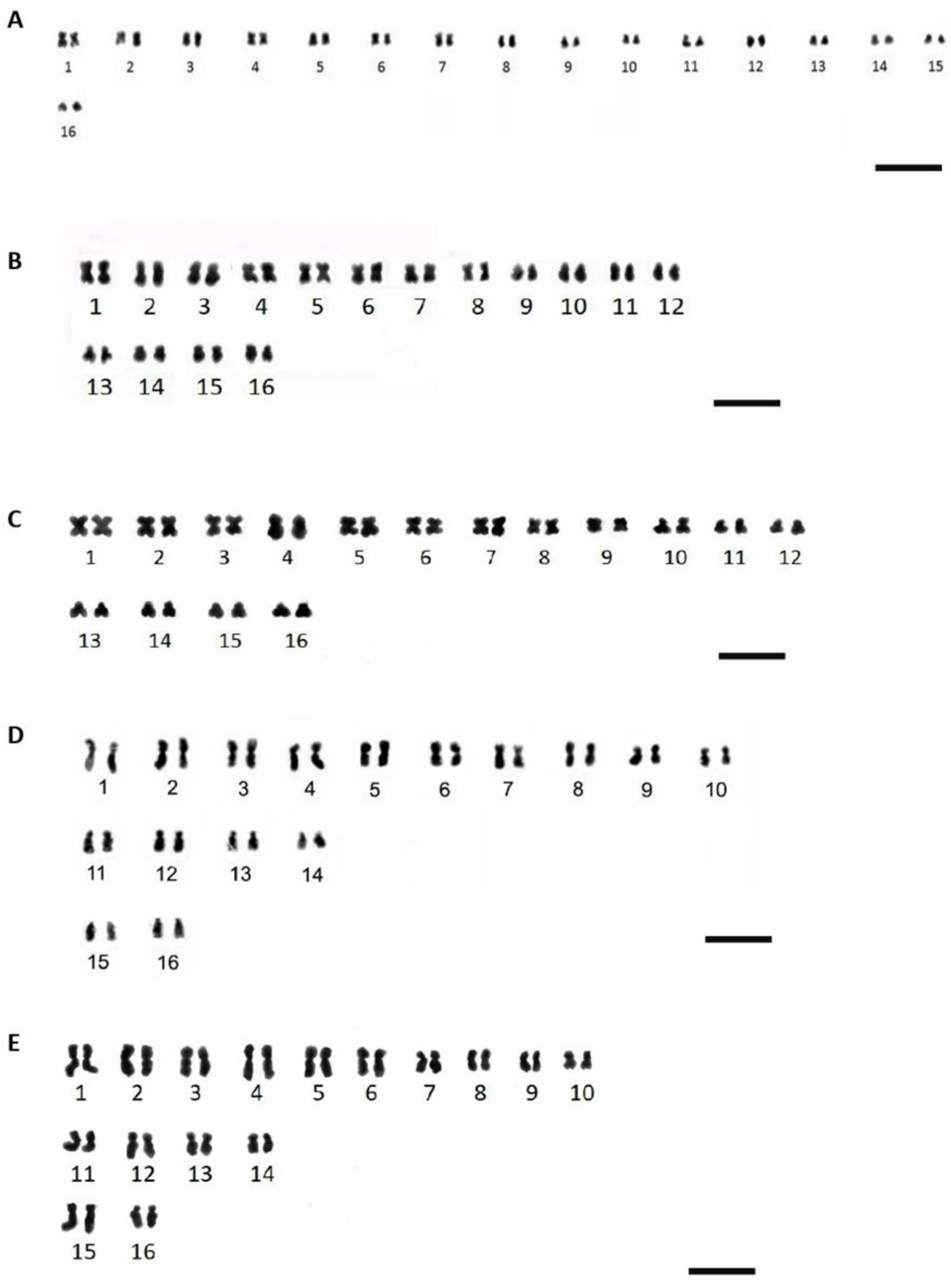

Figure 2. *Wasmannia auropunctata* karyotypes of populations from southeastern Bahia, Brazil: (A) Itacaré (1 to 15 = metacentric and 16 = submetacentric chromosomes; 2K = 30M+2SM); (B) Dário Meira and (C) Florestal (1 to 12 = metacentric, 13 to 16 = submetacentric chromosomes; 2K = 24M+8SM); (D) Ilhéus and (E) Jequié (1 to 10 = metacentric, 11 to 14 = submetacentric, 15 and 16 = subtelocentric chromosomes; 2K = 20M+8SM+4ST). Bar = 5μm.

population from the Amazon Forest.

Possibly, the degree of anthropization of the environment, together with the effective size of *W. auropunctata* populations studied (CHAPMAN; BOUKE, 2001; FOUCAUD et al. 2007, 2010; LORITE; PALOMEQUE, 2010; CHIFFLET et al., 2018), had a determining effect on the occurrence of karyotypes recorded. However, it is suggested that more cytogenetic studies be developed with the regional populations of *W. auropunctata*, in order to generate additional information that allows to increase the knowledge about the organization patterns of the karyotypes within the studied localities.

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