Biologia **66**/3: 535—542, 2011
Section Zoology
DOI: 10.2478/s11756-011-0040-3

The effect of induced changes in sexual asymmetry of honey bees (*Apis mellifera*) on swarming behaviour

Roman Linhart[1], Vítězslav Bičík[2]* & Jiří Vagera[3]

[1] *Zahradní 743, CZ-58303 Heřmanův Městec, Czech Republic*
[2] *Department of Zoology, Palacký University, Svobody 26, CZ-77146 Olomouc, Czech Republic; e-mail: vitezslav.bicik@upol.cz*
[3] *Department of Cell Biology and Genetics, Palacký University, Šlechtitelů 11, CZ-78371 Olomouc, Czech Republic*

Abstract: This study was built on the assumption that mother (queen) and workers (nurses) distribute their genes either through swarms (female biomass) or through the drones (male biomass). The swarming mood of the bee colonies was suppressed by an exactly defined increase in drone rearing. We studied the efficiency of reproductive investments (on genetic and energetic levels) of the mother and workers to the next generations. The equalization of fitness of the mother and nurses was achieved by a deliberately induced change in numerically stable sex asymmetry of a bee colony. A swarm was compensated with its energy demand and a volume ratio of distributed genetic information. The newly introduced term "reproductive investment complex" (RIC) includes the reproductive potential of the mother and reproductive energy of workers into care for the mother and for the brood. The number of individuals of one sex was closely connected with the weight of individuals of the oppposite sex. The described method of suppression of swarming mood was successfully tested on 60 honey bee colonies over seven years (2003–2009). A number of beekeepers that were acquainted with this method confirmed the success.

Key words: *Apis mellifera*; antiswarming drone rearing; biomass; reproductive investment complex (RIC); genes distribution

Introduction

The society of honey bee (*Apis mellifera* L., 1758) creates uniform entity. However, individual castes inside this entity struggle for maximization of their fitness. Evolutionary stable strategy of reproduction of bee colonies depends on swarming. However, swarming is a natural but economically undesirable phenomenon, which significantly reduces the world yield of bee products and increases the cost of their production. In Germany, about 30% of bee colonies reach the swarming mood and generally swarm out despite recently used antiswarming methods (Liebig 1998). In less developed countries, bee-keeping is more extensive and the percentage of swarming is probably higher. Flying swarms represent also a toxicological risk to the public and a veterinary risk for bee colonies.

Potentional overheating of a bee colony, insufficient room or pollen nutrition surplus are mentioned by beekeepers as the mechanisms initiating swarming. Nevertheless, wild bee colonies swarm in various conditions almost every year. A measure of swarming is understood as a characteristic of the whole colony, although it is not a genetically homogeneous system.

Theories describing the possible factors initiating the swarming of a bee colony, so far, have been focused on factors affecting the egg laying of the mother (queen). The biomass of drones has not been studied yet. The imbalance between the quantity of nurses and hatched larvae fed royal jelly is considered to be an initiating factor of the swarming mood (Přidal & Čermák 2003). The swarming can only occur if the bee colony approaches the maximum of its number and care potential outweighs above reproductive potentional of the mother (Fefferman & Starks 2006). They are based, for example, on studies on the size of the worker population but take no account of the drone biomass. The present models for eusocial hymenoptera find no reason for the absence of the male sex in the nonreproducing caste. Crozier (2008) assumes that the biological superiority of the females is a determinant for the social hymenoptera. In contrast to the traditional concepts, Lin et al. (2003) suggested that the swarming is induced by the common action of many selfish bee individuals and that the mother is not its direct cause. Emphasis was placed on the anatomical modifications of ovaries and on the pharyngeal gland functions of workers. Thompson et al. (2008) believe that the activation of ovaries in the workers can be conditioned epigenetically.

---

* Corresponding author

Significant feature of an eusocial diplo-haploid bee society is a noticeable asymmetry in the sex ratio and asymmetry in expression of the sexual behaviour is a significant feature of an eusocial diplo-haploid bee society (e.g., Boomsma & Franks 2006; Normark 2006). Obviously, there must be a mechanism that regulates the relative ratio between various castes. The swarming period was predicted also according to swarming sounds and their changes (Ferrari et al. 2008). As for the existence of the gene within natural selection it is the same whether it is spread by parental or sibling investments. The ratio of the caste members and their abundance is dependent on the workers. They determine and build the number of larger hexagonal cells meant for drones (where the mother lays unfertilized eggs) as well as the smaller cells from which workers emerge when fertilized eggs hatch. It is the nutrition which decides whether young females belong to the castes of workers or mothers.

In a strengthening colony, there is an asymmetry between the mother's reproductive potential (egg-laying) and a worker's care potential (care for brood). The mother first invests her reproductive energy into the production and egg-laying, then workers invest their reproductive energy into care for the brood. The mother and workers create, within the society, a functional complex which we called reproductive investment complex (RIC). Therefore a worker responsible for development stages is called the nurse.

An effective anti-swarming method is to schedule a split in the bee colony at an appropriate time so as to prevent overcrowding. However, this treatment is labour and time-consuming. Nevertheless, the initiation of the swarming mood can be only delayed and partially managed by making colony splits. So far, no attention has been given to research in the general mechanisms of the reproduction investment management at the level of a colony as a whole, whereas individuals are considered (according to the theory of selection between relatives) to be instruments for distribution of their own genes.

We, therefore, regard sterile workers as the reproductively active biomass that not only actively participates in the swarm forming but even differentiates before swarming its reproductive investment into the offspring of various castes. We examined how the ratio between males and females might be specified in this relationship, according to the amount of shared genes and whether individuals of the various investing castes (the mother and the workers) endeavour primarily to maximize their fitness (Hamilton 1964).

We therefore attempted to influence the reproductive behaviour of bees. We built on the assumption that the mother and nurses can distribute their genes either through swarms composed of the female biomass or through the drones – the male biomass. Each individual of the swarm and each drone share some genetic material with the mother and sister. In this way swarms and drones spread genes of mother and nurses outside the original bee colony.

This study emphasizes the fact that an unequal amount of energy fixed in the male and the female biomass is needed for the distribution of the same nurse gene copy by a male or a female individual. Gene copies of the investing individuals can be distributed from the maternal colony both, by swarms consisting of the female biomass (mother and workers) and the male biomass (drones).

To suppress the swarming mood, we used a deliberate increase in production of the male biomass which compensates for a potential swarm in energy demand and the volume of distributed genes. We tested in this case whether the bee colonies refrain from the swarming.

Material and methods

The experiments were carried out in 2001–2009 on 60 bee colonies of *Apis mellifera carnica* Pollman, 1879, Singer and Vigor breed. The experimental beehives were located in eastern Bohemia at an altitude of 309 m in the foothills of the Železné hory mountains.

During the years 2001 and 2002, we investigated the average weight of drones and workers. In total, 150 individuals of each caste were removed annually from six colonies chosen randomly from our 60 colonies. This sample size was sufficient for determining the average weight of both castes. As sampling was carried out late at night, the weight of the bees was not significantly affected by nectar gathered during the day. The bees were immobilized for a short time by the smoke of ammonium nitrate before counting.

The mean weight of saturated swarming workers was determined by sampling 150 individuals from swarms which took off. One wing of the mother of these potential swarming colonies was shortened such that the swarms would not fly far (often landing on the low vegetation relatively close to the original hive). The total weight of all swarms was determined by knocking the hives into woven sacks for weight recording.

In the years 2001–2002, we also determined the total weight of biomass of workers of non-swarming colonies and their numbers in order to obtain comparative data. Vapors of ammonium nitrate for immobilization of swarms and the whole colony of non-swarming bees were used as well. It was done in late evening hours when flying activity had finished.

Another method of quantifying the biomass was investigation of the egg-laid area of the combs by weighing and counting of individuals of both castes. The area of drone cells was established by placing a frame with squares of 1 dm^2 and determined from photographs. These data were important for optimizing the area of the drone comb, necessary for the suppression of swarming.

The gene copy distribution of a worker's investment into her offspring (both drones and workers) is based on the mean weight of the castes and calculations.

We simulated a model where all diploid female individuals are carriers of 100 genes and all haploid male individuals carry 50 genes. This is in order to express the absolute number of shared genes among individuals with the same ploidy level (e.g., 75) along with the same degree of relationship in percents (75%). The models are designed to describe the state in which the female offsprings of the mother are supersisters sharing 75% of the shared genetic information. This actually occurs in those sisters that are daughters of the same mother and father. The situation is more complicated because mothers copulate with more drones (Boomsma & Ratnieks 1996). However, we consider the most simple and probably evolutionarily original description of the state as a necessary starting point for explanation of the problem.

We do not operate with the real number of genes carried by particular individuals but with their relative volume ratios in genomes of the individuals, resulting from the ploidy level of the members of particular castes.

The experiments with antiswarming breed of drones were carried out during the years 2003–2009 in our 60 colonies. We used thin-walled frame hives with screened bottom boards. A similar type of hive is used in most countries. In the maximum size, the colonies occupied three frames. Each contained 12 combs in frame size 37 × 30 cm. Two frames were used as the brood combs and one as the honeycomb.

During the replacement of the winter long-lived workers' generation by the spring short-lived and obviously strengthening colonies (second half of April), two hive frames for building the drone cells were inserted into the center of a brood comb. From both sides, these frames were followed by at least two combs with workers' brood. This setup optimises the temperature for the drone brood. While the frames were built and egg-laid, two more frames were added into the second brood comb according to the same principles. After the end of the swarming period (around 10–15 July) the building frames with the drone brood were removed and processed to wax. The rate of the swarming, time demand for maintenance of a bee colony, honey yield and *Varroa destructor* Anderson et Trueman, 2000 infection degree were assessed in relation to the data from the previous period of our beekeeping in the traditional way.

Results

Genetical and etoecological aspects of the used method

The mother of the bee colony is able to lay more than 2,000 eggs per day. Three larvae of a new generation of workers can satisfy the care potential of each nurse. Nevertheless, they mature soon and need to take care of the triple number of larvae. To meet this care potential, the mother would have to triple egg-laying permanently with each generation of even more numerous worker caste. Over time, the mother is no longer able to increase her reproductive investment. The workers therefore diverts their nurturant potential into a few young mothers, swarm-forming workers and drones. The swarm-forming workers and the mothers are bred before swarming, while the drones are bred continuously throughout the reproductive period. In the total female biomass, the contribution of the old mother and her fertile daughters (young mothers) is insignificant.

The haploidity of drones leads to anomalies between relatives, that cause, for the mother and investing workers (nurse bees), unequal benefits from breeding of various castes. There is a competition between the mother and the nurses for the most effective distribution of their own genes. The rare occurrence of drones appears e.g. after the mother grows old and decreases concentration of mother's substance which castrates workers or after the death of the mother.

Schematic representation of absolute and relative fitness of the mother (Fig. 1) and the nurse (Fig. 2) originates from one mother mated by one drone. It is based on the volume of shared genes in the grades A1 and A2 at various ways of their distribution: A1 – first grade of reproductive energy acceptors and propagators of copies of the genes; A2 – second grade of reproductive energy acceptors and propagators of copies of the genes; RIC: the mother and the nurses – donors investing reproductive energy and distributing copies of their genes.

The volume and absolute value of genes shared across grades of acceptors show how the number of gene copies of the studied members of RIC decreases over time. The percentage values show the gene copies of the RIC members participation on the offspring genome of various ploidy level and sex formation.

Fig. 1 shows the mother in the donor position of the reproductive energy as a member of the RIC. This generation is identical with the F0 generation in the classic genealogical trees. The successive grades of the acceptors A1, A2 correspond to the generations F1, F2 in the classic genealogical tree.

Mother's reproductive interests are an antithesis of the workers' reproductive interests (Fig. 1). Mothers are paid off most by production of their sons, to whom they are relatively related in 100% at grade A1. Granddaughters of these sons are carriers of 50% relative relatedness to the mother and carry also 50% of the absolute number of genes at grade A2. The sons of worker-laid drones achieve also 50% of relative proportion of mother's genes but in absolute numbers they share only 25% of the genes. In terms of maximizing the mother's fitness, it can be concluded that the production of her own sons is the most beneficial for her. They are at once the mother's sons and nurses' brothers, thus their breeding is, in terms of the nurses fitness, the least efficient option. The breeding of individuals of various castes is not equally beneficial for the mother and the nurses. The mother and the nurses compete for the most effective distribution of their gene copies. But the bee colony (as a system based on cooperation) is important for all castes. Therefore, the interests of the mother and the workers must be, within the reproductive system, coordinated by the evolutionarily stable strategy whereas the genes of the mother are distributed as efficiently as the genes of the nurses. It ensures conciliation between the interests of sub-components of the RIC. The compromise is reached so that workers breed fewer drones than swarm-forming workers but they invest more energy into the biomass of bigger drones than into the workers' biomass. On the contrary, nurses increase their fitness by the amount of the female biomass. The same amount of the reproductive energy falls on the gene copy distributed by drones and workers. However, the balance between absolute numbers of distributed copies of mothers and nurses is not achieved. The antiswarming effect is achieved only with establishment of this equilibrium.

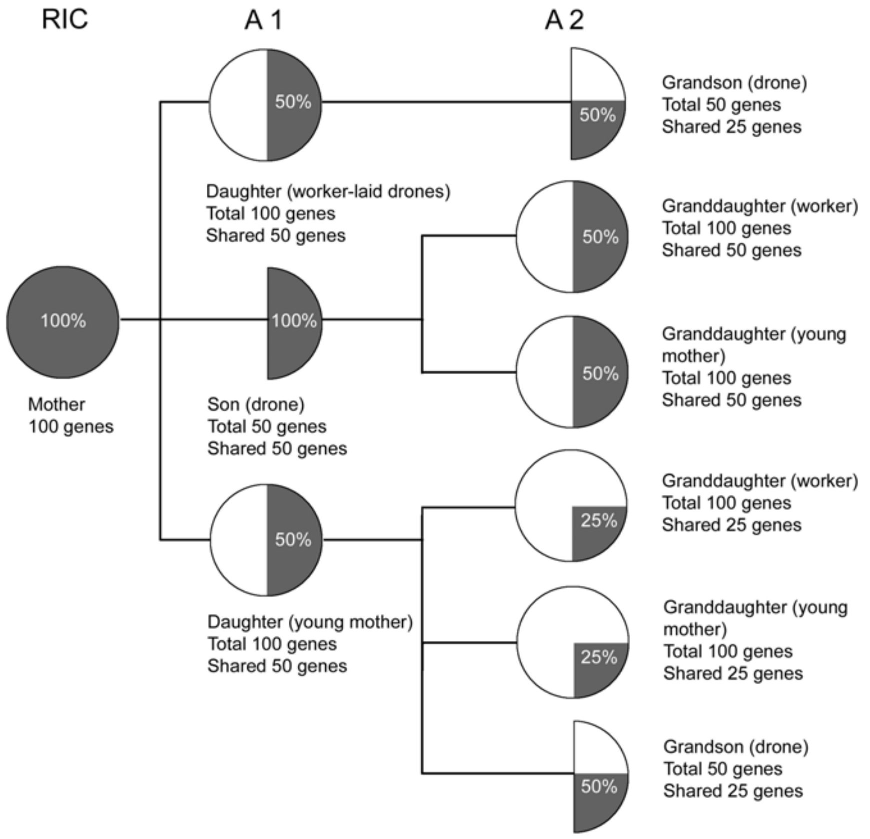

Fig. 1. Effectiveness of the mother's reproductive investment. ● – workers or mother, ◗ – drone. Dark – evaluated volume of genetic information.

The nurse is in the donor position of the reproductive energy as a member of the RIC (Fig. 2). Bred individuals, acceptors A1, are therefore, in terms of the classical comprehension of the relationship, her siblings or offspring. In this figure we distinguish the nurse's siblings from her lineal descent with the terms: brother, sister and son at the grade of A1. This emphasizes that the breeding of her brothers is not as useful as the breeding of her sons. However, they are equivalent acceptors of the nurse's reproductive energy. Therefore we defined them as the offspring of this nurse. Each of the reproductive energy donors sharing part of the same genes with the acceptor is considered as a parent. A bred nurse's sibling is her offspring at the same time. This view is conceived deliberately to abandon the difference between donor's investment into the lineal offspring and siblings' breed. With a view to conservation and genes distribution of the donor, it is indifferent whether it is sibling or parental investment. In the A2 generation, we therefore unify the terminology and use grandson, granddaughter in brackets for the nurse relatives

Investments into sisters are very beneficial for maximizing the nurse's fitness (Fig. 2). At A1 grade, the nurse is related in 75% to them. If her sisters (young mothers) produce drones, they will be, at the grade A2, related to the worker RIC in 75% as well. Investing into sisters (workers) is the same situation. Their relationship to the nurse reaches 75% at grade A1. The offspring of drone egg-laying bees (sisters) are also related in 75% at grade A2.

Within the antiswarming measures, we grossed up the male biomass to equate the absolute fitness values of both components of the RIC. When calculating the size of the drone breed we take into account the mean weight of the basic components of the male and female biomass.

The mean weight of a hived worker bee is 0.1 g. However, if a nectar-sucked swarm-forming worker weighs 0.15 g and a drone weighs 0.23 g, then 1.5 worker must be produced to achieve the same biomass as that of the drone (because $0.23/0.15 = 1.53$).

If the nurse puts as much energy into the breeding of the worker's biomass as the potential breeding of one drone, the 1.5 diploid worker would carry $75 + 37.5$ of the shared nurse gene copies, which is 112.5 genes. If a one haploid drone (brother) was bred from the same amount of energy, only 25 of the shared nurse gene copies would be distributed ($112.5/25 = 4.5$). The male biomass is, with the same energy investment, a 4.5 times worse distributor of nurses' gene copies than the female biomass.

We found that the workers breed actually 4.5 times fewer of drones than their reproductively active sisters. This is in compliance with the calculation above. The numerical and weight ratios of the sexes are such as to achieve the same degree of effectiveness of the investing worker genes distribution, via the male and female biomass (that is evolutionary stable strategy). Because

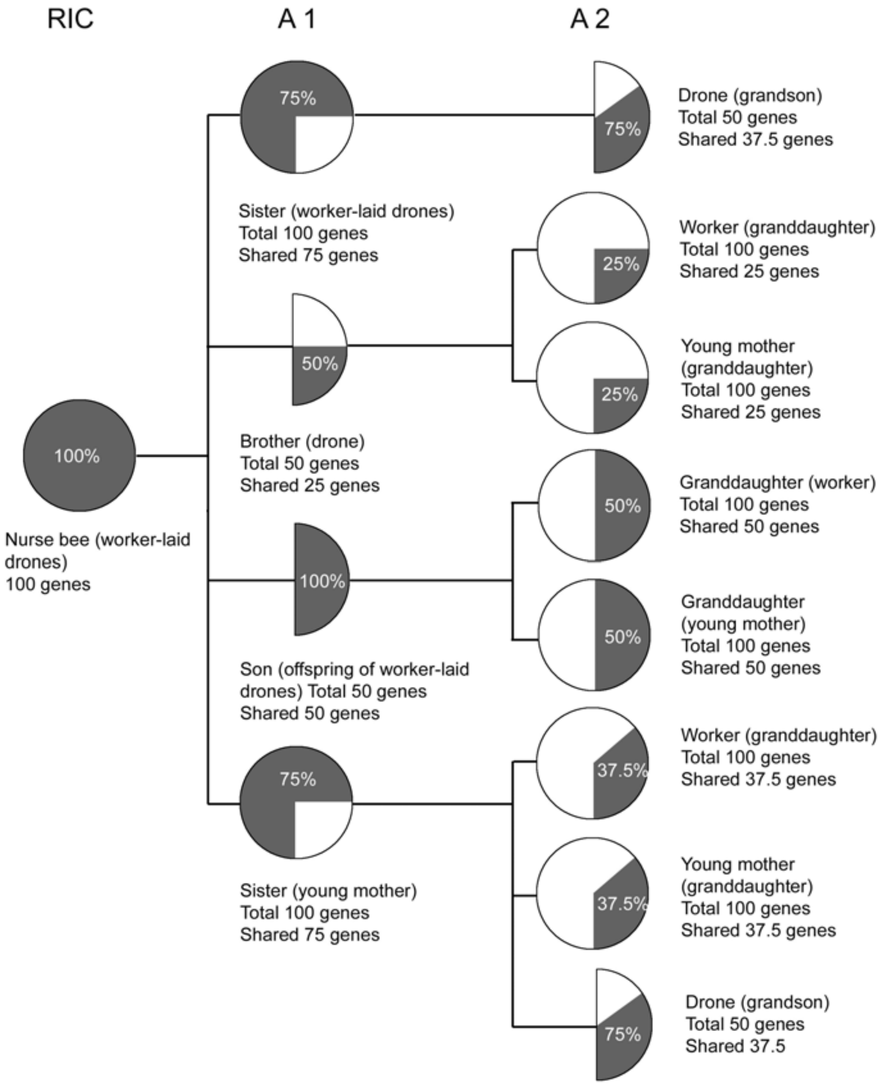

Fig. 2. Effectiveness of the nurse bee reproductive investment. ● – workers or mother, ◗ – drone. Dark – evaluated volume of genetic information.

drones are 4.5 times worse distributors of the nurses' gene copies per unit of invest energy than one-half of the swarm-forming workers' colony, they are produced 4.5 times less in the bee colony. However, they are 1.5 times heavier than swarm-forming workers. The numbers 4.5 and 1.5 are in ratio 3 : 1. This corresponds to the fact that the investing worker is on average three times more related to its supersister (75% relationship) than its brother – the drone (25% relationship).

Optimal rate of antiswarming drone rearing for the beekeeping practice

Let us introduce an example of practical application of the method. Ten thousand hived bees weigh about 1 kg. A very strong bee colony, comprising around 60,000 workers, weighs about 6 kg. This biomass is split into two parts before swarming. One part is ready to swarm out with the old mother or with the secondary swarm (swarm-forming bees), while the other half stays with the young queen in the original colony and is not part of the swarm. Approximately 30,000 bees, that are one-half of the female individuals from the colony, are prepared to swarm out. Drones are bred at a rate that is 4.5 times lower than the females in a normal colony. Hence, for the hypothetical half colony, 6,667 individuals are expected to be males (30,000/4.5 = 6,667). A wild bee colony would breed such a number of drones during the whole season. The colony invests into the primary swarm such amount of biomass that is one-half of its total female biomass before swarming. From the 6 kg worker colony, a 3 kg primary swarm is formed. Considering that the weight of a swarm-forming worker was increased from 0.1 g to 0.154 g, only 20,000 of the workers with total weight 3,000 g, would leave with the primary swarm. The remaining 10,000 swarm-forming bees would stay in the original colony with a young mother as a reserve biomass for the formation of the potential secondary swarm.

In this natural state, drones are bred against the total biomass of 30,000 swarm-forming workers. The ratio of the biomass is 3:1 in favour of the female biomass. So: 30,000 workers × 0.154 g = 4,620 g. When we multiply 6,667 drones with the drone weight

(0.232 g), we get the weight 1,546.74 g. So that, 4,500/1,546.74 = 2.99 (rounded off 3.00).

Other calculations can prove the equal investment rate of the same gene copies distribution: 30,000 workers × 0.154 g = 4,620 g. Then 4,620/75 = 61.6 for workers. For drones, it is: 6,667 × 0.232 g = 1,546.7 and 1,546.7/25 = 61.8.

Antiswarming drone rearing can be defined as the standard drone breeding rate, multiplied, in comparison with the natural state, as many times as the drones are worse nurse bee genes distributors than the swarm-forming worker bees at the level of an individual: 6,667 × 4.5 = 30,001. If we rear 30,000 drones to compensate for the biomass of 30,000 swarm-forming workers, their ratio in relation to the female biomass would be approximately 1 : 1.

The following calculation reveals that the ratio between male and female biomass within the antiswarming drone rearing: 30,000 workers × 0.154 = 4,620 g. 30,000 drones ×0.232 = 6,960 g. So, 6,960/4,620 = 1.50 for the drone biomass.

Assuming a model of 100 genes in the female genome, the carried genes ratio would be 3.00 in favor of workers, because 30,000 workers × 75 = 2,250,000 genes. 30,000 drones × 25 = 750,000 genes. It holds 2,250,000/750,000 = 3.00.

Another important antiswarming factor is the balance between genes distributed by the mother and workers in the production of an equal amount of the generative individuals. If the colonies in their natural state bred 30,000 workers and 4.5 times fewer drones (6,667), then the nurse would distribute 2,250,000 of genes via workers (30,000 × 75 genes) and 166,675 of genes via drones (6,521 × 25 genes). The mother would distribute 1,500,000 of genes via workers (30,000 × 50 genes) and 326,050 of genes via drones (6,667 × 50). This means that a nurse bee in the natural state distributes 1.32 times more copies of its genes than the mother (nurse: 2,250,000 + 166,675 = 2,416,675; mother: 1,500,000 + 333,350 = 1,833,350; 2,416,675/1,833,350 = 1.32 times more).

In the antiswarming drone rearing with 30,000 worker bees and 30,000 drones, a nurse would distribute 2,250,000 genes via workers (30,000 × 75 genes) and 750,000 genes via drones (30,000 × 25). The mother would distribute 1,500,000 of genes via workers (30,000 × 50) and 1,500,000 of genes via drones (30,000 × 50). This means that the mother and a nurse would distribute the same number of their gene copies in the antiswarming rearing – 3,000,000 (mother: 1,500,000 + 1,500,000; nurse: 2,250,000 + 750,000). Thereby, the balance in fitness between components of the RIC (the mother and a nurse) is established. Strengthening of the mother's fitness along with energy demand of the drone is the major factor for the suppression of the swarming mood. If we strengthen the mother's fitness, we would strengthen her dominance over the workers. The workers are not transformed into swarm-forming workers and the swarming is prevented at any level – energetic, fitness and hormonal.

The result of these calculations is a need to produce about 30,000 drones in the antiswarming drone rearing during the season. It is necessary to take into account the number of drone cells on a comb, the fact that the comb will be egg-laid on both sides and that each cell will be used for the drone larvae at least twice.

On the tract of 1 dm^2 of a comb, there are 250 of drone cells (30,000/250 = 120 dm^2) on one side. When the comb is double-sided, the same number of cells is found on 60 dm^2 (120/2). We use frames of internal dimensions 35 × 28 cm. The area of each is 9.8 dm^2 and 60/9.8 = 6.12 of the frame. When 6.12 is divided by two (since this area will be egg re-laid), we get the number 3.06 of the used frame (rounded off 3.00) The hive frames are never 100% filled with eggs and so swarms with weight of 3 kg are not the heaviest. Therefore, we use four frames.

Discussion and conclusion

During the seven years (2003–2009) of the application of described method (with antiswarming breed of drones) at 60 bee colonies we observed swarming only twice when the method was not strictly followed. In the past for the same number of bee colonies, when the standard method of colony splits was applied, an average of 15 swarms per year was recorded. Honey production in non-swarmed colonies has increased approximately by about 30%, while the production of wax increased by about 40%. The time needed for the maintaining of one bee colony has decreased from the average 160 minutes to about 60 minutes. We rear marked mothers in bee colonies so we are sure of the swarming rate. The increase in the productivity of the colonies is attributed to a higher temperature comfort since the drone biomass helps to heat the brood on combs and thousands of workers are released for harvesting. The opinions of some beekeepers (in verb.) that drones deprive the colony of honey were not confirmed. The drone larvae consume mainly the royal jelly, formed on the base of pollen proteins.

We assume evolutionary original state of bee colony – one mother fertilized by one drone as shown in Figs 1 and 2. In recent societies of honey bee we encoutered various factors influencing the degrees of kin and the intensity of spreading the genetic information. Our study stems from practical experience with antiswarming drone rearing proving that the principle is basically the same as in the evolutionary original state.

In the case of polyandry, which is the standard for honey bee (Oldroyd et. al. 1994; Neumann & Moritz 2000) even in the case of rare polygyny of some bee species (Rinderer et al. 1998; Haberl & Tautz 1999), the average rate of the genetic relationship between individuals in the population is lower. The kin can also be influenced in cases within bee society where the offspring of certain drones (subfamilies) are advantageous (Tarpy et al. 2004).

Another influencing factor can be the fact that if there is a mother in a colony, the eggs laid by workers-laid drones are eaten by the other workers (Montague & Oldroyd 1998; Visscher 1998). Nurses remove also larvae from which a diploid drone would be developed (Santomauro et al. 2004). Drones developed from the mother's eggs are bigger (they have to be strong to mate successfully) and have more quality sperm than drones of worker-laid drones (Gencer & Firatli 2005).

It is an important finding that the workers reach a higher level of fitness in grade A1 by breeding their own drones (sons) related in 100%, compared with breeding drones from the mother's eggs (her brothers) related in 50%. The mother-laid drones do not have this ability in relation to the nurse, because they share with her only the genes from the mother's side. Therefore, the workers tend to produce their own male offspring as workers-laid drones or to invest in breeding of young mothers and swarm-forming workers. Thus, they invest into that part of the female biomass that is actively involved in swarming.

The role of nurses in colonies of hymenopterous insects probably determines the number and weight proportions of individuals of the opposite sex. The ratio of drones to workers is determined by nurses. Drones also stimulate workers to more intensive activity besides being necessary for copulation purposes (Zeng & Yan 2004).

Bee colonies are not in terms of reproductive investment internally homogeneous systems and cannot be regarded as an integral unit in matters of reproduction. External expressions of the reproductive behaviour of a society as a whole (swarming) are influenced by internal antagonistic needs of mothers and nurse bees, resulting from the different strategies of maximizing their fitness. The fitness level of the mother can be therefore increased to the level of an average nurse by breeding the recommended number of drones, so that the swarming mood is suppressed. The distribution of gene copies through drones is very energy-demanding for nurses. This depletes the reproductive energy that would be invested into swarm forming in the natural state.

If a gene is considered a basic unit in natural selection (Dawkins 1976, 1982; Boomsma & Franks 2006), then, according to genocentric model it can be reached in a bee colony reconciliation between antagonistic parts of RIC on the basis of the same amount of reproductive investments.

Parameters of biomass correlate with the number of drones and workers in their natural state. There is a dependence on relationship aspects derived from the amount of the shared gene copies in relation to the investing nurse.

Hölldobler & Wilson (1995) reached a similar conclusion when studying quantitative ratios of the bred male and female fertile individuals in ant colonies. They studied whether the worker's investments into the various sex individuals vary on the basis of relationships. They found that the ratio of the bred sex is close to the value of 3 : 1, because young females are 3-times more related to workers than their brothers are. In this fact, we see the proof of the kin-selection theory (Hamilton 1964). Our current analysis suggests that a similar mechanism could hold for societies of other eusocial insect with the sex asymmetry. Elements of behaviour resembling a bee eusociality were found in ants, e.g., by Liebig et al. (1999).

Brief instructions for the application of our method were published (Linhart & Bičík 2007; Linhart et al. 2007) with an appeal to commercial and hobby beekeepers to broaden the testing of this method. About 40,000 organized beekeepers subscribe to the journal Včelařství in the Czech Republic and could read our contributions. So far, we have received 3,583 positive references from the Czech Republic and Slovakia, and many other beekeepers are going to apply this method. The method has been unsuccessful for a small number of beekeepers only. They have failed due to the lack of adherence to the strict instructions of the method. Searching for the causes of failure, we proved that the time terms or the instructions were not kept. We believe that the described antiswarming method is based on general biological principles. As it has been successfully tested under natural conditions of the Czech Republic, we expect its functioning in various apiculture technologies, in all geographical races of the honey bees and the related subspecies of the genus *Apis*. The health condition of our 60 experimental bee colonies was excellent during the seven years (2003–2009) of the testing of this method. They wintered during the years 2006–2008 in a good condition as well, in spite of the common significant losses resulting from the overgrowth of acarid *Varroa destructor* in the mild winters in those years. In some areas, the spring mortality reached up to 80% of the colonies. *Varroa* prefers drones, if there are enough drones it attacks workers at the second rate. However, it is important to monitor the acarid infection carefully, cut out the drone komb, and process to wax immediately, after the end of the swarming time. This method of swarming suppression is so effective that mothers rearing is not possible. Within its use, the colony tends to the so called quiet mother exchanges, which is also very much appreciated.

We believe that our work based on practical experience with antiswarming breeding of drones in honey bee brings also stimuli for theoretical studies dealing with biology of haplodiploidy insect.

Acknowledgements

The research was supported by grant no. 30314762 of the Czech Ministry of Education. The authors would like to thank L. Shirley, M. Wolfová and J. Koleček for valuable comments on an earlier draft. We thank both anonymous reviewers for their comments which improved the presentation of the results.

References

Boomsma J.J. & Franks N.R. 2006. Social insects: from selfish genes to self organisation and beyond. Trends Ecol. Evol. **21:** 303–308. DOI: 10.1016/j.tree.2006.04.001

Boomsma J.J. & Ratnieks F.L.W. 1996. Paternity in eusocial Hymenoptera. Philos. Trans. R. Soc. Lond. B Biol. Sci. **351:** 947–975. DOI: 10.1098/rstb.1996.0086

Crozier R.H. 2008 Advanced eusociality, kin selection and male haploidy. Austr. J. Entomol. **47:** 2–8.

Dawkins R. 1976. The Selfish Gene. Oxford University Press, Oxford, 224 pp.

Dawkins R. 1982. The extended phenotype. The gene as the unit of selection. W. H. Freeman and comp. Oxford, 307 pp.

Fefferman N.H. & Starks P.T. 2006. A modeling approach to swarming in honey bees (*Apis mellifera).* Insectes Sociaux **53:** 37–45.

Ferrari S., Silva M., Guarino M. & Berckmans D. 2008. Monitoring of swarming sounds in bee hives for early detection of the swarming period. Comput. Electron. Agric. **64:** 72–77.

Gencer H.V. & Firatli C. 2005. Reproductive and morphological comparisons of drones reared in quenright and laying worker colonies. J. Apic. Res. **44:** 163–167.

Haberl M. & Tautz D. 1999. Paternity and maternity frequencies in *Apis mellifera sicula.* Insectes Sociaux **46:** 137–145.

Hamilton W.D. 1964. The genetical evolution of social behaviour I and II. J. Theor. Biol. **7:** 1–52.

Hölldobler B. & Wilson E.O. 1995. Journey to the Ants: A Story of Scientific Exploration. Harvard University Press, Cambridge, Massachusetts, 198 pp.

Liebig G. 1998. Einfacht Imkern. Eigenverlag, Stuttgart, 210 pp.

Liebig J., Peeters C. & Hölldobler B. 1999. Worker policing limits the number of reproductives in a ponerine ant. Proc. R. Soc. Lond. B Biol. Sci. **266:** 1865–1870. DOI: 10.1098/rspb.1999.0858

Lin C.C., Chen T.W. & Lai C.C. 2003. The economics of honey bee swarming. Regional Science and Urban Economics **33:** 581–594.

Linhart R. & Bičík V. 2007. Med pomáhají tvořit i trubci [Even drones help to create honey]. Včelařství **5:** 125–126.

Linhart R., Bičík V. & Vagera J. 2007. Současná a nově navržená opatření proti rojení včely medonosné [Present and newly proposed measures against swarming of honey bee]. Včelařství **10:** 1–16.

Montague C.C. & Oldroyd B.P. 1998. The evolution of worker sterility in honey bees: An investigation into a behavioral mutant causing failure of worker policing. Evolution **52:** 1408–1415.

Neumann P. & Moritz R.F.A. 2000. Testing genetic variance hypotheses for the evolution of polyandry in the honey bee (*Apis mellifera* L.). Insectes Sociaux **47:** 271–279.

Normark B.B. 2006. Perspektive maternal kin groups and the origins of asymetric genetic systems – genomic imprinting, haplodiploidy, and parthenogenesis. Evolution **60:** 631–642.

Oldroyd B.P., Rinderer T.E. & Schwenke J.R. 1994. Subfamily recognition and task specialization in honey bees (*Apis mellifera* L.) (Hymenoptera, Apidae). Behav. Ecol. Sociobiol. **34:** 169–173. DOI: 10.1007/BF00167741

Přidal A. & Čermak K. 2003. O rojivosti včel [On swarming of honey bees]. Včelařství **56:** 112–115.

Rinderer T.E., Stelzer J.A., Oldroyd B.P. & Tingek S. 1998. Levels of polyandry and intracolonial genetic relationships in *Apis koschevnikovi.* J. Apic. Res. **37:** 281–287.

Santomauro G., Oldham N.J., Boland W. & Engels W. 2004. Canibalism of diploid drone larvae in the honey bee (*Apis mellifera*) is released by odd pattern of cuticular substances. J. Apic. Res. **43:** 69–74.

Tarpy D.R., Gilley D.C. & Seeley T.D. 2004. Levels of selection in social insects: a review of conflict and cooperation during honey bee (*Apis mellifera*) quen replacement. Behav. Ecol. Sociobiol. **55:** 513–523. DOI: 10.1007/s00265-003-0738-5

Thompson G.J., Kucharski R., Maleszka R. & Oldroyd B.P. 2008. Genome – wide analysis of genes related to ovary activation in worker honey bees. Insect Mol. Biol. **17:** 657–665. DOI: 10.1111/j.1365-2583.2008.00838.x

Visscher P.K. 1998. Colony integration and reproductive conflict in honey bees. Apidologie **29:** 23–45.

Zeng Z.J. & Yan W.Y. 2004. Chinese research suggests drones stimulate worker foraging. Am. Bee J. **144:** 232–233.

Received July 15, 2010
Accepted January 20, 2011