Chapter 3

Improvement and Selection of Honeybees Assisted by Molecular Markers

Maria Claudia Colla Ruvolo-Takasusuki,
Arielen Patricia Balista Casagrande Pozza,
Ana Paula Nunes Zago Oliveira,
Rejane Stubs Parpinelli,
Fabiana Martins Costa-Maia, Patricia Faquinello and
Vagner de Alencar Arnaut de Toledo

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62426

Abstract

Royal jelly is an important apiarian product for honeybees and has been used as an important ingredient to human health and healthy life style. Because of its wide use, there is great demand in their production. As royal jelly is a secretion of the cephalic glands of bees and it is produced at a certain age of the workers, it is necessary to perform the selection of producing queens to increase the amount produced. The employment of molecular markers is a tool that can be used to identify the genotypes of the best producers. Among the molecular markers, one of them called MRJP3 (Major Royal Jelly Protein 3) has been used in the Program of Improvement of *Apis mellifera* Royal Jelly Producing (PIAMRJP), State University of Maringá, Brazil. This molecular marker has been efficient in genotyping queens' royal jelly producers. Combined with classical breeding studies, the selection of queens assisted by MRJP3 marker has allowed to keep the selected genotypes for royal jelly production since 2006 (10 years). In this chapter, we present the main aspects of royal jelly, the hypopharyngeal glands, the major proteins of royal jelly and how it can be used as molecular markers.

Keywords: *Apis mellifera*, MRJP3, microsatellite, royal jelly, honeybee queen

1. Introduction

Genetic improvement in any organism has the objective of increasing the gene frequencies of the economic importance of loci to be selected in the population. In relation to bees, this means increasing the frequency of the number of colonies that produce above the average generation from which the selection was made.

The production of royal jelly and honey production are the result of the combined work of the workers [1], and therefore the entire colony becomes a unit of selection, where the assessment of improved queens is carried out by production workers' progeny [1,2]. Royal jelly production studies allowed to observe considerable variation in its production by Africanized honeybees [3,4]. These results show the need for selection of queens [5].

Selection of bees with genotypes involves the use of improved queens' replacement techniques and instrumental insemination and for molecular marker-assisted selection. There are few data in the literature linking molecular markers for the production of royal jelly. The identification and characterization of several loci of the major royal jelly proteins (MRJPs) allowed using one of the loci *Mrjp3* as a molecular marker for selection of *Apis mellifera* queens Africanized. Early studies by selecting queens and genotyping the best producers began in 2006, in the apiary of the State University of Maringá, Brazil. The first study associating the MRJP3 marker with royal jelly production was realized by [6].

High variability in major royal jelly proteins (MRJPs), especially MRJP3 to contain microsatellite regions, indicated a great potential of using these proteins, particularly microsatellite regions occurring in the *Mrjp3* gene as a marker for selecting studies for the improvement of the production of royal jelly. Subsequently, other researches were conducted using classic improvement parameters such as MRJP3 marker. The results to date have shown that this marker is important to genotype producing arrays of royal jelly.

Thus, this chapter shows the importance of royal jelly to honey bees and to human health, the importance of improving assisted by molecular markers and the results obtained with the selection of royal jelly producing queens and genotyped for MRJP3.

2. Royal jelly

Royal jelly is secreted by the mandibular and hypopharyngeal glands located at the head of honeybees [7]. Hypopharyngeal gland secretion has a clear, water-like consistency and is rich in protein, while the mandibular gland produces a white secretion with milky consistency [7, 8]. Royal jelly can be described as a viscous substance, white-yellowish or grayish white, slightly opalescent with a characteristic pungent odor, although not unpleasant or rancid (**Figure 1**) [9–10]. These glands have the highest growth rate and activity of worker nurses between days 10 and 14 [11–15]. The development of the glands can be influenced by internal factors of the colony such as offspring and population density and external factors such as foraging and enabling bees to adapt quickly to the colony [13,16–18].

http://dx.doi.org/10.5772/62426

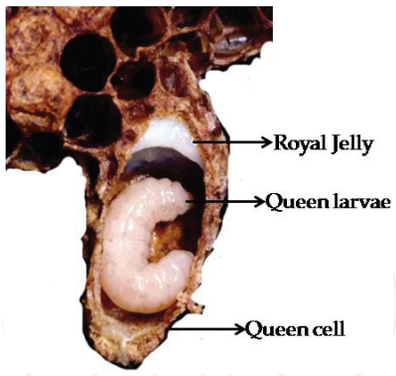

Figure 1 Queen cell with queen larvae of *A. mellifera* and royal jelly.

Royal jelly is a glandular secretion recognized for complex composition, containing minerals, proteins, amino acids, steroids, phenols, carbohydrates, vitamins, lipids, acetylcholine and other unknown substances [9,19]; it is important too in reproduction and development. Royal jelly is the larval food until the third day of development when it becomes the exclusive food of the queen throughout her life, guaranteeing fertility and increased longevity. From third day, worker larvae are fed a mixture of honey, pollen and water, known as brood food; drones receive food brood and royal jelly [20].

The average lifespan of queens of *A. mellifera* live is 1 to 2 years [21]; they become sexually mature 6 days after emergence, mate about 17 drones and store all of the sperm needed to fertilize eggs for the duration of their lifespan [22]. Few drones rear in the summer, but a slight rise in drone rearing occurs during swarming [20]. Queens can lay 1500–2000 eggs per day throughout their lives [23,24], depending on the needs of the hive and environmental factors, while a large number of workers (sterile females) are responsible for maintaining the hive.

Due to the fertility and longevity of queens, related to the exclusive feeding with royal jelly, studies have been conducted considering similar effects in humans. Some beneficial effects have been attributed to consumption of royal jelly, as elimination of physical and mental fatigue, appetite normalization activation of brain function, improved vision, increased resistance against viral infections and skin rejuvenation [10].

Owing to considerable amount of proteins, free amino acids, lipids, vitamins, sugars and bioactive substances such as 10-hydroxy-trans-2-decenoic acid and antibacterial protein 350 KDa proteins, royal jelly becomes an ingredient for various healthy foods [25]. Review carried out by [25] shows several studies have reported that the royal jelly exhibits beneficial physiological and pharmacological effects in mammals, including vasodilative and hypotensive activities, antihypercholesterolemic activity and antitumor activity.

3. Molecular marker Major Royal Jelly Protein 3

Royal jelly contains from 12 to 15% crude protein consisting of soluble proteins in water and water-insoluble proteins. The fraction of soluble proteins of royal jelly produced by the hypopharyngeal and mandibular glands contains several major proteins with molecular

weight between 47 and 80 kDa [26] besides a small amount of minor proteins such as antibiotics and peptides [27,28]. Those proteins constitute the main group of major royal jelly proteins. The MRJPs represent between 82 and 90% of the total proteins of larval jelly [19]. Some regions of MRJPs can be focused on amino acids rich in nitrogen, thus high levels of nitrogen would be stored in MRJPs. The availability of nitrogen can be critical to the rapid growth of young larvae, as well as for the development of the queen [29]. These observations support the hypothesis that MRJPs have an important role in the nutrition of bees [19].

Major royal jelly protein-3 can be visualized on denaturing SDS-PAGE electrophoresis in head extracts of worker nurses (10–14 days old) or royal jelly (**Figure 2**). The polymorphism was estimated by [30].

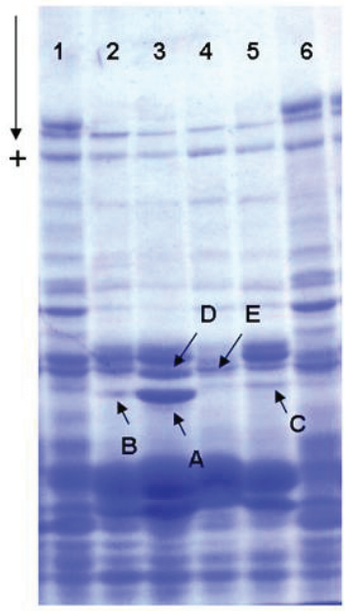

Figure 2 Denaturating SDS-PAGE electrophoresis showing MRJP3 polymorphism in extracts of head of *A. mellifera* nurse. A, B, C, D, E = alleles. Source: Baitala et al. (2013).

MRJPs genes encoding a group of proteins that have a common evolutionary origin with Yellow proteins of *Drosophila melanogaster* [31]. Genome of *Drosophila* encodes at least seven family members of Yellow proteins [32], whose loci are involved in the larval pigmentation [33], unlike the MRJPs that have nutritional function of larvae.

The genes encoding key proteins of royal jelly began to be identified in studies [34] and [35]. After these pioneering studies, several studies have been published in order to identify and characterize new genes encoding the MRJPs proteins [19,31,36,37]. The availability of the complete genome of *Apis mellifera* [38] made it possible to identify new genes encoding proteins of the family MRJP [29].

Since the first study were identified nine proteins in MRJPs *A. mellifera* (MRJP1, MRJP2, MRJP3, MRJP4, MRJP5, MRJP6, MRJP7, MRJP8, and MRJP9) besides an incomplete polypeptide, MRJPψ, encoded by a pseudogene. The genes encoding these proteins are located on chromo-

some 11 [29]. Classification of *A. mellifera* MRJPs has been performed based on the N-terminal sequences of purified protein and cDNA sequences available in the cDNA library.

Analysis using PCR and DNA sequencing showed that the different alleles of the gene encoding MRJP3 protein differ in length as a result of a varying number of repeating basic units in a region of the *Mrjp3* gene [31,36]. The authors attributed the polymorphism of these proteins is a consequence of the presence of a region with varied number of repetitive sequences in tandem (microsatellite). These markers are comprised of a variable number of identical sequences having from 15 to 100 base pairs, in tandem and repeated up to 50 times. The molecular differences in four types of MRJP3 have shown that the polymorphism of these proteins is linked to the size variability, which is determined genetically by bees from the same colony [19].

The *Mrjp3* is a polymorphic locus that has been identified by DNA sequencing five alleles and PCR analysis identified at least 10 alleles of different sizes [36]. This study also revealed a Mendelian inheritance and high variability of the genomic locus of MRJP3.

Although *A. mellifera* [19,29,31,35–37] and other bees of the genus *Apis* [39–43] having the MRJPs are characterized, data in the literature on the use of MRJPs as molecular markers for selection associated with the improvement of royal jelly production are still scarce.

4. *Apis mellifera* queens' selection using MRJP3 marker

The genetic improvement has the aim to increase the frequencies of desirable genes of the loci of economic importance to be selected in a population [44]. Thus, the genetic breeding of bees has the goal to increase the frequency of the number of colonies that produce above the average generation from which the selection was made. Selection of honeybees with superior genotypes involves the use of improved queens replacement techniques, instrumental insemination and molecular marker-assisted selection [45].

Selection of queens is carried out by genetic evaluation, which depends on the estimation of the components of (co)variance and genetic parameters for identification of genetically higher bees. Royal jelly production evaluated by Bayesian inference had a heritability estimate of 0.27% acceptance, 0.10 for the production of royal jelly per colony and 0.55 per dome [5]. The analyses performed by these authors showed that selection of queens can increase the production of royal jelly by colony, larval acceptance and production of royal jelly by the dome, and the external factors can modify the gene expression of individuals.

However, there are few data in the literature associating molecular markers for the production of royal jelly. One of the first molecular studies carried out to obtain DNA markers related to production of royal jelly was performed by [46]. These authors reviewed a total of 96 alleles produced for 10 microsatellite loci and according to the observed allele frequency for some alleles, it was possible to identify seven alleles that can be used as markers bees producing large quantities of royal jelly. The use of molecular markers, particularly microsatellites, can

contribute to detect polymorphisms that might be useful to identify colonies of bees with high productivity of royal jelly.

High variability of MRJPs proteins and especially the MRJP3 to contain microsatellite region shows a great potential to use MRJP family proteins as markers for selection of producing queens for improving the production of royal jelly. Use of MRJPs as molecular markers in studies of population genetics and as selection markers associated with the improvement of royal jelly production is still scarce. Some researches have shown that this molecular marker is efficient to be used in the selection of royal jelly-producing queens.

Africanized honeybees selected for royal jelly production showed high allelic variability for the locus *Mrjp3* (**Figure 3**), showing the potential of this marker for selection [6]. In this research analyses of multiple linear regressions with EPD (expected progeny differences) values for royal jelly production were performed. The variance analyses indicated that the *Mrjp3* repetitive region influenced the genetic value of queen's offspring for royal jelly production. The determination coefficient (R2) 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. Authors concluded that the three alleles present a considerable genetic effect on the variation of royal jelly production.

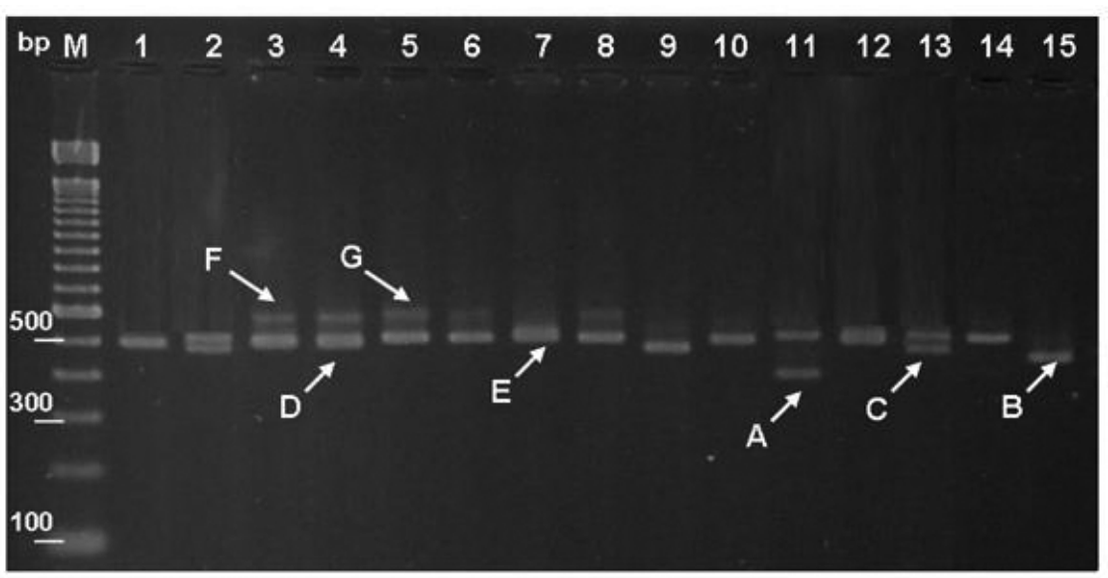

Figure 3 Molecular marker MRJP3. Number = *A. mellifera* DNA. A, B, C, D, E, F, G = *Mrjp3* alleles. M = molecular weight marker. Source: Baitala et al (2010).

Continuing the process of selection and the Program of Improvement of *Apis mellifera* Royal Jelly Producing (PIAMRJP), State University of Maringá, Brazil, alleles of the locus *Mrjp3* descendants queens, those selected by [6], were evaluated in 2011 [47]. Results showed that the royal jelly-producing queens had a high degree of genetic diversity and excess homozygous alleles. The highest frequencies were estimated for *Mrjp3 D* and *E* alleles 0.3357 and 0.3107,

http://dx.doi.org/10.5772/62426

respectively, showing that the selection process of queens royal jelly producing these alleles are being maintained and only the *C* allele had a low frequency of 0.0321.

Results obtained by [47] confirm those obtained by [6], the locus *Mrjp3* and their alleles *C*, *D* and *E* influence the genetic value for producing royal jelly; however, the real role of MRJP3 these bees has not yet been identified. The sequencing of *Mrjp3* of *A. mellifera* Africanized alleles in PIAMRJP was performed [48]. Homology and identity of these sequences were compared with the sequences deposited in the database for *A. mellifera* (**Figure 4**). Alleles *Mrjp3* detected showed high identities with alleles deposited in BLAST system. Alleles *Mrjp3 C, D* and *E* are being maintained in the genome of the selected matrices queens.

High similarity among the *Mrjp*3 alleles analyzed and those described in other studies show that the *Mrjp3* locus is conserved among species and subspecies of *Apis*. Similar results were obtained by [40]. These authors found that there are high similarity sequences and intron-exon have the same structure between four species *A. mellifera, A. cerana, A. dorsata* and *A. florea.*

The selection of royal jelly-producing queens may be promoting a selection of these reproduction bees, can alter the genetic characteristics of a given population, can be influenced by the process of transmission of these genes generation to generation [49]. However, it is important to maintain a degree of genetic variation, which results in a larger potential response to selective improvement [50].

In addition to the continuous genotyping of royal jelly–producing queens to locus *Mrjp3*, we developed a study to see if the mitochondrial DNA (mtDNA) of Africanized bees *A. mellifera* maintained in the breeding program have African or European origin. This research was performed by [51], using matrices producing royal jelly.

Mitochondrial DNA was analyzed using the molecular marker PCR-RFLP with specific primers and restriction enzymes to European and African honey bees. Analyses were performed with workers' daughters of royal jelly-producing queens in 2013, seven years after the beginning of the PIAMRJP started in 2006. After this period of selection and analysis of genetic parameters, alleles *C, D and E* are being maintained in queens, evidencing the role in royal jelly production. Queens selected for royal jelly production showed predominance of African mtDNA; therefore, genes of maternal origin are African. Use of microsatellite markers and mtDNA can be used in bee improvement programs to ensure the genetic origin of queens and verify the efficiency of Program of Improvement of *Apis mellifera* Royal Jelly Producing [51].

The employment of molecular markers in selection programs and improvement of honey bees for royal jelly production is efficient because it allows keeping genotypes of interest to ensure the highest productivity of the hives. The microsatellite marker MRJP3 has shown good results as a tool to verify the genotypes of producing matrices, facilitating identification and maintenance of the hives in the apiary of the Program of Improvement of *Apis mellifera* Royal Jelly Producing.

```
A.m.protein             ------------------------------------------------CAATCAGAATGCT 13
A.m.protein3            ------------------------------------------------CAATCAGAATGCT 13
A.m.protein3-like       ----------------------------CTGGCAATCAGAATGCTGGCAATCAGAATGCT 32
A.m.carnicaprotein3     --------------------GGAAGATATCACAATCAGAATGCTGGCAATCAGAATGCT 39
MRJP3-C                 ATTATCATTTTGCCTGTTTACCATTCCTCTTGTTATCATTCTGTCTGTTACCATTTTGCT 60
                                                                    * **   ****

A.m.protein             GGCAATCAGAATGCTGACAATCA----GAATGCTGACAATCAGAATGCTAACAATCAGAA 69
A.m.protein3            GGCAATCAGAATGCTGACAATCA----GAATGCTGACAATCAGAATGCTAACAATCAGAA 69
A.m.protein3-like       GGCAATCAGAATGCTGACAATCA----GAATGTTGACAATCAGAATGCTAACAATCAGAA 88
A.m.carnicaprotein3     GGCAATCAGAATGCTGACAATCA----GAATGCTGACAATCAGAATGCTAACAATCAGAA 95
MRJP3-C                 TGTTATCATTTTGTCTATTACCATTTTGCTTGTTATCATTCTGTTTGTTACCATTTTGTC 120
                         *  ****   **   *  * **    *  ** *  ** ** *  ** ** ** *  *

A.m.protein             TGCTGATAATCAGAATGCTAACAAACAAAATGGTAATAGACAAAATGATAACAGACAGAA 129
A.m.protein3            TGCTGATAATCAGAATGCTAACAAACAAAATGGTAATAGACAAAATGATAACAGACAGAA 129
A.m.protein3-like       TGCTGATAATCAGAATGCTAACAAACAAAATGGTAATAGACAAAATGGTAACAGACAGAA 148
A.m.carnicaprotein3     TGCTGATAATCAGAATGCTAACAAACAAAATGGTAATAGACAAAATGATAACAGACAGAA 155
MRJP3-C                 TGTTACCATTTTGCTTGTTATCATTCTGTCTGTTACCATTTTGCTTGTTATCATTCTGTC 180
                        ** *   * *  *  ** ** **  *    ** **  *       ** ** **  * *

A.m.protein             TGATAACAAGCAAAATGGTAACAGACAGAATGATAACAAGCAAAATGGTAACAGACAGAA 189
A.m.protein3            TGATAACAAGCAAAATGGTAACAGACAGAATGATAACAAGCAAAATGGTAACAGACAGAA 189
A.m.protein3-like       TGATAACAAGCAAAATGGTAACAGACAGAATGATAACAAGCAAAATGGTAACAGACAGAA 208
A.m.carnicaprotein3     TGATAACAAGCAAAATGGTAACAGACAGAATGATAACAAGCAAAATGGTAACAGACAGAA 215
MRJP3-C                 TGTTACCATTTTGCTTGTTATCATTCTGTCTGTTACCATTTTGCTTGTTATCATTCTGTC 240
                        ** ** **       ** ** **  * *  ** ** **       ** ** **  * *

A.m.protein             TGATAACA--------AGCAAAAT-------GGTAACAGACAAAATGGTAACAA------ 228
A.m.protein3            TGATAACA--------AGCAAAAT-------GGTAACAGACAAAATGGTAACAA------ 228
A.m.protein3-like       TGATAACA--------AGCAAAAT-------GGTAACAGACAGAATGATAACAAGCAAAA 253
A.m.carnicaprotein3     TGATAACA--------AGCAAAAT-------GGTAACAGACAGAATGATAACAAGCAAAA 260
MRJP3-C                 TGTTATCATTTTGTCTATTACCATTTTGTTTGTTAGCATTCTGATTATCAGCATTCTGAT 300
                        ** ** **        *  *  **       * ** **  *  * *   * **

A.m.protein             ---------ACAGAATGATAACAAGCAAAATGGTAACAGACAGAATGATAACAAGAGGAA 279
A.m.protein3            ---------ACAGAATGATAACAAGCAAAATGGTAACAGACAGAATGATAACAAGAGGAA 279
A.m.protein3-like       TAGTAACAGACAGAATGATAACAAGCAAAATGGTAATAGACAAAATGGTAACAAACAGAA 313
A.m.carnicaprotein3     TGGTAACAGACAGAATGATAACAAGCAAAATGGTAACAGACAAAATGGTAACAAACAGAA 320
MRJP3-C                 TGTTAGCATTCTGATTGTCAGCATTCTGATTGTCAGCATTCTGATTGCCAGCATTCTGAT 360
                                 * ** **  * **  *  * **  *  *  *  * **  * **    **

A.m.protein             TGGTAACAGGCAAAATGATAAT-------------CAA---------------------- 304
A.m.protein3            TGGTAACAGGCAAAATGATAAT-------------CAA---------------------- 304
A.m.protein3-like       TGATAACAAGCAAAATGGTAATA----------GACAAAATGATAACAAGCAAAATGGTA 363
A.m.carnicaprotein3     TGATAACAAGCAAAATGATAATA----------GACAAAATGATAACAAGCAAAATGGTA 370
MRJP3-C                 TGCCAGCATTCTGATTGCCAGTCTCTTCCGGTTGCCGAGCTGTT--CCTGCATTGTGATC 418
                        **  * **  *  * **  * *            * *

A.m.protein             ------------------------------------------------
A.m.protein3            ------------------------------------------------
A.m.protein3-like       ACAGACAGAATGATAA------------------------------- 379
A.m.carnicaprotein3     ACAGACAGAATGATAACAAGAGGAATGGTAACAGGCAAAATGATAAT 417
MRJP3-C                 ACTTCCCGGAAGGGA------------------------------- 433
```

Figure 4 Alignment of sequences similar to the *Mrjp3 C* allele performed using ClustalW2 (EMBL-EBI); sequences include *A. mellifera* major royal jelly protein mRNA, complete cds (GU434675.1); *A. mellifera* major royal jelly protein 3 (*Mrjp3*), mRNA (NM_001011601.1); *A. mellifera carnica* major royal jelly protein 3 *(Mrjp3)* gene, complete cds (AY663104.1); and PREDICTED: *A. mellifera* major royal jelly protein 3-like (LOC727045), partial mRNA **(**XM_001122757.2). "*" = nucleotides identical in all of the aligned sequences. Source: Casagrande-Pozza (2011).

http://dx.doi.org/10.5772/62426

Author details

Maria Claudia Colla Ruvolo-Takasusuki[1*], Arielen Patricia Balista Casagrande Pozza[1], Ana Paula Nunes Zago Oliveira[1], Rejane Stubs Parpinelli[2], Fabiana Martins Costa-Maia[3], Patricia Faquinello[4] and Vagner de Alencar Arnaut de Toledo[2]

*Address all correspondence to: mccrtakasusuki@uem.br

1 Biotechnology, Genetics and Cell Biology Department, State University of Maringá, Av. Colombo, Maringá, PR, Brazil.

2 Animal Science Department, State University of Maringá, Av. Colombo, Maringá, PR, Brazil.

3 Federal University of Technology – Paraná, Dois Vizinhos, PR, Brazil

4 Federal University of Goiás – Rodovia GO, Ceres, GO, Brazil

References

[1] Collins A.M.; Rinderer T.E.; Harbo J.R.; Brown M.A. Heritabilities and correlations for several characters in the honey bee. The Journal of Heredity, 1984;75:135–140.

[2] Rinderer T.E. Measuring the heritability of characters of honeybees. Journal of Apicultural Research, 1977;16:95–98.

[3] Mouro G.F.; Toledo V.A.A. Evaluation of *Apis mellifera Carniolan* and Africanized honey bees in royal jelly production. Brazilian Archives of. Biology and Technology. 2004;47:469–476.

[4] Garcia R.H.C.; Nogueira-Couto R.H. Produção de geléia real por abelhas *Apis mellifera* italianas, africanizadas e descendentes de seus cruzamentos. Acta Scientiarum Animal Sciences, 2005;27(1):17–22.

[5] Faquinello P.; Toledo V.A.A.; Martins E.N.; Oliveira C.A.L.; Sereia M.J.; Costa-MaiaF. M.; Ruvolo-Takasusuki M.C.C. Parameters for Royal Jelly Production in Africanized Honeybees. Sociobiology, 2011;57(3):495–509.

[6] Baitala T.V.; Faquinello P.; Toledo V.A.A.; Mangolin C.A.; Martins E.N.; Ruvolo-Takasusuki M.C.C. Potential use of major royal jelly proteins (MRJPs) as molecular markers for royal jelly production in Africanized honeybee colonies, Apidologie, 2010;41:160–168.

[7] Haydak M.H. Honey bee nutrition. Annual Review of Entomology, 1970;15:143–156.

[8] Beetsma J. The process of queen-worker differentiation in the honeybee. Bee World, 1979;60(1):24–39.

[9] Garcia-Amoedo L.H.; Almeida-Muradian L.B. Physicochemical composition of pure and adulterated royal jelly. Química Nova, 2007;30(2):257–259.

[10] Koshio S.; Almeida-Muradian L.B. de. Aplicação da CLAE para determinação do ácido 10-hidróxi-2-decenóico (10-HDA) em geléia real pura e adicionada a mel brasileiro. Química Nova, 2003;26(5):670–673.

[11] Takenaka T.; Kaatz H.H. Protein synthesis by hypopharyngeal glands of worker honey bees. In: Eder J; Rembold H (eds.). Chemistry and Biology of Social Insects. München: Peperny, 1987. pp. 166–167.

[12] Crailsheim K.; Stolberg E. Influence of diet, age and colony condition upon intestinal proteolytic activity and size of the hypopharyngeal glands in the honeybee (*Apis mellifera* L.*).* Journal of Insect Physiology, 1989;35:595–602.

[13] Huang Z.Y.; Otis G.W. Factors determining hypopharyngeal gland activity of worker honey bees. Insectes Sociaux, 1989;36(4):264–276.

[14] Knecht D.; Kaatz H. H. Patterns of larval food production by hypopharyngeal glands in adult worker honey bees. Apidologie, 1990;21:457–468.

[15] Naiem E.S.; Hrassnigg N.; Crailsheim K. Nurse bees support the physiological development of young bees (*Apis mellifera* L.). Journal of Comparative Physiology, 1999;169:271–279.

[16] Simpson J.; Riedel I.B.M.; Wilding N. Invertase in the glands hypopharyngeal of the honeybee, Journal of Apicultural Research, 1968;7:29–36.

[17] Brouwers E.V.M. Measurement of hypopharyngeal gland activity in the honeybee, Journal of Apicultural Research, 1982;21:193–198.

[18] Silva de Moraes R.L.M.; Cruz-Landim C. Influência da densidade populacional no comportamento dos núcleos das glândulas hipofaringeanas de *Apismellifera* (Hymenoptera, Apidae). Naturalia, 1984;9:27–33.

[19] Schmitzová J.; Klaudiny J.; Albert S.; Schröder W.; Schreckengost W.; Hanes J.; Júdová J.; Simúth J. A family of major royal jelly proteins of the honeybee *Apis mellifera* L. Cellular and Molecular Life Sciences, 1998;54:1020–1030.

[20] Winston M. The biology of the honey bee. Cambridge: Harvard University Press, 1991. 294 p.

[21] PageRE; PengCYS Aging and development in social insects with emphasis on the honey bee, *Apis mellifera* L. Experimental Gerontology, 2001;36:695–711.

[22] Woyke J. Natural and artificial insemination of queen honey bees. Pszczelnicze Zeszyty Nauwoke, 1960;4:183–273.

[23] Merrill J.H. Observations on brood-rearing. American Bee Journal, 1924;64:337–338.

[24] Nolan WJ. The brood-rearing cycle of the honey bee. Bulletin of U.S. Department of Agriculture, 1925;1349:1–56.

[25] Oršolić N. Royal jelly: component efficiency, analysis, and standardization. Arh Hig Rada Toksikol. 2013;64(3):445–461. doi: 10.2478/10004-1254-64-2013-2332.

[26] Hanes J.; Smúth J. Identification and partial characterization of the major jelly protein of the honeybee (*Apis mellifera* L.). Journal of Apicultural Research, 1992;31:22–26.

[27] Fujiwara S.; Imai J.; Fugiwara J.; Yaeshima T.; Kobayashi K.A. potent antibacterial protein in a royal jelly. Journal of Biological Chemistry, 1990;265:11333–13337.

[28] Biliková K.; Wu G.; Simúth J. Isolation of peptide fraction from honeybee royal jelly as anti faul brood factor. Apidologie, 2001;32:175–283.

[29] Drapeau M.D.; Albert S.; Kucharski R.; Prusko C.; Maleszka R. Evolution of the Yellow/Major royal jelly protein family and the emergence of social behavior in honey bees. Genome Research, 2006;16:1385–1394.

[30] Baitala T.V.; Nakasugui L.F.; Cantagalli L.B.; Toledo V.A.A.; Ruvolo-Takasusuki M.C.C. Major royal jelly protein-3 polymorphism in Africanized honeybee colonies producing royal jelly. Magistra, 2013;25(1):30–36.

[31] Albert S.; Bhattacharya D.; Kaludiny J.; Schmitzová J.; Simúth J. The family of major royal jelly proteins and its evolution. Journal of Molecular Evolution, 1999a;49:290–297.

[32] Maleszka R.; Kucharski R. Analysis of Drosophila yellow-B cDNA reveals a new family of proteins related to the royal jelly proteins in the Honeybee and to an orphan protein in an unusual bacterium *Deinococcus radiodurans*. Biochemical Biophysics and Research Communications, 2000;270(3):773–776.

[33] Walter M.F.; Black B.C.; Afshar G.; Kermabon A.Y.; Wrigth T.R.F.; Biessmann H. Temporal and spatial expression of the yellow gene in correlation with cuticle formation and DOPA decarboxylase activity in *Drosophila* development. Developmental Biology, 1991;147:32–45.

[34] Klaudiny J.; Hanes J.; Kulifajova J.; Albert S.; Simúth J. Molecular cloning of two cDNAs from the head of the nurse honey bee (*Apis mellifera* L.) coding for related proteins of royal jelly. Journal of Apicultural Research, 1994;33:105–111.

[35] Albert S.; kaudiny J.; simúth J. Newly discovered features of the updated sequence of royal jelly protein RJP57-1; longer repetitive region on C-terminus and homology to *Drosophila melanogaster* yellow protein. Journal of Apicultural Research, 1996;35:63–68.

[36] Albert S.; Klaudiny J.; Simúth J. Molecular characterization of MRJP3, highly polymorphic protein of honeybee (*Apis mellifera*) royal jelly. Insect Biochemistry and Molecular Biology, 1999b;29:427–434.

[37] Albert S.; Klaudiny J. The MRJP/YELLOW protein family of *Apis mellifera*: Identification of new members in the EST library. Journal of Insect Physiology, 2004;50:51–59.

[38] The Honey Bee Genome Sequencing Consortium. Insights into social insects from the genome of the honeybee *Apis mellifera*. Nature, 2006;443(26):931–49.

[39] Albert S.; Schmitz J. Characterization of major royal jelly protein-like DNA sequences in *Apis dorsata*. Journal of Apicultural Research, 2002;41(3–4):75–85.

[40] Srisuparbh D.; Klinbunga S.; Wongsiri S.; Sittipraneed S. Isolation and Characterization of Major Royal Jelly cDNAs and Proteins of the Honey Bee (*Apiscerana*). Journal of Biochemistry and Molecular Biology, 2003;36(6):572–579.

[41] Albertová V.; Su S.; Brockmann A.; Gadau, J; Albert S. Organization and Potential Function of the *mrjp3* Locus in Four Honeybee Species. Journal of Agricultural and Food Chemistry, 2005;53:8075–8081.

[42] Imjongjirak C.; Klinbunga S.; Sittipraneed S. Cloning, expression and genomic organization of genes encoding major royal jelly protein 1 and 2 of the honey bee (*Apis cerana*). Journal of Biochemistry and Molecular Biology, 2005;38(1):49–57.

[43] Su S.; Albert S.; Chen S.; Zhong B. Molecular cloning and analysis of four cDNAs from the heads of *Apis ceranacerana* nurse honeybees coding for major royal jelly proteins. Apidologie, 2005;36:389–401.

[44] Bergmann J.A.G. Avaliação genética. In: PEREIRA J.C.C. (eds.). Melhoramento genético aplicado à produção animal. 3. ed. Belo Horizonte: FEPMVZ, 2001. pp. 502–515.

[45] Harbo J.R.; Rinderer T.E. Breeding and genetics of honeybees. Beekeeping in the United States. 2005. Disponível em: http://maarec.cas.psu.edu/bkCD/HBBiology/breeding_genetics.htm. Acess in 11/19/2007.

[46] Chen S; LI J.; Zhong B.; Su S. Microsatellite analysis of royal jelly producing traits of Italian honeybee (*Apis mellifera* Liguatica). Acta Genetica Sinica, 2005;32(10):1037–1044.

[47] Parpinelli R.S.; Ruvolo-Takasusuki M.C.C.; Toledo V.A.A. MRJP microsatellite markers in Africanized *Apis mellifera* colonies selected on the basis of royal jelly production. Genetics and Molecular Research, 2014;13(3):6724–6733. DOI: http://dx.doi.org/10.4238/2014.August.28.16

[48] Casagrande-Pozza A.P.B. Sequenciamento dos alelos das proteínas principais da geléia real de abelhas *Apis mellifera* africanizadas. UEM: Universidade Estadual de Maringá, Maringá, 2011.52 p. Dissertação (Mestrado em Zootecnia).

[49] Falconer D.S. (1987) Introdução à genética quantitativa, Universidade Federal de Visçosa, Minas Gerais.

[50] Page R.E., Kerr W.E. Honey bee genetics and breeding, in: Spivak M., Fletcher D.J.C., Breed M.D. (Ed.), The "African" honey bee, Colorado: Westview Press, 1991; pp. 157-186.

[51] Oliveira A.P.N.Z. Seleção de abelhas *Apis mellifera* L. Africanizadas produtoras de geleia real por marcadores moleculares. UEM: Universidade Estadual de Maringá, Maringá, 2012. 41p. Dissertação (Mestrado em Genética e Melhoramento).

IntechOpen

IntechOpen