

Major royal jelly proteins (MRJPs) of Honeybees, Functions, and Biological Activities

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ABSTRACT

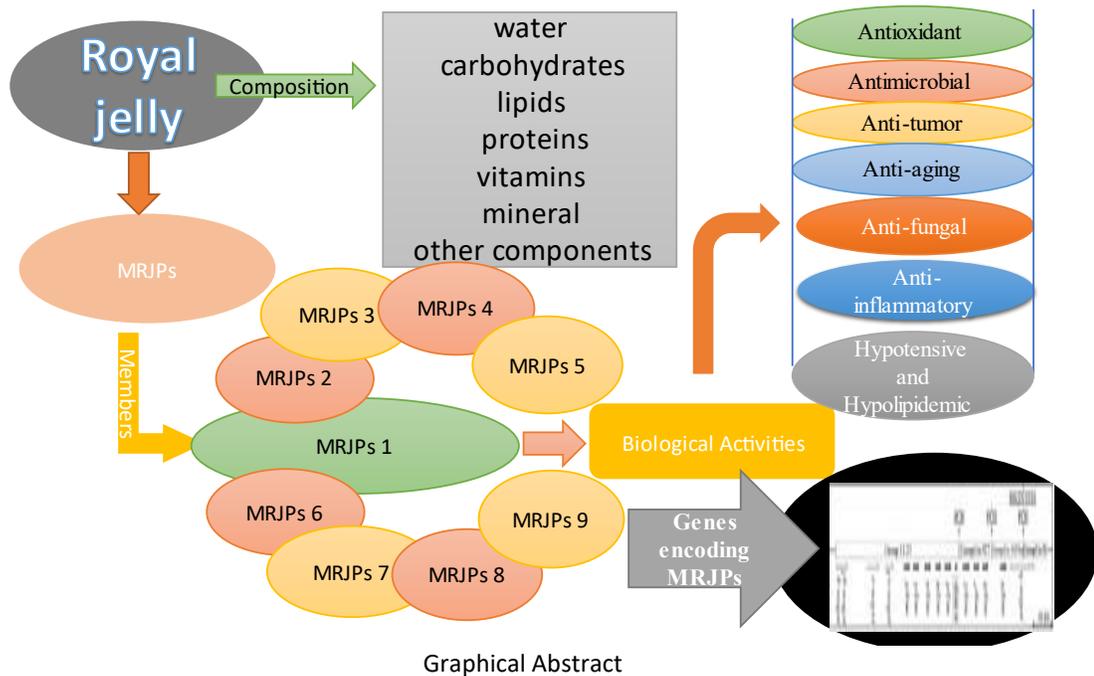
Honeybees are important to produce honey and many other valuable products such as royal jelly, pollen, bee wax, and bee venom. Royal jelly is a special milky substance secreted by the pharyngeal glands of the heads of young worker honeybees. As a mixture, royal jelly contains a variety of organic and inorganic components. Nutrition to queens and three-day-old larvae affects the behavior and regulates the physiological functions of individual bees. In addition, royal jelly proteins perform amazing biological functions for other organisms. This paper reviews the Classification, Functions, and different MRJPs members. Further, the Nutritional and health benefits of members of Major Royal Jelly Proteins (MRJPs), the Systematic evolution of MRJPs, and the expression of the MRJPs gene in honeybees. The content and functions of each member of the MRJPs protein family are not the same, and even the expression of the same member in different tissues leads to changes in the physiological functions. The breadth and depth of application of royal jelly products.

Keywords: Honeybee, pharyngeal glands, Royal jelly, MRJPs

INTRODUCTION

Royal jelly is a special milky substance secreted by the pharyngeal glands of the heads of young worker bees in bee colonies and used to feed queen bees and larvae (Simúth, 2001; Srisuparbh *et al.*, 2003; Kunugi and Ali., 2019; Ali and Kunugi, 2020). As a mixture, royal jelly contains a variety of organic and inorganic components such as protein, amino acids, fatty acids, sugars, vitamins, acetylcholine, insulin, etc. (Howe *et al.*, 1985; Chen and Chen, 1995; Cornara *et al.*, 2017). Among them, protein is the most abundant organic component in royal jelly, accounting for 11% to 14% of the total amount of royal jelly. The protein in royal jelly is divided into water-soluble protein and non-water-soluble protein. Water-soluble protein accounts for 46% to 89% of the total protein content. It is the main component of protein in royal jelly and is called major royal jelly proteins (MRJPs) (Schmitzová *et al.*, 1998; Chen Shenglu, 2001; Qu *et al.*, 2008; Buttstedt *et al.*, 2013; Qiu *et al.*, 2020).

Royal jelly has important biological functions and medical health effects, which may be closely related to MRJPs. For example, in terms of biological functions, MRJPs are involved in determining the hierarchical differentiation of queen bees in bee colonies (Drapeau *et al.* 2006), provide the necessarily available food for larval development, and may play a role in the regulation of bee behavior (Albertová *et al.*, 2005; Robinson *et al.*, 2005). In addition, MRJPs have important healthcare effects on other animal bodies, mainly exhibited as efficient antibacterial activity, strong immune activity, and promotion of special cell production (Shen *et al.*, 2007). MRJPs is a protein family that contains multiple proteins or peptide members with high homology (the consistency of amino acid sequence reaches more than 60% to 70%), and the molecular weight is mostly 49 to 87 kDa (Alu'datt *et al.*, 2018; Drapeau *et al.*, 2006; Shen *et al.*, 2007). This study aims to highlight the functions of different MRJPs members, further the importance of MRJPs in nutritional and health benefits for human life, and different MRJPs gene expressions in honeybees.



Graphical Abstract

Composition of MRJPs:

Royal jelly was developed and used before the 17th century, and people regarded it as a treasure for strengthening the body and prolonging life. In 1852, Wetherill analyzed the chemical components of royal jelly for the first time and found that royal jelly is a natural substance with a very complex composition (Wetherill, 1852). Albert *et al.* (1999b) found that about 50% of the dry material components of royal jelly are proteins. Schmitzová *et al.* (1998) found that 82% to 90% of the water-soluble proteins in royal jelly belong to a protein family with high homology, namely MRJPs. The high degree of homology between members is mainly manifested in the N-terminal sequence and its cDNA sequence. So far, 10 members of the MRJPs family (MRJPs1~9 and MRJP-5) have been verified at the protein, among which MRJPs1~5, which was discovered earlier, have been well studied. It was found that members of the protein family have biochemical functions like ovalbumin. Therefore, it has been proposed that the members of the protein family have biochemical functions like ovalbumin. The MRJPs protein should be named albumins, that is, apalbumin1 represents MRJP1, apalbumin2 represents MRJP2, etc. (Xia *et al.*, 2006). Smith *et al.* (2004) believe that this naming method is more realistic because these proteins are not only present in royal jelly, but the food of worker bees also contains certain proteins of this series. However, this naming method is not recognized by most researchers. To avoid confusion in professional terminology, most research articles have always followed the traditional naming method.

MRJPs are mainly secreted by the hypopharyngeal glands of the feeding bees in the bee colony. The hypopharyngeal glands are exocrine glands and are located on the head of the worker bee. They are a pair of grape-shaped vesicular glands (Li *et al.*, 2008). The existence of the MRJPs gene can be identified by constructing a library of expression sequence tags (EST) of the pharyngeal glands of honeybees. MRJPs are the most well-studied among European bees. Klaudivny *et al.* (1994a) for cloning and sequence analysis of the cDNA of two proteins (MRJP3 and MRJP4) in the royal jelly of European bees. The cDNA full lengths of MRJP1 and MRJP2 were also measured successively in 1998 and 1999 (Júrová *et al.*, 1998; Schmitzová *et al.*, 1998; Biliková *et al.*, 1999). Especially after the whole genome sequencing of honeybees was completed, bioinformatics methods were used to verify that the MRJPs protein family is encoded by 9 genes, and these 9 genes are arranged in a 60 kb cycle sequence, and the consistency has reached more than 60% (Drapeau *et al.*, 2006). Some members of the main protein of Royal jelly are highly polymorphic, for example, MRJP3 has 5 different isomer members (Drapeau *et al.*, 2006). The composition and function activity of Royal jelly as shown in Figure 1.

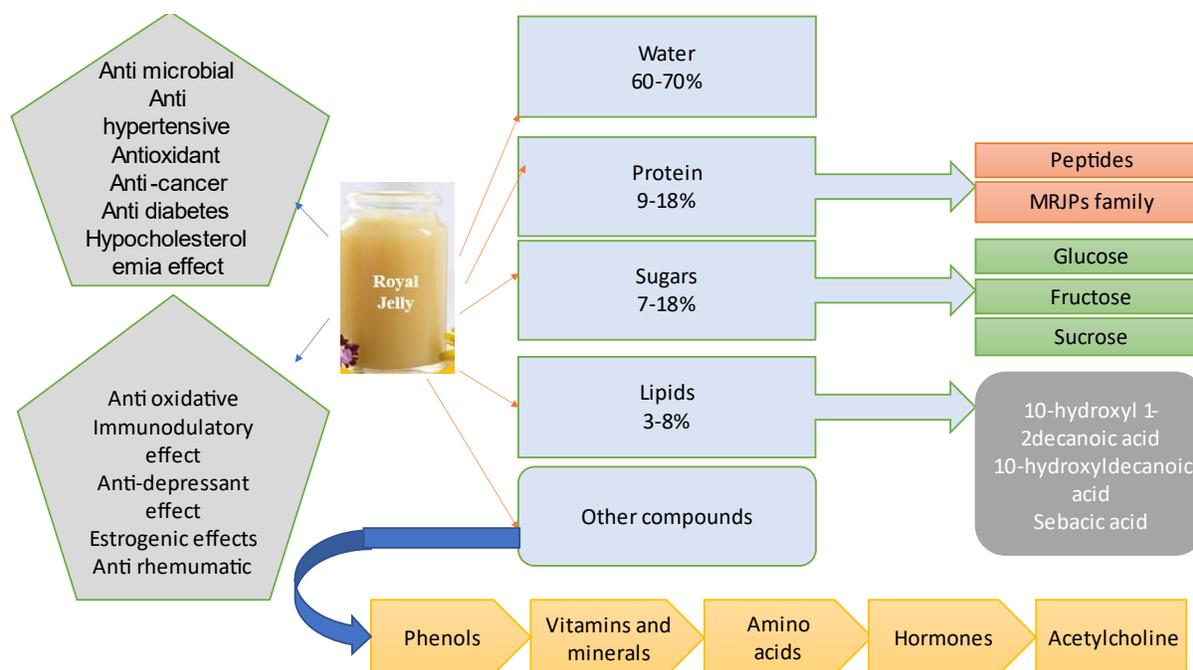


Fig 1. The composition and following functional activities of the royal jelly compound (Reprinted from Balan *et al.*, 2020).

Different MRJPs members:

Scientists have identified 10 members of MRJPs through a variety of molecular biology pathways, namely MRJPs1-9 and MRJP. Among them, MRJP1s-5 is a relatively well-researched protein member, accounting for about 82% of the total protein of Royal jelly (Santos *et al.*, 2005).

MRJP1:

MRJP1 is a weakly acidic glycoprotein with molecular weight, and its isoelectric point pI is 4.0 - 6.3. The total length of the gene sequence encoding this protein is 3,038 bp and contains 6 exons and 5 introns (Biliková *et al.*, 2002; Malecová *et al.*, 2003; Peixoto *et al.*, 2009; Tamura *et al.*, 2009b). The MRJP1 protein is highly conserved. For example, a study by Malecova *et al.* (2003) found that there were 7 mismatches during the replication of the MRJP1 gene, but it did not cause its amino acid sequence to mutate. MRJP1 is the most abundant in the main protein of royal jelly, accounting for 31% and 48% of the water-soluble protein in royal jelly (Simúth, 2001; Srisuparbh *et al.*, 2003). The mRNA of MRJP1 is expressed in large quantities in the hypopharyngeal glands of both feeding honeybees, and the presence of MRJP1 is found in both pollen and honey (Simúth, 2001; Won *et al.*, 2008).

At the same time, in the case of eliminating cross-contamination, Tamura *et al.* (2009) used polyclonal antibodies of MRJP1 to detect the presence of MRJP1 in the mushroom body, optic lobe, and tentacle lobe nerve fibers of the European bee brain. According to Simúth (2001), it was reported MRJP1 in the royal jelly of European bees has different forms, including monomer form (55 kDa), oligomer form (290, 350, and 420 kDa, etc.), and polymer form fatty acids (non-water-soluble). Among them, the oligomer contains 54 amino acid residues, which are composed of 5 MRJP1 monomers and 1 apisimin peptide segment in an aqueous solution, relying on structural characteristics to complete the self-assembly (Biliková *et al.*, 2002), but its detailed subunit structure needs further X-ray diffraction analysis to obtain its protein crystal structure. The MRJP1 oligomer is a thermally stable protein. Its advanced structure will not be destroyed at 56°C, and denaturation will occur when treated with urea, indicating that the subunits are connected by non-covalent bonds (Tamura *et al.*, 2009).

The content of MRJP1 oligomers varies with changes (Tamura *et al.*, 2009), There are also studies reporting that the content of 57 kDa protein decreases with changes in storage temperature and time (Kamakura *et al.*, 2001a; Kamakura and Fukushima, 2002), indicating that the large presence of MRJP1 in royal jelly is not only the reason for its high stability.

MRJP2:

The molecular weight of MRJP2 is 49~51 kDa, and it is a weakly alkaline glycoprotein, which has a moderate content in the main protein of royal jelly, which is about 16% (Srisuparbh *et al.*, 2003; Tamura *et al.*, 2009a). MRJP2 is mainly synthesized and secreted in the pharyngeal glands of feeding bees, but there are reports that MRJP2 is also expressed in the brains of worker bees (Thompson *et al.*, 2006).

Interestingly, MRJP2 did not show polymorphism in European bees, but it has a certain level of polymorphism in the *Apis cerana* (Su *et al.*, 2005), indicating that the genetic differences in MRJPs at the species level are more obvious.

MRJP3:

MRJP3 is a weakly alkaline glycoprotein with a molecular weight of 60 to 70 kDa, accounting for 26% of the total amount of the main protein (Schmitzová *et al.*, 1998; Srisuparbh *et al.*, 2003). MRJP3 is the earliest protein in MRJPs, determined by cDNA cloning and sequencing, and its cDNA length is 380~550 bp (Klaudiny *et al.*, 1994a). MRJP3 is a protein with a high degree of polymorphism. There are 5 isomers, all of which have similar N-terminal sequences and exhibit length polymorphism on SDS-PAGE electrophoresis. Through sequence analysis of the cDNA of MRJP3, it is believed that the length polymorphism of MRJP3 stems from the existence of a variable duplication region in the open reading frame (ORF) of its C-terminal (VNTR) (Albert *et al.*, 1999b). In addition, Albert and Klaudiny (2004) also detected single-nucleotide polymorphisms (SNPs) in the MRJP3 gene. The main reasons for the high degree of polymorphism of MRJP3 are replication slippage of gene fragments and gene mutations, which probability of mutation will not be reduced because the male bee is haploid; MRJP3 gene is not expressed in haploid drone individuals; the variable duplication area of MRJP3 may be a neutral gene (Wolff *et al.*, 1991; Albert *et al.*, 1999b). Therefore, based on the above reasons, the high degree of polymorphism of the MRJP3 gene can be used to study the genetic structure of bee populations, which can be identified by ordinary PCR, which is simple and efficient. However, it should be noted that the MRJP3 polymorphism signal detected by 7% to 8% SDS-PAGE, and the use of antiserum in the MRJP3 repeat region for immunological research can obtain clear polymorphism results (Albert *et al.*, 1999b). MRJP3 belongs to one of the more highly expressed members of the MRJPs family, it was found that the mRNA content of MRJP3 in 8-day-old worker bees accounts for 8% of the total mRNA (Klaudiny *et al.*, 1994a). There is a certain correlation between the higher level of polymorphism of the MRJP3 gene and the production of royal jelly in bee colonies. Therefore, this polymorphism site has been developed as a detection marker for the genetic polymorphism of bees and the production of royal jelly of different bee species (Baitala *et al.*, 2010).

MRJP4:

MRJP4 is a weakly alkaline glycoprotein and contains many important amino acid components (Schmitzová *et al.*, 1998; Li *et al.*, 2007). Compared with MRJPs1~3 and MRJP5, the expression of MRJP4 in the hypopharyngeal glands of worker bees is relatively small, so the identification of MRJP4 is more difficult (Klaudiny *et al.*, 1994b; Ohashi *et al.*, 1997, 1999). Schmitzová *et al.* (1998) reported that neither SDS-PAGE electrophoresis nor chromatographic techniques can detect the presence of MRJP4 at the protein level and can only use cDNA clone sequencing to reveal its existence. Sano *et al.* (2004) first identified MRJP4 at the protein level through 2D-PAGE.

MRJP5:

MRJP5 accounts for about 9% of the total amount of royal jelly protein. It is a weakly alkaline glycoprotein and contains more important amino acid components (Srisuparbh *et al.*, 2003; Li *et al.* 2008). The level of MRJP5 polymorphism is also high, it contains 3 peptide repeats, and it exhibits length polymorphism on the SDS-PAGE. Its isomers are mainly concentrated between 77 and 87 kDa. This highly polymorphic information site can also be used for genetic research of bee colonies (Srisuparbh *et al.*, 2003).

MRJPs 6-9:

MRJPs6 to 9 of only MRJP7, is an acidic proteins, and the rest are weakly alkaline proteins (Tamura *et al.*, 2009a). Unlike other members of the MRJPs family, MRJP8 and MRJP9 are expressed in bee venom glands and do not have nutritional functions. For example, by cloning the cDNA of MRJP9 and conducting phylogenetic studies, researchers believe that the appearance of MRJP9 predates the nutrient-rich repeat regions of the protein family and the oldest

member of the MRJPs family (Albert and Klaudiny, 2007; Peiren *et al.*, 2008). MRJP8 does not express secretion in the hypopharyngeal glands of worker bees, and Alberte and Klaudiny (2004) can only find its existence through the EST library on the head of worker bees. MRJP8 and MRJP9 have many similarities, Phylogenetic analysis clustered them together at the base of the MRJPs evolutionary tree, indicating that they are the oldest protein in the MRJPs family, both MRJP8 and MRJP9 lack repeat regions that evolved later. These repeat regions are the main nutritional value of royal jelly (Albert and Klaudiny, 2007). MARJP may be a pseudogene, that is, an invalid allele. The researchers predicted that it may encode an incomplete polypeptide but did not find its transcript (Drapeau *et al.*, 2006).

Functions of MRJPs

The first is in terms of nutritional function. It is generally believed that MRJPs are the main source and nitrogen necessary for the growth of honeybee larvae. The pentapeptide repeating unit in MRJP3 stores a wealth of available nitrogen (Albertová *et al.*, 2005), nitrogen is an important component of biological macromolecules, such as nucleic acids and proteins, which are essential for the rapid growth of bee larvae and the high reproductive capacity of queen bees. The tripeptide repeat unit of MRJP5 contains methionine residues, which is an important area for storing sulfur (Drapeau *et al.*, 2006). The microarray method is used to find that MRJP2 and MRJP7 are expressed at high levels in their brains in new emergent honeybee (Thompson *et al.*, 2006). Due to certain differences in the composition of the royal jelly of Asian bees and European bees, when the royal jelly of Asian bees is used to feed the larvae of European bees, it is impossible to make them develop into king bees, on the contrary (Takenaka and Takenaka, 1996). Therefore, MRJPs, an important active ingredient in royal jelly, may be one of the controlling factors for the development of bee colonies. In addition, studies have proved that the glycosylation site at the N-terminal of the protein is essential for communication between cells or tissues. Therefore, MRJP1, which has an N-terminal glycosylation site in the mushroom body of the bee brain, plays an important role in the communication ability of bees. The mushroom body is the visual, motor, and olfactory center of bees, and the neural network in it forms the information processing and memory center of the brain. If the expression of MRJP1 in the mushroom body is reduced, it will directly affect the acquired learning ability of worker bees (Kucharski *et al.*, 1998; Peiren *et al.*, 2008). The third aspect is the influence of MRJPs on the physiology of bees. During the process of collecting pollen and nectar from the outside world, bees can easily infect microorganisms on plants. Under normal circumstances, to avoid infection, the inherent cellular immune response of bees is mediated by a series of antibacterial polypeptides or peptides in tissues, blood cells, and hemolymph. However, antibacterial proteins in MRJP1 are innate and can be produced in the hypopharyngeal glands without any immune stimulation of bees (Fontana *et al.*, 2004). The main role of members of the yellow family who are closely related to MRJPs is to promote the sexual maturity and development of individuals of different genders (Gilliland *et al.*, 2005).

Nutritional and health benefits of MRJPs

MRJPs have many important nutritional and health functions, such as MRJP1 can stimulate the DNA synthesis of the original cultured mouse liver cells, promoting cell propagation, and maintaining liver cell activity (Kamakura *et al.*, 2001). Protein isolated from royal jelly with the same N-terminal sequence as MRJP1 can stimulate human mononuclear cell production (Kimura *et al.*, 2003); MRJP1 can stimulate the growth of lymphocytes in serum media and has an anti-fatigue function in mice (Kamakura *et al.*, 2001); Peptides obtained by gastrointestinal protease decomposition of MRJP1 can effectively inhibit the activity of human vasoconstrictor type I converting enzyme (Matsui *et al.*, 2002). Fontana *et al.* (2004) speculated that MRJP1 is the precursor of Roslisin and Jelleines and said that MRJP2 also has a certain resistance and anti-microbial (Feng *et al.*, 2021). Glycoprotein with a molecular weight of 70 kDa (MRJP3) has a strong anti-allergic ability, Wound Healing, and it exhibits effective immunomodulatory ability (Okamoto *et al.*, 2003; Minegaki *et al.*, 2020); MRJPs protein has a strong antioxidant activity after hydrolysis by N-terminal hydrolase, such as inhibiting the peroxidation reaction of linoleic acid (Guo *et al.*, 2009). Different Major royal jelly proteins (MRJPs) and their biological activities as shown in (Table 1, Figure 2). The MRJPs family affects the body's immune system. It is reported that MRJP1 and MRJP2 are allergens that cause food allergies. They can stimulate macrophages in mice to release TND- α , while MRJP3 has strong strain resistance and can inhibit the levels of interleukin 4 (IL-4), IL-2, and INF- γ (Tamura *et al.*, 2009). In addition, the complexity of the protein composition and the lack of continuous data on the expression of related genes make the physiological and biological properties of these proteins difficult to predict, hindering the study of related protein functions in royal jelly (Santos *et al.* 2005).

Table 1. Major royal jelly proteins (MRJPs) and their biological activities.

Biological Activity	MRJPs	References
Antioxidant	mrjp-mix	(Nagai & Inoue, 2004; Guo <i>et al.</i> , 2009; Xin <i>et al.</i> , 2016)
	mrjp1-7	(Park <i>et al.</i> , 2020)
Antimicrobial	mrjp1-7	(Park <i>et al.</i> , 2020)
	mrjp1	(Brudzynski <i>et al.</i> , 2015; Brudzynski & Sjaarda, 2015)
	mrjp1	(Bucekova and Majtan, 2016; Vezetu <i>et al.</i> , 2017)
	mrjp2 and mrjp4	(Bilikova <i>et al.</i> , 2009, Kim and Jin, 2019; Park <i>et al.</i> , 2019; Park <i>et al.</i> , 2020)
	mrjp2	(Feng <i>et al.</i> , 2021)
Anti-tumor	mrjp2	(Abu-Serie & Habashy, 2019)
Hypotensive and Hypolipidemic	mrjp1, mrjp2, and mrjp3	(Kashima <i>et al.</i> , 2014)
	mrjp1	(Fan <i>et al.</i> , 2016)
	mrjp1	(Matsui <i>et al.</i> , 2002)
	rj proteins, mrjp1 and mrjp3	(Sato <i>et al.</i> , 2021)
	mrjp1	(Tahir <i>et al.</i> , 2020)
Cell proliferation, Growth-promoting and Wound Healing	mrjp-mix	(Chen <i>et al.</i> , 2016; Jiang <i>et al.</i> , 2018; Park <i>et al.</i> , 2020)
	mrjp1	(Kimura <i>et al.</i> , 2003; Tamura <i>et al.</i> , 2009; Moriyama <i>et al.</i> , 2015)
	mrjp1	(Kimura <i>et al.</i> , 1996, Watanabe <i>et al.</i> , 1996, Wan <i>et al.</i> , 2018)
	mrjp2, mrjp3, and mrjp7	(Lin <i>et al.</i> , 2019)
	mrjp3	(Minegaki <i>et al.</i> , 2020)
Anti-aging	mrjp-mix	(Xin <i>et al.</i> , 2016, Jiang <i>et al.</i> , 2018)
	mrjp1	(Detienne <i>et al.</i> , 2014)
Neuroprotective		
	mrjp-mix	(Chen <i>et al.</i> , 2017)
Reproductive and Fertility	mrjp-mix	(Xin <i>et al.</i> , 2016, Liu <i>et al.</i> , 2020)
Anti-inflammatory and Immune-Modulatory	mrjp1 and mrjp2	(Thien <i>et al.</i> , 1996; Šimúth <i>et al.</i> , 2004, Majtán <i>et al.</i> , 2006; Rosmilah <i>et al.</i> , 2008; Majtán <i>et al.</i> , 2010; Hayashi <i>et al.</i> , 2011; Bilal and Azim, 2018)
	mrjp3	(Okamoto <i>et al.</i> , 2003, Kohno <i>et al.</i> , 2004)

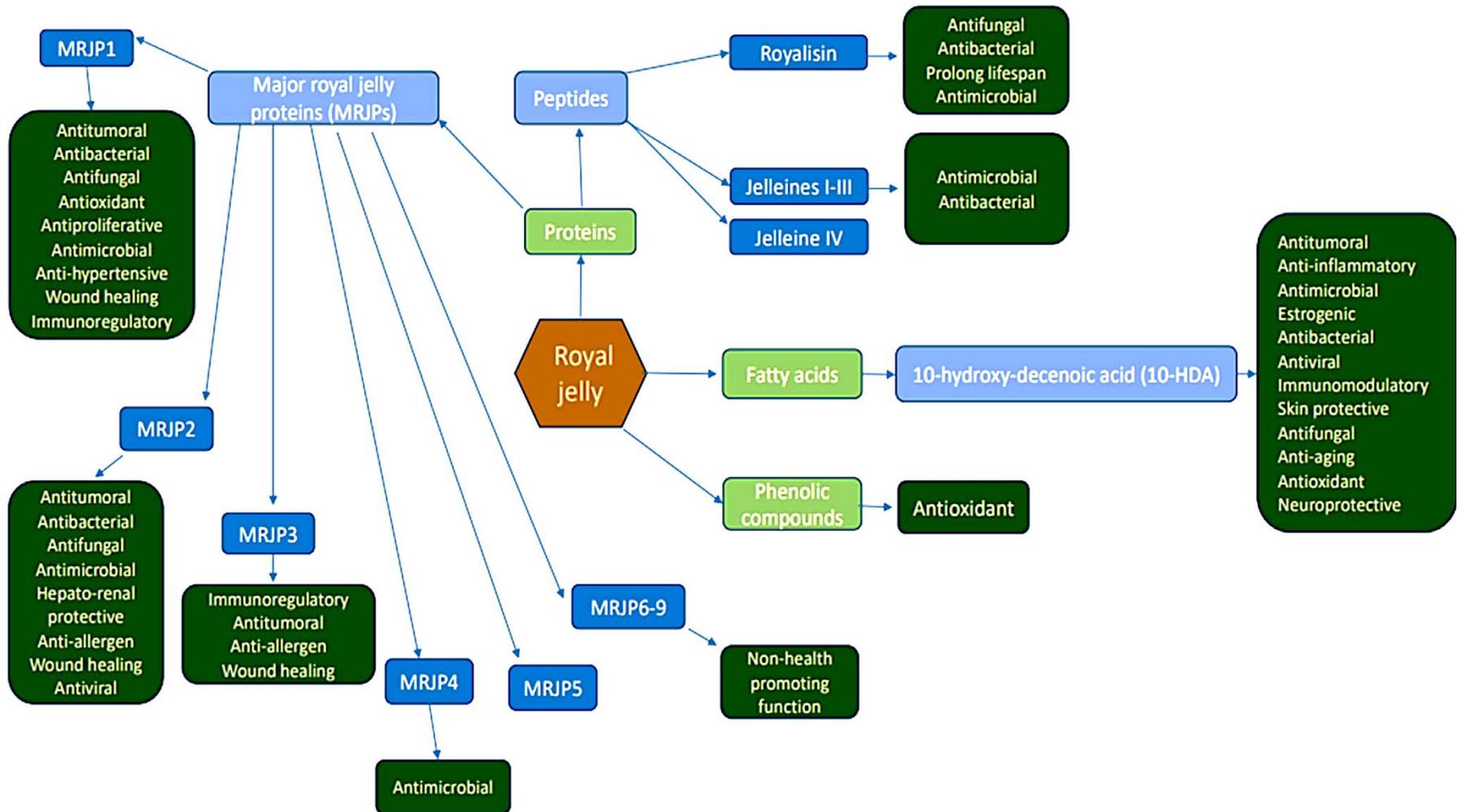


Fig 2. Summary of the chemical composition and biological activities of royal jelly, along with their effects on aged skeletal muscle and possible underlying mechanism (Reprinted from Bagameri *et al.*, 2022)

Systematic evolution of MRJPs:

MRJPs evolved from an ancient protein family (YELLOW). The two have high homology and are often classified as the MRJP/YELLOW family. The members of this family perform different physiological functions and show a high degree of conservation (Albert and Klaudiny, 2004). The yellow gene family is widely distributed, such as in all arthropods (Millipedes) and some marine bacteria, among which the yellow gene in fruit flies is the most fully studied. However, compared with its precursor, the MRJPs gene is only in the bee genus *Apis*. Therefore, it can be inferred that the origin of the MRJPs gene is late (Maleszka and Kucharski, 2000; Drapeau et al., 2006). Albert and Schmitz (2002) used PCR primers designed from European bees to detect MRJP3 and MRJP5 gene sequences that are highly homologous to bees in the large bee *Apis dorsata* and speculated that the proteins expressed have similar functions. Therefore, the evolutionary history of their MRJPs protein family can be traced back to the common ancestor of European bees and big bees 2.2 billion years ago. The YELLOW/MRJP protein is a multi-functional protein system. The modes of action of the members are interrelated. Many of the members play a role in the reproductive and maturation process of insects. At the same time, the evolutionary process of the MRJPs protein family is consistent with the formation of bee social habits (Drapeau et al., 2006). The function of the YELLOW protein has shown a high degree of conservatism in its evolution, while bee MRJPs have undergone a certain level of genetic differentiation and functional divergence in their evolution. Even between MRJPs proteins with higher homology, their physiological functions are not the same (Albert and Klaudiny, 2004).

The gene coding region of each member of MRJPs1~9 contains 5 introns, the structure is very similar, and the different members have the same translation stage, indicating that this gene family may have evolved from a common precursor (Drapeau et al., 2006). Therefore, there is a hypothesis that the MRJPs gene is produced by multiple replications that occurs almost simultaneously because multiple replication mechanisms often produce gene clusters at the same site (Albert et al., 1999; Wagner et al., 2003). There is also an argument that the MRJPs gene is formed by the accumulation of genetic variations at an original gene site. However, the evolutionary tree constructed based on DNA sequence shows that the MRJPs gene is more closely related to yellow-e3, and the latter statement is rejected. There are three reasons: first, yellow-e3, as a promoter, is located on one side of the 10 MRJPs gene members (Figure 3); second, among all yellow-like genes in bees, only the intron/exon structure and protein amino acid sequence of yellow-e3 are most similar to MRJPs; third, and most importantly, microarray data show that the functional similarity between Yellow-e3 and MRJPs even exceeds the functional similarity between yellow-e3 and other Yellow members (Drapeau et al., 2006). The repeating region of MRJPs appeared very early in the evolution of its gene family, and this repeating fragment in the *mrjp3* gene is especially elongated. The nitrogen content in MRJPs is proportional to the length of its repeating area. Therefore, Albertová et al. (2005) believe that based on providing more effective nutrients for queen bees and larvae, through a long-term natural selection mechanism, the MRJPs secreted by the pharyngeal glands of worker bees have evolved into nutrient-rich repeating areas.

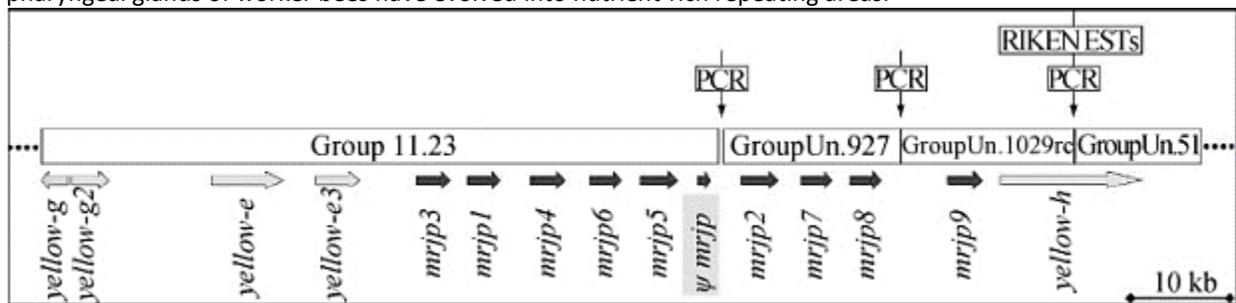


Fig 3. Genomic landscape of genes encoding MRJPs (Reprinted from Drapeau et al., 2006)

The expression of the MRJPs gene:

The whole genome sequence information of western bees contains the spatial structure between genes. For example, the five genes encoding members of the YELLOW family are located on the flanks of the MRJPs family genes. The intron/exon structure of the MRJPs gene and the protein sequence information expressed, it was found that the replication of the MRJPs gene was started by yellow-e3 as an initiator (Drapeau et al., 2006).

The expression of MRJPs in European bees is different in different types, developmental stages, genders, and organizations. MRJP1 and MRJP3 are not only expressed in worker bees, but also in queen bees and drones. The MRJPs family genes are expressed throughout the developmental stages of bees, such as embryos, larvae, and

pupae, but their expression is lower than that of adult bees (Drapeau *et al.*, 2006). There are also differences in the expression of the MRJPs gene over time. MRJPs are found in the pharyngeal glands of 1-day-old worker bees, while the typical royal jelly is synthesized in the pharyngeal glands of 3-day-old worker bees. The expression of MRJP5 is highest in larvae within 5 days of age, but then gradually decreases, as the expression of MRJP5 cannot be detected after 26 days of age, this situation is completely different from that of MRJP1 (Kucharski *et al.*, 1998). The expression differences of MRJPs in different tissues of bees are also more obvious. For example, MRJP1 is expressed in the brains of mature worker bees, indicating its role in determining the individual behavior of bees. MRJP8 and MRJP9 can be expressed in the bee venom glands of adult bees, etc., but their role is not yet clear (Drapeau *et al.*, 2006). Drapeau *et al.*, (2006) these expression differences of MRJPs members show that although gene members are relatively close in the genome, their expression regulation is not consistent. Overall, the nine gene members of MRJPs are not only expressed in large quantities as nutrients in the pharyngeal glands of worker bees. The special expression methods they have evolved precisely prove that the members of the protein family have their own independent and unified functional value.

There is an age-dependent role phenomenon in worker bees in bee colonies, and the change in the division of labor is accompanied by changes in the secretion function of the pharyngeal glands. The pharyngeal glands of feeding bees mainly secrete royal jelly containing MRJPs to feed queen bees and larvae. With the increase in worker bees' daily age (from feeding bees to field bees), their pharyngeal glands stop secreting royal jelly and instead secrete metabolic enzymes (including α -glucosidase, amylase) that catalyze the hydrolysis of carbohydrates to convert nectar into honey (Feng *et al.*, 2009). In principle, we can detect a decrease in the expression level of the target gene during or after this transformation process, and this transformation can also be reversed. In addition, the pharyngeal glands of worker bees will synthesize different MRJPs members according to the age of the species. For example, the expression of MRJPs1~4 and MRJP7 in feeding bees is significantly higher than that of breeding bees, and the expression of MRJP6 in both is lower. The reason for this result requires further research (Liu *et al.*, 2010). The expression volume and expression time of the MRJPs gene are precisely regulated. Bozic and Woodring (2000) believe that juvenile hormones are involved in the regulation of changes in the secretion function of the pharyngeal glands. The above research on the regulation of MRJPs gene expression helps us to better understand the differences between homologous genes and their role in the ontogeny of social insects. The MRJPs gene is also an important way to study the molecular mechanism of the phenomenon of bee day-age polymorphism.

CONCLUSION

By summarizing and analyzing the relevant research progress of the main protein of royal jelly in bees, there are mainly the following different aspects of research that deserve special attention. The behavioral characteristics of bee colonies are mainly social, generational overlap, and parental nurturing. These social behaviors are mostly controlled and regulated by members of the MRJPs family in royal jelly. The regulatory mechanism has become a hot topic in current research, but also a difficult point. The protein component in royal jelly has important nutritional value and has become a good food supplement for people to prolong their lives. Therefore, the mass production model of the target protein component needs to be established urgently, and the separation and purification of the functional protein in royal jelly or the cloning of the main protein gene of royal jelly and effectively increasing its expression is an effective way. Further, the content and functions of each member of the MRJPs protein family are not the same, and even the expression of the same member in different tissues leads to changes in the physiological functions. Therefore, in-depth research on a single member of MRJPs needs to be carried out urgently, especially at the molecular and cellular levels. The results in this area will help to prove the occurrence and regulatory mechanism of bee social behavior, as well as expand the breadth and depth of application of royal jelly products. In short, with the development of genetic engineering, proteomics and modern separation technology, the functions, and structures of the members of the MRJPs family will be clarified one by one. This is undoubtedly of great significance to further clarify the physiological and biological activities of the protein components of royal jelly and expand its scope of application.

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