# Geographical Influences on Content of 10-Hydroxy-*trans*-2-Decenoic Acid in Royal Jelly in China

WEN-TING WEI, <sup>1</sup> YUAN-QIANG HU, <sup>2</sup> HUO-QING ZHENG, <sup>1</sup> LIAN-FEI CAO, <sup>3</sup> FU-LIANG HU, <sup>1,4</sup> and H. RANDALL HEPBURN<sup>5</sup>

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**ABSTRACT** The content of 10-hydroxy-*trans*-2-decenoic acid (10-HDA), a marker compound in royal jelly (RJ), is the most important criterion in grading RJ for commercial trade and varies with its origin. To identify the effect of geographical origin on 10-HDA content in RJ, 138 samples were collected from 19 provinces of China (divided into three groups) produced by either *Apis mellifera ligustica* Spinola, 1806 or a hybrid of *A. m. ligustica* and *Apis mellifera carnica* Pollman, 1879 and analyzed for moisture, sugar, crude protein, ash, acid, and 10-HDA concentration. The results show that RJ from western China has a significantly higher 10-HDA level ( $2.01 \pm 0.05\%$ ) than those from northeastern ( $1.87 \pm 0.05\%$ ) and eastern ( $1.75 \pm 0.03\%$ ) China. RJ secreted by hybrid bees contained more 10-HDA ( $1.89 \pm 0.03\%$ ) than that secreted by *A. m. ligustica* ( $1.78 \pm 0.03\%$ ). The 10-HDA content of RJ produced during flowering of rape (*Brassica campestris* L.), lime (*Tilia amurensis* Ruprecht), and vitex (*Vitex negundo* L. variety *heterophylla* (Franch.) Rehder) was 1.92, 1.80, and 1.68\%, respectively. The results would be helpful during the process of price determination of RJ by providing some basis of geographical, bee strain, and botanical information for commercial trade.

**KEY WORDS** royal jelly, 10-hydroxy-*trans*-2-decenoic acid, geographical origin, bee strain, botanical origin

Royal jelly (RJ) is a secretion of the hypopharyngeal glands of nurse worker bees, Apis mellifera ligustica Spinola, and other species of Apis. It plays a critical role in caste determination because those larvae that are fed copious amounts of RJ develop into large fertile gueens rather than into smaller sterile workers (Townsend and Lucas 1940). Numerous studies have revealed some exceptional biological properties of RJ, including antibacterial and anti-inflammatory activities, vasodilative and hypotensive activities, disinfectant actions, antioxidant, antihypercholesterolemic, and antitumor activities (Viuda-Martos et al. 2008). Therefore, there is a considerable commercial market for RJ, especially in China and Japan (Krell 1996), and it is used in many sectors such as the pharmaceutical, food, cosmetic, and manufacturing industries (Sabatini et al. 2009). China is the main producer in the world (Sabatini et al. 2009, Daniele and Casabianca 2012) with an annual RJ yield of  $\approx$ 3,000 tons, and this comprises 95% of the product in world trade (Zheng et al. 2011).

0, South Africa. (Genc and Aslan

RJ consists of 60-70% moisture (Karaali et al. 1988, Sabatini et al. 2009), 9-18% proteins (Karaali et al. 1988), 3-8% lipids (Karaali et al. 1988, Sabatini et al. 2009), 6-18% hydrocarbons (Sesta 2006, Daniele and Casabianca 2012), and 0.8-3.0% minerals (Sabatini et al. 2009) on a wet weight basis. Among these components, 10-hydroxy-trans-2-decenoic acid (10-HDA) is a marker used for the commercial trade and stands as the base of some biological functions of RJ. The content of 10-HDA in RJ is its international standard of its quality of RJ and directly determines the price of RJ on the international market. The wholesale price of RJ in China has risen to approximately US\$20/kg. In some cases, a 1% increase in 10-HDA translates in an increase of US\$1.5/kg in price. The price in international trade is higher and fluctuates from US\$20 to US\$40/kg mainly based on the 10-HDA content according to China Customs statistics (China Chamber of Commerce for Import & Export of Medicines & Health Products [CCCMHPIE] 2013). According to Chinese quality standards, RJ should contain no <1.4% of 10-HDA, and RJ with no <1.8% is classified as a premium product (General Administration of Quality Supervision, Inspection and Quarantine of People's Republic of China [AQSIQ] 2008). The 10-HDA content of crude RJ is commonly required to be 1.8% for international trade.

The content of 10-HDA in commercial RJ varies considerably between 1.4 and 3.4% in wet matter (Genc and Aslan 1999, Antinelli et al. 2003, Garcia-

<sup>&</sup>lt;sup>1</sup> Department of Special Economic Animal Science, College of Animal Sciences, Zhejiang University, Hangzhou 310058, China.

<sup>&</sup>lt;sup>2</sup> Apicultural Technological Association of Jinyun County, Zhejiang Province 321400, China.

<sup>&</sup>lt;sup>3</sup> Institute of Animal Husbandry and Veterinary Science, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China.

<sup>&</sup>lt;sup>4</sup> Corresponding author, e-mail: flhu@zju.edu.cn. <sup>5</sup> Department of Zoology and Entomology, Rhodes University, Gra-

hamstown 6140, South Africa.



Henan

(5)

Hubei

(14)

Jiangsu

Zhejiang

(16)

Fujian (3)

Anhui (8

(8)

Shaanxi

(2)

Guizhou

Fig. 1. Distribution of sampling locations. The northeastern group (pink) contains four provinces with cold weather; the western group (yellow) has four provinces well above sea level, cold, and arid; the eastern group (blue) includes 11 provinces at low elevations with relatively warm weather. The numbers in parentheses indicate the sample size of each province. (Online figure in color.)

Sichuan

(15)

Yunnar

Amoedo and Almeida–Muradian 2003, Ferioli et al. 2007, Sabatini et al. 2009) and can be affected by geographical origin (Antinelli et al. 2003, Ferioli et al. 2007), bee strain (Wu et al. 2010), harvesting time (Zheng et al. 2010), and so on. Geographical origin is particularly noteworthy among the above factors.

Several studies have detected some possible, but uncertain, evidence of great variations in 10-HDA content in RJ samples from different locations. Antinelli et al. (2003) reported on two freshly harvested samples from France and Thailand with 2.6 and 1.8% of 10-HDA, respectively. In the RJ from Brazil, the 10-HDA content of seven samples produced in Sao Paulo State ranged from 1.58 to 3.39% with a mean 2.53% in wet matter (Garcia-Amoedo and Almeida-Muradian 2003), and is thus analogous to that of French RJ. A study on Turkish RJ showed significant differences within the country, with a mean concentration of 2.09% 10-HDA in RJ from the western part (two samples) compared with the central region (two samples) where it was 0.65% higher (Genc and Aslan 1999). Ferioli et al. (2007) reported a higher amount of 10-HDA in Italian RJ than in RJ originating from outside Europe. In their study, eight Italian RJ samples were harvested in the Emilia-Romagna region and compared with six extra-European samples, of which two were from Australia, one from China, one from America, and the geographical origin of the rest two was unknown. However, systematic research is still needed because of the lack of large sample sizes in the afore-mentioned studies. Moreover, the effect of geographical origin was not a primary aim in the above cited work, so that possible relationships between geography and other factors, for example, bee strain, botanical origin, and climate, were not demonstrated. As a result, effects of geography on RJ composition would be more direct and credible when botanical influence was controlled, for example, comparing RJ produced on rape (Brassica campestris L.) pollen with different geographical information. In this study, 138 RJ samples from 19 provinces of China were analyzed to identify possible geographical differences in the 10-HDA content.

## Materials and Methods

Samples. In total, 138 RJ samples produced either by *Apis mellifera ligustica* or a hybrid of *A. m. ligustica* and *Apis mellifera carnica* Pollman, 1879 (*A. m. ligustica* by *A. m. carnica*) during various nectar flows were harvested in 19 provinces between March 2008 and June 2009. These areas (Fig. 1) represent >90% of the regions where commercial RJ is produced in China.

Table 1. Chemical properties of RJ from different geographical areas (mean  $\pm$  SE)

Group	Northeastern group	Western group	Eastern group	Total
N	28	29	81	138
Total sugar (%)	$12.73 \pm 0.30$	$13.58 \pm 0.24$	$13.20 \pm 0.13$	$13.18 \pm 0.11$
Crude protein (%)	$14.11 \pm 0.23$	$13.93 \pm 0.24$	$14.25\pm0.10$	$14.16 \pm 0.09$
Ash (%)	$1.08 \pm 0.02$	$0.99 \pm 0.02$	$0.96 \pm 0.02$	$0.99 \pm 0.01$
Acidity (ml/1N NaOH/100 g)	$36.65 \pm 0.48$	$36.41 \pm 0.51$	$36.04 \pm 0.29$	$36.24 \pm 0.22$
Moisture (%)	$65.15 \pm 0.30$	$64.43 \pm 0.25$	$64.72 \pm 0.21$	$64.75 \pm 0.15$
10-HDA (%)	$1.87\pm0.05$	$2.01\pm0.05$	$1.75\pm0.03$	$1.83\pm0.02$

The sampling areas were divided into three groups based on climate and elevation (Fig. 1). The first group (38° 43' N to 53° 33' N) comprised northeastern China, including Inner Mongolia, Heilongjiang, Jilin, and Liaoning provinces, which are characterized by a high latitude and extremely cold weather with an average temperature of  $5.68^{\circ}$ C and reaching as low as  $-30^{\circ}$ C in winter. The average elevation of this group is around 300 m, and the mean annual precipitation is 614.9 mm. The second group (21° 8′ N to 39° 19′ N) consisted of provinces in western China, including Qinghai, Sichuan, Yunnan, and Guizhou, which are located on the Qinghai-Tibet Plateau and Yunnan-Guizhou Plateau and are well above sea level with an average elevation of  $\approx$ 2,500 m. As a result, this group is characterized by drought and cold with an annual average temperature lower than 10°C. The third group  $(23^{\circ} 30' \text{ N to } 42^{\circ} 37' \text{ I})$ N) comprises the provinces of eastern China, including Hebei, Beijing, Shanxi, Shaanxi, Shandong, Henan, Anhui, Hubei, Jiangsu, Zhejiang, and Fujian. The climate of the "eastern group" is much warmer (the annual average temperature is ≈16°C) and more humid (the mean annual precipitation is >1,250 mm) than that of the "northeastern group" because protective high mountains form a boundary between the two regions and block the cold winds originating from the arctic air mass. When compared with the "western group," the eastern group mainly consists of low-lying plains with an average elevation lower than 200 m. Eight samples were produced in four different provinces by selected bees bred from A. m. ligustica for high RJ production ("Zhejiang Royal Jelly Bee"); five samples belong to western group (one from Yunnan and four from Guizhou province) and three belong to eastern group (two from Anhui and one from Jiangsu province). Fifty-six of the 138 samples were produced during the rape flowering season to which three belong to the northeastern group, 21 to the western group, and 32 the eastern group, respectively. Seventeen samples of the 138 samples were derived from honey bees for aging on vitex (Vitex negundo L. variety heterophylla (Franch.) Rehder) flowers while nine samples were produced on lime (Tilia amurensis Ruprecht) pollen. All samples were immediately frozen at -18°C after harvesting and transported to the laboratory on ice.

Chemical Analyses. The analyses of moisture, crude protein, total sugar, acidity, ash, and 10-HDA followed the methods for the quality standard of RJ published in 2008 by the AQSIQ (GB 9697–2008; AQSIQ 2008). Moisture was measured by weight loss after drying in a vacuum drying oven. The quantity of crude protein was calculated by multiplying the content of nitrogen, determined by Kjeldahl's method, by 6.25 (Association of Official Analytical Chemists 1984). The total amount of sugar was determined by Fehling's method after hydrolyzing the sample in HCl (6 mol/liter) for 10 min and calculating the glucose equivalent. Acidity was determined by titration with NaOH and presented in terms of neutralization by ml/1N NaOH/100 g sample. Mineral content was determined at 750°C after preheating and adding concentrated sulfuric acid. 10-HDA was determined by high-performance liquid chromatography (HPLC; Bloodworth et al. 1995).

Statistical Analyses. The data of all independent variables obtained from chemical analyses of 138 royal jellies were analyzed with One-Sample Kolmogorov-Smirnov test and were determined to be normally distributed. One-way analysis of variance (ANOVA) was used to compare the means of each variable from different geographic origins; this was followed by Fisher test for post hoc, least significant differences (LSDs) where necessary. To identify a possible effect of geographic origin, bee strain, and their interactions on 10-HDA content, a univariate linear model test was performed. Further, we compared the 10-HDA content of RJ secreted by the same strain (A. m. ligustica or A. m. ligustica by A. m. carnica) from different geographical groups using One-way ANOVA followed by Fisher test for post hoc, LSDs when necessary. Fifty-six RJ samples derived from honey bees foraging on rape flowers were divided into two groups based on bee strain. 10-HDA concentrations were compared among the three geographical groups within each strain by one-way ANOVA. All statistics was performed with SPSS 16.0 for windows (SPSS Inc. 2007).

### Results

**Composition of RJ.** The descriptive statistics for total sugar, protein, ash, moisture, 10-HDA content, and acidity are shown in Table 1. There were significant differences detected in 10-HDA (F = 12.188; df = 2, 135; P = 0.000), sugar (F = 3.186; df = 2, 135; P = 0.044), and ash (F = 7.498; df = 2, 135; P = 0.001) content among the three geographic groups. However, there were no significant differences among the other three parameters—moisture (F = 1.237; df = 2, 135; P = 0.293), protein (F = 0.993; df = 2, 135; P = 0.373), and acidity (F = 0.609; df = 2, 135; P = 0.545).

Moisture changed little among regions, suggesting that the concentration of other components in wet



Fig. 2. Percentage of 10-HDA by weight in RJ of different geographical and bee strain origins. The numbers within the bar indicate the number of samples. The error bars denote the SEMs. Different letters on the column indicate significant differences within the same strain. (Online figure in color.)

matter could be used directly for comparisons. RJ produced in the western part of China contained the most 10-HDA, followed by RJ from the northeastern region, with RJ from the eastern area having the lowest values  $(t_{W-N} = 0.151; df = 135; P = 0.032; t_{N-E} = 0.104;$  $df = 135; P = 0.032; t_{W-E} = 0.226; df = 135; P = 0.000).$ The western group had significantly higher amounts of sugar (13.58%) than the northeastern group (12.73%); t = 0.177; df = 135; P = 0.013); however, the eastern group contained 13.20% sugar and could not be distinguished from the other two ( $t_{N-E} = 0.081$ ; df = 135; P = 0.094;  $t_{W-E} = 0.064$ ; df = 135; P = 0.171). The ash content of the northeastern group (1.08%) was slightly, but significantly, higher than those of the western, 0.99% (t<sub>N-W</sub> = 0.169; df = 135; P = 0.017), and eastern groups, 0.96% (t<sub>N-E</sub> = 0.185; df = 135; P = 0.000) (Table 1).

Content of 10-HDA in RJ Varies With its Geographic Origin. Geography (F = 14.607; df = 2, 129; P = 0.000) and bee strain (F = 4.048; df = 1, 129; P = 0.046) both influenced 10-HDA concentration in RJ, but their interaction did not (F = 1.243; df = 2, 129; P = 0.292). Geography was the dominant factor in the total variance (mean square of geography = 0.812, whereas mean square of strain = 0.225), indicating that geographical origin was the primary factor influencing the 10-HDA content.

For RJ secreted by A. m. ligustica, there were significant differences between groups ( $t_{W-N} = 0.186$ ; df = 70; P = 0.028;  $t_{N-E} = 0.264$ ; df = 70; P = 0.024;  $t_{W-E} = 0.423$ ; df = 70; P = 0.000; Fig. 2). The highest concentration of 10-HDA (2.03%) was observed in RJ produced in western China, followed by those from northeastern China at 1.83%. The eastern group RJ samples had only 1.66% 10-HDA on average, which was far lower than the RJ of the western group. In RJ produced by A. m. ligustica by A. m. carnica bees, the



Fig. 3. Percentage of 10-HDA by weight in rape-derived RJ of different geographical and bee strain origins. The numbers in the bars indicate sample sizes. There was no rape RJ produced by *A. m. ligustica* in northeastern China. The error bars denote the SEMs. Different letters above the columns indicate significant differences within the same strain. (On-line figure in color.)

western group again contained the highest average content of 10-HDA (2.04%), followed by the northeastern (1.94%) and eastern groups (1.84%). However, a significant difference in RJ was only found between the western and eastern groups (t = 0.341; df = 59; P = 0.008).

**Content of 10-HDA in Rape RJ.** Fig. 3 shows the 10-HDA content of rape-derived RJ with different geographical and bee strain origins. The variance of 10-HDA within RJ produced by hybrid bees was smaller when compared with those produced by *A. m. ligustica*, but their average content was similar. RJ in the western group had more 10-HDA than the others, regardless of strain.

In RJ produced by *A. m. ligustica*, the average content of 10-HDA of the western group, 2.19%, was 27% higher (F = 23.289; df = 1, 25; P = 0.000) than that in eastern group, 1.72%. The content of 10-HDA was 1.82, 2.04, and 1.89% for northeastern, western, and eastern groups, respectively, produced by hybrid bees.

Botanical Origin Influences the 10-HDA Content in RJ. The average 10-HDA content of RJ produced during lime, vitex, and rape nectar flows differed (F = 5.984; df = 2, 79; P = 0.004; Fig. 4). RJ originating from rape pollen contained a significantly higher content of 10-HDA (1.92%) than RJ from vitex pollen (1.68%; t = 0.260; df = 79; P = 0.001) and was similar to that from lime pollen (1.80%; t = 0.188; df = 79; P = 0.182). 10-HDA content did not differ in RJ produced in lime and vitex flowering seasons (t = 0.175; df = 79; P = 0.276).

### Discussion

The chemical components of RJ may vary considerably with geographical, botanical, seasonal, strain of



Fig. 4. Percentage of 10-HDA by weight in RJ derived from different botanical origins. The error bars denote the SEMs. Different letters above the columns indicate significant differences between different botanical origins. (Online figure in color.)

honey bee, and other factors (Genc and Aslan 1999, Antinelli et al. 2003, Garcia–Amoedo and Almeida– Muradian 2003, Ferioli et al. 2007, Sabatini et al. 2009). However, these factors have not previously been analyzed on an individual basis. For example, the possible effect of geographical origin on 10-HDA was derived from several independent references, in which the sample size was rather too small to give precise evidence so that the results were not comparable. The chemical variation in the RJ of previous studies usually involved several of these factors. We aimed to determine the possible effects of strain, geography, and botanical origin on 10-HDA content in RJ.

Our data clearly indicate that bee strain influences the quantity of 10-HDA independently and this effect does not interacting with other environmental factors. RJ produced by A. m. ligustica by A. m. carnica bees contained more 10-HDA than that by A. m. ligustica bees (Figs. 2 and 3). These data are in agreement with the work of Wu et al. (2010), in which they found more 10-HDA in RJ from A. m. carnica than in the RJ of the Italian bee, A. m. ligustica. The reason for the difference was at least partially because of the large yield of RJ production by Italian bees in China. After the secretory ability of bees has reached its upper limit (Plettner et al. 1998), the quantity of 10-HDA will be constant thereafter, so even if the production increases, the relative content of 10-HDA to the total volume decreases. The average 10-HDA content in eight samples produced by Zhejiang Royal Jelly Bee was 1.62%, which was less than the total average content 1.83%; this result thus supports this explanation. However, five of these eight samples with a mean 10-HDA content of 1.69% were from the western group and the remaining three with 1.50% of 10-HDA were from eastern China. This difference in 10-HDA content corresponds with that of the general results,

indicating that the special high RJ production lineage did not affect the comparison between different geographical groups. The fact that the variance within the RJ produced by hybrid bees was smaller than that produced by *A. m. ligustica* might be attributable to the adaptability of the hybrid bees in various environments. Our results indicate that hybridization with *A. m. carnica* bees may elevate the 10-HDA concentration of RJ in *A. m. ligustica*.

The average content of 10-HDA varied in RJ produced on lime, vitex, and rape nectar flows, indicating differences in botanical origins could also affect the content of 10-HDA of RJ, possibly through fatty acids in bee pollen, which are the precursors of 10-HDA (Plettner et al. 1998), and which vary considerably in kind and proportions depending on their botanical origins (Villanueva et al. 2002, Bastos et al. 2004).

Influence of strain on RJ composition was separated from that of environment by comparing the 10-HDA content in RJ from the same strain (Fig. 2), and botanical effects were removed by analyzing RJ produced from pollen of the same plant (rape) at different locations (Fig. 3). The quantity of 10-HDA tended to be higher in areas at high latitudes and elevation above sea level where the temperatures were considerably lower than at the lower elevation of the warm, eastern region. Four of the five RJ samples containing the highest concentrations of 10-HDA were produced in Sichuan Province, which belongs to the western group; the remaining one was from the northeastern group. In contrast, the five RJ samples containing the least 10-HDA were all produced in eastern China.

In conclusion, the geographical origin and honey bee strain, and the botanical origin were all found to be associated with the 10-HDA content of RJ. Our basic data presented here may be applicable to the commercial production of RJ by assisting determining the prices of RJ from different sources.

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