

## Are Drones Injured during Storage in Own and Stranger Queenright Colonies (*Apis mellifera carnica*)?

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### ABSTRACT

The aim of this study was to check whether honey bee drones are also injured. We also compared the degree of injury to drones in own and stranger colonies. Drones were stored in mailing cages in their own colonies and stranger colonies. The number of injuries and the death rate were checked twice, after 3 and 7 days of storage. In total, over 4,608 drones were examined. Nine different types of injuries were observed for the drones, with leg injuries being the most common – lack of segments of tarsus (ca. 70-75% of all injuries). Other types of injuries included black arolia, missing arolia, wing and antenna injuries. The research showed that drones stored in bee colonies suffer injuries just like queens and worker bees do, though to a significantly lesser extent. This study also showed that storage of drones in mailing cages resulted in very high mortality of 62 to 75%.

**Keywords:** Honey bee, Drone storage, Mailing cages, Mortality.

### INTRODUCTION

In an emergency, bees defend their nest. The stimuli that encourage aggression in bees include the alarm pheromone, vibrations, carbon dioxide, hair, and dark colors (Crane, 1990). Aggressive tendencies depend on the function that bees perform in the colony (guards, soldiers, or foragers), as well as their age (Alaux *et al.*, 2009). Honeybees defend their nests not only against predators and parasites, but also against honey bees from other colonies that may rob their food supplies. The guards' acceptance threshold varies depending on the number of bees that sneak in. When more stranger bees with a full honey stomach try to invade the nest, their behavior becomes less liberal (Boch *et al.*, 1970).

Drones can freely access all colonies, regardless of their origin, as shown by numerous studies on drone drifting.

According to some authors, only 10% of drones visit other colonies (Buttler, 1939; Lewieniec, 1954; Witherell, 1965; Skowronek and Kruk, 1998), while others claim that between 50 and 80% of drones drift (Free, 1958).

Workers regulate the number of adult drones in the colony (Free and Williams, 1975). Drones are evicted in autumn (Ribbands, 1953; Ruttner, 1956) mainly by "unemployed foragers" (Free and Williams, 1975), but workers can also behave aggressively towards males outside the foraging season.

Aggressive behavior in bees may take the form of injuries. Injuries to queens stored in queenless colonies were first observed by Woyke *et al.* (1956). Further research conducted by Jasiński (Jasiński, 1984; Jasiński, 1986; Jasiński, 1987; Jasiński, 1995) provided a classification of such injuries: (I) Changes in the color of arolia, (II) Missing leg segments or missing whole

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legs, (III) Arolium deformations and partial or complete loss of arolia and claws, (IV) Other injuries (frayed wings and loss of antennae or antenna segments). Other researchers also described leg paralysis, probably resulting from stings (Gerula and Bienkowska, 2002; Gerula, 2006).

Queens stored in “queen banks” suffer primarily from leg injuries (Jasiński, 1995; Jasiński and Fliszkiewicz, 1995; Loc *et al.* 1996; Wilde *et al.* 1997). According to Jasiński (1995), injuries from group II, III, and IV negatively influence the queens’ motor and sensory abilities and disqualify them as high-quality breeding material. Even a small number of queens (Gerula and Bienkowska, 2002; Jasiński, 1984; Jasiński, 1986) stored in one colony are exposed to injuries from worker bees (Jasiński, 1988; Jasiński and Fliszkiewicz, 1997a, Woyke, 1988). Injuries to queens were observed regardless of the age of the workers attending to them (Jasiński and Fliszkiewicz, 1997b) and the presence of brood in the bee colony.

Queens, workers and drones have tarsal glands on their feet (Lensky and Slabeski, 1981). The structure of the glands is similar for all bee forms. The drone tarsal gland secretion also differs chemically from the female’s, and its biological effects are still obscure (Lensky *et al.* 1985) The tarsal gland secretion by queen, in the presence of 9-oxodec-2-enoic-acid inhibits the construction of queen cells in colony (Lensky and Slabeski, 1981). Injuries to queens’ legs disrupt the production of the tarsal gland secretion. Queens with arolia injuries do not leave traces of this secretion on glass (Woyke *et al.*, 1956). It is assumed that the secretion of tarsal glands can serve different purposes in the three bee castes, since some differences in the chemical composition in queens, workers, and males were observed (Lensky *et al.*, 1984). Worker bees caged in nurse colonies are also injured (Jasiński and Kawecki, 1992; Madras-Majewska, 2009; Zajdel *et al.*, 2014), regardless of whether a queen is present in the colony (Madras-Majewska, 2009) or

whether the workers come from their own or stranger colonies (Zajdel *et al.*, 2014).

Drones are isolated and stored in colonies until the insemination of queens. Male individuals are usually placed in isolation cages or queen excluder cages, which the workers can enter to feed the drones, ensuring higher survival rate and better condition of the drones.

The aim of this research was to verify whether, and to what extent, drones stored in colonies are injured by bees from the nurse colonies. In addition, we aimed to check to what extent storage of drones in mailing cages affects their survivability.

## MATERIALS AND METHODS

The experiment was conducted during two summer seasons (from June to July), in the apiary of the Apiculture Division at the Warsaw University of Life Sciences on drones from Carniolan bee colonies (*Apis mellifera carnica*).

### Drones’ Injuries during Storage in Bee Colonies

Drones’ acceptability and survivability are affected by such factors as the strength of the colony (Currie and Jay, 1988), the race of the nursing bees, the condition of the queen and the foraging conditions (Crane, 1990; Free, 1957), which is why colonies of one race, *A. Mellifera carnica*, were selected for the experiment. The colonies were of medium strength (6–7 brood combs, 10–12 combs occupied by bees, 2 combs with pollen bread) with young egg-laying queens, and outside the foraging season they were fed with sugar syrup.

The study was conducted in 24 bee colonies (13 in 2018 and 11 in 2010). One-half of the drones used in this study originated from their own colonies (i.e. from the colonies in which the research was conducted), and the other half from stranger colonies (i.e. from colonies other than the banking colony). We chose young drones (with a “soft” body and light hair). Drones were collected from combs in the middle of

the nest and not from external combs, where live older drones matured to mating flights. They were put into plastic mailing cages with slots in one side, which were used in similar studies involving queens and workers (Gerula and Bieńkowska, 2002; Jasiński and Fliszkiewicz, 1997a; Zajdel *et al.*, 2014). One side of each cage had 27 slots (2.5×11 mm), which bees from the nurse colonies used to interact with the drones. This size of the slots enabled the workers to feed the drones. The use of small hole mesh cages could lead to higher mortality of drones, as was the case for queens (Woyke *at al.*, 1956). The food chambers of the cages were filled with “bee candy”. Drones were put into cages without workers to eliminate the possibility of the drones' being injured by workers inside the cages. Six drones from one type of colony, either own or stranger, were placed in each cage. Each frame held 16 cages arranged in four lines (1 series), containing drones from one group (own or stranger colony). Two frames were placed in one colony at the same time: one with own drones and another one with stranger drones (Figure 1).

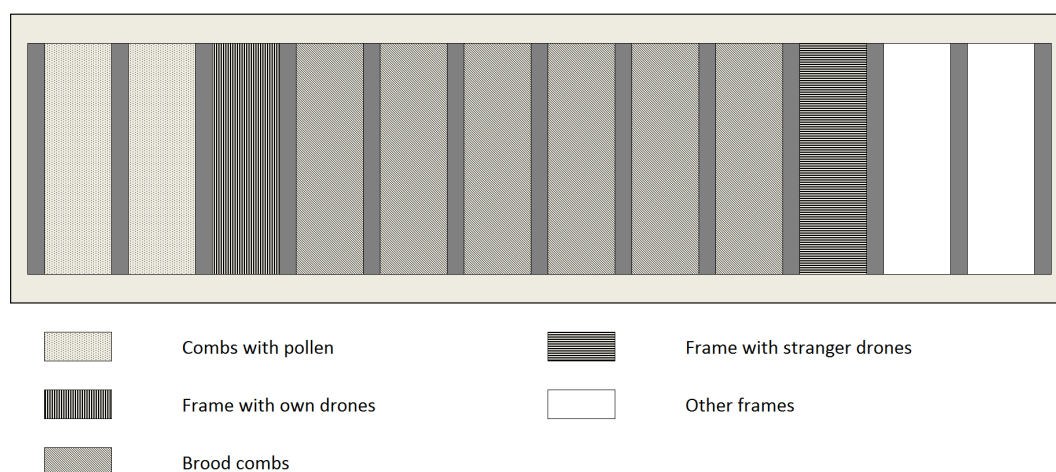
The frames with drones were placed behind the last brood comb of the colonies in the afternoon, when the acceptability of drones is the largest (Currie and Jay, 1988). A total of 172 drones were introduced into the colony at one time. Placement of such a

number of drones in colonies positively influences their acceptance by workers and reduces losses (Currie and Jay, 1988).

We expected a high mortality rate of the drones placed in cages without workers that would attend to them, so the study was carried out on a large number of individuals (4,608). In total, 48 series in both types of colonies were performed over two years. A different number of repetitions was performed in different colonies, and the colonies were not compared to one another.

The number of injuries and the death rate were checked twice, after 3 and 7 days of storage. The drones were anaesthetized with carbon dioxide for the checks for ca. 1 min until all the drones stopped moving. Drones were checked under a stereoscopic microscope with a variable magnification range of 10-16X. We paid particular attention to their legs, antennae and wings. During the first check (on the third day), dead drones were removed from the cages. Living drones were again placed in cages and stored in colonies for 4 more days. On the seventh day, after the second check, the drones were released from the cages. After each stage, the number of injured and dead bees was determined.

SPSS 17.0. and STATGRAPHICS Centurion software was used to conduct statistical analyses. Kolmogorov-Smirnov and Shapiro-Wilk tests revealed that the



**Figure 1.** Arrangement of combs in a colony during storage of own and stranger drones.

distribution of data was not normal. In order to standardize the analysis of the data and facilitate its interpretation for all comparisons, non-parametric tests were employed: Kruskal-Wallis and Mann-Whitney U tests (between drones from own and stranger colonies) and the Wilcoxon test (within one group of drones, after 3 and 7 days of storage). We compared the level of injuries of body parts (legs, segments of tarsus, arolia, wings and antennae) for drones stored in own and stranger colonies using the Chi-square test,  $P < 0.05$ .

## RESULTS

In own colonies, injured drones were found in 35 out of 384 cages (9.2%, 62 individuals) after 3 days and in 56 cages (14.6%, 66 individuals) after 7 days of storage. In stranger colonies, injured drones were found in 36 out of 384 cages (9.4%, 50 individuals) after 3 days and in 64 cages (16.7%, 53 individuals) after 7 days of storage. Only cages with injured drones were considered for further statistical analysis (Zajdel *et al.*, 2014).

Nine types of injuries were identified in drones stored in colonies: Five were missing tarsus segments (from 1 to 5 segments, Figure 2), the other being black arolium (Figure 3), lack of arolium, jagged wings (Figure 4) and injured antennae (Figure 5).

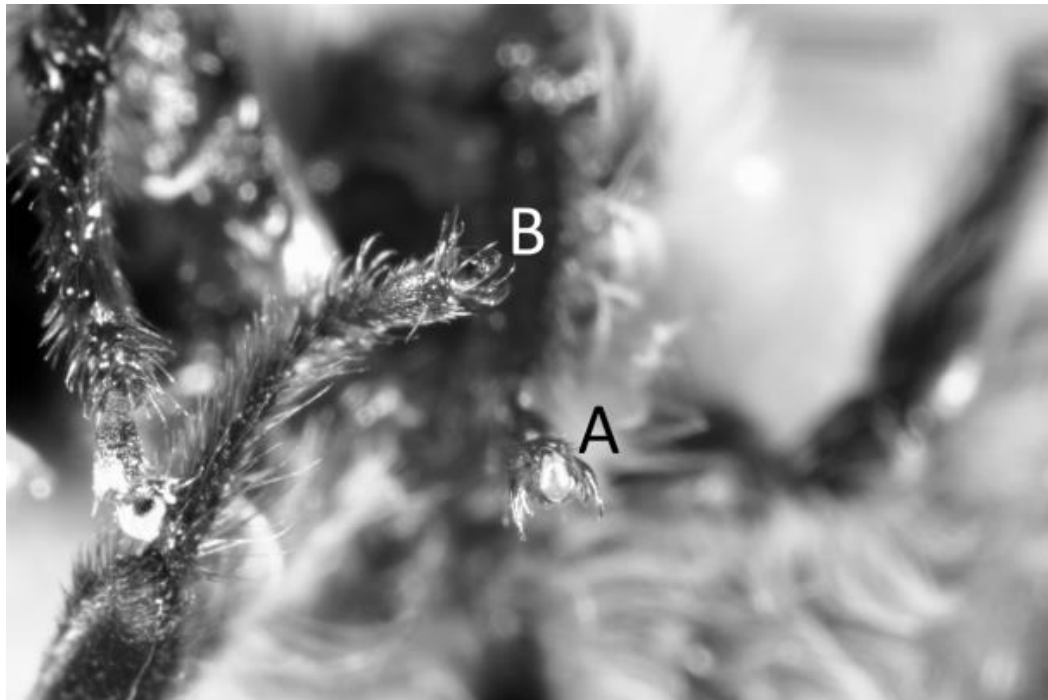
As shown in Table 1, the duration of storage and the origin of the drones had no significant impact on the number of injured bees. In both colony types, the most common observation was one injured drone per cage.

Over 40% of leg injuries to drones stored in both colony types were injuries to the 3<sup>rd</sup> pair of legs. Legs of the 2<sup>nd</sup> pair were injured least frequently. No differences were found in the frequency of leg injuries to own and stranger drones (Figure 6).

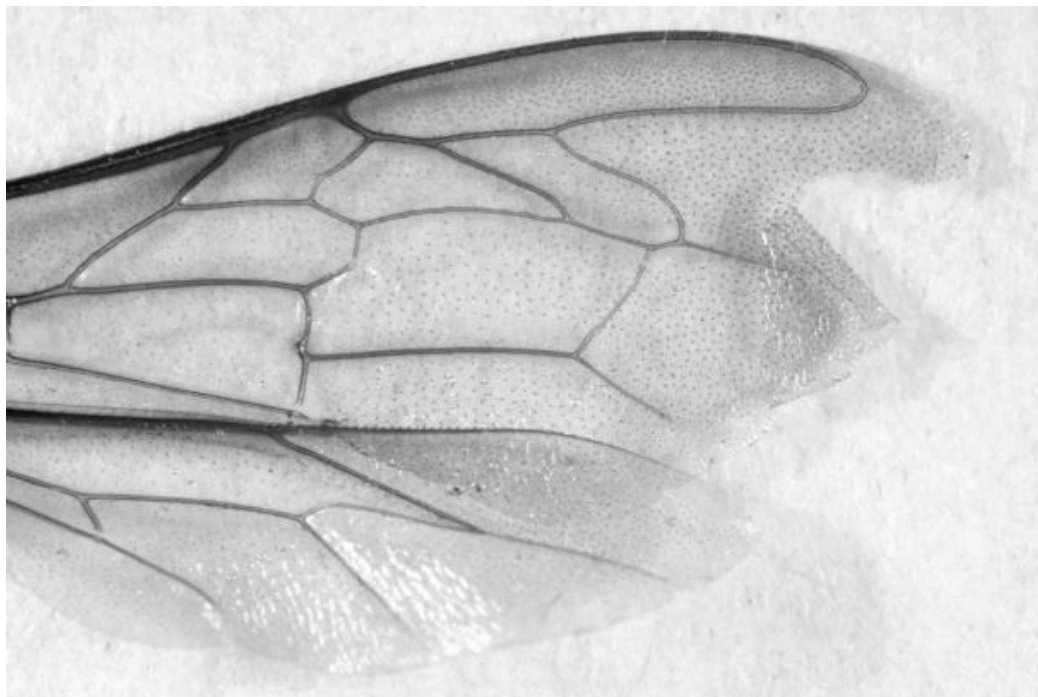
No drone was found to have the entire leg missing; the injuries were limited to lack of 1 to 5 tarsal segments (Figure 7). The most frequent injury suffered by drones stored in both types of colonies was the lack of the last tarsal segment (over 30% of leg injuries). The least frequent injury was the lack of the whole tarsus (6.9-8.8% of individuals, Figure 7). No significant differences were found, however, in the



**Figure 2.** Lack of four segments of tarsus.



**Figure 3.** Normal arolium (A) and black arolium (B).



**Figure 4.** Jagged wings.



**Figure 5.** Injured antenna.

**Table 1.** The number of injured drones stored in own colonies or stranger colonies for 3 and 7 days, respectively, calculated per cage.

Groups of drones	No of days stored	Min-Max (No)	Min-Max (%)	Mean $\pm$ SE <sup>a</sup>	Median	Modal	Skewness
Own	3	1–2	16.7–33.4	1.15 $\pm$ 0.09 a	1	1	3.09 $\pm$ 0.4
	7	1–2	16.7–33.4	1.25 $\pm$ 0.06 a	1	1	1.43 $\pm$ 0.3
Stranger	3	1–3	16.7–50	1.15 $\pm$ 0.09 a	1	1	3.34 $\pm$ 0.4
	7	1–3	16.7–50	1.21 $\pm$ 0.06 a	1	1	1.9 $\pm$ 0.3

<sup>a</sup> The same letters mean no differences between the mean values in individual groups, Kruskal-Wallis test,  $P > 0.05$ .

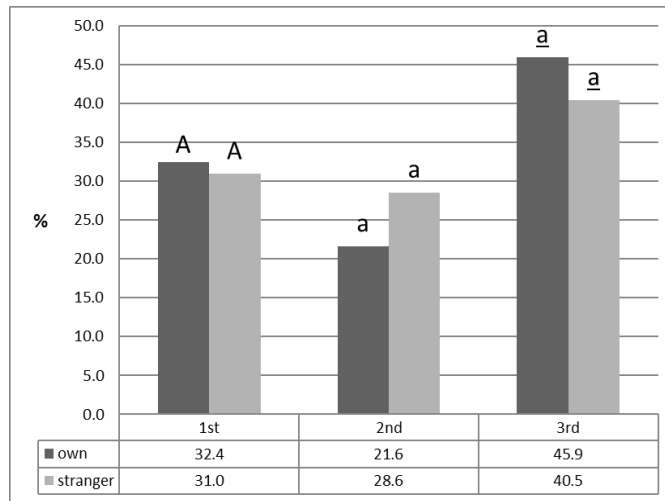
frequency of injuries to tarsal segments of own and stranger drones (Figure 7).

Almost  $\frac{3}{4}$  of the observed injuries suffered by drones, both in own and stranger colonies, were missing tarsus segments (Figure 8). Arolia were found missing more frequently in own than in stranger drones ( $\chi^2 = 3.709$ ,  $df = 1$ ,  $P = 0.04$ , Figure 8). The frequency of wing injuries was similar for both groups of drones. Only drones stored in stranger colonies had missing antenna

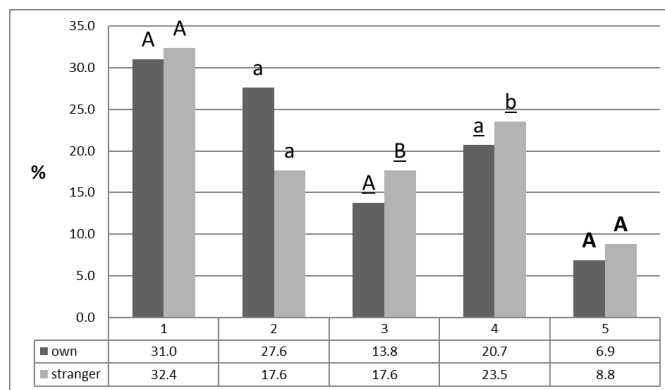
segments (2% of the injured drones, Figure 8).

The mortality of drones placed in mailing cages was very high (62–75% of individuals on average) regardless of the storage time and colony type (Table 2). Modal values in the examined drone groups reached the maximum level (6 drones), which means that in most cages all the drones died.

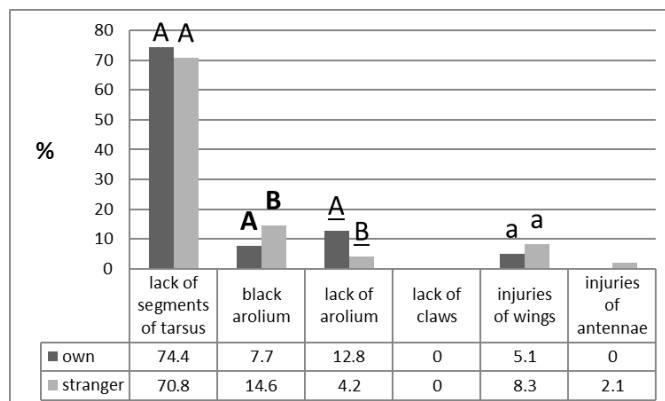
In many cases, injured drones survived the 7-day storage period, which shows that the injuries did not affect the mortality and the



**Figure 6.** Frequency (%) of injuries to the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> pair of legs among drones (in cages with injured drones) stored for 7 days in own colonies or stranger colonies (different types of letters indicate significant differences, Chi-square test,  $P < 0.05$ ).



**Figure 7.** Percentages of missing tarsus segments (axes 1 to 5) among drones (in cages with injured drones) stored for 7 days in own colonies or stranger colonies (different types of letters indicate significant differences, Chi-square test,  $P < 0.05$ ).



**Figure 8.** Percentage summary of all types of injuries among drones (in cages with injured drones) stored for 7 days in own colonies or stranger colonies (different types of letters indicate significant differences, Chi-square test,  $P < 0.05$ ).

**Table 2.** Mortality of drones stored in own colonies or stranger colonies for 3 and 7 days, calculated per cage.

Groups of drones	No of days stored	Min-Max (No)	Min-Max (%)	Mean±SE <sup>b</sup>	Median	Modal	Skewness
Own	3	1–6	16.7 – 100	3.95±0.09 A*a**	4	6	-0.21±0.13
	7	1–6	16.7 – 100	4.51±0.08 Ba***	5	6	-0.78± 0.13
Stranger	3	1–6	16.7 – 100	3.73 ±0.09 Aa	4	6	-0.14±0.13
	7	1–6	16.7 – 100	4.45±0.08 Ba	6	6	-0.66±0.13

<sup>b</sup> Different letters indicate significant differences between the mean values in individual groups:

A\* (CAPITALS): Comparison of ranks in the same group of bees after being stored in colonies for 3 and 7 days (Kruskal-Wallis test  $P < 0.05$ ).

a\*\* (small letters): Comparison of ranks in different groups of bees after being stored in a colony for 3 days (Mann-Whitney U test,  $P > 0.05$ ).

a\*\*\* (small bold letters): Comparison of ranks in different groups after being stored in a colony for 7 days (Wilcoxon test,  $P > 0.05$ ).

main cause of death was insufficient care provided by worker bees.

## DISCUSSION

Drones are placed in cages and stored in colonies for a short time (before the insemination). The drones are usually stored in queen excluder cages, which can be entered by worker bees. In our study, we used mailing cages (Gerula, 2006; Zajdel, 2012) to confirm that drones are injured by workers from the nurse colony.

Injuries were suffered both by drones stored in their own and in stranger colonies, just like in the case of queens or workers stored in similar conditions (Gerula, 2006; Zajdel, 2013; Zajdel *et al.*, 2014). Bees from the colony bit the drones' body parts (tarsi, antennae, wings) protruding from the cages, injuring them. This suggests that it is not the drones' origin but their placement in cages that provokes aggressive behavior in bees from the nurse colonies.

The study shows that drones stored in colonies are injured, but very infrequently (only 5.4% of own drones and 4.5% of stranger drones). Similar frequency of injuries was observed in the case of workers stored in their own colonies (5.3%), while

workers stored in stranger colonies were injured almost twice as frequently (9.9%) (Zajdel *et al.*, 2014). In the case of queens, injuries were found in as many as half of them (Jasiński, 1988), most of them being disabling injuries (Jasiński, 1995; Jasiński and Fliszkiewicz, 1997b).

For queens stored in colonies, between 17 (Gerula, 2006) and 26 different types of injuries were observed (Jasiński, 1995; Jasiński and Fliszkiewicz, 1995), and 11 in workers (Zajdel *et al.*, 2014). We found only 9 types of injuries for drones. The number and type of injuries suffered by representatives of various bee castes most probably depends on differences in the chemical composition of the tarsal gland secretion of queens, workers and drones (Lensky *et al.*, 1984). Bees leave a footprint at the place where their foot touches the surface (Buttler *et al.*, 1969). Workers use footprints to mark the hive entrance (Butler *et al.*, 1969), while the queen marks its presence on combs (Lensky and Slabezki, 1981). According to FederLe *et al.* (2001) the adhesion of the arolium to smooth surfaces is enabled by a thin liquid. It can be hypothesized that pheromones are secreted from the arolium (Asperges *et al.*, 2017). This substance also inhibits the construction of queen cells (Lensky and Slabesky 1981).



Woyke (1988) found out that queens with damaged arolia did not leave footprints while walking on glass. This shows that injuries to queens' legs inhibit production of the tarsal gland secretion.

The rate of secretion by the queen's tarsal glands was about 13 times higher than by those of the workers (Lensky and Slabesky, 1981). It is probably for this reasons that queens were found to have suffered from much more arolium injuries (85% of injuries) than other types of injuries (Jasiński, 1984; Jasiński, 1986; Jasiński, 1987).

Drones' arolia were injured much less frequently than in the case of queens, i.e. at the level of 18.2-20.5% (in stranger and own colonies, respectively), but much more frequently than in the case of workers, only 2-4% of which suffered such injuries (Zajdel, 2012).

The most frequent type of injuries suffered by drones were missing tarsal segments (over 70%), similar to worker bees (74-93%) (Zajdel, 2012). In the case of stored queens, this type of injuries was observed only in 32% of the specimens (Gerula, 2006). Drones stored in own colonies and workers stored in colonies suffered wing injuries with a similar frequency (ca. 5%, Zajdel, 2012). Queens stored in colonies suffered only sporadic wing injuries (Gerula, 2006). Injuries consisting of missing antenna segments were found only in drones stored in own colonies (2.1%) and were equally rare as in the case of the other castes – workers (Zajdel, 2012) and queens (Gerula, 2006).

In the cages used in the present research, the contact between the drones and workers was significantly restricted. The drones and workers interacted only through slots in one of the cage sides, which negatively affected the feeding process and resulted in high mortality of the drones after just 3 days of storage (62-75%). Mailing cages are definitely not suitable for storing drones, as too many of them die inside.

## CONCLUSIONS

Drones placed in cages and stored in colonies are injured by bees from the nurse colonies. Drones stored in their own, and in stranger colonies, suffer injuries to the same extent. The most common type of injury found in drones is missing tarsal segments (62-75% of all injuries). Other types of injuries observed in drones related to arolia (black or missing arolium), antennae and wings. Storing drones in colonies for a long time results in high mortality. Mailing cages should not be used to store drones, as most of them die after just 3 days of storage.

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of Worker Bees (*Apis mellifera carnica*)  
Stored in Own and Stranger Queenright  
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## آیا زنبورهای نر در طی نگهداری در کلونی های ملکه دار خودی یا بیگانه ( *Apis mellifera carnica* ) آسیب می بینند؟

ب. زاجدل، ز. جاسینسکی، و ک. کوچارسکا

### چکیده

هدف این پژوهش بررسی این مطلب بود که آیا زنبورعسل نر هم آسیب می بیند یا خیر. در این پژوهش، ما درجه آسیب دیدگی زنبور نر را در کلونی خودی با بیگانه مقایسه کردیم. زنبورهای نر در داخل قفس های پستی (mailing cage) در کلونی خودشان و کلونی بیگانه نگهداری شدند. سه روز و ۷ روز بعد از قرار دادن در قفس پستی، تعداد آسیب ها و مرگ و میر کنترل شد. در کل، بیش از ۴۶۰۸ زنبور نر مورد بررسی قرار گرفت. ۹ نوع آسیب مختلف برای زنبورهای نر مشاهده شد که در میان آنها، آسیب های مربوط به پا ( نداشتن قسمتی از پنجه) از همه بیشتر بود ( تقریباً ۷۵-۷۰٪ از موارد). آسیب های دیگر شامل آرولیوم سیاه (black arolia)، فقدان آرولیوم، و صدمه به بال و شاخک بود. پژوهش نشان داد که زنبورهای نر نگهداری شده در کلونی همانند ملکه و زنبورهای کارگر آسیب می بینند، هرچند که مقدار این آسیب ها به طور معناداری کمتر است. نیز، این پژوهش نشان داد که نگهداری زنبورهای نر در قفس های پستی منجر به مرگ و میر زیاد در حد ۶۲-۷۵٪ می شود.