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REVIEW ARTICLE

A review of methods used in some European countries for assessing the quality of honey bee queens through their physical characters and the performance of their colonies

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Summary

The term "quality" in relation to queens and drones refers to certain quantitative physical and / or behavioural characters. It is generally believed that a high quality queen should have the following physical characteristics: high live weight; high number of ovarioles; large size of spermatheca; high number of spermatozoa in spermatheca; and be free from diseases and pests. It is, however, also known that the performance of a honey bee colony is the result of its queen's function as well as of that of the drones that mated with her. These two approaches are often considered together and give a general picture of the queen production technique and selection. Here we describe the most common and well known anatomical, physiological, behavioural and performance characters related to the queens, as measured in different European countries: the live weight of the virgin queen (Bulgaria); the live weight of the laying queen (Bulgaria, Italy); the diameter and volume of spermatheca (Bulgaria, Greece, Slovenia); the number of ovarioles (Greece, Italy, Slovenia); the weight of ovaries (Slovenia); the number of spermatozoa in spermatheca (Italy, Poland, Slovenia); the brood pattern (Bulgaria, Greece); the egg laying ability / fecundity (Bulgaria); the brood production (Croatia, Serbia); the colony population development (Croatia, Serbia, Slovakia); the honey production (Croatia, Denmark, Serbia, Slovakia); the hygienic behaviour (Croatia, Denmark, Serbia, Slovakia); the defence behaviour (Croatia); the calmness / sitting on the comb (Croatia, Denmark); and swarming (Croatia, Denmark). The data presented fit well with the findings of the same characters in the literature, and in general they support the argument for the term "quality characters". Especially for the weight of the queen, the number of ovarioles, the volume of the spermatheca and the number of spermatozoa, data per country proved its own accuracy by repetition through the years. We also report that when instrumentally inseminated queens are kept under mass production conditions (in small cages in queen banks and with low number of attendants) they can transfer the semen to their spermatheca and clear their oviducts more efficiently when they have been inseminated with small amounts of semen in two or three sequences (but not four), compared to those inseminated with the same amount of semen at once (Poland). Furthermore, we had an inside view of the sanitary conditions of the colony: a. through the health status of the queen (nosema plus virus analysis) (Slovenia); and b. evaluating the nosema load of worker bees (Denmark) and of the queens (Greece). This is the first step to summarize this type of diverse data for such an important issue. The knowledge acquired can be used to fill in the existing gaps in the breeding or queen evaluation systems of each country in order to facilitate standardization of methodology for comparable results.

Revisión de los métodos usados en algunos países europeos para la evaluación de la calidad de las abejas reinas a través de sus caracteres físicos y el desarrollo de sus colonias

Resumen

El término "calidad" en relación con las reinas y los zánganos se refiere a ciertos caracteres físicos y / o conductuales cuantitativos. En general se cree que una reina de alta calidad debe tener las siguientes características físicas: un elevado peso vivo; elevado número de ovariolas; gran tamaño de la espermateca; elevado número de espermatozoides en la espermateca; y estar libre de enfermedades y plagas. Sin embargo se sabe también, que el rendimiento de una colonia es el resultado tanto de la función de su reina, como de los zánganos que se aparearon con ella. Estos dos enfoques se consideran a menudo juntos y dan una idea general de la técnica de producción y selección de la reina. Aquí se describen los caracteres anatómicos, fisiológicos, conductuales y de rendimiento más comunes y mejor conocidos de las abejas reinas, medidos en diferentes países europeos: el peso vivo de la reina virgen (Bulgaria); el peso vivo de la reina fecundada (Bulgaria, Italia); el diámetro y el volumen de la espermateca (Bulgaria, Grecia, Eslovenia); el número de ovariolas (Grecia, Italia, Eslovenia); el peso de los ovarios (Eslovenia); el número de espermatozoides en la espermateca (Italia, Polonia, Eslovenia); el patrón de cría (Bulgaria, Grecia); la capacidad de puesta de huevos / fecundidad (Bulgaria); la producción de cría (Croacia, Serbia); el desarrollo de la población de la colonia (Croacia, Serbia, Eslovaquia); la producción de miel (Croacia, Dinamarca, Serbia, Eslovaquia); el comportamiento higiénico (Croacia, Dinamarca, Serbia, Eslovaquia); el comportamiento de defensa (Croacia); la docilidad / asentamiento en el panal (Croacia, Dinamarca); y el enjambrazón (Croacia, Dinamarca). Los datos presentados se ajustan bien con los encontrados para los mismos caracteres en la literatura, y en general, apoyan los argumentos para el término "caracteres de calidad". Especialmente para el peso de la reina, el número de ovariolas, el volumen de la espermateca y el número de espermatozoides, los datos por país, probaron su propia exactitud al repetirse a través de los años. Se registró también que cuando las reinas inseminadas artificialmente se mantienen bajo condiciones de producción en masa (en pequeñas jaulas en los bancos de reinas y con bajo número de asistentes) éstas pueden transferir el semen a su espermateca y despejar sus oviductos de manera más eficiente cuando han sido inseminadas con pequeñas cantidades de semen en dos o tres secuencias (pero no cuatro), que cuando han sido inseminadas con la misma cantidad de semen en una sola vez (Polonia). Además, tuvimos un aspecto detallado de las condiciones sanitarias de la colonia: a. a través del estado de salud de la reina (nosema más análisis de virus) (Eslovenia); y b. la evaluación de la carga de nosema de las abejas obreras (Dinamarca) y de las reinas (Grecia). Este es el primer paso en la síntesis de este tipo de datos diversos para un tema tan importante. Los conocimientos adquiridos pueden servir para llenar los vacíos existentes en los sistemas de cría o de evaluación de la reina de cada país, con el fin de facilitar la estandarización de la metodología para obtener resultados comparables.

Keywords: honey bee, queen quality, breeding, spermatheca, ovary, ovariole, spermatozoa, performance, population, brood, honey production, swarming, defence, calmness, disease prevalence

Introduction

In social insects, the quality of the reproductive individuals, in honey bees (*Apis mellifera* L.) the queen and drones, indisputably affects the colony's performance. Reproduction and fertilization of the queen is a crucial stage in the life cycle of a honey bee colony as it determines its future fitness. Characteristics such as productivity, development, swarming, aggressiveness and resistance to diseases are all influenced by the genetic origin and quality of the queen as well as by characters of the drones with which she mates and various other environmental factors (e.g. food supply and nutrition, colony requirements, rearing season) (Avetisyan, 1961; Winston, 1987; Gencer *et al.*, 2000; Guler and Alpay, 2005; Akyol *et al.*, 2006; Al-Ghzawi and Zaitoun, 2008; Meixner *et al.*, 2014).

Table 1. Reports of correlations between various quality characters of honey bee queens and other factors affecting them.

Relation between quality characters	Positive correlation	No correlation
Weight of the queen - number of ovarioles	Weaver, 1957; Avetisyan, 1961; Woyke, 1971; Szabo, 1973; Wen-Cheng and Chong-Yuan, 1985; Gilley *et al.*, 2003	Corbella and Concalves (1982); Hatch *et al.*, 1999; Jackson *et al.*, 2011
Weight of the queen - diameter of spermatheca	Akyol *et al.*, 2008; Kahya *et al.*, 2008; Bieńkowska *et al.*, 2009	
Weight of the grafted larvae - number of queen ovarioles	Hoopingarner and Farrar, 1959; Woyke, 1971	
Number of ovarioles - diameter spermatheca	Weaver, 1957; Woyke, 1971	Jackson *et al.*, 2011
Number of ovarioles - brood production	Avetisyan, 1961	
Weight of the queen - brood production	Makarov, 1969; Akyol *et al.*, 2008	
Weight of the queen - early start of egg-laying	Taranov, 1974; Siuda & Wilde, 2006	Skowronek *et al.*, 2002
Weight of the queen fecundity -	Harbo, 1986	
Age of larvae used for grafting - number of ovarioles	Jordan, 1960; Woyke, 1960, 1964, 1971; Szabo and Townseed, 1974	
Age of larvae used for grafting - size of spermatheca	Jordan, 1960; Woyke, 1960, 1964, 1971; Szabo and Townseed, 1974; Gilley *et al.*, 2003; Tarpy *et al.*, 2000	
Age of larvae used for grafting - weight of queen	Tarpy *et al.*, 2000	
Size of the spermatheca - number of spermatozoa	Woyke, 1966; Bieńkowska *et al.*, 2008	

Commercialization of queen breeding requires the mass production of large numbers of high quality queens (Büchler *et al.*, 2013). But what does quality stands for? And how is it assessed? Some of the quality traits are related solely to the queen, such as her physical and physiological characters, whilst others are linked also to the quality of the mating and eventually to the performance of the respected colonies. It is generally believed that a high quality queen should have the following measurable characters: heavy live weight; high weight of ovaries; high number of ovarioles; large size of spermatheca; high number of spermatozoa in spermatheca; early start of egg-laying; high number of eggs laid per day (fecundity); quality of the brood; and be free from diseases and pests.

All of these characters are affected by external factors, including queen age and mating success (number and quality of available drones). For example, one of the most obvious characters of a high quality queen is its fecundity, which is higher in younger queens (Winston, 1980, 1987). A queen with high fecundity enables a colony to have a large worker population which will in consequence be highly productive. Indeed, several studies have shown that colonies headed by young queens (one or two years old) are up to 30% more productive than colonies headed by older ones in respect of brood production and honey yield (Avetisyan, 1961; Woyke, 1984; Genç, 1992; Kostarelou-Damianidou *et al.*, 1995; Akyol *et al.*, 2008). Inferior egg laying activity of older queens and reduction in sperm availability can cause a problem for overwintering, as the lower number of eggs laid in the autumn may result in colony collapse in winter and early spring due to insufficient number of worker bees (Kaftanoğlu, 1987; Genç, 1992; Tarpy *et al.*, 2000; Akyol *et al.*, 2008). Consequently, a starting higher number of ovarioles would presumably allow a queen to continue laying high number of eggs for longer and a larger spermatheca would allow for higher sperm storage.

So when referring to queen quality, we are including a combination of quantitative physical characters (such as most of the ones mentioned above) and many authors have found positive correlations between some of these quality parameters (Table 1), thus enabling measurement of only few parameters to assess the quality of a queen. It should be noted that the study by Hatch *et al.* (1999) in which the positive correlations noticed by others were not observed, was however performed on queens naturally produced by the colony during emergency queen rearing, and not reared by a breeder using common queen rearing techniques. According to Hatch *et al.* (1999) worker bees are actively involved in regulating production of new queens and preferentially rear queens starting from eggs. In artificial queen production (used in the other quoted studies) only uniform aged larvae are used, and thus pressure by the worker bees to select among differently aged developing queens is probably less accentuated. These differences in the rearing methods could explain the contrasting results.

Many studies on queen quality were developed to investigate the differences between naturally mated or instrumentally inseminated queens, and many of the characters listed above were used in these studies (Taranov, 1974; Szabo *et al.*, 1987; Medina and Goncalves, 2000; Skowronek *et al.*, 2002; Jasiński *et al.*, 2006; Siuda and Wilde, 2006). In instrumentally inseminated queens additional factors may influence queen quality, such as movement of the queen in the mating nucleus and the higher number of nursing bees in mating nuclei (Cobey *et al.*, 2013). These factors influence the successful migration of semen through the oviducts to the spermatheca (Woyke, 1979), as semen retention in the oviducts could be harmful and sometimes fatal to the queens (Vesely, 1970; Bieńkowska *et al.*, 2006, 2008, 2011).

As well as the above described physical characters, quality of a queen can be assessed indirectly through the performance of the colony she is heading. Accordingly, the colonies headed by a high quality queen should show the following performance characters: low swarming tendency; high hygienic behaviour; high brood production; high population production; high honey and pollen production; low aggressiveness; and resistance to diseases.

Many authors have investigated the link between the physical characters of the queen and the performance of the colony using various behavioural attributes such as brood production, honey production, colony weight and survival rate of the queens (Harbo and Szabo, 1984; Vesely, 1984; Konopacka, 1987; Boigenzahn and Pechhacher, 1993; Kostarelou-Damianidou *et al.*, 1995; Cobey, 1998; Collins, 2000; Pritsch and Bienefield, 2002; Al-Qarni *et al.*, 2003; Rhodes and Somerville, 2003; Rhodes *et al.*, 2004; Skowronek *et al.*, 2004, Akyol *et al.*, 2008).

As commercial honey bee breeding is increasing in Europe, the demand for evaluation of the quality of the queens on the market is also increasing. In this paper we review the research results from many countries in the field of queen quality control. Firstly we report on the different methodology used in some European countries and the characters taken into account for the direct and indirect evaluation of queen quality and secondly, the extent to which this methodology is used in practice, and results from application of the methods. Each European country is presented independently and in alphabetical order.

Standard methods used

For physical characters

Density of worker brood

- Bulgaria: A pattern of rhomb-comb (10 cells x 10 cells) containing 100 brood cells. The rhomb comb should not contain more than 10 empty cells (10%).
- Greece: two wooden circles of a 7.5cm diameter, measuring 167 complete worker brood cells are used (Greek modification of Collins, 2000) or both circles should contain > 98% worker brood cells and < 10% empty cells. Cells containing honey or pollen are excluded from the total count.

Fecundity of the queen

- Bulgaria: The worker brood cells in the colony are estimated by a netting frame (5sm / 5sm). Then the average fecundity for a 24 hour period is calculated by divided the number of worker brood cells with 12. The queen should lay more than 2000 eggs in this.

Weight of the queen

- Bulgaria, Italy: Live weight (mg) of virgin queens: only queens less than eight hours old are used. During May-August period twenty to thirty virgin queens per reproduction apiary are studied twice for this parameter. The live weight should not be less than 190 mg.
- Bulgaria: Live weight (mg) measurement of laying queens: ten randomly sampled queens that are laying on the day of sampling are examined twice during the same period (May-August). The live weight should not be less than 230 mg.

Weight of the ovaries

- Slovenia: Ovaries were weighted immediately after dissection using micro balance.

No of ovarioles

- Greece. The laying queens are transferred to the laboratory alive. After freezing they are dissected in an insect saline solution (Berger and Abdalla, 2005; Carreck *et al.*, 2013). The right ovary is located and removed and the ovarioles are counted following the procedure described in Rhodes and Somerville (2003) (Greece) or Eckert (1934) (Italy). Threshold for ovariole number was set to 130.
- Slovenia: Ovaries were fixed for 24 h in a 10% neutral buffered formalin and they were then dehydrated in an ascending series of alcohol and xylene and finally embedded in wax as described by Gregorc and Bowen (1999). Sections of 7µm were cut on a Leica microtome, floated on distilled water (40°C) and collected on cleaned slides. Tissue sections were then dewaxed by immersion in xylene and rehydrated in a series of alcohols and in distilled water. Tissue sections were further stained using Mayer's haematoxylin and Eosin, and finally mounted in Paramount aqueous mounting medium. Stained tissues sections of one ovary from each queen was randomly selected and examined using Zeiss stereomicroscope, digital photo camera was used to take pictures. Ovarioles in ten tissue sections from each ovary were counted using computer program Lucia.Net. Ovarioles were counted under 200 x magnification using Zeiss stereomicroscope.

Diameter of spermatheca

- Bulgaria, Greece: The spermatheca is isolated with the tracheal net around it and the diameter is calculated as the average of two crossed dimensions measured with a graduated microscope lenses or special software of an Image Analysis System. The diameter of the spermatheca should not be less than 1.2 mm.

No of spermatozoa in spermatheca

- Italy, Poland, Slovenia: The spermatheca is crushed in 0.5 ml of 0.9% saline solution. For further dispersion of spermatozoa 4.5 ml distilled water is added. The spermatozoa are then counted in 25 Bürker counting chambers (Koeniger *et al.*, 2005).

Presence of semen in oviducts

- Poland: Residue of semen in oviducts was classified when semen was present in at least one oviduct, versus no residue in either oviduct.

Presence of *Nosema* spp. spores in queens

- Greece: The alimentary canal of the queen is removed, placed in a 200µl of distilled water and homogenized. A sample of the suspension is examined under the haemocytometer slide for the presence of *Nosema* spp. spores. If *Nosema* spp. spores are found, their number should not exceed 500,000.
- Slovenia: Sampled queens' midguts were macerated in 1 ml PBS and a drop of suspension was examined under 400 x magnification and *Nosema* spp. identified. Spores were counted using Bürker's haemocytometer. Molecular determination and differentiation between *N. apis* and *N. ceranae* spores was conducted using 100µl of the spore suspension solutions according to the OIE Terrestrial Manual 2008.

For colony development or performance

Amount of brood and population

- Croatia: Colony development cycle is calculated from the data on number of combs occupied with bees and the combs containing brood.
- Serbia: The surface of comb covered by bees or brood was measured by dividing the comb in 10 vertical parts (five parts per side), a method described by Kulinčević *et al.* (1990), and also described in The Regulations on 'How to measure the breeding livestock traits' (Službeni Glasnik RS 21/96). The numbers represent the average of values recorded for each trait from all frames of the colony.
- Slovakia: Spring development is assessed by a four point system and estimated as hive strength (number of individuals and brood) by comparing the tested hives at the beginning of the production season at the date set usually in April or May according to the weather development. Four point system is used, where 4 points are given to the strongest colonies with quick and fluent development, 3 points to colonies with suitable spring growth and 2 or 1 points to colonies with insufficiently slow spring development and low brood areas.

Calmness (Ruttner, 1988)

- Croatia: Calmness behaviour shows how the bees are sitting on the comb, while the colony is examined and it is scored according to a 4 point system: 4 - very quiet on the honey and brood combs, 3 - quiet on combs, 2 - bees are moving on the combs, 1 - bees abandon the combs or hives.
- Denmark: Comb behaviour: 5 - very calm bees / bees are moving quietly on the frame, even if they are provoked / easy to get off the frames and don't fly up, 4 - calm bees / bees are a little restless on the frame / bees are flying up, when removed from the frame, 3 - nervous bees / bees are restless on the frame and flying, even when unprovoked, 2 - bees turbulent / bees are running on the frame / lots of bees are flying up, even when unprovoked, 1 - bees are very turbulent / lots of bees are flying up, even when unprovoked.

Gentleness (Ruttner, 1988)

- Croatia, Slovakia: Gentleness is evaluated during the whole year and the average is calculated from each inspection at the end of the productive season. It is assessed also according to a 4 point system: 4 - very moderate / protection from stinging or smoke is not needed, 3 - moderate / there is no need for protection, just little smoke / beekeeper is not attacked by bees, 2 - aggressive / smoke and protection clothes (veil, gloves) are necessary, 1 - very aggressive / the work is not possible without help of smoker, face protection and gloves are necessary / stinging occurs even at greater distance around apiary.
- Denmark: Defence behaviour: 5 - no stings, even without the use of smoker, 4 - no stings, but smoker is used, 3 - 1-3 stings unprovoked, 2 - 4-10 stings unprovoked, 1 - aggressive / lots of stings.

Swarming tendency (Ruttner, 1988)

- Croatia, Slovakia: Tendency for swarming- it is assessed by a 4 point system at the end of the season: 4 - colonies shows no inclination to swarming throughout the season / no queen cells occurred, 3 - queen cells can be found during a routine inspection / after extension of the hive by supers and destroying of existing queen cells, workers do not continue with their building, 2 - swarming can be managed just by creating artificial swarms, 1 - testing colony swarmed.
- Denmark: Swarming is graded from 1-5 (from low to high): 5 - no indication of swarming at all, 4 - small queen cells with eggs seen, but no swarm control management has been done / no swarming, 3 - queen cells with eggs seen / one swarm control management has been done. / no swarming, 2 - several swarming control managements have been undertaken, 1 - swarming.

Honey production

- Croatia: Honey production per colony is estimated in kg and recorded at each extraction of honey. Average honey production of the testing apiary is shown as 100. The score of each queen is calculated as a percentage of honey above or below average, and shown as relative value (%).
- Denmark: Honey production is compared to the average production of the apiary.
- Serbia: Nectar crop (= productivity) is measured by using a

modified Szabo method (Szabo, 1982). The weight of the colonies is recorded on day 1 and day 3 of the foraging season during acacia nectar flow.

- Slovakia: Honey extracted from the colony is weighted. Honey left in the colony is estimated and added to this amount.

Hygienic behaviour

- Hygienic test is done in the spring or summer, when there is enough brood – from May to August using the "pin-killed test" (Spivak and Downey, 1998; Büchler *et al.*, 2013). The number of cells with removed brood is counted after 12, 18, 24 and 36 hours (Slovakia) or after 24 and 48 hours in (Serbia) or 8, 12 and 24 hours (Croatia) or only 24 hours (Denmark). The average time in hours which the bees need to remove all killed brood is counted.

Infestation of *Nosema* spp. in the colony

- Denmark: The breeders collect 60 bees from the entrance of the colonies early in Spring (before the new bees hatch).

Varroa tolerance

- Slovakia: Optimal varroa drop-out is between 20 and 100. The percentage of mites damaged by bees is also counted.

Virus analysis

- Slovenia: Sampled queens' midgut are pooled and analysed for the presence of ABPV, BQCV, DWV and SBV viruses (de Miranda *et al.*, 2013).

Bulgaria

Introduction

A new actualized and detailed programme for honey bee selective breeding and queen rearing was developed and approved by the Ministry of Agriculture in Bulgaria in 1999. The main goal of that programme is to preserve the local Bulgarian honey bee. The breeding programme is carried out by the National Apiculture Breeding Association, with the support of the Ministry of Agriculture (MoA) and the financial support of State Fund 'Agriculture'. The National Apiculture Breeding Association was founded in 2001 with the aim of setting up a new scientific and organizational system of honey bee breeding and reproduction in Bulgaria. This includes the necessary infrastructure for the breeding process, named gene bank, selection centres, testing research apiaries, reproduction apiaries etc.

The biological and productive quality control includes determination and estimation of the queen fertility by quantification of: a. brood quantity and quality; b. winter resistance; c. swarming inclination; d. aggressiveness; e. honey and wax productivity; g. hygienic behaviour. Accordingly, honey bee colonies are chosen for breeding when they show high queen fertility, high honey productivity, good wintering ability, low aggressiveness, good hygienic behaviour.

The reproduction of the selected queens is carried out in registered reproduction centres. Through the above system the National Apiculture Breeding Association controls the activities of honey bee breeding and reproduction in more than 140 breeding apiaries and 12 registered queen reproduction centres in the country. Beekeepers can buy queens for their needs from the reproduction apiaries, all of which receive initial breeding material (instrumentally inseminated queens) from the 12 registered centres.

The following characters and thresholds are used for the characterization of the quality of the queens in the laboratory of the National Apiculture Breeding Association: density of worker brood; fecundity of the queen; weight of the queen (virgin and laying); and diameter of spermatheca.

Results

Here we report on activity carried out in the period 2008-2009. Data concerning diameter of spermatheca and season variability in live weight (mg) of virgin and laying queens for four reproduction apiaries are given in Tables 2, 3 and 4, respectively. According to the protocol diary data of the reproduction apiaries for the local Bulgarian honey bee *A. m. macedonica,* 70-80% of the queens tested reach the thresholds for the parameters above and although the queens reared in June had the highest mean weight at emergence (Table 3), the mean weight of laying queens in June were lower than the mean weights of laying queens in May and July (Table 4) probably due to the different rate of honey bee colony's development.

Croatia

Introduction

The breeding programme in Croatia was initiated in 1997 and describes the main breeding goals: increase of yields; tolerance to diseases (with emphasis on *Varroa destructor*); calmness on the combs, gentleness, decrease of swarming tendency and good spring development, characteristics for Carniolan bees *A. m. carnica* (Dominiković *et al.*, 1997). Currently, there are 33 queen breeders, with a total annual production of about 30,000 queens.

Queen quality control

The queen breeder is responsible for the selection of breeder queens, in accordance to the general rules of the breeding programme. The breeder carries out the evaluation of his colonies as a first level of performance test. Recordings are performed for each mother queen and drone producing colonies and noted on a "queen card". At the end of the season, the breeder sends the performance data to the Breeding Association.

Table 2. Diameter of spermatheca (mm) of Bulgarian honey bee queens from four reproduction apiaries: Cv% - coefficient of variability; $S\bar{x}$% - punctuality index; range (minimum and maximum values).

Regions	**n**	**Range**	$\bar{X} \pm S\bar{x}$	**Cv%**	$S\bar{x}$ **%**	**% queens not accepted due to bad quality**
Ralevo	11	1.15 - 1.40	1.270 ± 0.020	5.039	1.594	10.0
Smolyan	12	1.10 - 1.35	1.210 ± 0.024	6.348	2.012	30.0
Dimovtci	13	1.20 - 1.35	1.270 ± 0.013	3.150	0.996	7.6
Turgovishte	10	1.15 - 1.30	1.230 ± 0.013	3.252	1.028	10.0

Table 3. Seasonal variability in live weight (mg) of virgin queens of Bulgarian honey bees: Range (minimum and maximum); Cv% - coefficient of variability; $S\bar{x}$% - punctuality index

Month	**n**	**Range**	$\bar{X} \pm S\bar{x}$	**Cv%**	$S\bar{x}$**%**
April	176	180 - 235	202.8 ± 3.3	7.90	1.65
May	176	180 - 238	203.8 ± 3.5	8.21	1.71
June	176	179 - 236	225.7 ± 2.8	7.32	1.24
August	176	178 - 238	203.5 ± 5.1	10.57	2.49
September	176	184 - 242	212.2 ± 2.8	7.70	1.34

Table 4. Seasonal variability in live weight (mg) of laying queens of Bulgarian honey bees: Range (minimum and maximum); Cv% - coefficient of variability; $S\bar{x}$% - punctuality index

Month	**n**	**Range**	$\bar{X} \pm S\bar{x}$	**Cv%**	$S\bar{x}$**%**
April	55	198 - 284	250.5 ± 6.4	10.56	2.56
May	55	232 - 320	272.7 ± 5.1	7.75	1.88
June	55	230 - 294	261.0 ± 5.2	6.95	2.01
July	55	232 - 292	264.2 ± 6.5	7.83	2.46

Independent test

From each queen breeder at least 12 queens are distributed to the independent testing yards (regular queen buyers) in the same climatic region for testing. The origin and testing yard of queens are known only to the organizers of the test. All reports containing the results of the average scores for each performance trait are published for general use by queen breeders and public (Dražić *et al.*, 2001; 2003; Kezić *et al.*, 2001; Svečnjak *et al.*, 2008; CAA, 2009).

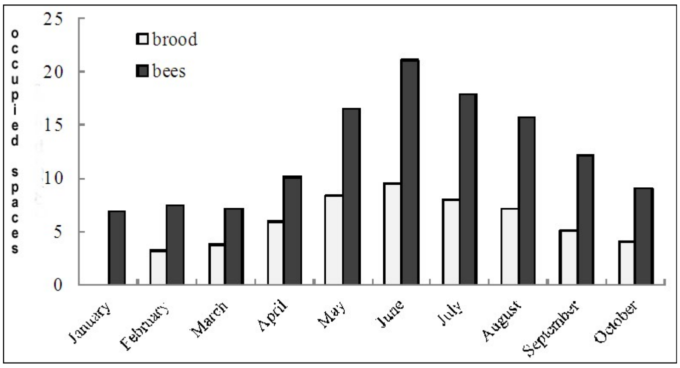

Fig. 1. Average strength of test colonies in continental region.

Results and discussion

Carniolan bee colonies have intensive spring development and maintain a high number of bees during summer (Figs 1 and 2). However, colony development depends on the season (temperature, pasture, dry or rainy periods) and region.

Bees in the Mediterranean region have a short winter break in brood cycle (maximum one month) (Hatjina *et al.*, 2014). A second break, which can be longer than a month, is recorded in the majority of colonies during hot and dry summer season. To avoid negative effects on colonies, almost half of the beekeepers move their colonies to the mainland during the warmest period.

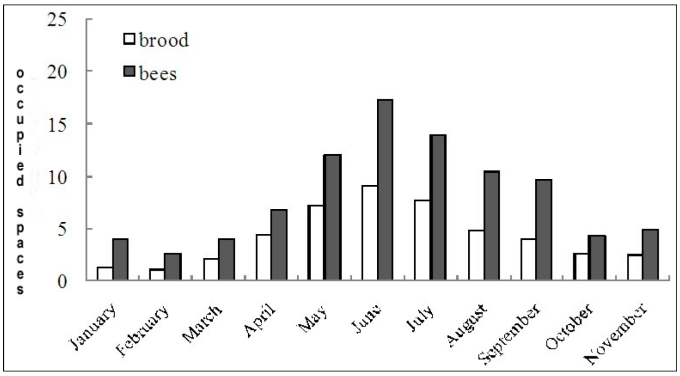

Fig. 2. Average strength of test colonies in Mediterranean region.

Behavioural traits

Behavioural traits of the Carniolan bee are presented in Table 5. The Carniolan bee is known as non-aggressive and calm on the combs during hive manipulation. These traits have to be preserved and improved through the breeding programme. Swarming tendency is a major negative characteristic of Carniolan bees. Care is being taken in order to decrease the intensity of this trait. The results of the swarming tendency demonstrate high variability between years.

Hygienic behaviour

Hygienic behaviour is shown in Table 6. Tolerance to diseases has been emphasized in the breeding programme for years. During the last 10 years, several practical methods for data collection of varroa tolerance traits have been used. Initially, mites fallen on the bottom boards after chemical treatment and calculation of varroa population development were used. Later, natural mite fall was introduced, as well as the pin-

Table 5. The mean scores for behavioural traits of tested colonies during five consecutive years.

Testing year	N	Defence		Calmness on comb		Swarming	
		mean	sd	mean	sd	mean	sd
2004	121	3.30	0.47	3.30	0.48	3.36	0.71
2005	66	3.49	0.56	3.33	0.62	3.59	0.74
2006	124	3.31	0.73	3.29	0.73	3.51	0.67
2007	156	3.13	0.70	3.04	0.74	2.59	0.97
2008	155	3.25	0.58	3.25	0.48	3.41	0.76

Table 6. Average number of punched sealed cells after 8, 12 and 24 hours in a hygienic test.

Testing year	N	Average number of sealed cells after		
		8 hours	12 hours	24 hours
2004	2	12.00	7.50	0.00
2005	8	13.40	8.40	0.00
2006	13	25.45	9.55	0.00
2007	63	32.64	12.77	0.36
2008	78	14.55	8.73	3.49

Table 7. Average (kg±sd) and relative honey production (RHP; %) of the test queens in period from 2004 to 2009 in continental and Mediterranean regions of Croatia.

Test year	Continental region			Mediterranean region		
	N	Honey (kg ± sd)	RHP (%)	N	Honey (kg ± sd)	RHP (%)
2004	46	33.70 ± 15.69	101.97	16	15.83 ± 2.72	99.88
2005	55	36.53 ± 19.35	97.99	39	14.01 ± 10.74	95.33
2006	88	39.13 ± 24.01	98.89	14	16.50 ± 12.11	97.90
2007	118	37.46 ± 24.52	99.62	17	14.65 ± 6.94	101.24
2008	131	28.70 ± 10.84	100.57	7	24.43 ± 9.52	104.02
2009	122	30.97 ± 10.98	99.99	10	12.90 ± 7.06	100.39

test for hygienic behaviour (adjusted method of Newton and Ostasiewski, 1986). A total of 50 sealed cells in stage of pink eyes of bee pupae are punched with an entomological needle (Büchler *et al.*, 2013).

Queen breeders are very cautious to express improvement of varroa tolerance in breeding stock, due to high expectations from beneficiary beekeepers. Concern is that beekeepers will relax attention for varroa infestation, which could cause severe mortalities, and as such, a negative reputation for queen breeder.

Honey production

Honey production is in the core interest of beekeepers when they estimate queen quality (Table 7). Honey production of queens in test is compared only with other colonies at the same location. Climate and pasture conditions differ among regions, and therefore it is difficult to compare honey yields between apiaries or seasons.

Denmark

Introduction

Danish beekeeping is relatively small. The Danish Beekeepers Association had 4,296 members in October 2011. From this we estimate the total number of beekeepers as 4,700 beekeepers, in a population of 5 million people. Commercial queen rearing is carried out by approximately 20 breeders. The number of colonies is estimated to be approximately 170,000.

Several honey bee subspecies and strains have been used over the years in Denmark. Different breeds have been favoured over time. Until the 20th century the native black bee (*A. m. mellifera*) was the dominant subspecies. The black bee has been forced back by the introduction of other types of bees and today only exists as a small population of approximately 200 colonies on the small island of Læsø (Kryger and Jespersen, 2011).

Performance tests by the Danish Beekeepers Association

Since 1990, the Danish Beekeepers Association has offered Danish queen breeders testing of queens for sale, regular open mated offspring of the breeding queens. The queens are tested in 10 different apiaries, evenly distributed all over the country. The queens are tested under routine beekeeping conditions. Ideally these testers represent the normal queen consumer. The identity of each queen is unknown to the testers and the queen breeders do not know who the test beekeepers are.

Nosema spp. levels have been at the focus of Danish bee breeding for many years. *Nosema* spp. has been the biggest threat of Danish beekeeping, probably due to the long, but mild winters, with a high humidity from the nearby sea. However, in the late 1980s a Danish school for difficult youngsters, offered to make digital analysis of bee samples for *Nosema* spp. spores for a price of €3.50 per sample. Since the early 1990s this has been performed annually every spring on offspring of all potential breeder queens. The breeders must collect exactly 60 bees in early April before the hatching of young bees, and send these for analysis. The method is rapid and the results from the approximate 400 colonies tested are available before the start of the breeding season in mid May. The digital camera system is unable to differentiate between *N. cerana* and *N. apis*. However, *N. cerana* was detected in bees from 2003 in Denmark (Klee *et al.*, 2007) and is widespread over the country as in the neighbouring countries (Vejsnæs, 2011). Breeder queens showing any signs of *Nosema* spp. in these tests are excluded from further breeding. *Nosema* spp. is today regarded as an overcome problem of Danish beekeeping even with the presence of *N. ceranae* (Fig. 3).

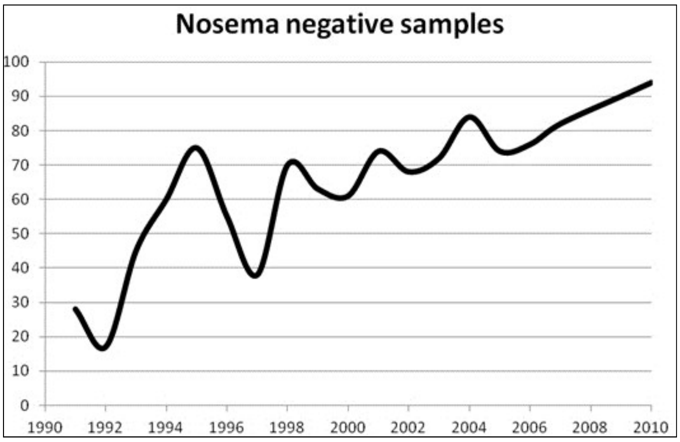

Fig. 3. The percentage of *Nosema* spp. spore negative samples in Denmark has increased since the start of the breeding programme from 20-40% negative samples to over 90% in the last few years.

Hygienic behaviour was only included in the Danish breeding programme during the late 1990s following a few years with increasing number of outbreaks of American foulbrood (AFB), from 50 to 60 between 1991-1995, suddenly the numbers rose to 130 cases. Over the years the number of empty cells has increased to a level where it seems necessary to decrease the hours in the hive to 24 hours, to get better measurements. In 2010 more than half of the 225 colonies clean out 80% or more of the dead brood compared to 40% in 2001, and 1 of 5 cleaned out 100% in 2010. All breeders do the testing in the same week, and the results are made public before the next season. Since the 2001 the number of outbreaks of AFB in Denmark has decreased, and in 2010 only 33 cases were reported. It is, however, difficult to assess the influence of breeding on this fall in outbreaks.

Evaluation

The trait selection is focussed on the following characters during the season: Honey production; swarming; temper; and comb behaviour. The results of the queen breeder tests are published every year in the Danish Beekeepers magazine in the springtime. All records are averaged over the apiaries to calculate a relative number for each trait. Hence it is possible for a queen user to choose queens with traits that seems most suitable for his stock, depending on his preference for low swarming tendency, or honey production. For honey production, typically the variation is more pronounced than for the other traits, probably due to finer scale it is measured on. In 2010 the average yield was 62.8 kg, higher than usual, with the best line producing 1.52 times this amount, and the poorest honey producer giving 0.72 times this. For swarming, gentleness and calmness the range is from 0.9 to 1.14 times the average, 0.92 to 1.12 times, and 0.89 to 1.13 times respectively. In Denmark there is no comparison in place to follow the progress over the years, in the behavioural traits. While it is possible to see gain in gentleness and swarming behaviour, when compared to the bees before the establishment, the progress was so rapid in the first years that several revision of the scale was implemented.

Considerable variation between various strains of individual breeders results from the frequent introduction of subspecies from much of the world.

Greece

Introduction

No national or controlled breeding system yet exists in Greece. Newly mated queens tend to be sold very quickly after the start of egg laying without allowing evaluation of their performance for a long period. It is thus, very essential to evaluate the quality of the commercially produced queens in a quick and a precise way during the first days of oviposistion. The Laboratory for Verification of Honey Bee Queens' Quality which belongs to the Hellenic Institute of Apiculture (of the Hellenic Agricultural Organization 'DEMETER') and was established in September 2007, according to specifications of ELOT EN/ISO 17025 in order to meet the above demands and to promote the use of high quality local queens. For the determination of the quality of young laying queens, the following biological and physiological parameters are measured: density of worker brood cells in an area of a 7.5cm diameter circle; percentage of empty brood cells in the same brood area; the number of ovarioles in one ovary; the diameter of spermatheca and the presence of *Nosema* spp. spores in the queen's alimentary canal (for detailed protocol see Hatjina, 2012).

Results and discussion

Data represents evaluations on bee breeders during 2007, 2008 and 2009 only once per year and for a specific month of produced queens. The tested queens from populations C, D, E, F and G were of local origin (*A. m. macedonica* or its hybrids). The queens from population B were found different from the above, and were thought to belong to an ecotype of *A. m. macedonica* (Hatjina *et al.*, 2008; research in progress). Finally the queens of population A were also found to be different, and it is believed that they belong to *A. m. cecropia* (research also in progress).

Between ten to seventeen queens per breeder were dissected in June 2007, in July 2008 and in July 2009. The percentages of the empty brood cells for all queens tested are presented in Fig. 4. Mosaic brood appearance declares a problematic queen and the results of the three years presented here show that the brood of the queens tested had a uniform appearance with very few empty cells (far below 10%). Spottiness is the first sign of a diseased or a poorly fertilized queen and although bee breeders do not measure it, they take the appearance of the brood into account when they access the quality of the queen. The same applies for the number of drone brood cells. A good queen never lays drone eggs that eventually will be developed to drone cells in the middle of the brood area.

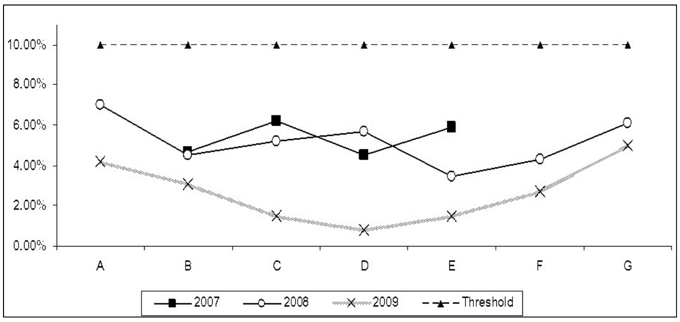

Fig. 4. Percentages of empty brood cells in a circle area of 7.5 cm diameter.

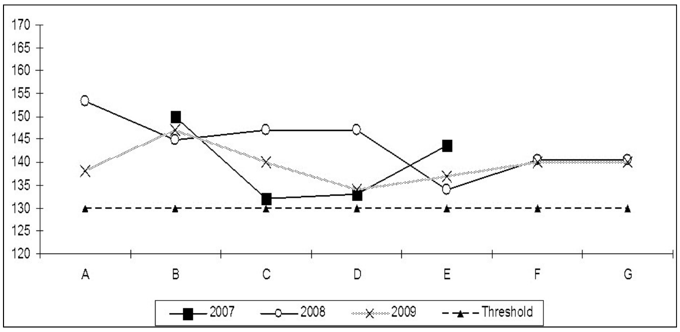

Fig. 5. Average number of ovarioles for each population tested.

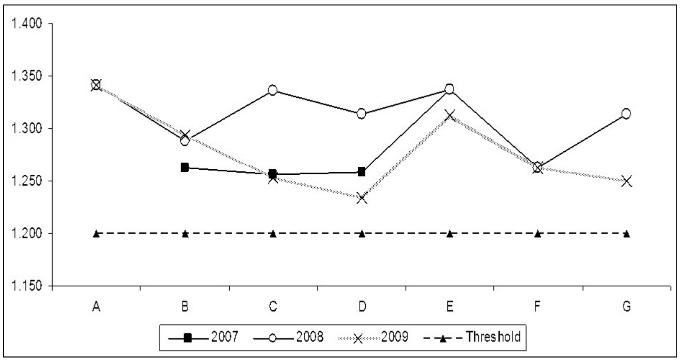

Fig. 6. Average diameter of spermatheca of all queens tested.

The numbers of ovarioles are presented in Fig. 5. All queens examined had ovariole number well above the threshold, but there were fluctuations between the years regarding the same bee breeder. At the same time, the diameter of spermatheca was also well above the threshold and with minor fluctuations between the years (Fig. 6) assuming that spermatheca volume is accordingly high and full of active and alive spermatozoa. However, as no direct measurements are taken for the viability of the spermatozoa in the spermatheca, an indirect measurement of this parameter is the appearance of the brood. According to Collins (2000), queens with less than 42% live spermatozoa produced brood with > 20% empty cells and became 'drone layers' more quickly than usual.

Nosema spp. spores were found only in some cases to be as high as several thousand spores, and in the remainder no spores were found in the alimentary canals of the queens. Health is a very important issue regarding the quality parameters of queens, as both queens and accompanying workers can transmit *Nosema* spp. to new colonies. On average, all queens tested had characteristics above the threshold allowing us to consider them as 'good' quality queens. Some fluctuations existed among the years, for some populations, as expected for a very dynamic organism as the honey bee queen, whose reproduction is affected by many practical, biological, genetic and environmental parameters. The fluctuations between the years also show the potential for improvement of the tested parameters taking into consideration the different aspects of the queen's reproductive biology.

Considering also the existence of some genetic differences between the populations tested, it is very important for Greek scientists and bee breeders to determine: a. if these differences are continuously reflected on the above measured characteristics; b. which are the characteristics of each distinct population/ ecotype of the Greek honey bees; and c. how the above measured characteristics correlate with the performance of the queen later in her life, a performance that refers to brood and population dynamics as well as to honey production, disease prevalence etc. Furthermore, with the continuation of the function of the accredited laboratory, more parameters are considered to be used in assessing the quality of honey bee queens, such as the number of alive spermatozoa in the spermatheca, the weight of the ovaries, and the number of *Nosema* spp. spores on the accompanied bees. The new parameters will give added value to the methodology used by the Accredited Laboratory and they will help to complete the physiological profile of the queens' quality.

Italy

Introduction

Routine quality control of queens used in the Italian National Breeding Programme is carried out by performance testing and estimation of breeding values with the BLUP Animal Model adapted for honey bees (Bienefeld *et al.*, 2007). However, occasional assessment of physiological parameters of queens produced by registered breeders is carried out. The aim of the work presented in this article was to compare instrumentally inseminated (II) and naturally mated (NM) queens by analysing their physiological qualities as expressed by the standard parameters of ovariole number, queen weight and sperm number. We chose to examine two-year old queens because most studies on II queens have focused on younger queens (Cobey, 2007). The queens belonged to two groups: 54 NM queens, produced by 4 breeders listed in the National Register of Queen Bee Breeders; 44 II queens, produced at the CRA-API laboratory using strains from the same breeders as above. II queens had a single insemination with 8 µl of semen from drones of a number of colonies located in the experimental apiary of the CRA-API.

Results and discussion

Ovariole number

As expected, no difference was found in ovariole number in differently mated queens (Table 8) ($t = 0.05$, $P = 0.96$). (The high average number of ovarioles found in this study confirms the results of several authors who compared *A. m. ligustica* to other races: Casagrande-Jaloretto *et al.* (1984) found that *A. m. ligustica* in Brazil had a greater number of ovarioles than *A. m. caucasica* and Africanized honey bee, the latter confirmed by Thuller *et al.* (1998); Chaud-Netto and Bueno (1979) found that *A. m. ligustica* had a significantly greater number of ovarioles compared to *A. m. scutellata*.

Queen weight

This parameter can vary considerably due to egg laying intensity and various other factors and mechanisms (genetic, biochemical) that affect egg laying and queen status at the time of assaying. Our results however did not produce significant differences between the two mating type groups (Table 9) ($t = 1.66$, $P = 0.1$) thereby highlighting the absence of influence of the instrumental insemination procedure (including anaesthesia) on weight increase, resulting from full development and maturation of the egg laying organs.

Sperm number in spermatheca

The number of sperms remaining in the spermatheca was significantly higher ($t = 3.47$, $P < 0.01$) in the NM queens, with an average of 2.78 million spermatozoa against 1.91 million in II queens (Table 10). Literature reports approximately 3-5 million spermatozoa reaching the spermatheca as the result of a single insemination with 8-10 µl of semen (Mackensen, 1964; Woyke, 1960; Konopacka and Bienkowska, 1995; Bienkowska *et al.*, 2008, 2011). In naturally mated queens, Woyke (1960) reported finding an average value of 5.3 million spermatozoa in the spermatheca after mating flights. It has also been reported that sperm number in the spermatheca decreases with queen age (Page, 1986; Szabo and Heikel, 1987). Our current study noted a considerable variability with a coefficient of variation (CV) of 41.4% in NM and 51.3% in II (for ovariole numbers the CV = 9.8% in NM and 11.7% in II; for queen weight CV = 10% in NM and 13.2% in II). The high variability is easily explained in NM queens given the numerous variables governing mating flights but is less easily explained in II queens unless we assume subjective factors causing different spermatheca filling performance from insemination onwards.

Relation between queen weight and ovariole number

A statistically significant correlation between queen weight and ovariole number was found for the II queens ($N = 27$, $R^2 = 0.325$, $P < 0.01$) (Fig. 7) but not for the NM queens ($N = 47$, $R^2 = 0.002$, $P = 0.766$), (Fig. 7). The relationship between body weight and ovary size has been underlined by some authors (Eckert, 1934; Komaroff and Alpatov, 1934; Burmistrova, 1965; Kahya *et al.*, 2008) but not by others (Corbella and Gonçalves, 1982). Our data suggest that ovaries of II queens are on average heavier than those of NM queens. The weight of ovaries does not only depend on the number of ovarioles but also on the number and development stage of eggs in ovarioles, which in turn depend on the rate of egg laying activity of a queen. It therefore seems that our II queens were laying more actively than NM queens.

Table 8. Mean ovariole numbers in 2 year old naturally mated (NM) and instrumentally inseminated (II) queens.

Type of mating	n	Right Ovary	Left Ovary	Total (Mean ± SE)	Min	Max
NM	47	174	173	346 ± 4.96	285	424
II	27	173	173	346 ± 7.82	284	442

Table 9. Mean (± SE) weight of 2 year old NM and II queens. *Means are not significantly different.

Type of mating	n	Weight (mg)	Min	Max
NM	54	221 ± 3.09*	160	270
IIS	43	223 ± 4.5*	175	293

Table 10. Residual number (mean ± SE) of spermatozoa in the spermatheca of 2 year old NM and II queens. *Means are significantly different.

Type of mating	n	N° of spermatozoa (millions)	Min	Max
NM	46	2.78 ± 0.17*	0.7	6
IIS	42	1.91 ± 0.16	0.20	4.5

Relation between sperm number and ovariole number

No correlation was found between the two characters in NM queens ($N = 39$, $R^2 = 0.000$, $P = 0.898$), while ovariole number in II queens was significantly correlated with residual sperm number ($N = 26$, $R^2 = 0.243$, $P = 0.011$) (Fig. 8). In NM queens the initial number of spermatozoa depends on the numerous variables influencing the mating flight, while in II queens the amount of introduced semen is known (in this study 8 µl), and this factor could influence the residual sperm number two years later.

Furthermore spermatheca size cannot be ignored: the number of spermatozoa transferred from oviducts to spermatheca has been shown by Woyke (1960) to be correlated with the volume of the spermatheca rather than the amount of semen received. Given the significant correlation between residual semen and ovariole number only in II queens, we can assume a correlation between spermatheca size and ovary development defined as number of individual components (ovarioles). Thus reproductive organ development is reciprocally limited, at least from a volume-quantity standpoint. The results obtained by Corbella and Goncalves (1982) and Phiancharoen *et al.* (2010), which show no connection between spermateca volume and number of ovarioles in NM queens, does not contrast with our data which refers to II queens.

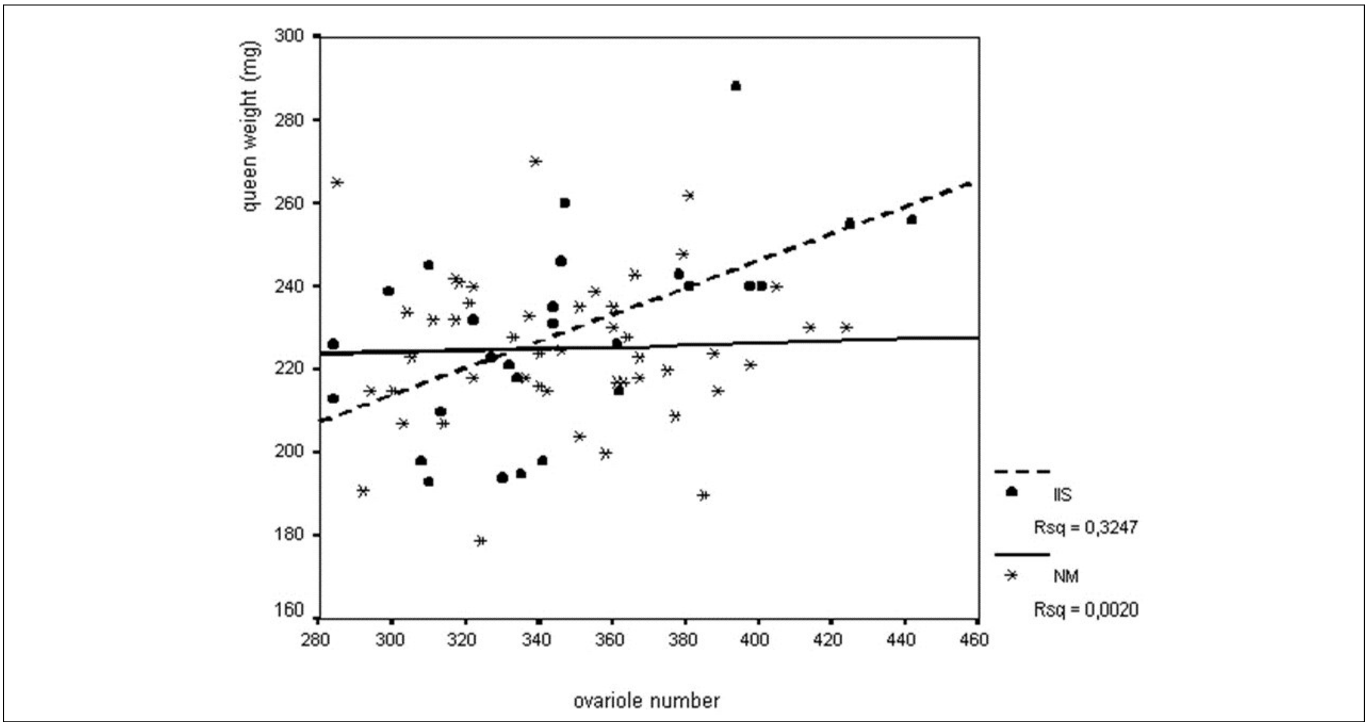

Fig. 7. Relation between queen weight and ovariole number.

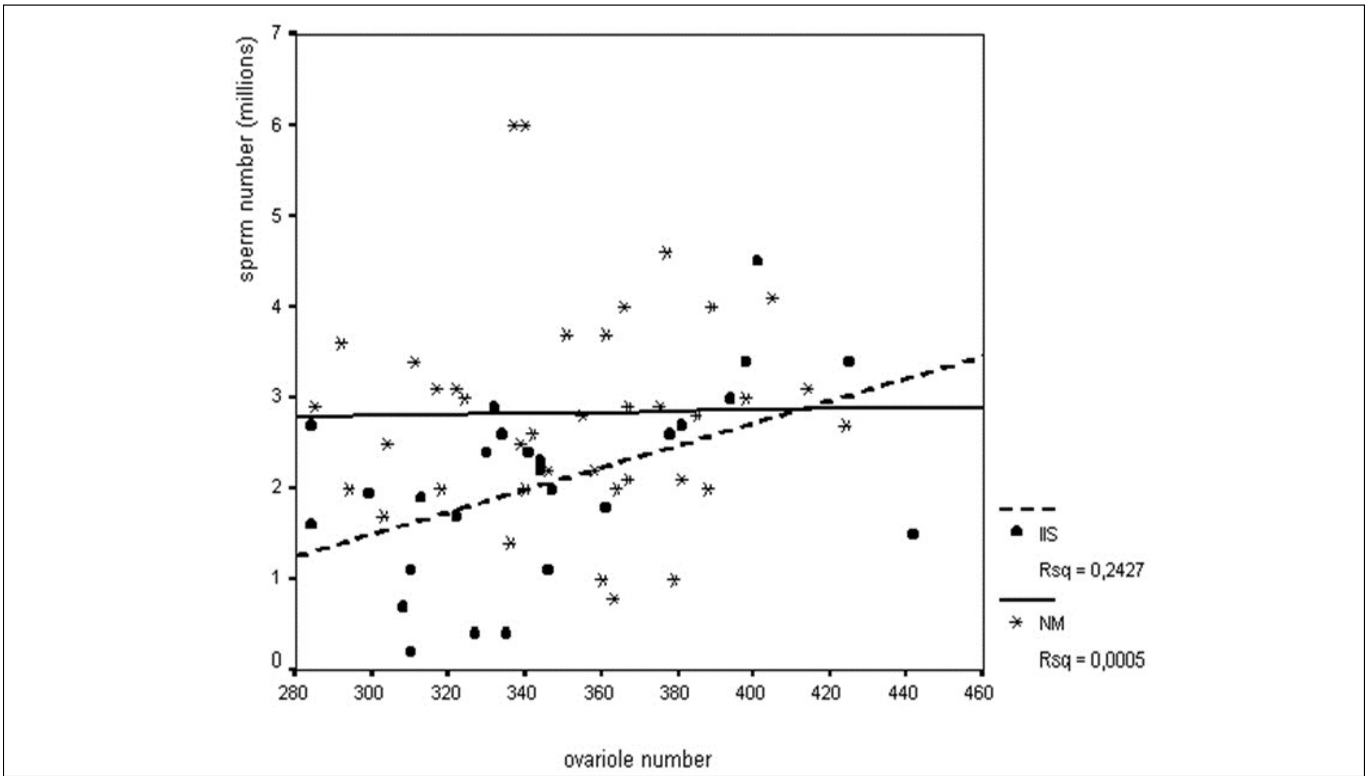

Fig. 8. Relation between sperm number and ovariole number.

Conclusions

The results obtained showed that for queens with natural mating, which are the vast majority of the commercial queens produced by professional queen breeders in Italy, the quality of the tested queens, in terms of ovariole numbers, queen weight, and semen in the spermatheca, was considered suitable, by comparison to existing data in the literature, and could represent the baseline for future *in vivo* assessment of Italian queen bees.

Poland

Introduction

In Poland honey bee breeding has been regulated by governmental law on farm animal management and reproduction for over 30 years. Breeding of three subspecies of bees is conducted: *A. m. mellifera* - commonly called national bee or local (black bees); *A. m. carnica* and *A. m. caucasica*. Since 2000 the national Black Bee Genetic Resources Conservation Programme has been carried on for four lines of *A. m. mellifera:* Asta, Pólnocna (North Bee), Kampinoska and Augustowska. A total of 57 programmes are run for genetic improvement of the different lines within the subspecies. Breeding activities take place in 90 breeding apiaries. Instrumental insemination is used for the fertilization of all queens.

The selection of breeding material is conducted in breeding as well as in selected production apiaries. Except for useful traits like honey yield, wintering and spring development, there are also evaluated biological traits such as swarming, wintering and gentleness. Morphological measurements based on the length of proboscis, cubital index and width of 3rd tergite are used for racial identification of the bees (Bouga *et al.*, 2011). The final evaluation is expressed as a sum of points obtained by each queen of tested colonies.

The above system is used for evaluation of queens that have been accepted or develop normally in their colonies. However, over 80 thousand instrumental inseminated (II) honey bee queens are sold to beekeepers annually, without a previous test of their egg laying capacity (Troszkiewicz, 2006) or other aspects. Here we present a recent study to evaluate the effect of various factors on the quality of II queens in mass production apiaries. The novelty in this study is that the inseminated queens were kept in very small cages in a queen bank with low number of attendant bees as is the practice in mass production apiaries and not in nucleus or in hives as has been demonstrated before.

Methodology

The tested factors were weight of queens just after emergence and just after insemination, dose of semen injected, as well as repetition of insemination in conjunction with dose of semen. The evaluated characters affected by these factors were the status of oviducts, and number of spermatozoa entering the spermatheca. Size of spermatheca was also measured as a queen quality character. The study was conducted in the apiary of the Apiculture Division in Pulawy, during the year 2007-2008. A total number of 641 daughter queens of *A. m. carnica*, Marynka line, were produced. One group of queens (n = 358, group A) were first weighed just after emergence and then inseminated once with 8 µl of semen collected from drones of the same line, when they were 14-21 days old. The remaining 283 queens (group B) were divided into smaller groups and were inseminated once or at intervals with 6 µl or 8 µl of semen from drones of the same line. After insemination, all queens were put in plastic transport cages along with 25 workers and stored in a queen bank. After 48 hours of storage in the bank, the queens were evaluated according to the above characters. Surviving ones were killed and then dissected to examine their oviducts for residue of semen and to count the number of spermatozoa that entered the spermatheca.

Results

Evaluation data of the 358 queens from group A is summarized in Table 11. The mean weights of the queens just after emergence and during insemination were 199.5 mg (min = 125mg and max = 255 mg) and 170.3 mg (min = 115mg and max = 218 mg) respectively. Eight of these queens (2.2%) were found dead at the time of the evaluation, while 67 queens (18.7%) still had semen in their oviducts (Table 11). A positive correlation (r = +0.66) was found between the weight at emergence and the weight at the time of insemination. The average volume of spermatheca was 0.82 mm^3 which significantly correlated with the weight of queens at emergence (r = +0.40) and during insemination (r = +0.45) (Table 11). The 67 queens that still had semen in their oviducts had a slightly smaller size of spermatheca and a significantly lower number of spermatozoa in the spermatheca (Table 11).

A high proportion (79%) of queens in group A (n = 283) had oviducts free from semen and were further classified as heavy, normal or light weight (Table 12). Significant differences ($P < 0.05$) were found between the characteristics of the three classes of queens, with heavier queens having the largest size of spermatheca and the highest number of spermatozoa in the spermatheca (Table 12).

Of the total 283 queens inseminated with different doses of semen, 13 (4.6%) died within two days. Nearly 87% of these queens had empty oviducts, and 8.8 % still had semen in their oviducts (Table 13). The number of queens with empty oviducts was significantly higher when the queens were inseminated several times (twice or thrice) with small amounts of semen compared to those inseminated only once with 6 µl or 8 µl (Table 13). But a little more than half (62.1%) of queens inseminated four times with 2 µl semen cleared their oviducts completely.

The number of spermatozoa in the spermatheca of queens with completely empty oviducts was higher in queens inseminated with 8 µl of semen instead of 6 µl but not significantly (Table 13). Also, when the same dose of semen was divided into small amounts and the queens were inseminated with small doses of semen in sequence, the number of spermatozoa entering into the spermatheca increased. This increase was significant when the queens were inseminated 2 or 3 times, but not 4. When comparing the different doses of semen and the amount of spermatozoa in the spermatheca, a significantly higher number of spermatozoa filled the spermatheca of the queens inseminated 4 times with 2 µl of semen, but in this group the percentage of queens with clear oviducts was 62.1% - significantly lower than in other groups (Table 13).

Table 11. The characters of queens that were inseminated once with 8 µl semen (group A). a, b –Significant differences at P ≤ 0.05; Sd – Standard deviation.

The queen	Number of queens		Weight at emergence (mg)		Weight at insemination age (mg)		Size of spermatheca (mm^3)		Number of spermatozoa in spermatheca (x 1000)	
	N	%	mean	Sd	mean	Sd	mean	Sd	Mean	Sd
with empty oviducts	283	79.0	201 a	19.8	172 b	17.5	0.85 a	0.15	4.007 b	1.40
with remaining semen in oviducts	67	18.7	193 a	21.8	166 b	17.6	0.81 a	0.13	2.873 a	1.09
died soon after insemination	8	2.2	-	-	-	-	-	-	-	-
Total	358	100	199.5	20.5	170.3	18.0	0.82	0.19	3.705	1.51

Table 12. Characteristics of different queen weight classes (group A). a,b,c, – Significant differences at P ≤ 0.05.

Class of queen	N	%	Weight at emergence (mg)		Weight at Insemination age (mg)		Size of spermatheca (mm^3)	Number of spermatozoa (X 1000)
			mean	Range	mean	Range	mean	Mean
Light	57	20.1	173.7 a	125 - 185	154.0 a	119 - 183	0.75 a	3.556
Moderate	137	48.4	197.4 b	186 - 210	171.8 b	115 - 212	0.85 b	4.069
Heavy	89	31.4	223.9 c	211 - 250	183.1 c	131 - 218	0.92 c	4.201
Total	283	100	201.0	125 - 250	171.8	115 - 218	0.85	4.007

Table 13. The percentages of single and multiple inseminated queens with empty oviducts 48 hours after last insemination and the number of spermatozoa in spermatheca of theses queens (group B). a,b,c,d – Significant differences at P ≤ 0.05, (after ArcSin transformation).

Dose of semen	No of queens	Queens with empty oviducts				Queens with semen filled oviducts		Dead queens	
		N	%	Number of spermatozoa (millions)	Sd	N	%	N	%
1 x 6 µl	30	25	83.3 ab	3.494 a	1.08	4	13.3ab	1	1.3a
2 x 3 µl	61	57	93.4 c	3.953 ab	1.29	2	3.3a	2	3.3a
3 x 2 µl	38	36	94.7 c	4.007 b	1.07	-	-	2	5.3b
1 x 8 µl	25	21	84.0 ab	3.902 ab	0.78	4	16.0ab	-	-
2 x 4 µl	100	90	90.0 c	4.009 b	1.40	5	5.0a	5	5.0b
4 x 2 µl	29	18	62.1 a	5.269 c	1.56	10	34.5b	1	3.4a
Total Average	283	247	87.3	3.319	1.38	25	8.8	13	4.6

Discussion

The above findings confirmed that the weight of the queen at the time of insemination is significantly lower compared to the weight at the time of emergence (Taranow, 1973; Nelson and Gary, 1983; Skowronek *et al.*, 2002, 2004). The reduction of body weight at the time of insemination could be due to preparation for mating flight (Eid *et al.*, 1980). A positive correlation was found between the weight of the queen at emergence and the size of spermatheca later on as well as number of spermatozoa entering into the spermatheca. Woyke (1960) and Bieńkowska *et al.* (2008) have also reported the positive correlation between the number of spermatozoa and the size of the spermatheca. The higher weight of queens at emergence is associated with larger size of spermatheca, a larger amount of semen entering the spermatheca and empty oviducts. Similarly the larger size of spermatheca has been found to be correlated with empty oviducts and hence, higher number of spermatozoa entered into the spermatheca when inseminated. It has also been found that a queen with a lower body weight at emergence had not only significantly smaller size of spermatheca but also could not pass all the semen from the oviducts into the spermatheca when inseminated with 8 µl of semen at once. Therefore, in a queen which is heavier at emergence a higher number of spermatozoa passes into the spermatheca compared to a lighter queen. Similar findings were reported by Avetisyan (1961), Woyke (1971), Szabo (1973), Gilley *et al.* (2003), Akyol *et al.* (2008), Bieńkowska *et al.* (2008, 2009).

In the mass production of instrumentally inseminated queens, the queens are most often kept in small cages with about 25 attendant bees in queenless colonies. These are not the optimal conditions for queens after insemination. Keeping queens in small cages after insemination results in high number of individuals which do not clear the excess of semen from their oviducts. Thus, queens kept this way, have lower number of spermatozoa in the spermatheca (Vesely, 1969, 1970; Woyke, 1979, 1983, 1989; Woyke and Jasinski, 1978, 1980, 1982, 1985; Gontarz *et al.*, 2005; Bieńkowska and Panasiuk, 2006; Bieńkowska *et al.*, 2008). However, for economic reasons, it is the only acceptable method in the commercial production of instrumentally inseminated queens. In this study, the average percentage of queens with some semen residue in their oviducts was 8.8%. However, depending upon the dose of semen used and the manner of insemination the percentage ranged from 0% to 34.5%. Furthermore, the findings of the experiment have revealed that under the mass production conditions (queens kept in small cages, in queens banks with low number of attendants) significantly more queens with empty oviducts and still with a high number of spermatozoa in the spermatheca are those inseminated with small amounts of semen in 2 or 3 sequences (but not 4), and not those inseminated with the same amount of semen at once. Therefore the quality of honey bee queens in mass production apiaries is determined largely by the body weight at emergence and the insemination mode (amount of semen, doses and sequence).

Serbia

Introduction

Several local ecotypes of *A. m. carnica* have been identified in Serbia (Mladenović, 2006; Georgijeva, 2008, Rašić, 2009; Nedić *et al.*, 2014). A programme on the selection, reproduction and breeding of Serbian ecotypes of *A. m. carnica* has been ongoing since 2001, with the collaboration of 6 selection centres, which were specifically set up in Radmilovac, (Belgrade University's property), Belgrade (APICENTER), Knjaževac (East Serbia -TIMOMED), Kraljevo (West Serbia- Pčelica Stojanović), Vranje (South Serbia) and Vršac (North Serbia).

A line selection in open population is conducted in all the centres, based on the evaluation of qualitative and quantitative characteristics of the colonies over a two year period. The following traits are used to evaluate the performance of colonies with selected queens every year: the frame area covered by bees and brood, the honey and pollen yield, the appearance of brood, the temperament of bees and some parameters related to disease resistance, such as symptoms of chalkbrood (*Ascosphaera apis*) and of the parasitic mite *Varroa destructor* and as well as hygienic behaviour.

The data refer to the evaluation cycle 2008-2009. The information concerning the number of bees and brood surface in one autumn and two spring inspections were taken into consideration. The data presented here relates to 4 selection lines from each centre, which were monitored from the moment of their introduction into selection to the moment of their replacement.

Data analysis was performed by using a Two-factor Analysis of Variance using the year and line as factors. The data was processed with Statistica 6 software.

Results and discussion

The data collected from 150 colonies are presented in Table 14 (surface of brood, surface covered by bees and nectar crop).

Surface of frames covered by bees

In the first spring inspection there were differences among lines and between centres. The development of all the lines in Vranje centre in 2009 was significantly inferior compared to 2008 and to the development of Kraljevo lines in 2009 ($p < 0.01$) (Table 14). In the second spring inspection there were statistically significant differences only among the individual lines within the centres, and for each centre between the two seasons 2008 and 2009 ($p < 0.01$). The autumn inspection showed that Kraljevo lines had significantly larger surface covered by bees compared to the Vranje lines ($p < 0.01$).

Size of brood surface

In the first spring inspection there were significant statistical differences among lines from the two centres in both years ($p < 0.01$). Kraljevo centre selection lines had much more intense development in 2008 than in 2009 and more intense than those in Vranje centre. The second spring inspection in 2008 showed that Kraljevo selection lines had a slightly faster development then selection lines from Vranje. The autumn inspection showed that Kraljevo lines had significantly larger sizes of brood surface than those in Vranje ($p < 0.01$). Results of the hygienic test performed during 2008 showed that no significant differences were exhibited in the ability of removing dead pupae between the different lines in each selection centre or among the selection centres ($p > 0.05$). The highest percentage of removed brood in 24 hours was observed in I to 92% again in Kraljevo line IV (92%), followed by Vranje line I (91%).

Conclusions

The domestic Carnolian bee (*A. m. carnica*) from west Serbia (Kraljevo) showed better productivity in most of the examined indicators compared to the lines from south Serbia (Vranje). The colonies in Kraljevo selection centre had significantly larger surfaces covered by bees and brood surfaces, in both autumn and first spring inspection, compared to the colonies from Vranje selection centre. However, the performance of the colonies in removing the dead pupae, as well as the achieved crops in acacia flow were not significantly different in the first year of observation. This result implies more favourable agro-ecological conditions during spring and winter in the area of the Kraljevo centre, but it did not bring about strongly significant differences in colony productivity between the centres. This is explained with the existence of compensation ability for development of bees from South Serbia. The observation of the productivity of individual lines showed that one line from Kraljevo selection centre (Line 4) was prominent for most of the monitored parameters compared to the lines from the same centre and to Vranje centre, which suggests an exceptional quality of selection material.

Table 14. Quantitative data (average surface covered by bees, the average surface of brood and the average Acacia nectar crop) collected during a complete evaluation cycle (2008-2009) from two Serbian selection centres

	Line		2008							2009				
			First spring inspection		**Second spring inspection**		**Crop (kg)**	**Autumn**		**First spring inspection**		**Second spring inspection**		**Crop (kg)**
			Bees	**Brood**	**Bees**	**Brood**		**Bees**	**Brood**	**Bees**	**Brood**	**Bees**	**Brood**	
Kraljevo selection center	1	N° of colonies	13	13	13	13	13	13	13	13	13	12	12	11
		Average	2.98	4.88	8.63	6.15	3.46	2.98	2.75	2.5	3.85	5.37	5.68	22.82
		Standard deviation	0.95	1.26	3.26	0.97	2.54	0.74	0.39	0.9	1.01	1.26	1.2	6.63
	2	N° of colonies	13	13	13	13	13	11	11	10	10	8	8	11
		Average	2.15	4.26	6.78	5.82	3.77	2.65	2.55	2.18	2.77	5.1	4.95	21.64
		Standard deviation	0.7	1.05	2.97	0.93	1.92	0.62	0.45	0.56	1	2.2	1.64	6.79
	3	N° of colonies	11	11	11	11	11	9	9	13	13	10	10	10
		Average	2.23	4.1	7.73	5.59	3.09	2.56	2.29	2.47	3.4	4.98	5.96	24.05
		Standard deviation	0.98	1.3	3.64	1.33	1.87	0.45	0.48	0.74	0.8	1.07	1.05	7.13
	4	N° of colonies	12	12	12	12	12	10	10	10	10	10	10	9
		Average	3.03	5.05	7.03	6.48	4.33	2.68	2.93	2.81	3.41	5.28	5.58	26.67
		Standard deviation	1.02	0.94	1.54	0.58	1.78	0.57	0.62	0.4	0.81	1.46	1.6	3.28
Vranje selection center	1	N° of colonies	10	10	10	10	10	10	11	7	7	7	7	7
		Average	2.79	2.56	4.3	4.22	2.9	2.06	0.23	1.03	2.06	4.11	5.79	22.86
		Standard deviation	1.26	1.24	2.35	1.84	1.43	0.72	0.19	0.34	0.52	1.05	0.83	2.97
	2	N° of colonies	7	7	7	7	7	7	7	6	5	5	5	5
		Average	3.21	2.93	4.14	5.09	2.64	1.36	0.2	1.02	1.56	3.54	3.98	18.8
		Standard deviation	1.28	1.31	1.76	1.61	1.25	0.58	0.08	0.33	0.47	1.85	1.91	5.81
	3	N° of colonies	8	8	8	8	8	8	8	7	7	7	7	7
		Average	2.5	2.18	3.75	4.8	3.06	1.5	0.18	0.87	1.93	3.74	4.94	20
		Standard deviation	0.35	0.05	0.58	0.73	1.8	0.35	0.05	0.28	0.77	1.45	1.99	3.87
	4	N° of colonies	8	8	8	8	8	8	8	6	6	6	6	6
		Average	1.89	2.23	4.64	4.98	3.19	1.01	0.23	1.03	2.02	3.97	4.83	20.83
		Standard deviation	0.41	0.1	0.45	0.39	1.46	0.2	0.1	0.35	0.39	0.8	0.49	1.94

Slovakia

Introduction

On the territory of Slovakia, around 250,000 honey bee colonies of the *A. m. carnica* (sometimes recognized as Carpathian Carnica sub-population or "Slovakian" Carnica) are kept by more than 15,500 beekeepers (data from Central Registry of Beehives for 2012). The import of other honey bee races has been illegal for a number of decades. Morphometric methods are mainly used to verify race purity. The main Slovakian lines of the Carniolan bee include: Kosicanka; Carnica Sokol; Vojnicanka; Tatranka; Sitnanka and several non-registered lines. Four imported carniolan lines are allowed for use in registered breeding stations, as well as for selling to beekeepers: Troiseck; Sklenar; Singer (Austria) and Vucko (Ukraine). Breeding of bees is performed in three breeding and in 28 reproduction stations (apiaries), in a total population of about 4,730 colonies. The owners of the colonies carry out performance tests of the breeding colonies according to Institute of Apiculture Liptovský Hrádok guidelines.

The testing stations for the independent testing of queen quality were set up recently in 2006. Honey bee queen quality evaluation is based on data regarding honey yield, gentleness, swarming tendency, behaviour on the combs, spring development, hygienic behaviour and varroa tolerance. Eighty eight queens were tested in all stations during 2009. All queens were evaluated according to their progeny; minimum 4 daughters are evaluated. Their sisters are evaluated by the breeder on his apiary.

Honey yield is the most rated indicator, usually it cannot be less than 80% of the tested group average and less than 60% of the control group average. Testing parameters within the group of accompanying features includes gentleness, seating on combs, swarming tendency and development, are evaluated separately. These parameters are assessed by pointing system, points average under 2 in each category is considered as insufficient, but these values are not considered as strictly as honey indicator. Average values of pin test and percentage of damaged mites is assessed to monitor if no abnormalities has occurred – in pin-killed test there is limit an 30 hours to remove all damaged brood, percentage of damaged mites from whole drop-out should be more than 2%.

Results

In the tables are summarized main data from testing of queens obtained from several breeders of Slovakian lines of Carniolan bees between the years 2006 and 2007. In 2006, nine queens were introduced for testing, and in 2007 eleven queens were tested. As an example one testing station was chosen, which is located in Livovska Huta, with an altitude of 850 m. These are the first data from the queen testing in Slovakia, as the official testing stations were established in 2006. The tested queens were delivered in 2006 and 2007 and were coded. The control group in both cases consisted from 20 colonies of the line Carnica Sokol. Based on the data from Tables 15 and 16, the queens delivered for testing in 2006 coded as TEST 060021, 060026, 060028, 060029, 060030 and 060033 are recommended for further breeding work and selling to the commercial apiaries, while the queens coded as 060022, 060023 and 060024 are not recommended for future breeding. The main reason is weak honey harvest, while the queen colony coded as 060023 also shows insufficient accompanying features. The colonies with queens coded as 060022, 060023 and 060024 showed also bad results in the evaluation of hygienic behaviour – more than 30 hours in average. Based on the data from Tables 17 and 18, among the queens delivered for testing in 2007 only queen coded as TEST074778 is not recommended for future breeding, the reason is weak honey yield. The accompanying parameters of each tested colony received sufficient results, as well as data on hygienic behaviour (each colony less than 30 hours in each tested period) and percentage of damaged mites (more than 2% of the total drop-out).

Table 15. Honey harvest between the years 2007 – 2009 – queens delivered in 2006.

Queen code	year 2007 (kg)	year 2008 (kg)	year 2009 (kg)	Total (kg)	%
TEST060021	93.00	32.00	0.00	125.00	85.15
TEST060022	72.00	19.00	0.00	9100	61.99
TEST060023	71.00	0.00	0.00	71.00	48.36
TEST060024	77.00	16.00	0.00	93.00	63.35
TEST060026	75.00	23.00	23.00	121.00	82.42
TEST060028	85.00	23.00	67.00	175.00	119.21
TEST060029	55.00	28.00	70.00	153.00	104.22
TEST060030	59.00	29.00	66.00	154.00	104.90
TEST060033	66.00	24.00	24.00	114.00	77.65
Average tested queens colonies	72.56	24.25	50.00	146.81	100.00
Average control group	82.00	23.00	52.00	157.00	106.94

Table 16. Accompanying features of tested colonies – average points from whole period of testing – queens delivered in 2006.

Queen code	Gentleness	Behaviour on the comb	Swarming tendency	Spring development
TEST060021	2.92	2.92	4.00	3.60
TEST060022	2.60	2.60	4.00	3.30
TEST060023	1.85	1.85	3.50	3.20
TEST060024	3.00	3.00	3.50	3.47
TEST060026	3.00	3.00	4.00	3.47
TEST060028	3.00	3.00	4.00	3.73
TEST060029	2.77	2.77	3.50	3.20
TEST060030	3.62	3.62	3.25	3.47
TEST060033	2.38	2.38	4.00	3.00

Table 17. Honey harvest between the years 2008 – 2009 – queens delivered in 2007.

Queen code	year 2008 (kg)	year 2009 (kg)	Total (kg)	%
TEST074101	35.00	68.00	103.00	130.53
TEST074202	28.00	59.00	87.00	110.25
TEST074303	26.00	48.00	74.00	93.78
TEST074404	30.00	57.00	87.00	110.25
TEST074575	21.00	64.00	85.00	107.72
TEST074677	32.00	65.00	97.00	122.93
TEST074778	21.00	31.00	52.00	65.90
TEST074879	27.00	42.00	69.00	87.44
TEST074949	36.00	32.00	68.00	86.18
TEST075047	31.00	38.00	69.00	87.44
TEST075148	19.00	58.00	77.00	97.58
Average tested queens colonies	27.82	51.09	78.91	100.00
Average control group	33.00	64.00	97.00	122.93

Table 18. Accompanying features of tested colonies – average points from whole period of testing – queens delivered in 2007.

Queen code	Gentleness	Behaviour on the comb	Swarming tendency	Spring development
TEST074101	3.42	3.42	4.00	3.56
TEST074202	3.29	3.29	3.83	3.38
TEST074303	3.42	3.42	3.58	3.50
TEST074404	3.63	3.63	4.00	3.50
TEST074575	3.46	3.46	3.83	3.50
TEST074677	3.42	3.42	3.83	3.63
TEST074778	3.42	3.42	3.75	3.13
TEST074879	3.29	3.29	4.00	3.25
TEST074949	3.38	3.38	3.33	3.75
TEST075047	3.29	3.29	3.75	3.50
TEST075148	3.58	3.58	3.42	3.44

Conclusions

Testing parameters which are used are based on traditional methods used previously in queen breeding and reproduction stations. However, questions such as: what rate to assign to each monitored factor, as well as which is the precise methodology of indexing of monitored parameters, are still unclear and discussed. A very important factor with good heritability seems to be the hygienic behaviour (Boecking *et al.*, 2000) combined with measuring of varroa mite population growth and survival tests (Büchler *et al.*, 2010; Dietemann *et al.*, 2013). Recent studies have shown strong negative genetic correlations between the contributions of both queens and workers to economically important traits (e.g. honey production). The most advantageous method currently available for evaluating breeding values in other animals, the Best Linear Unbiased Prediction (BLUP)-Animal Model, has been adapted to the peculiarities of honey bee genetics and reproduction, as mentioned in studies of Bienefeld *et al.* (2007).

Slovenia

Introduction

Geographically, Slovenia is situated between the Alps, the Pannonian Flatland, the Dinaric Area and the Adriatic Sea. The Carniolan honey bee *A. m. carnica* Pollman 1879 is the indigenous species and has been preserved in all geographic areas. Queen producers are conducting tests of honey bee colonies and performing selection of breeder colonies. Field production tests of queens and evaluations of the breeding values are performed for queen mothers. Diagnosis and prevention of honey bee diseases in queen rearing apiaries are conducted as part of extension service for beekeepers. Activities in queen rearing apiaries aim at preserving the variability of the race in the geographic areas and at improving the breeding quality of honey bee. The evaluation of morphological and ethological characteristics of bees in queen rearing apiaries is important for the selection and breeding of queens.

The quality of the queen produced, can be predicated on a number of variables that a colony often cannot control and it relates to rearing techniques. During queen rearing the majority of conditions can be controlled and thus rearing conditions and optimum queen quality is of great importance. Thus standard values should be monitored and established. The aim of present work was to monitor the queen quality parameters in Slovenian queen breeders' apiaries and to obtain potential standards for high quality queens.

Materials and methods

Honey bee queens were collected from queen breeding stations during the rearing seasons 2006 and 2008. In the year 2006, 324 queens were sampled from 27 apiaries and in the year 2008, 288 queens were sampled from 24 apiaries. Queens originated from five different regions: Prekmurje, Štajerska, Gorenjska, Dolenjska and Central region. From each apiary twelve sister queens were sampled each sampling years. Queens were mated using typical technology for each apiary. Nine out of twelve queens from each apiary were dissected using stereo-microscope and prepared for further morphological analyses in respect of queen weight, ovary weight, spermatheca volume, sperm number, ovariole number and number of *Nosema* spp. spores. Three remaining queens were used for molecular analyses in order to detect bee viruses.

Table 19. Average weights of queens and ovaries.

	Year 2006 (n = 324)				Year 2008 (n = 288)			
	mean	± SD	Min	Max	mean	± SD	Min	Max
Queen weights (mg)	208.40	15.31	184.70	233.30	209.50	11.96	153.70	262.20
Ovary weights (mg)	69.83	11.08	53.30	86.89	73.05	9.83	38.70	126.20

Table 20. Number of spermatozoa and volume of spermatheca.

	Year 2006 (n = 239)				Year 2008 (n = 171)			
	mean	± SD	Min	Max	mean	± SD	Min	Max
No. of spermatozoa (x 10^6)	3.30	1.68	0.80	6.01	4.96	1.14	3.34	6.69
Volume of spermatheca (mm^3)	-	-	-	-	0.890	0.106	0.744	1.070

Results

Weight of queens and ovaries, number of ovarioles

The number of ovarioles in year 2006 varied from 134.39 to 180.63, with an average of 160.94 (± 14.97) while the number of ovarioles in year 2008 varied from 125.46 to 172.21 with an average of 149.09 (± 15.57). No correlation was found between queen weight and ovariole number (r = 0.1423), or among the ovary/queen weight and ovariole number (r = 0.2270). However, a correlation was established between the weight of queens and the weight of ovaries (r= 0.7434). Average queen weight and ovary weight in 2006 and 2008 is shown in Table 19.

Number of spermatozoa in the spermatheca

Table 20 shows the average number of spermatozoa in spermatheca of queens in year 2006 and 2008. Volume of spermatheca from year 2008 is also shown. The correlation coefficient (r) between spermatozoa number and volume of spermatheca was 0.1960.

Presence of *Nosema* spp. spores and differentiation of *N. apis* and *N. ceranae*

No spores were found in queens from year 2006. However, in year 2008, 4.6% of queens and 75.5% of their attendants workers were found to be positive and results of molecular tests confirmed *N. ceranae* in all positive worker samples. In 2008, the number of spores was 680,000 in the queens and 8.66 million in attendants, on average. The highest number of spores counted was 27.43 million and the least 1.29 million.

In 2006, ABPV and DWV were the most frequent viruses, while in 2008 DWV and the BQCV were the most common.

Discussion

Body size and reproductive performance are known to be correlated in both male and female insects (Reiss, 1989). Established queens weights for both years seems to be useful standard value for breeders as the queen weight is dependent on its reproductive activity at the time of its removal from a mating nucleus (Nelson and Gary, 1983). In our previous experiments egg laying queens were 1.2 x heavier and their ovaries were heavier at the level of 2.7 x, than those of the non ovipositing queens (Gregorc *et al.*, 2008). Our tested queens were sampled from mating apiaries removed directly from mating nuclei. They were fully developed and reproductive and thus ready for introduction into productive honey bee colonies. Weight of tested queens in both years, were 208 and 209 mg respectively. The first standard for queen weights was thus established and further sampling will be performed in order to monitor the reared queens characteristics. In our analyses we found 160 ovarioles in queens in 2006 and 150 ovarioles in 2008. Variations in numbers of ovarioles between queens reared in both years could be reduced in further breeding efforts.

The level of *Nosema* spp. spores in the digestive systems of all the workers does not correlate with mating success of their queens or the location of their mating station. The viruses present in queens had no effect on observed characteristics and further experiments should be perform in order to establish potential effects of viruses infection in queens on their reproductive performance. Studies of reproductive performance in female insects have often focused on direct measures of gamete production such as egg size, egg number and ovary volume (Wickman and Karlsson, 1969; Berrigan, 1991).

Results of the current survey will be a potential standard for queen characteristics determinations in future queen rearing practice. Together with survey of queens infections the entire queen quality parameters as a result of rearing practice will be considered and applied into beekeeping practice. Results of the survey will help queen breeders and specialists in extension service in improving rearing technologies and mating conditions in queen rearing apiaries.

General conclusions and perspectives

The use of standard, high-quality queens is a prerequisite for any research on colony development and behaviour, as well as for economically successful beekeeping. In this work we have reviewed several methods for assessing queen quality accompanied by examples from different countries (they do not represent the only methods used in each country). The presented data also represent a validation of each methodology, as they include original data records for two or three years. Different countries, influenced by beekeeping traditions and socio-economic systems, apply different protocols and standards for assessing the physical and performance characters which could determine the ‘quality’ of a mated queen. Breeding programmes exists in many countries, but each one is dealing with the quality or the characters the queen is transferring to her progeny in a different way. The same evaluation methods are applied in a similar or a modified way to meet the requirements of each country’s needs. Instrumental Insemination is commonly used in some countries while almost completely absent in others.

It has been made obvious that the quality of a queen is neither a single attribute nor even a group of attributes. It is rather the collective result of several groups of attributes such as: a. the physiological and biological ones that have been influenced by the reproduction process (body size and wing length, weight, number of ovarioles, diameter of spermatheca); b. the physiological and biological ones that have been influenced by the fertilisation process (empty oviducts, number of sperm in spermatheca, time of onset of oviposition, genetic variability of sperm); c. the behavioural/performance attributes of the queen which reflect the inherited traits by both, the queen and the drones with which she has mated, but have also been influenced by the environmental conditions (honey production, colony development, aggressiveness, swarming, hygienic behaviour, disease prevalence). Obviously common beekeeping practices can also affect the outcome. Nevertheless, the genetic origin of the queen as well as the genetic origin of the drones is the base line of the whole reproductive process of the reproductive female offspring (Bar-Cohen *et al.*, 1978) and the means of expressing the performance characters.

Most of the research work performed on the ‘quality’ of the queens refers to physical characters (as those mentioned in Table 1), and here we report on several of them, as weight of the queen, diameter of spermatheca, weight of the ovaries, number of ovarioles. Some others have also been reported to give information for queen reproductive ‘quality’ such as standard morphological measures of thorax width, head width, and wing lengths (Weaver, 1957; Fischer and Maul, 1991; Dedej *et al.*, 1998; Hatch *et al.*, 1999; Gilley *et al.*, 2003; Dodologlu *et al.*, 2004; Kahya *et al.*, 2008) and their correlations (Delaney *et al.*, 2010) as well as some physiological and reproductive determinations such as vitellogenin amounts and effective paternity frequency (Delaney *et al.*, 2010).

Here we have described the most common and well known anatomical, physiological, behavioural and performance characteristics: the weight of the queen at the time of the emergence is regarded as one of the most important characters and it has been associated with the number of ovarioles, size of spermatheca, brood production (see Table 1). It is also differentiated among the genotypes and rearing seasons (Koc and Karacaoglu, 2011). In this study it has also been shown that the weight of II queens is not different of that of naturally mated queens (Italy), and that it is positively associated with the diameter of spermatheca as well as the number of spermatozoa entering the spermatheca and empty oviducts (Poland) as it has also been found by Avetisyan (1961), Woyke (1971), Szabo (1973), Gilley *et al.* (2003), Akyol *et al.* (2008), Bieńkowska *et al.* (2008, 2009).

The density and appearance of worker brood is undoubtedly a characteristic of a successfully mated queen as well as of a queen with viable sperm in her spermatheca (examples from Bulgaria and Greece) as it has been demonstrated by Colins (2000). Low density of brood gives a spotted appearance on the comb, indication of a diseased colony or queen failure or inbreeding. Recent work has shown that queen failure could be attributed to pesticide use which can reduce the viability of sperm even by 50% (Pettis, 2013).

The number of ovarioles is a character remaining unchanged for the queen's entire life and it is related to the origin as well as to breeding conditions of a queen (Avetisyan, 1961; Woyke, 1971; Szabo, 1973; Wen-Cheng and Chong-Yuan, 1985; Gilley *et al.*, 2003). Woyke (1987) also showed that the number of ovarioles was significantly correlated not only with the weight of the queens and the age of larvae but also with external features on the wings, such as the number of bristles. The more ovarioles, the more eggs the queen can potentially lay. It has also been shown that the number of ovarioles is related to the weight of the emerging queen as this is also an outcome of the queen's origin and rearing conditions. However, it is neither related to the weight of the queen nor to the weight of the ovaries later on as the weight of the old laying queens is recorded under changeable conditions (as has also been shown in this study through the examples from Slovenia and Italy). However, in none of the studies here is shown any relationship between the number of ovarioles and others productive characters, as brood production for example, a relationship that needs to be shown or proven.

A queen's quality is not only a function of her own reproductive potential but also a result of her mating success which is determined by assessing the number of stored sperm in a queen's spermatheca (Lodesani *et al.*, 2004; Al-Lawati *et al.*, 2009), and this character is being evaluating in several examples in this study (Italy, Poland, Slovenia). It also has been shown that in these examples sperm numbers are in accordance with previous studies and this trait is been used more and more to evaluate the reproductive success of the queen. However, the number of sperm does not prove its viability, which could be shown in a direct way thought the spottiness or the density of brood, as mentioned before.

In connection with the numbers of sperm, the diameter of spermatheca is also evaluated as a morphological character of the queen, influenced by its mating success. Sometimes, the diameter of the spermatheca is used instead of sperm counts as it is easier to do (Bulgaria, Greece, Slovenia) based on the assumption that larger spermatheca enables more sperm to be stored. Most of the previous research has shown that the diameter of spermatheca is related to the weight of the queen and the age of the larva used for this queen. However, Woyke (1966) and Bieńkowska *et al.,* (2008) have also shown that larger spermatheca is associated with higher number of sperm. Therefore, the size can be used instead of the number of sperms as a character for queen quality. Delaney *et al.* (2010) have also shown that thorax width was positively correlated with both stored sperm number and mating frequency, which suggests that queens with larger thoraces are predisposed to mate with a greater number of drones, therefore having more sperm but also enhancing the genetic diversity in the colony for better adaptation, and tolerance or resistance to diseases (Hamilton, 1987; Sherman *et al.*, 1988; Schmid-Hempel, 1998; Palmer and Oldroyd, 2003; Tarpy, 2003; Cremer *et al.*, 2007; Seeley and Tarpy, 2007). Woyke (1960) reported that the oviducts of naturally mated queens were empty after 24 hours whereas those of instrumentally inseminated ones were full until 48 hours. However, not all instrumentally inseminated queens were able to empty their oviducts, phenomenon which can lead to death of the queen after some time. One of factors which can prevent proper migration of semen into the spermatheca and cause semen retention in the oviducts of a queen is the lack of sufficient workers to take care of the queen (Vesely, 1965, 1971; Woyke, 1979; Jasiński, 1984; Harbo, 1985; Gontarz *et al.*, 2005), phenomena that do not apply when the queens are placed in large colonies or nuclei. Queens confined to cages after insemination and later introduced into individual colonies are slower to begin egg laying and store fewer sperm cells (Wilde, 1994). However, because this is the usual practice in mass production apiaries, is important to know the successful transfer of semen in the spermatheca and if the insemination practice has any effect on this. The example of Poland in this study has shown that also under these circumstances insemination of queens in smaller doses of semen and in sequence (2 or 3 times maximum) results in more sperm to be stored in spermatheca, than insemination with a large amount at once. Therefore, insemination method can influence the quality of the queen.

Determining all possible physical characters on the queens themselves is not always possible as it is destructive; also in the absence of behavioural information (Rhodes and Somerville, 2003; Anderson, 2004; Hatch *et al.*, 1999; Tarpy *et al.*, 2000; Gilley *et al.*, 2003), therefore measurements are performed on a set of performance characters via the progeny of the queen indicating also inherited characteristics such as honey production, aggressiveness, swarming and hygienic behaviour (Bienefeld and Pirchner, 1990; Jevtić *et al.*, 2012). There are many studies which evaluated both instrumentally inseminated queens and naturally mated queens and the connection between performance and their physical characters using various behavioural attributes such as brood production, honey production, colony weight and survival rate of the queens (Harbo and Szabo, 1984; Vesely, 1984; Konopacka, 1987; Boigenzahn and Pechhacher, 1993; Kostarelou-Damianidou *et al.*, 1995; Cobey, 1998; Collins, 2000; Pritsch and Bienefield, 2002; Al-Qarni *et al.*, 2003; Rhodes and Somerville, 2003; Rhodes *et al.*, 2004; Skowronek *et al.*, 2004, Akyol *et al.*, 2008). These are the same characters taken into account as the bottom-line in most breeding and selection programmes in order to choose which queens to reproduce, according to the aims of the breeding program. At the Apimondia symposium "Controlled mating and selection of the honey bee" held in Lunz in 1972, technical recommendations for methods to evaluate the performance of bee colonies were developed (including honey production, swarming tendency, calmness, and hygienic behaviour) (Ruttner, 1972) and since these same methods with several modifications have been used as selection criteria for the queens (Croatia, Denmark, Serbia, Slovakia). More detailed descriptions of these methods and their implementation on the rearing and selection of honey bee queens can be found on the *BEEBOOK* paper on queen rearing (Büchler *et al.*, 2013).

In respect of the life span of the queen, this character is not usually taken into account during evaluation of a 'good queen'. However, it is essential to know that a 'high' quality queen will also live or lay eggs for more than one year, as the average time for replacing a queen is two years. Queen replacement is costly, but as it had been previously documented, colonies headed by young queens (1 to 2 years old) are up to 30% more productive than colonies headed by older ones in respect of brood production and honey yield (Avetisyan, 1961; Woyke, 1984, Genç, 1992, Kostarelou-Damianidou *et al.*, 1995, Akyol *et al.*, 2008). Therefore, a good queen should be very productive till end of the second year of her life. Furthermore, the confinement of the queens in cages during and after insemination can also have an effect on queen life, as it can induce injuries to the legs of the queens done by the workers (Gerula, 2007), which can eventually lead to supersedure.

Honey production seems to be the main goal of most breeding programmes existing. It has been selected for even before 1960s in different parts of the world (Soller and Bar-Cohen, 1967; Oldroyd and Goodman, 1990; Calderon and Fondrk, 1991) and its evaluation is performed as honey harvested for each season (Croatia, Denmark, Slovakia), or as the difference in the weight of the colonies over three days (Serbia). It is however, believed that honey production is increased as adult population and especially brood area are increased. Accordingly brood and population measurements give an insight of the colony strength and its potential for honey production. At the same time there are conflicting reports on the relative importance of queen and worker characters to honey production. Soller and Bar-Cohen (1967), Sugden and Furgala (1982); Nelson and Gary (1983) and Szabo (1982) computed a very high genetic correlation between brood area and honey yield, but this was not proven in Bar-Cohen *et al.* (1978).

Nevertheless, an apparently 'good' quality queen in terms of physiological parameters cannot be assessed as such in terms of biological parameters if she is currying a high load of 'pathogens'. The sanitary condition of a queen is reflecting the sanitary condition of the nucleus and obviously of the condition of the whole reproduction unit. Several studies have measured these parasites in queens but none has fully investigated how they may impact a queen's reproductive quality. The health status of the queen as well as the ability of the colony to prevent the appearance of certain diseases is of increasing value in breeding schemes, showing as well the inheriting ability of the queen traits and for this reason we mention this separately (examples: Denmark, Greece, Slovenia). Furthermore, Seeley and Tarpy (2007) have found that colonies headed by multiple-drone inseminated queens had markedly lower disease intensity and higher colony strength at the end of the summer relative to colonies headed by single-drone inseminated queens. Hygienic behaviour is recognized as a natural antiseptic defence against the brood diseases, American foulbrood and chalkbrood, and perhaps against varroa (Boecking and Spivak, 1999; Evans and Spivak, 2010; Spivak and Reuter, 2001; Wilson-Rich *et al.*, 2009), therefore an important inherited trait to be determined via the progeny of a queen.

The methods described here are not the only ones that exist, but they are the ones used as important examples in order to cover as many of different methods as possible. There are some more physiological characters that have not been addressed in this review. Characters that could try to evaluate the reared queens in a more detailed way, such as the time of oviposition, number of live spermatozoa in the spermatheca, the effective paternity frequency or even the degree of parasitism (Delanay *et al.*, 2010). However, the main characters employed in practice in most of the European countries have been mentioned already. The most appropriate morphological, physiological and behavioural traits of the queen will be the standards in the future queen rearing practice. This is also one of the goals of the RNSBB (Research Network for Sustainable Bee Breeding), to recommend the best standards for queen rearing in the new millennium.

Two critical points need to be further discussed: a. how the above measured biological and physiological attributes correlate with the performance of the queen later in her life; and b. whether and to what extent some of the physiological attributes can be used alone in the absence of the behavioural/ performance attributes in order to accurately access the queen quality. As already noted, the existing data already show a positive trend in the correlation between the biological/ physiological characteristics and the performance. It is therefore, the first step to summarize this type of diverse data for such an important issue. The knowledge acquired can be used to fill in the existed gaps in the systems of each country in order to facilitate standardization of methodology for comparable results. The discussion has not been finalised, but it is the future work to determine this issue to a definite point. Nonetheless, we also believe that what is very important is that all physiological characters described in this work are characterised by the following features: easy to access in one season, repeatable, traceable, practical, no need of explicitly of equipment. Because of all the above features, is possible that the biological/ physical characteristics of the queens can be used as a quick and accurate methodology under the chance of the full evaluation of a breeding scheme.

Putting together all the aspects of performed results of the work will give valuable insights into the factors influencing the colony phenotype and will contribute in establishing the most appropriate behavioural and physiological standards for the queen produced by the beekeeping industry. Results of the queen rearing surveys will also contribute into establishing queen quality certification for queen breeding protocols in different countries. However, we also believe that further studies are needed to establish the relationship between the physical characters of a queen with the performance of the colony this queen is heading later on.

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References

AL-LAWATI, H; KAMP, G; BIENEFELD, K (2009) Characteristics of the spermathecal contents of old and young honey bee queens, *Journal of Insect Physiology*, 55(2): 117-122. http://dx.doi.org/10.1016/j.jinsphys.2008.10.010

AKYOL, E; YENINAR, H; SAHINLER, N; GULER, A (2006) The effects of additive feeding and feed additives before wintering on honey bee colony performances, wintering abilities and survival rates at the East Mediterranean region. *Pakistan Journal of Biological Sciences*, 9(4): 589-592.

AKYOL, E; YENINAR, H; KORKMAZ, A; ÇAKMAK, I (2008) An observation study on the effects of queen age on some characteristics of honey bee colonies. *Italian Journal of Animal Sciences*, 7: 19-25. http://dx.doi.org/10.4081/ijas.2008.19

AL-GHZAWI, M; ZAITOUN, S (2008) Origin and rearing season of honey bee queens affect some of their physiological and reproductive characteristics. *Entomological Research*, 38(2): 139-148. http://dx.doi.org/10.1111/j.1748-5967.2008.00151.x

ANDERSON, D (2004) Improving queen bee production. *Rural industries Research and Development Corporation. Publication No. 04/153 Project No. CSE-85A.*

AVETISYAN, G A (1961) The relation between interior and exterior characteristics of the queen and fertility and productivity of the bee colony. *Proceedings of XVIII International Beekeeping Congress:* p. 44-53.

BAR-COHEN, R; ALPERN, G; BAR-ANAN, R (1978) Progeny testing and selecting Italian queens for brood area and honey production. *Apidologie*, 9: 95-100.

BERGER, B; ABDALLA, FC (2005) Programed cell death during ovarian differentiation in queens of *Apis mellifera* Linnè (Hymenoptera, Apini) *Brazilian Journal of Morphological Science*, 22(1): 1-4.

BERRIGAN, D (199l) The allometry of egg size and number in insects. *Oikos*, 60: 313-321. http://dx.doi.org/10.2307/3545073

BIENEFELD, K; PIRCHNER, F (1990) Heritabilities for several colony traits in the honey bee (*Apis mellifera carnica*). *Apidologie*, 21: 175-183.

BIENEFELD, K; EHRHARDT, K; REINHARDT, F (2007) Genetic evaluation in the honey bee considering queen and worker effects - a BLUP - animal model approach. *Apidologie*, 38(1): 77-85. http://dx.doi.org/10.1051/apido:2006050

BIEŃKOWSKA, M; PANASIUK, B (2006) Influence of the diameter of the inseminating needle tip on the results of bee queens' fertilization. *Journal of Apicultural Science*, 50(2): 137-145.

BIEŃKOWSKA, M; WĘGRZYNOWICZ, P; PANASIUK, B; GERULA, D; LOC, K (2008) Influence of the age of honey bee queens and dose of semen on condition of instrumentally inseminated queens kept in cages with 25 worker bees in bee colonies. *Journal of Apicultural Science*, 52(2): 23-34.

BIEŃKOWSKA, M; PANASIUK, B; GERULA, D; WĘGRZYNOWICZ, P (2009) Weight of honey bee queens and its effect on the quality of instrumentally inseminated queens. In *Proceedings of 41th International Apicultural Congress, Montpellier, France, 15-20 September, 2009.* p 135.

BIEŃKOWSKA, M; LOC, K; PANASIUK, B; WĘGRZYNOWICZ, P; GERULA, D; (2011) Effect of semen dose and application on the performance of instrumentally inseminated honey bee queens kept with 25 worker bees. *Journal of Apicultural Science*, 55(1): 99-109.

BOECKING, O; SPIVAK, M (1999) Behavioural defences of honey bees against *Varroa jacobsoni* Oud., *Apidologie,* 30: 141-158.

BOECKING, O; BIENEFELD, K; DRESCHER, W (2000) Heritability of the varroa specific hygienic behaviour in honey bees (Hymenoptera Apidae). *Journal of Animal Breeding and Genetics*, 117(6): 417-424.

BOIGENZAHN, C; PECHHACKER, H (1993) Über die Art der Anpaarung. *Bienenvater*, 114: 151-152.

BOUGA, M; ALAUX, C; BIENKOWSKA, M; BÜCHLER, R; CARRECK, N L; CAUIA, E; CHLEBO, R; DAHLE, B; DALL'OLIO, R; DE LA RÚA, P; GREGORC, A; IVANOVA, E; KENCE, A; KENCE, M; KEZIC, N; KIPRIJANOVSKA, H; KOZMUS, P; KRYGER, P; LE CONTE, Y; LODESANI, M; MURILHAS, A M; SICEANU, A; SOLAND, G; UZUNOV, A; WILDE, J (2011) A review of methods for discrimination of honey bee populations as applied to European beekeeping. *Journal of Apicultural Research*, 50(1): 51-84. http://dx.doi.org/10.3896/ibra.1.50.1.06

BÜCHLER, R; BERG, S; LE CONTE, Y (2010) Breeding for resistance to *Varroa destructor* in Europe. *Apidologie*, 41: 393-408. http://dx.doi.org/10.1051/apido/2010011

BÜCHLER, R; ANDONOV, S; BIENEFELD, K; COSTA, C; HATJINA, F; KEZIC, N; KRYGER, P; SPIVAK, M; UZUNOV, A; WILDE, J (2013) Standard methods for rearing and selection of *Apis mellifera* queens. In *V Dietemann; J D Ellis; P Neumann (Eds) The COLOSS* BEEBOOK*, Volume I: standard methods for* Apis mellifera *research*. *Journal of Apicultural Research*, 52(1): http://dx.doi.org/10.3896/IBRA.1.52.1.07

BURMISTROVA, N D (1965) Directed changes in the number of ovarioles in queen honey bees. *Pschelovodstvo*, 85: 15.

CAA (2009) *Annual Report for 2008*. Croatian Agricultural Agency, Zagreb, Croatia.

CALDERONE, N W; FONDRK, M K (1991) Selection for high and low, colony weight gain in the honey bee, *Apis mellifera*, using selected queens and random males. *Apidologie*, 22: 49-60.

CARRECK, N L; ANDREE, M; BRENT, C S; COX-FOSTER, D; DADE, H A; ELLIS, J D; HATJINA, F; VANENGELSDORP, D (2013) Standard methods for *Apis mellifera* anatomy and dissection. In *V Dietemann; J D Ellis; P Neumann (Eds) The COLOSS* BEEBOOK*, Volume I: standard methods for* Apis mellifera *research*. *Journal of Apicultural Research*, 52(4): http://dx.doi.org/10.3896/IBRA.1.52.4.03

CASAGRANDE-JALORETTO, D C; BUENO, O C; STORT, A C (1984) Numero de ovariolos em rainhas de *Apis mellifera*. *Naturalia*, 9: 73-79.

CHAUD-NETTO, J; BUENO, O C (1979) Number of ovarioles in workers of *Apis mellifera adansonii* and *Apis mellifera ligustica*: a comparative study. *Journal of Apicultural Research*, 18: 260-263.

COBEY, S W (1998) Comparison of colony performance of instrumentally inseminated and naturally mated honey bee queens. (Proceedings of American Bee Research Conference, Colorado Springs, CO, USA). *American Bee Journal*, 138: 292.

COBEY, S W (2007) Comparison studies of instrumentally inseminated and naturally mated honey bee queens and factors affecting their performance. *Apidologie*, 38: 390-410. http://dx.doi.org/10.1051/apido:2007029

COBEY, S W; TARPY, D R; WOYKE, J (2013) Standard methods for instrumental insemination of *Apis mellifera* queens. In *V Dietemann; J D Ellis; P Neumann (Eds) The COLOSS* BEEBOOK*, Volume I: standard methods for* Apis mellifera *research*. *Journal of Apicultural Research*, 52(4): http://dx.doi.org/10.3896/IBRA.1.52.4.09

COLLINS, A (2000) Survival of honey bee (Hymenoptera: Apidae) spermatozoa stored at above-freezing temperatures. *Journal of Economic Entomology*, 93: 568-571. http://dx.doi.org/10.1603/0022-0493-93.3.568

CORBELLA, E; GONCALVES, L S (1982) Relationship between weight at emergence, number of ovarioles and spermathecal volume of Africanized honey bee queens (*Apis mellifera* L.). *Revista Brasileira de Genetica*, 5(4): 835-840.

CREMER, S; ARMITAGE, S A O; SCHMID-HEMPEL, P (2007) Social immunity. *Current Biology*, 17: R693-R702.

DEDEJ, S; HARTFELDER, K; AUMEIER, P; ROSENKRANZ, P; ENGELS, W (1998) Caste determination is a sequential process: effect of larval age at grafting on ovariole number, hind leg size and cephalic volatiles in the honey bee (*Apis mellifera carnica*), *Journal of Apicultural Research*, 37: 183-190.

DELANEY, D A; KELLER, J J; CAREN, J R; TARPY, D R (2010) The physical, insemination, and reproductive quality of honey bee queens (*Apis mellifera*). *Apidologie*, 42: 1-13. http://dx.doi.org/10.1051/apido/2010027

DE MIRANDA, J R; BAILEY, L; BALL, B V; BLANCHARD, P; BUDGE, G; CHEJANOVSKY, N; CHEN, Y-P; GAUTHIER, L; GENERSCH, E; DE GRAAF, D; RIBIÈRE, M; RYABOV, E; DE SMET, L VAN DER STEEN, J J M (2013) Standard methods for virus research in *Apis mellifera.* In *V Dietemann; J D Ellis; P Neumann (Eds) The COLOSS* BEEBOOK, *Volume II: standard methods for* Apis mellifera *pest and pathogen research. Journal of Apicultural Research*, 52(4): http://dx.doi.org/10.3896/IBRA.1.52.4.22

DIETEMANN, V; NAZZI, F; MARTIN, S J; ANDERSON, D; LOCKE, B; DELAPLANE, K S; WAUQUIEZ, Q; TANNAHILL, C; FREY, E; ZIEGELMANN, B; ROSENKRANZ, P; ELLIS, J D (2013) Standard methods for varroa research. In *V Dietemann; J D Ellis; P Neumann (Eds) The COLOSS* BEEBOOK*, Volume II: standard methods for* Apis mellifera *pest and pathogen research. Journal of Apicultural Research*, 52(1): http://dx.doi.org/10.3896/IBRA.1.52.1.09

DODOLOGLU, A; EMSEN, B; GENE, F (2004) Comparison of some characteristics of queen honey bees (*Apis mellifera* L.) reared by using Doolittle method and natural queen cells. *Journal of Applied Animal Research*, 26: 113–115. http://dx.doi.org/10.1080/09712119.2004.9706518

DOMINIKOVIĆ, Z; BRENCE-LAZARUS, T; BUBALO, D; DRAŽIĆ, M; KEZIĆ, N (1997) *Program gojidbenog stvaranja pčela u Republici Hrvatskoj*. Hrvatski stočarsko selekcijski centar.

DRAŽIĆ, M; BUBALO, D; BERG, S; BÜCHLER, R; PECHHACKER, H; KEZIĆ, N (2001) Selekcija pčela tolerantnih na varoozu. Zbornik sažetaka. 37. znanstveni skup hrvatskih agronoma s međunarodnim sudjelovanjem. *Opatija*, 19-23: 291.

DRAŽIĆ, M; JAKOPOVIĆ, I; BUBALO, D; KEZIĆ, N (2003) Gospodarske odlike selekcioniranih matica sive pčele (*A. m. carnica*) u Hrvatskoj. *Priopćenja sa XXXVIII Znanstvenog skupa hrvatskih agronoma*. Žimbrek, Tito (ur.). Zagreb: Agronomski fakultet, 182.

ECKERT, J E (1934) Studies in the number of ovarioles in queen honey bees in relation to body size. *Journal of Economic Entomology*, 27: 629-635.

EID, A A; EWIES, M A; NASR, M S (1980) The weight of the newly emerged honey bee queen as an index of its potential productivity. *Bulletin of Faculty of Agriculture, University of Cairo, Egypt*. 29: 91-111.

EVANS, J D; SPIVAK, M (2010) Socialized medicine: individual and communal disease barriers in honey bees. *Journal of Invertebrate Pathology*, 103: 62-72. http://dx.doi.org/10.1016/j.jip.2009.06.019

FISCHER, F; MAUL, V (1991) Untersuchungen zu aufzuchtbedingten königinnenmerkmalen. *Apidologie*, 22: 444-446.

GENÇ, F (1992) A study on determination of the effects of rising different ages queens on colony performance. *Proceedings of 1st Beekeeping Seminar East Anatolia, Erzurum, Turkey.* pp. 76-95.

GENÇER, H V; SHAH, S Q; FIRATLI, Ç (2000) Effects of supplemental feeding of queen rearing colonies and larval age on the acceptance of grafted larvae and queen traits. *Pakistan Journal of Biological Sciences*, 3(8): 1319-1322.

GEORGIJEV, A; MLADENOVIĆ, M (2008) Izvori varijacija biološko-produktivnih osobina pčelinjeg društva. *XVI Naučno savetovanje sa međunarodnim učešćem"Kvalitetom i selekcijom u pčelarstvu ka Evropi*", str.26-32, Zemun.

GERULA, D (2007) Observations of body injuries of artificially inseminated honey bee queens inflicted in the subsequent stages of rearing and during their introduction into colonies. *Journal of Apicultural Science*, 51(2): 5-17.

GILLEY, D C; TARPY, D R; LAND, B B (2003) Effect of queen quality on interactions between workers and duelling queens in honey bee (*Apis mellifera* L.) colonies. *Behavioural Ecology and Sociobiology*, 55(2): 190-196. http://dx.doi.org/10.1007/s00265-003-0708-y

GONTARZ, A; BIEŃKOWSKA, M; LOC, K (2005) Effect of queen caging conditions on insemination results. *Journal of Apicultural Science*, 49(1): 5-15.

GREGORC, A; BOWEN, I D (1999). In-situ localization of heat-shock and histone proteins in honey bee (*Apis mellifera* L.) larvae infected with *Paenibacillus larvae. Cell Biology International*, 23: 211-218. http://dx.doi.org/10.1006/cbir.1999.0344

GREGORC, A; LOKAR, V; SMODIŠ ŠKERL, M (2008) Testing of the isolation of the Rog-Ponikve mating station for Carniolan (*Apis mellifera carnica*) honey bee queens. *Journal of Apicultural Research*, 47(2): 138-142. http://dx.doi.org/10.3896/IBRA.1.47.2.09

GULER, A; ALPAY, H (2005) Reproductive characteristics of some honey bee (*Apis mellifera* L.) genotypes. *Journal of Animal and Veterinary Advances*, 4: 864-870.

HAMILTON, W D (1987) Kinship, recognition, disease, and intelligence: constraints of social evolution. In *Y Ito; J L Brown; J Kikkawa (Eds). Animal societies: theory and facts.* Japanese Scientific Society; Tokyo, Japan. pp. 81-102.

HARBO, J R (1985) Instrumental insemination of queen bees - Parts 1 & 2. *American Bee Journal*, 125: 197-202; 282-287.

HARBO, J R (1986) Oviposition rates of instrumentally inseminated and naturally mated queen honey bees *(Hymenoptera: Apidae). Annals of the Entomological Society of America*, 79: 112-115.

HARBO, J R; SZABO, T I (1984) A comparison of instrumentally inseminated and naturally mated queens, *Journal of Apicultural Research*, 23: 31-36.

HATCH, S; TARPY, D R; FLETCHER, D J C (1999) Worker regulation of emergency queen rearing in honey bee colonies and the resultant variation in queen quality. *Insectes Sociaux*, 46: 372-377

HATJINA, F (2012) Greek honey bee queen quality certification. *Bee World*, 89: 18-20.

HATJINA, F; HARISTOS, L; BOUGA, M; KOSTARELOU, M; EMMANOUEL, N (2008) Quality characteristics of produced honey bee queens in Greece. *Proceedings of the Third European Conference of Apidology, 8-11 September 2008, Belfast UK.* pp. 50-51.

HATJINA, F; COSTA, C; BÜCHLER, R; UZUNOV, A; DRAZIC, M; FILIPI, J; CHARISTOS, L; RUOTTINEN, L; ANDONOV, S; MEIXNER, M D; BIENKOWSKA, M; DARIUSZ, G; PANASIUK, B; LE CONTE, Y; WILDE, J; BERG, S; BOUGA, M; DYRBA, W; KIPRIJANOVSKA, H; KORPELA, S; KRYGER, P; LODESANI, M; PECHHACKER, M; PETROV, P; KEZIC, N (2014) Population dynamics of European honey bee genotypes under different environmental conditions. *Journal of Apicultural Research*, 53(2): 233-247. http://dx.doi.org/10.3896/IBRA.1.53.2.05

HOOPINGARNER, R; FARRAR, C L (1959) Genetic control of size in queen honey bees. *Journal of Economic Entomology*, 52(4): 547-548.

JACKSON, J T; TARPY, D R; FAHRBACH, S E (2011) Histological Estimates of Ovariole Number in Honey Bee Queens, *Apis mellifera*, Reveal Lack of Correlation with other Queen Quality Measures Histological estimates of ovariole number in honey bee queens, *Apis mellifera*, reveal lack of correlation with other queen quality measures. *Journal of Insect Science*, 11: 82. http://dx.doi.org/10.1673/031.011.8201

JASIŃSKI, Z (1984) Porownanie efektivnošći stosowanzch w Polsce metod przechowzwania matek po sztucznym unacienianiu.XXI *Naukowa Konferencja Pszczelarska Pulawy 9-10 Marca*. pp. 8-9.

JASIŃSKI, Z; PRABUCKI, J; WILDE, J; WOYKE, J; CHUDA-MICKIEWICZ, B; SIUDA, M; MADRAS-MAJEWSKA, B; SAMBORSKI, J; BRATKOWSKI, J; JOJCZYK, A; BĄK, B (2006) Study on factors accelerating oviposition of instrumentally inseminated queen bees. *Proceedings of the Second European Conference of Apidology, Prague, Czech Republic, 10-16 September 2006.* p. 57.

JEVTIĆ, G; ANDJELKOVIĆ, B; LUGIĆ, Z; RADOVIĆ, J; DINIĆ, B (2012) Heritability of production characteristics of regional populations of honey bees from Serbia. *Genetica*, 44(1): 47-54. http://dx.doi.org/10.2298/GENSR 1201047J

JORDAN, R (1960) Die Zucht der Konigin ausgehend vom Ei. *Bienenvater*, 81(1): 3-7, 44.

KAHYA, Y; GENÇER, Y; WOYKE, J (2008) Weight at emergence of honey bee (*Apis mellifera caucasica*) queens and its effect on live weights at the pre and post mating periods. *Journal of Apicultural Research*, 47(2): 118-125. http://dx.doi.org/10.3896/IBRA.1.47.2.06

KEZIĆ, N; BUBALO, D; DRAŽIĆ, M (2001) Uzgoj sive pčele (*Apis mellifera carnica* Pollman, 1879) u Hrvatskoj. Zbornik radova: *Biološka raznolikost u stočarstvu Republike Hrvatske*, HAZU Zagreb, 18. do 19, str. 141-145.

KLEE, J; BESANA, A M; GENERSCH, E; GISDER, S; NANETTI, A; TAM, D Q; CHINH, T X; PUERTA, F; RUZ, J M; KRYGER, P; MESSAGE, D; HATJINA, F; KORPELA, S; FRIES, I; PAXTON, R J (2007) Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. *Journal of Invertebrate Pathology*, 96: 1-10. http://dx.doi.org/10.1016/j.jip.2007.02.014

KOÇ, A U; KARACAOĞLU, M (2011) Effects of queen rearing period on reproductive features of Italian (*Apis mellifera ligustica*), Caucasian (*Apis mellifera caucasica*), and Aegean ecotype of Anatolian honey bee (*Apis mellifera anatoliaca*) queens. *Turkish Journal of Veterinary and Animal Sciences*, 35(4): 271-276.

KOENIGER, G; KOENIGER, N; TINGEK, S; PHIANCHAROEN, M (2005) Variance in spermatozoa number among *Apis dorsata* drones and among *Apis mellifera* drones. *Apidologie*, 36: 279-284. http://dx.doi.org/10.1051/apido:2005009

KOMAROFF, P M; ALPATOV, W W (1934) Biiträge Zur Kenntnis der Variabilität der Honigbiene. I. Das Gewicht und das Genitalsystem der Königin als Rassenmerkmale. *Archive. Bienenkde*, 15: 11-20.

KONOPACKA, Z (1987) Biological quality of instrumentally inseminated queens, *Proceedings of XXX1st International Apicultural Congress, Warsaw, Poland.* pp. 163-167.

KONOPACKA, Z; BIENKOWSKA, M (1995) The results of queen insemination with the semen stored in glass capillaries. *Pszcelnicze Zeszyty Naukowe*, 39: 213-217.

KOSTARELOU-DAMIANIDOU, M; THRASYVOULOU, A; TSELIOS, D; BLADENOPOULOS, K (1995) Brood and honey production of honey bee colonies requeened at various frequencies. *Journal of Apicultural Research*, 34(1): 9-14.

KULINČEVIĆ, J; ALEKSOVSKI, S; KRUNIĆ, M (1990) Selekcija autohtone medonosne pčele (*Apis mellifera L.*) na produktivnost. Arhiv za polj. *Nauke*, 51: 15-26.

LODESANI, M; BALDUZZI, D; GALLI, A (2004) Functional characterization of semen in honey bee queen (*A. m. ligustica* S.) spermatheca and efficiency of the diluted semen technique in instrumental insemination. *Italian Journal of Animal Science*, 3: 385-392.

MACKENSEN, O (1964) Relation of semen volume to success in artificial insemination of queen honey bees. *Journal of Economic Entomology*, 57(4): 581-583.

MAKAROV, Y U L (1969) *Biologically and economically useful characters of far Eastern bees and their selection.* Timiryazev Academy of Agriculture; Moscow, USSR. 18 pp.

MEDINA, L M; GONÇALVES, L S (2000) Effect of weight at emergence of Africanized (*Apis mellifera* L.) virgin queens on their acceptance and beginning of oviposition, *American Bee Journal,* 141: 213-215.

MEIXNER, M D; BÜCHLER, R; COSTA, C; FRANCIS, R M; HATJINA, F; KRYGER, P; UZUNOV, A; CARRECK, N L (2014) Honey bee genotype and environment interactions. *Journal of Apicultural Research*, 53 (2): 183-187. http://dx.doi.org/10.3896/IBRA.1.53.2.01

MLADENOVIĆ, M (2006) Medna produktivnost nekih selekcijskih linija matica u Srbiji. *XIV Naučno savetovanje sa međunarodnim učešćem"Zaštita i proizvodnja domaće pčele i meda* : 7-13. Zemun.

NEDIĆ, N; FRANCIS, R M; STANISAVLJEVIĆ, L; PIHLER, I; KEZIĆ, N; BENDIXEN, C; KRYGER, P (2014) Detecting population admixture in the honey bees of Serbia. *Journal of Apicultural Research*, 53 (2): 303-313. http://dx.doi.org/10.3896/IBRA.1.53.2.12

NELSON, D L; GARY, N E (1983) Honey productivity of honey bee colonies in relation to body weight, attractiveness and fecundity of the queen. *Journal of Apicultural Research*, 22(4): 209-213.

NEWTON, D C; OSTASIEWSKI, N J (1986) A simplified bioassay for behavioural resistance to American foulbrood in honey bees (*Apis mellifera* L.). *American Bee Journal*, 126: 278-281.

OLDROYD, B P; GOODMAN, R D (1990) On the relative importance of queens and workers to honey production. *Apidologie*, 21(2): 153.

PAGE, R E (1986) Sperm utilization in social insects. *Annual Review of Entomology*, 31: 297-320.

PALMER, K A; OLDROYD, B P (2003) Evidence for intra-colonial genetic variance in resistance to American foulbrood of honey bees (*Apis mellifera*): further support for the parasite/pathogen hypothesis for the evolution of polyandry. *Naturwissenschaften*, 90: 265-268. http://dx.doi.org/10.1007/s00114-003-0418-3

PETTIS, J S, (2013) Influence of pathogens and agro-chemicals on honey bee longevity and social behaviour. *Proceedings of the XXXIII International Apicultural Congress, Kiev, Ukraine, 29 October - 3 November 2013.*

PHIANCHAROEN, M; PIRK, C W W; RADLOFF, S E; HEPBURN, R (2010) Clinal nature of the frequencies of ovarioles and spermathecae in Cape worker honey bees, *Apis mellifera capensis*. *Apidologie*, 41: 129-134. http://dx.doi.org/10.1051/apido/2009054

PRITSCH, G; BIENEFELD, K (2002) Comparison of performance of bee colonies with naturally mated and artificially inseminated queens (*A. m. carnica*). *Apidologie*, 33: 513-514. http://dx.doi.org/10.1051/apido:2002032

RAŠIĆ, S; MLADENOVIĆ, M; NEDIĆ, N; BOŽIČKOVIĆ, A; STOJANOVIĆ, Z (2009) Analiza nekih obeležja linija medonosnih pčela u selekciji. *XVII Naučno savetovanje sa međunarodnim učešćem "Kvalitet meda i selekcija medonosne pčele*", 7 februar, Poljoprivredni fakultet Beograd: 70-79.

REISS, M J (1989) *The allometry of growth and reproduction.* Cambridge University Press; Cambridge, MA, USA.

RHODES, J W; SOMERVILLE, D C (2003) Introduction and early performance of queen bees, Report : Rural Industries Research & Development Corporation, NSW Agriculture Pub# 03/049. Project: DAN-182A.

RHODES, J W; SOMERVILLE, D C; HARDEN, S (2004) Queen honey bee introduction and early survival of queen age at introduction. *Apidologie*, 35: 383-388. http://dx.doi.org/10.1051/apido:2004028

RUTTNER, H (1972) Technical recommendations for methods of evaluating performance of bee colonies. In *F Ruttner. Controlled mating and selection of the honey bee.* Apimondia Publishing House; Bucharest, Romania. pp. 87-92.

RUTTNER, F (1988) *Breeding techniques and selection for breeding of the honey bee*. British Isles Bee Breeders Association / Ehrenwirth Verlag; Munich, Germany.

SHERMAN, P W; SEELEY, T D; REEVE, H K (1988) Parasites, pathogens, and polyandry in social Hymenoptera. *American Naturalist*, 131: 602-610.

SCHMID-HEMPEL, P (1998) *Parasites in social insects*. Princeton University Press; Princeton, NJ, USA. 392 pp.

SEELEY, T D; TARPY, D R (2007) Queen promiscuity lowers disease within honey bee colonies. *Proceedings of the Royal Society of London.* B274: 67-72.

SIUDA, M; WILDE, J (2006) Effect of queen body weight on start of oviposion. *International Apicultural Scientific Conference in centenary of Jan Dzierżon's death*. Puławy 25-27.04.2006. Materiały konferencyjne: 88-92.

SKOWRONEK, W; KRUK, C; KŁOPOT, J (2002) Factors affecting oviposition of artificially inseminated honey bee queens. *Journal of Apicultural Science*, (46)2: 85-95.

SKOWRONEK, W; BIEŃKOWSKA, M; KRUK, C (2004) Changes of honey bee queen weight of honey bee queens during their maturation. *Journal of Apicultural Science*, 48(2): 61-68.

SOLLER, M; BAR-COHEN, R (1967) Some observations on the heritability and genetic correlation between honey production and brood area in the honey bee. *Journal Apicultural Research*, 6: 37-43.

SPIVAK, M; DOWNEY, D (1998) Field assays for hygienic behaviour in honey bees (Hymenoptera: Apidae). *Journal of Economic Entomology*, 91: 64-70.

SPIVAK, M; REUTER, G S (2001) *Varroa jacobsoni* infestation in untreated honey bee (Hymenoptera: Apidae) colonies selected for hygienic behaviour. *Journal of Economic Entomology*, 94: 326-3. http://dx.doi.org/10.1603/0022-0493-94.2.326

SUGDEN, M A; FURGALA, B (1982) Evaluation of six commercial honey bee (*Apis mellifera L*) stocks used in Minnesota. *American Bee Journal*, 122: 283-286.

SVEČNJAK, L; HEGIĆ, G.; KEZIĆ, J; TURŠIĆ, M; DRAŽIĆ, M; BUBALO, D; KEZIĆ, N (2008) The state of beekeeping in Croatia. *Journal of Central European Agriculture*, 3: 475-482.

SZABO, T I (1973) Relationship between weight of honey-bee queens (*Apis mellifera* L.) at emergence and at the cassation of egg laying. *American Bee Journal*, 113(7): 250-252.

SZABO, T I (1974) Behavioural studies on queen introduction in the honey bee II. Effect on age and storage conditions of virgin queens on their attractiveness to workers. *Journal of Apicultural Research*, 26(2): 73-78.

SZABO, T I (1982) Correlations between colony traits in the honey bee. *American Bee Journal*, 112: 611-616.

SZABO, T I; HEIKEL, D T (1987) Numbers of spermatozoa in spermathecae of queens aged 0 to 3 years reared in Beaverlodge, Alberta. *Journal of Apicultural Research*, 26: 79-82.

TARANOV, G F (1973) Vies matok i ich kacestwo. *Pćelovodstvo*, 53 (11): 16-18.

TARANOV, G F (1974) Condiciones para mejorar la calidad de las reinas en las granjas apícolas especializadas. *Apiacta,* 9: 8-10.

TARPY, D R; HATCH, S; FLETCHER, D J C (2000) The influence of queen age and quality during queen replacement in honey bee colonies. *Animal Behaviour*, 59: 97-101.

TARPY, D R (2003) Genetic diversity within honey bee colonies prevents severe infections and promotes colony growth. *Proceedings of the Royal Society of London*, B 270: 99–103.

THULLER, R H C; MALASPINA, O; BUENO, O C; CHAUD-NETTO, J (1998) Number of ovarioles in workers descendant from crossings between Africanized and Italian honey bees, *Apis mellifera* L.: Comparison among backcrosses and ancestors colonies. *Anais da Sociedade Entomológica do Brasil*, 27: 237-243.

TROSZKIEWICZ, J (2006) Hodowla pszczół w minionym trzydziestoleciu. XXIV Ogólnopolskie Dni Pszczelarza, *Kluczbork*: 21-28.

VEJSNÆS, F (2011) *Nosema Ceranae* almindeligt udbredt i Danmark. *Tidsskrift for Biavl*, 8/2011: 229.

VESELY, V (1965) Spermaverhltung in lateralen Oviducten künstlich besamter besamter Bienenköniginnen*. Proceedings of XXIIth International Apicultural Congress, München, Germany.* p. 604.

VESELY, V (1970) Retention of semen in the lateral oviducts of artificially inseminated honey-bee queens (*Apis mellifera* L.). *Acta Entomologica Bohemoslovaca*, 67: 83-92.

VESELY, V (1971) Untersuchung des verzögerten Soermaverbleibs in den Eileitern besamter Königinnen. *Der XXIII Bienenzuchtenkongr. In Moskau. Apimondia* Verlag. Bukarest: 442.

VESELY, V (1984) Der Einfluss der Kunstlichen Besamug auf die Leistungszuvht. *Bienenvater*, 105: 332-335; 366-370.

WEAVER, N (1957) Effects of larval age on dimorphic differentiation of the female honey bee. *Annals of Entomological Society of America*, 50: 283-294.

WEN-CHENG, H; CHONG-YUAN, Z (1985) The relationship between the weight of the queen honey bee at various stages and the number of ovarioles, eggs laid and sealed brood produced. *Honey Bee Science*, 6: 113-116.

WICKMAN, P; KARLSSON, B (1969) Abdomen size, body size and the reproductive effort of insects. *Oikos*, 56: 209-214. http://dx.doi.org/10.2307/3565338

WILDE, J (1994) The effects of method of keeping queen honey bees after instrumental insemination on their performance. *Acta Academiae Agriculturae ac Technicae Olstenensis, Zootechnica*, 39: 153-166.

WILSON-RICH, N; SPIVAK, M; FEFFERMANN, N H; STARKS, P T (2009) Genetic, individual, and group facilitation of disease resistance in insect societies, *Annual Review of Entomol*ogy, 54: 405-423. http://dx.doi.org/10.1146/annurev.ento.53.103106.093301

WINSTON, M L (1980) Swarming, afterswarming, and reproductive rate of unmanaged honey bee colonies (*Apis mellifera*). *Insects Sociaux*, 27: 391-398.

WINSTON, M L (1987) *The biology of the honey bee*. Harvard University Press. 281 pp.

WOYKE, J (1960) Naturalne i sztuczne unasienianie matek pszczelich. *Pszczelnicze Zeszyty Naukowe*, 4(3/4): 183-275.

WOYKE, J (1966) Wovon hangt die Zahl der Spermien in der Samenblase der auf naturlichem Wege begatteten. Koniginnen ab. *Z. Bienenforsch*, 8(7): 236-247.

WOYKE, J (1971) Correlations between the age at which honey bee brood was grafted, characteristics of the resultant queens, and results of insemination. *Journal of Apicultural Research*, 10(1): 45-55.

WOYKE, J (1979) Effect of the access of worker honey bees to the queen on the results of instrumental insemination. *Journal of Apicultural Research*, 19(2): 136-143.

WOYKE, J (1983) Dynamics of entry of spermatozoa into the spermatheca of instrumentally inseminated queen bees. *Journal of Apicultural Research*, 22(3): 150-154.

WOYKE, J (1984) Correlation and interaction between population, length of worker life and honey production by honey bees in a temperate region. *Journal of Apicultural Research*, 23(3): 148-156.

WOYKE, J (1987) Can the number of ovarioles in the ovaries be estimated by external characters of living queens? *Proceedings of XXXIst International Apicultural Congress, Warsaw, Poland.*

WOYKE, J (1989) Results of instrumental insemination. In *R F A Moritz (Ed.). The instrumental insemination of queen bees.* Apimondia; Bucharest, Romania. pp. 85-91.

WOYKE, J; JASIŃSKI, Z (1978) Influence of age of drones on the results of instrumental insemination of honey bee queens. *Apidologie*, 9(3): 203-212.

WOYKE, J; JASINSKI Z (1980) Influence of the number of attendant workers on the results of instrumental insemination of honey bee queens kept at room temperature. *Apidologie*, 11: 173-179.

WOYKE, J; JASINSKI, Z (1982) Influence of the number of attendant workers on the number of spermatozoa entering the spermatheca of instrumentally inseminated queens kept outdoors in mating nuclei. *Journal of Apicultural Science*, 21: 129-133.

WOYKE, J; JASIŃSKI Z (1985) Comparison of the dynamics of entry of spermatozoa into the spermatheca of instrumentally inseminated queen honey bees kept under different conditions. *Pszczelnicze Zeszyty Naukowe*, 29: 377-388.