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Original article

Potential use of major royal jelly proteins (MRJPs) as molecular markers for royal jelly production in Africanized honeybee colonies*

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Abstract – The present study determined the genetic variation at *mrjps* loci (*mrjp3*, *mrjp5* and *mrjp8*) and evaluated the potential use of MRJPs as molecular markers for higher royal jelly production in Africanized honeybee colonies. The three analyzed loci produced a total of 17 alleles. This high allelic polymorphism indicated these loci could serve as genetic markers. The potential use of MRJPs as molecular markers for royal jelly production was evaluated by analyses of multiple linear regressions with EPD (expected progeny differences) values for royal jelly production. The variance analyses indicated that the *mrjp3* repetitive region influenced the genetic value of queen's offspring for royal jelly production. The determination coefficient (R^2) for the significant alleles of the repetitive region of *mrjp3* indicated that 36.85% of the EPD variation is explained by the variation of *C*, *D* and *E* alleles. Therefore, these three alleles present a considerable genetic effect on the variation of RJ production.

***Apis mellifera* / *MRJP-3* / genetic variability / polymorphism / EPD**

1. INTRODUCTION

Royal jelly (RJ) is secreted by nurse bee hypopharyngeal glands, playing an important role in larval development. Worker bee larvae are fed with RJ for 3 days and only larvae that develop into queen bees are continuously fed with large quantities of RJ (Schmitzová et al., 1998). RJ is always supplied directly to the queen or to the larvae as soon as it is secreted; it is not stored. The only situation in which harvesting becomes feasible is during queen rearing, when the larvae destined to become queen bees are abundantly supplied with RJ (Garcia and Nogueira-Couto, 2005; Nogueira-Couto and Couto, 2006). The queen larvae can not consume the food as fast as it is provided and RJ accumulates in the queen cells. RJ commercial production is, therefore, dependent on a hive's capability to rear a large numbers of queens.

RJ provides essential amino acids, lipids, vitamins, acetylcholine (Ach) and other nutrients of the larval food and queen food (Schmitzová et al., 1998; Simúth, 2001; Garcia-Amoedo and Almeida-Muradian, 2007). Major royal jelly proteins (MRJPs) constitute 82–90% of total RJ protein in the honeybee (Schmitzová et al., 1998). MRJPs have been extensively studied and characterized in *Apis mellifera*. Nine *A. mellifera*

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loci encoding MRJPs (*mrjp1-mrjp9*) and a pseudogene encoding an incomplete polypeptide (*mrjp-ψ*) have been identified (Klaudiny et al., 1994; Albert et al., 1996, 1999a, b; Schmitzová et al., 1998; Albert and Klaudiny, 2004; Drapeau et al., 2006). MRJP3 and MRJP5 displayed size polymorphism with molecular weight ranges between 60–70 and 77–87 kDa, respectively (Srisuparbh et al., 2003). The highly polymorphic repeat length character of *mrjp3* and *mrjp5* can be used as highly informative loci in the genetic studies of honeybee colonies (Beye et al., 1998).

It is important and lucrative for the beekeeper to breed queens whose daughters produce higher quantities of royal jelly. China is the largest producer of RJ with an annual production of around 2000 tons, corresponding to 90% of the world's output (Li et al., 2003). The higher RJ producing bees have made a great contribution to the world's RJ production (Chen et al., 2005). However, genetic characteristics of RJ production remain unknown. Very little is known about molecular markers for RJ production in *A. mellifera*. Chen et al. (2005) analyzed genetic variations at 10 microsatellite loci to determine molecular characteristics of different honeybee populations from Italy and China. According to allele frequency, seven alleles at six loci were probably molecular markers for high RJ production.

The purpose of the present study was to determine genetic variation at *mrjps* loci (*mrjp3*, *mrjp5* and *mrjp8*) and evaluate the potential use of MRJPs as molecular markers to increase RJ production in Africanized honeybees. In addition, the genetic structure of Africanized honeybee in relation to these genetic markers was determined. With this information, it will be possible to create strategies so that beekeepers can increase RJ production.

2. MATERIAL AND METHODS

2.1. Honeybee samples

Africanized *A. mellifera* nurses were collected at Iguatemi's Experimental Farm-Apiary, a branch of Maringá State University. A total of 34 mini-hives producing RJ were sampled. The brood frame was shaken and the remaining bees were collected as nurse bees. Africanized queens of mini-hives originated from 15 mother queens. Gonçalves and Kerr (1970), Kerr et al. (1970a, b) and Akyol et al. (2008) recommended the use of queens weighing more than 200 mg at emergence. Therefore only queens with an initial weight above 180 mg were introduced in the mini-hives. The queens introduced in each mini-hive were mated naturally during flight.

Honeybees from two RJ production cycles (cycle I and cycle II) were evaluated. Production cycle is the queen evaluation period for RJ production. Each production cycle begins with the introduction of a new queen (a mother queen's daughter) in a mini-hive and ends with the RJ production quantification. After the queen's evaluation period, a new queen of the same mother colony was introduced to go on with the process and initiate a new cycle. Both molecular analyses and the evaluation of RJ production were undertaken for 23 mini-hives for cycle I and 11 mini-hives for cycle II.

2.2. Preparation of genomic DNA

We used a modified protocol of Bardakci and Skibinski (1994) to extract genomic DNA from 10 nurse bees of 34 mini-hives. The thoraxes were homogenized individually in extraction buffer (50 mM Tris-HCl pH 8.0, 50 mM EDTA pH 8.0, 100 mM NaCl and 1% SDS) and proteinase K (25 μg/mL). DNA concentration and DNA quality was certified in 0.8% agarose gel in TAE 1X buffer (0.04 M Tris-acetate, 0.001M EDTA, pH 8.0) stained with ethidium bromide solution (0.5 mg/mL).

2.3. PCR amplifications

PCR reactions were performed with genomic DNA using the following primers. For the amplification of the repetitive region of *mrjp3* (Albert et al., 1999b): forward: ATG TAA TTT TGA AGA ATG AAC TTG; reverse: TGT AGA TGA CTT AAT GAG AAA CAC. For the amplification of the repetitive region of *mrjp5* (Albert et al., 1999a): forward: AGA CTC TTC AAA CGG TCG TTG C; reverse: CTG TAA TTT CAT ACT TAA AGC CAT. For the amplification of the locus *mrjp8* (Klaudiny et al., 1994): (forward: TTG CGA AGT GAA TGG ATC; reverse: TTA TTT TTG GCA ACC ACT TCG).

Standard PCR analyses were performed in a 20 μL of a mixture containing Tris-KCl 1X (Tris-HCl 20 mM pH 8.4 and KCl 50 mM), 0.5 μM of

each *primer*, 0.1 mM of each dNTP, one unit of *Taq* DNA Polymerase (Invitrogen). The specific concentrations of $MgCl_2$ for *mrjp3*, *mrjp5* and *mrjp8* primers were respectively 1.7; 1.5; and 3.0 mmol/L. For amplification with the *mrjp3* primer this was 10 ng/μL genomic DNA, with the *mrjp5* and *mrjp8* primers were 20 ng/μL. The mixture was incubated in a thermocycler model "Techne TC-512®". The protocol for the amplification of the *mrjp3* and *mrjp5* repetitive regions was: an initial denaturation for 2 min at 94 °C followed by 30 cycles of 30 s at 94 °C, 30 s at 54 °C and 1 min at 72 °C. The reaction was completed after 10 min at 72 °C. The protocol for the amplification of the *mrjp8* repetitive region was: an initial for 5 min at 94 °C followed by 35 cycles of s at 94 °C, 50 s at 50 °C and 100 s at 72 °C. The reaction was completed at 10 min at 72 °C. Reaction products were size-separated by electrophoresis on 2% agarose gel (MS-8 BioAmerica-Inc) at 60 volts using TBE 0.5X buffer (0.045 M Tris-borate, 0.001 M EDTA, pH 8.0). The PCR products were stained with ethidium bromide solution (0.5 mg/mL) and visualized under ultraviolet light. A 100 bp DNA ladder (Invitrogen) was used to determine the size of the generated fragments.

2.4. Royal jelly production

The developmental cycle of an Africanized honeybee worker takes 20 days from egg to emergence of the adult (Kerr et al., 1972; Garófalo, 1977) and the mean of life span is 24.4 days (Winston and Katz, 1981). Thus, the 34 mini-hives were submitted to RJ production tests 50 days after the introduction of a new queen. Each mini-hive had approximately 20 000 to 25 000 honeybees.

To produce RJ, a round of queen production was initiated, but was interrupted 66–72 hours after grafting. Each mini hive was composed of two nucs, with five frames below the queen excluder and four frames above, plus two bar frames with 14 cups each. The RJ was collected 66–72 hours after grafting and its production by the mini-hive (g) measured in an analytical scale (0.001 g). The cups were returned to mini-hives for cleaning by worker bees and the new grafting was performed. Larvae were grafted twice a week during one month. After the queens' evaluation period, a new queen was introduced to give sequence to the cycle. Those data were used for the EPD (expected progeny differences) estimate.

2.5. Data analyses

Allele frequencies, heterozygosity, F_{IS} and F_{ST} were computed using the POPGENE 1.31 (Yeh et al., 1999) software.

Expected progeny differences (EPDs) provide estimates of an animal genetic breeding value. An EPD predicts the difference in the performance of a future product of a certain father or mother, compared with the other parents' progeny, where both are created under the same condition. EPDs values for RJ production were obtained by MTGSAM (multiple trait Gibbs sampling in animal models) software (Van Tassel and Van Vleck, 1995).

EPDs provide estimates of an animal genetic breeding. EPDs values for RJ production indicate the capacity of the queen in transmitting her traces for RJ production to her progeny.

EPDs values were analyzed in function of the allele frequencies in each locus, by multiple linear regression according to the following equation:

$$y_i = b_0 + \sum_{j=1}^{k} b_j X_j + e_i$$

where: y_i is the EPD value for RJ production from colony i; b_0 is the constant; b_j is the regression coefficient for EPD of the RJ in function of the frequency of allele j in colony; X_j is the frequency of allele j; e_i is the random error associated to y_i. Path analysis was used to explore the relationship among the significant alleles in the determination of the EPD for RJ. Multiple linear regression and path analysis was performed with the software SAEG 5.0 (systems of statistical and genetic analyses) developed by UFV (Federal University of Viçosa, 1993).

3. RESULTS

3.1. Genetic analysis at *mrjp* loci in Africanized honeybee producing royal jelly

The three analyzed loci were polymorphic and produced a total of 17 alleles (Fig. 1). Seven alleles (size range from 410 bp to 610 bp) have been observed at *mrjp3* repetitive region, six (570–720 bp) at *mrjp5* repetitive region and four (360–420 bp) at *mrjp8* locus. Table I shows the allele frequencies in

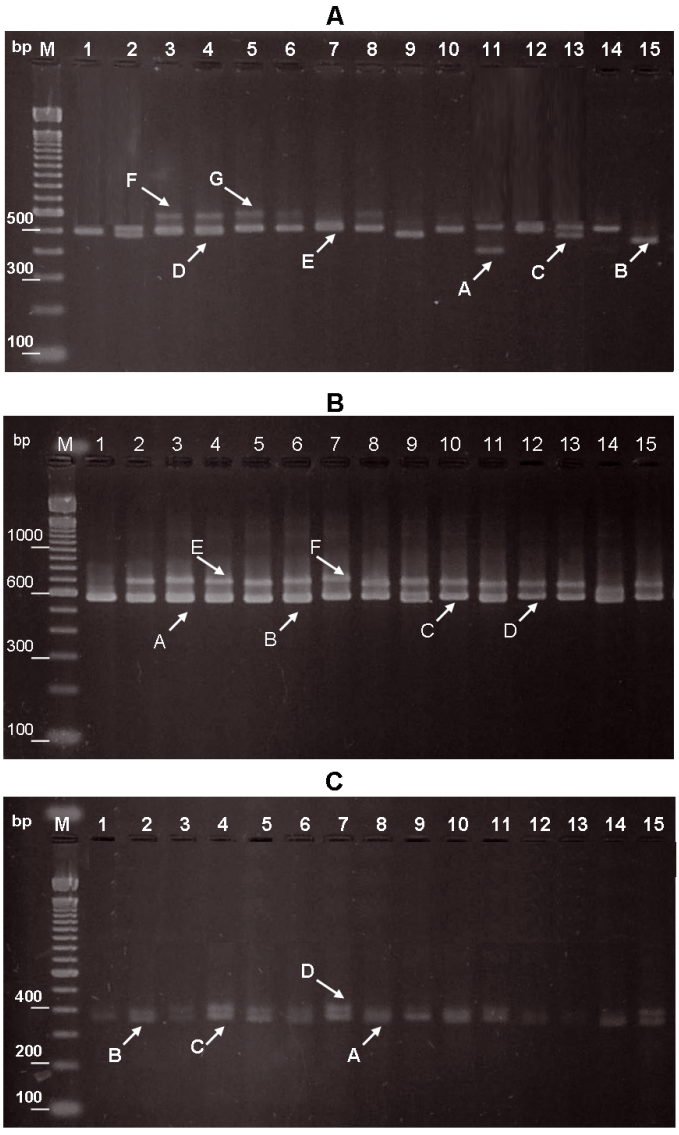

Figure 1. Electrophoresis profile of *mrjps* loci of the Africanized honeybee colonies. (A) Fragments amplified for the *mrjp3*, (B) Fragments amplified for the *mrjp5*, (C) Fragments amplified for the *mrjp8* M = DNA molecular weight marker (DNA Ladder-Invitrogen). Arrows indicate the alleles. Numbers of lanes (1–15) = different genotypes of Africanized honeybee for *mrjps* loci (A = *mrjp3*, B = *mrjp5*, C = *mrjp8)*.

the analyzed loci for the RJ production cycles (cycle I and cycle II).

Expected Progeny Differences (EPDs) in Africanized honeybees producing RJ are shown in Table I. The highest value of EPD observed was 2.1055 and the lowest value was –1.8866. A positive EPD is desirable when selecting for traits such as high RJ production.

The variance analyses were significant ($P < 0.05$) for the *mrjp3* repetitive region, and the best fit equation was observed in *C*, *D* and *E* alleles (Tab. II). Those analyses indicate that

Table I. EPD values for mini-hives, allele frequencies for the *mrjps* loci (*mrjp3, mrjp5* and *mrjp8*) in the Africanized honeybee colonies producing royal jelly.

Mini-hive	EPD	*Locus mrjp3* Allele frequency							*Locus mrjp8* Allele frequency						*Locus mrjp5* Allele frequency			
		A	*B*	*C*	*D*	*E*	*F*	*G*	*A*	*B*	*C*	*D*	*E*	*F*	*A*	*B*	*C*	*D*
Cycle I																		
MR1	–1.4369	0.00	0.00	0.15	0.25	0.35	0.20	0.05	0.05	0.5	0.20	0.05	0.2	0.00	0.25	0.20	0.25	0.30
MR3	–0.8623	0.10	0.00	0.05	0.35	0.50	0.00	0.00	0.25	0.15	0.20	0.20	0.05	0.15	0.40	0.05	0.2	0.35
MR4	0.02632	0.00	0.00	0.05	0.50	0.35	0.10	0.00	0.05	0.40	0.05	0.00	0.20	0.30	0.20	0.25	0.15	0.40
MR5	0.8617	0.00	0.00	0.00	0.70	0.20	0.05	0.05	0.20	0.35	0.05	0.10	0.30	0.00	0.15	0.25	0.10	0.50
MR8	–1.8866	0.10	0.05	0.40	0.40	0.00	0.05	0.00	0.05	0.35	0.30	0.10	0.10	0.10	0.05	0.45	0.40	0.10
MR9	0.01345	0.00	0.00	0.15	0.60	0.05	0.20	0.00	0.05	0.40	0.10	0.25	0.05	0.15	0.00	0.25	0.50	0.25
MR10	0.14139	0.05	0.15	0.20	0.30	0.30	0.00	0.00	0.25	0.25	0.25	0.05	0.15	0.05	0.25	0.15	0.20	0.40
MR11	–0.6162	0.00	0.00	0.05	0.80	0.05	0.05	0.05	0.05	0.20	0.25	0.15	0.25	0.10	0.00	0.15	0.50	0.35
MR12	–0.6949	0.00	0.00	0.70	0.20	0.00	0.10	0.00	0.15	0.30	0.05	0.00	0.45	0.05	0.00	0.20	0.65	0.15
MR14	1.06297	0.10	0.00	0.00	0.65	0.20	0.05	0.00	0.00	0.55	0.15	0.00	0.10	0.20	0.10	0.30	0.35	0.25
MR15	–0.5729	0.00	0.00	0.28	0.61	0.11	0.00	0.00	0.25	0.2	0.10	0.05	0.05	0.35	0.15	0.25	0.30	0.30
MR20	0.52344	0.00	0.10	0.35	0.20	0.30	0.05	0.00	0.15	0.35	0.10	0.15	0.20	0.05	0.10	0.40	0.20	0.30
MR22	–0.0008	0.05	0.00	0.25	0.65	0.00	0.00	0.05	0.05	0.50	0.15	0.05	0.05	0.20	0.00	0.15	0.55	0.30
MR23	0.89801	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.50	0.00	0.56	0.00	0.44	0.22	0.33	0.17	0.28
MR24	–0.7633	0.00	0.00	0.40	0.55	0.00	0.00	0.05	0.10	0.15	0.30	0.25	0.15	0.05	0.00	0.20	0.60	0.20
MR26	0.14648	0.00	0.05	0.50	0.40	0.00	0.00	0.05	0.05	0.50	0.05	0.00	0.35	0.05	0.05	0.20	0.35	0.40
MR27	2.10547	0.00	0.00	0.05	0.65	0.30	0.00	0.00	0.15	0.15	0.20	0.05	0.35	0.10	0.25	0.15	0.35	0.25
MR28	1.01723	0.00	0.00	0.25	0.70	0.05	0.00	0.00	0.15	0.40	0.00	0.25	0.10	0.10	0.10	0.25	0.35	0.30
MR29	1.2832	0.00	0.00	0.20	0.20	0.45	0.10	0.05	0.20	0.30	0.15	0.10	0.10	0.15	0.28	0.22	0.28	0.22
MR30	–1.0357	0.00	0.00	0.00	0.85	0.05	0.10	0.00	0.20	0.40	0.00	0.30	0.00	0.10	0.05	0.05	0.75	0.15
MR31	0.96293	0.00	0.00	0.13	0.75	0.12	0.00	0.00	0.30	0.15	0.10	0.25	0.20	0.00	0.05	0.30	0.45	0.20
MR32	0.35273	0.00	0.00	0.38	0.25	0.38	0.00	0.00	0.15	0.35	0.10	0.20	0.15	0.05	0.10	0.20	0.65	0.05
MR34	–0.8772	0.00	0.00	0.50	0.40	0.10	0.00	0.00	0.30	0.20	0.15	0.10	0.10	0.15	0.35	0.15	0.30	0.20
Cycle II																		
MR10	–1.2172	0.00	0.00	0.60	0.35	0.05	0.00	0.00	0.30	0.20	0.05	0.00	0.40	0.05	0.15	0.40	0.15	0.30
MR11	–1.6141	0.05	0.00	0.35	0.30	0.05	0.25	0.00	0.25	0.20	0.15	0.10	0.30	0.00	0.20	0.10	0.40	0.30
MR12	0.14305	0.00	0.00	0.20	0.60	0.20	0.00	0.00	0.00	0.50	0.10	0.00	0.05	0.35	0.00	0.20	0.40	0.40
MR13	–1.1729	0.00	0.00	0.65	0.20	0.15	0.00	0.00	0.15	0.25	0.10	0.15	0.25	0.10	0.05	0.20	0.45	0.30
MR22	0.2863	0.00	0.00	0.8	0.10	0.10	0.00	0.00	0.10	0.30	0.30	0.00	0.20	0.10	0.15	0.20	0.30	0.35
MR23	0.97138	0.00	0.00	0.00	0.35	0.45	0.2	0.00	0.00	0.30	0.20	0.15	0.15	0.20	0.15	0.20	0.45	0.20
MR30	–0.7371	0.11	0.00	0.33	0.33	0.22	0.00	0.00	0.05	0.40	0.05	0.20	0.20	0.10	0.15	0.25	0.35	0.25
MR31	0.88406	0.1	0.00	0.20	0.20	0.50	0.00	0.00	0.15	0.20	0.15	0.00	0.40	0.10	0.25	0.20	0.35	0.20
MR33	0.43309	0.00	0.00	0.45	0.30	0.25	0.00	0.00	0.30	0.15	0.15	0.00	0.30	0.10	0.20	0.25	0.15	0.40
MR35	1.0554	0.00	0.00	0.30	0.65	0.05	0.00	0.00	0.05	0.20	0.35	0.15	0.15	0.10	0.15	0.20	0.25	0.40
MR36	0.33311	0.00	0.00	0.25	0.70	0.05	0.00	0.00	0.20	0.40	0.15	0.20	0.05	0.00	0.00	0.35	0.30	0.35

Table II. Variance analysis to fitted equation to EPD and MRJPs alleles in Africanized honeybee colonies. *P*-value < 0.05 = significant, *** = not significant.

Variance sources	F	*P*-value
MRJP3	5.84	0.0029
MRJP5	0.23	***
MRJP8	0.88	***

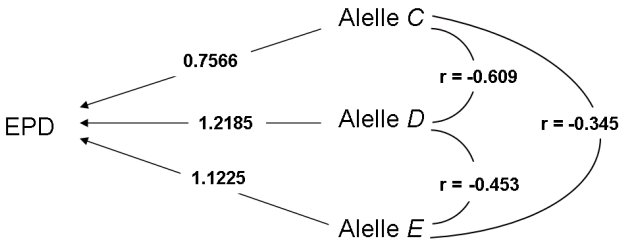

Figure 2. Path analysis diagram showing the direct effects of *C*, *D* and *E mrjp3* alleles and their interrelations (r = correlations) in the EPD for royal jelly production in Africanized honeybee colonies.

the *mrjp3* repetitive region influences the genetic value of the queen for RJ production. The variance analyses for the *mrjp5* repetitive region and *mrjp8* loci were not significant, giving indications that these loci probably have low effect on the genetic value for the RJ production (Tab. II).

The determination coefficient (R^2), detected in *mrjps* loci analyzed, indicated that 52.84% of the EPD variation is explained by the variation of all alleles. The R^2 for the significant alleles of the repetitive region of *mrjp3* indicated that 36.85% of the EPD variation was explained by the variation of *C*, *D* and *E* alleles. Therefore, these three alleles had a high effect on the variation of RJ production genetic value.

The path analysis showed that the *mrjp3* alleles *C*, *D* and *E* presented positive direct effects on EPD (Fig. 2), indicating that the higher RJ production had direct contributions from these gene products.

3.2. Genetic structure in Africanized honeybees using *mrjps* loci

Observed and expected heterozygosity (Ho and He respectively) are shown in Table III. The average heterozygosity values, for the cycles I and II of RJ production, were 0.5838 and 0.5819 respectively, indicating a high genetic diversity on these loci. The high genetic diversity indicates a great potential for selection. However, a great difference was observed between Ho and He for the *mrjp3* repetitive region in both RJ production cycles, showing that the heterozygosity is decreasing in this locus. The reduction in Ho suggests that these alleles can be used as molecular markers for RJ production. These results show that it is important to conduct additional studies on the *mrjp3* repetitive region to evaluate its potential as an efficient molecular marker for RJ production.

The F_{IS} value (0.2025) indicated an excess of homozygotes that can be explained by the selection of queens for RJ production. The low F_{ST} value (0.0068) indicates that the nurse honeybees of the two RJ production cycles are not genetically differentiated.

4. DISCUSSION

Polymorphism of the *mrjp3* locus is due to the inherent ability of the *mrjp3* repetitive region to generate new alleles or through selection (Albert et al., 1999b), something that

Table III. Population structure in Africanized honeybee colonies producing royal jelly (RJ): observed heterozygosity (Ho) and expected heterozygosity (He).

RJ production cycle	Locus	Ho	He
Cycle I	*mrjp3*	0.2857	0.6544
	mrjp5	0.8035	0.8022
	mrjp8	0.6623	0.7212
	Mean	0.5838	0.7259
	Std Dev	0.2677	0.0740
Cycle II	*mrjp3*	0.1193	0.6860
	mrjp5	0.8455	0.8101
	mrjp8	0.7810	0.7324
	Mean	0.5819	0.7428
	Std Dev	0.4019	0.0627
Total	Mean	0.5827	0.7347
	Std Dev	0.3100	0.0662

will be evaluated in future sequencing studies. Six different *mrjp3* alleles were identified in a single honeybee colony. In experiments with different colonies from different locations, previous authors identified more than 10 different alleles ranging from 380 to 550 bp (Albert et al., 1999b).

Changes in the allelic frequencies, observed in different RJ production cycles, could be indicative that selection of these alleles was occurring. Chen et al. (2005) investigated genetic variation at 10 microsatellite loci and based on allele frequencies they suggested that seven alleles have a positive correlation in the RJ yield.

The evaluation of queens is carried out by the production of their offspring (Rinderer, 1977; Collins et al., 1984). The offspring's genetic merit is dependent on the breeding value of its parents (Bergmann, 2001). RJ production shows the offspring's workers phenotype, being influenced by the genotype-environment interaction. Real genetic potentiality of those honeybees will be known by minimizing environmental variations.

The expected difference between the progeny of an individual and the original population is one-half the individual's breeding value, and can be estimated as expected progeny differences (EPD), which is one-half the animal's estimated breeding value or genetic value. Thus, the EPD values reflect the extent to which the queens tested in the mini-hives transmitted their RJ production traces to their progeny.

If the selected individuals are genetically superior to the population average for a trait, then their offspring will be expected to perform above average for that trait (Bergmann, 2001). With the results obtained in this study, a strategy to intensify the RJ production would be to increase the frequency of *C*(~480 bp), *D* (~510 bp) and *E* (~530 bp) alleles in the RJ producing colonies. This in turn will require genotyping of queens using their male offspring. Then, the queens with superior genetic value for RJ production can be selected and produce queen offspring, whose colonies will be RJ high producers. However, it is important to emphasize that only 36.85% of genetic variation was used in this process. With the genotyping of males, which provide sperm to be used for the instrumental insemination of queens, genetic control of the crosses can be attained.

In summary, our data suggest that the *mrjp3* alleles (*C*, *D* and *E*) influence the genetic value of queens for RJ production. The identification of three alleles of the *mrjp3* repetitive region brings new perspectives in the use of these molecular markers for queen selection to increase RJ production. However, it is recommended that studies with homozygous colonies for the *C*, *D* and *E* alleles be made to measure their effect in RJ production and verify their interaction.

The findings reported in this paper have important implications for future research on the use of the *mrjp3* repetitive region as a molecular marker set in a queen selection program to increase RJ production in Africanized honeybees. These findings have relevance for beekeepers, given that demand for RJ has been increasing.

4.1. Population structure

Ho reduction for *mrjp3* indicates that there is a great potential in using this region as a molecular marker for RJ production. These results were consistent with the variance analyses, indicating that the repetitive region of

mrjp3 influences the queen's genetic value for RJ production.

The analyzed Africanized honeybees producing RJ show high genetic diversity indicating that they have selection potential. Furthermore, the high degree of allelic variability observed for the analyzed loci may also serve as genetic markers for population genetic studies on Africanized honeybees.

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Utilisation potentielle des protéines majeures de la gelée royale comme marqueurs moléculaires pour la production de gelée royale dans les colonies de l'abeille africanisée.

***Apis mellifera* / *MRJP-3* / variabilité génétique / polymorphisme / EPD**

Zusammenfassung – Die mögliche Nutzung der Hauptproteine des Gelée Royale (Major Royal Jelly Protein, MRJP) als molekularer Marker für die Gelée royale-Produktion in Völkern der Afrikanisierten Honigbiene. Gelée royale (GR) stellt die wichtigste Quelle an essentiellen Aminosäuren, Lipiden, Vitaminen, Acetylcholin (AcH) und anderen Nährstoffen im Larven- und Königennenfutter dar. Die Hauptproteinbestandteile des Gelée Royale (Major Royal Jelly Proteins, MRJPs) stellen 82–90 % des Gesamtproteingehalts von GR dar und sind bereits eingehend untersucht und charakterisiert. Die genetische Variabilität in der repetitiven Region von MRJP kann als möglicher Marker für die Selektion von Hongbienen in der GR-Produktion genutzt werden. Wir untersuchten hier die genetischen Varianten in drei verschiedenen MRJP-Genen (*mrjp3*, *mrjp5* und *mrjp8*) und die Möglichkeit, diese als Marker für die GR-Produktion bei Afrikanisierten Honigbienen zu nutzen. Ammenbienen Afrikanisierter Bienenvölker wurden in der Bienenhaltung der Landesuniverstät Maringá in Brasilien aus insgesamt 34 Minivölkern gesammelt, die hinsichtlich der GR-Produktion bewertet wurden. Die Königinnen dieser Minivölker stammten von 15 Mutterköniginnen ab und wurden für die Produktion von Larven zur Königinnen-Tochter-Produktion genutzt. Diese Königinnen waren natürlich verpaart und wurden anschließend in die Minivölker eingeführt. Anhand von jeweils zwei Zyklen wurden die Bienenvölker hinsichtlich ihrer GR-Produktion bewertet. Mittels genomischer Primer wurden die repetitiven Regionen der *mrjp3*, *mrjp5* und *mrjp8* Gene amplifiziert. Die drei Loci waren polymorph und wiesen insgesamt 17 Allele auf (Abb. 1). Für die *mrjp3* repetitive Region fanden wir sieben Allele mit einer Längenvariation von 410 bis 610 Basenpaaren (bp), für die *mrjp5* repetitive Region waren es sechs (570–720 bp) und am *mrjp8* Locus waren es vier Allele (360–420 bp). Ein positiver Zuchtwert (Expected Progeny Difference, EPD) ist vorteilhaft für die Selektion von Merkmalen wie der GR-Produktion. Der Bestimmungskoeffizient (R^2) für die signifikanten Allele der repetitive Region von *mrjp3* zeigte, dass 36,85 % der EPD-Variation durch die Allele *C*, *D* und *E* erklärt werden kann. Diese drei Allele haben einen starken Effekt auf den genetischen Wert der GR-Produktion. Der F_{IS} Wert (0,2025) zeigt einen Homozygotenüberschuss an, der durch die bereits erfolgte Selektion der Königinnen für die GR-Produktion erklärt werden kann. Der niedrige F_{ST} Wert (0,0068) deutet an, dass die Ammenbienen der zwei Zyklen der GR-Produktion genetisch nicht differenziert sind. Diese Ergebnisse weisen darauf hin, dass Untersuchungen mit Völkern, die für die Allele *C*, *D* und *E* homozygot sind, durchgeführt werden sollten, um ihre Effekte auf die GR-Produktion messen und ihre Interaktionen bestimmen zu können. Des Weiteren zeigt die Untersuchung auf, dass die *mrjp3* repetitive Region als molekularer Marker für die Selektion von Königinnen von Imkern in einem Zuchtprogramm zur Steigerung der GR-Produktion in Afrikanisierten Bienen genutzt werden kann.

***Apis mellifera* / *MRJP-3* / genetische Variabilität / Polymorphismus / EPD**

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