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Short Communication

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Requeening queenright honey bee colonies with queen cells in honey supers

Leslie A. Holmes[1,*], Jeffery D. Kearns[1], Lynae P. Ovinge[2], Patricia Wolf Veiga[3], Shelley E. Hoover[1]

[1]Department of Biological Sciences, University of Lethbridge, 4401 University Drive, Lethbridge, AB T1K 3M4, Canada, [2]Alberta Beekeepers Commission, 11434-168 Street #102, Edmonton, AB T5M 3T9, Canada, [3]National Bee Diagnostics Centre, Northwestern Polytechnic, PO Box 1118, Beaverlodge, AB T0H 0C0, Canada *Corresponding author, mail: Leslie.Holmes@uleth.ca

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Many Canadian beekeepers replace a subset of their honey bee queens annually. However, introducing a new queen to a honey bee colony is a management practice with a high degree of uncertainty. Despite the consensus that it is most effective to introduce queens to queenless colonies, some commercial beekeepers claim success with introducing queen cells into the honey super of queenright colonies. We tested the success rate of this practice by introducing queen cells to 100 queenright colonies in southern Alberta during a honey flow. The genotypes of the resultant offspring drones were determined using the microsatellite marker A76 to identify their laying queen mothers. Our results show that new queens successfully supersede original queens in 6% of queenright colonies, suggesting that the practice does not result in the new queen taking over leadership in most colonies. Additionally, supersedure by daughter queens is more common (13%) than new queen supersedure when introducing queen cells to queenright colonies during a honey flow. However, there could be a benefit to the practice of requeening queenright colonies with queen cells in honey supers if the colonies that accepted a new queen (whether a daughter of or unrelated to the old queen) were colonies with a failing queen.

Key words: Apis mellifera, queen cell introduction, mitochondrial DNA, queen supersedure

Introduction

In the United States and Canada, beekeepers cite poor-quality queens as one of the most common causes of colony losses (Seitz et al. 2015, CAPA 2021) and report needing to replace half their queens each season (Amiri et al. 2017). Beekeepers frequently wish to requeen colonies with laying queens present (queenright) because searching for and removing original queens prior to requeening is time consuming and not always successful. Queen introduction methods vary, but the ability to requeen queenright colonies without having to search for and remove original queens would be ideal for beekeepers, saving them time and labor. However, experiments from the 1970s and 1980s showed that requeening queenright colonies with queen cells or virgins is not typically successful (Boch and Avitabile 1979, Jay, 1981). For example, Szabo (1982) found only 13% of queens from queen cells introduced to queenright colonies successfully superseded, supporting the recommendation to dequeen colonies before requeening, which can have up to 90% success rates (Boch and Avitabile 1979).

Despite poor outcomes when requeening queenright colonies, some commercial beekeepers in Alberta perform queenright queen cell introductions with enough success to continue the practice. These beekeepers typically introduce queen cells to honey supers of strong double brood chamber colonies during honey flows and note higher colony survival in bee yards where this technique is practiced. Their anecdotal success with this technique begets the question of whether the success of requeening queenright colonies has changed over time. Beekeeping practices and the environment have both changed since these early studies were published. For example, patterns of pesticide use have changed, new pesticides have been developed, and new pests and pathogens have emerged in apiculture (most notably the introduction of *Varroa* and associated viruses to Canada) (Goulson et al. 2015). Earlier experiments on queenright queen introductions involved marking original queens before introducing unmarked new queens or cells into colonies (Szabo 1982). In this study, we propose to use a genetic marker to confirm which queen (i.e., the original, newly introduced, a daughter queen, or a combination of queens) is producing offspring after a new queen cell introduction. This project tests whether the success of queen cell introductions to honey supers during a honey flow has changed over the past 40 years, given environmental change, and improved knowledge of honey bee biology and beekeeping best management practices.

Materials and Methods

Working with a commercial beekeeper, we used 100 queenright honey bee colonies located in canola pollination fields north of

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Rainier, Alberta, in 2020 for this study. Before a new queen cell was introduced to a colony, 10 drone pupae and 50 broodnest worker bees (where sampling drone pupae was not possible) were sampled, frozen, and sent to the National Bee Diagnostic Centre in Beaverlodge, Alberta, for maternal source genetic testing to profile the matriline of each colony's original queen. We preferentially sampled drone pupae because they are haploid, carrying only maternal genes. Where possible, drone pupae were sampled over drone adults to reduce the chances of sampling individuals that may have drifted from another colony (Currie and Jay 1991).

A single open-mated queen was used to supply larvae (a mix of half and super-sisters), which were grafted into queen cups to produce 100 queen cells. These cells were subsequently introduced to the 100 queenright colonies. Queen cells were introduced between 2 frames in the highest honey super fully occupied by adult bees. Four days after their introduction, the queen cells were checked for successful emergence based on the appearance of the cell (i.e., a circular hole cut at the tip of the cell for successful emergence versus being torn down or irregular holes in the side of the cell). We sampled 10 drone pupae and 50 broodnest worker bees (where sampling drone pupae was not possible) from each colony 6 wk later (i.e., after enough time had passed for worker population turnover) and sent the samples to the National Bee Diagnostic Centre to profile the matriline of each sample. For colonies where drone pupae were not available 6 wk after queen cell introduction, an additional sample consisting of young worker bees was taken from the broodnest a week later (i.e., 7 wk after queen cell introduction) because, in the absence of drone brood, 6 wk may be a conservative sampling window for population turnover if delays in mating and oviposition using this queen replacement occurred. Three possible requeening outcomes were identified using these data: (i) the original queen was not superseded and remained laying in the colony for at least 6 wk, (ii) the new queen cell was accepted, and the new unrelated queen was actively laying 6 wk later, and (iii) the new queen cell was not accepted but the original queen was superseded by a daughter queen.

Matriline Analysis

For all samples (i.e., pre- and postintroduction colony profiles from individual worker bees and drone pupae), DNA extractions were performed using the NucleoSpinTissue kit following manufacturer instructions (Macherey-Nagel, Düren, Germany). For genotyping, PCRs were performed to amplify microsatellite marker A76 using 3 µl of DNA, template, 2× AccuStartTM II PCR Supermix (Quantabio, Beverly, USA), and 2 µl of fluorescent-tagged primers (e.g., A76-Forward [5′GCCAATACTCTCGAACAATCG3′] and A76-Reverse [5′GTCCAATTCACATGTCGACATC3′]), for a total reaction volume of 50 µl. Reactions were run in a thermocycler under the following conditions: 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 58 °C, and 45 s at 72 °C, with a final elongation for 3 min at 72 °C and held at 4 °C. PCR products were sent to Psomagem (Maryland, USA) for fragment analysis, using 500 LIZ size standard. Microsatellite markers were analyzed using GeneMapper v6 (Applied Biosystems), and scoring assignments were verified by visual inspections. A single hypervariable microsatellite A76 was chosen to distinguish the diversity in matrilines based on the findings published by Estoup et al. (1994). In this study, a number of fathers and maternal genotypes were detected, and the A76 microsatellite marker was able to distinguish the different genotypes in the sampled population of drone pupae and broodnest worker bees from each colony.

Using the preintroduction matriline profiles of original queens (Table 1), queen genotype was inferred for individual samples as per Mendelian segregation for each colony. Populations of drone pupae and broodnest worker bees matching both heterozygous queen alleles were assigned to the original queen, whereas populations matching only one heterozygous queen allele were assigned to a daughter queen, and populations with no matching alleles were inferred to be a newly introduced queen. We performed a chi-square goodness of fit test to determine whether, over all 100 colonies, the number of supersedure outcomes was randomly distributed between original queen vs. new queen cell queen vs. daughter queen.

Results

Two days post-queen cell introductions, we observed that 58 queens successfully exited their queen cells; 13 had not emerged and were found to have dead larvae or pupae inside; and 29 queen cells were torn down, and therefore, we did not know the outcome of these 29 queen cells at this time. Six weeks post-queen cell introductions, 85 colonies were queenright. The 15 queenless colonies included 13 colonies with nonviable queen cells and 2 colonies that had torn down queen cells at the initial inspection. In addition, samples from 3 queenright colonies were removed from analysis because the mother was not successfully determined. Thus, based on 82 queenright colonies sampled 6 wk after queen cell introductions, genetic testing on drone pupae and broodnest worker bees showed the original queen was the mother of the sampled offspring in 66 (80.5%) of the queenright colonies, her daughter queen in 11 (13.4%), and a new unrelated queen from the introduced cell in only 5 (6.1%) of the colonies (Fig. 1; $\chi = 82.71$, df = 2, $P < 0.001$). Genotypes of each queen inferred from sampled drone pupae and broodnest worker bees 6 and 7 wk post-queen cell introduction are presented in Table 1.

Discussion

Our results agree with previous studies (Boch and Avitabile 1979, Jay 1981, Szabo 1982), showing that queen cell introductions in the presence of laying queens are not commonly successful, even during a strong honey flow with introductions away from broodnests where original queens are likely to be found. However, practicing queenright queen cell introductions could still benefit colonies (and beekeepers) if the 6% of colonies that accepted a new queen needed a new queen but, for some reason, were reluctant or unable to rear a replacement daughter queen. For example, worker bees may be stimulated to accept a new queen cell if their current queen is of lower quality (e.g., diseased or old). Furthermore, if the additional 13% of colonies that reared daughter queens were also in need of new queens and were stimulated by a queen cell introduction to rear their own daughter queens, then beekeepers could potentially benefit from queen cell introductions to a subset (19%) of queenright colonies. While this study did not include queenless colonies, queenless colonies are often present in a yard, and therefore, beekeepers might further benefit from requeening their queenright colonies with queen cells if any of their colonies are queenless.

Commercial beekeepers may benefit from queen cell introductions to honey supers of queenright colonies if the costs associated with requeening queenright colonies with queen cells make up for the lower success rate. Commercial beekeepers that do not employ this practice of requeening will typically requeen their colonies annually or biannually with mated queens purchased from queen breeders, a practice that requires removing the original queen prior to the introduction of a new queen. The cost of producing a queen cell and a mated queen is less than $5 and $20, respectively, but the cost of purchasing a mated queen is $40–50 (Bixby et al. 2020). Thus, it

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Table 1. Genotype of queens deduced by their progeny (drone pupae and brood nest worker bees) using A76 microsatellite (A76-forward [5′GCCAATACTCTCGAACAATCG3′] and A76-reverse [5′GTCCAATTCACATGTCGACATC3′]). Drone pupae and broodnest worker bees were sampled from 100 queenright colonies prior to queen cell introductions to determine the genotypes of the original queens. Drone pupae and broodnest worker bees were sampled again 6 wk post-queen cell introduction, and 7 wk post-queen cell introduction to determine queen genotype of the laying queen at each sampling time. Only queenright colonies at "post queen cell introduction" sampling time are included.

	Pre-queen cell introduction sampling		Six weeks post-queen cell introduction sampling		Seven weeks post-queen cell introduction sampling	
Colony ID	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
#2	204	248	204	248	No sample	No sample
#3	248	284	248	284	248	284
#5	204	255	204	255	204	255
#6	260	282	260	282	260	282
#8	245	283	245	283	245	283
#10	248	332	248	332	248	332
#11	270	339	270	339	270	339
#12	207	240	207	240	207	240
#13	207	338	207	338	No sample	No sample
#14	260	345	260	345	No sample	No sample
#15	260	345	282	333	282	333
#16	204	284	204	333	No sample	No sample
#17	207	226	207	226	207	333
#19	204	333	204	333	No sample	No sample
#20	207	283	207	283	No sample	No sample
#24	207	340	207	340	333	339
#25	207	339	207	339	207	339
#26	207	247	207	247	207	247
#27	207	247	207	247	207	247
#28	207	283	207	283	207	283
#29	207	270	207	270	207	248
#30	270	339	270	339	No sample	No sample
#31	270	339	270	339	270	339
#32	204	339	204	339	204	339
#33	207	283	207	283	No sample	No sample
#34	247	260	247	260	247	260
#35	245	353	245	353	245	353
#36	226	257	226	257	226	257
#37	226	260	226	260	226	260
#38	207	257	207	257	207	257
#40	248	251	248	251	248	251
#41	232	329	232	329	232	329
#42	226	257	226	257	226	240
#43	207	283	207	165	No sample	No sample
#46	207	338	226	329	226	329
#47	207	257	207	257	207	257
#48	207	282	207	282	No sample	No sample
#49	232	280	268	280	268	280
#50	207	247	253	282	253	282
#52	248	270	248	270	248	270
#53	248	248	248	248	248	248
#54	243	338	243	338	No sample	No sample
#55	210	248	210	248	No sample	No sample
#56	210	248	210	248	No sample	No sample
#58	284	357	284	357	284	357
#59	232	340	232	340	232	340
#60	270	340	270	340	270	340
#61	284	353	284	353	284	353
#62	232	360	232	360	232	360
#64	245	260	204	260	204	260
#65	207	283	207	283	No sample	No sample
#66	251	257	251	257	No sample	No sample
#67	226	340	226	340	No sample	No sample
#68	255	339	255	339	255	339
#69	207	264	207	282	207	282
#70	204	260	204	260	204	260

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Table 1. Continued

	Pre-queen cell introduction sampling		Six weeks post-queen cell introduction sampling		Seven weeks post-queen cell introduction sampling	
Colony ID	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
#71	207	264	207	264	207	264
#72	207	284	207	284	No sample	No sample
#73	207	242	207	242	207	242
#74	207	251	207	251	No sample	No sample
#75	210	282	210	282	No sample	No sample
#76	226	276	226	276	226	276
#77	207	253	207	253	207	253
#78	210	342	210	342	No sample	No sample
#79	207	329	207	329	No sample	No sample
#80	207	242	207	242	207	242
#81	232	242	232	242	232	242
#82	207	277	207	277	207	277
#83	226	243	226	243	No sample	No sample
#84	210	255	210	255	210	255
#85	245	284	245	284	No sample	No sample
#86	259	332	259	332	No sample	No sample
#87	276	284	276	284	No sample	No sample
#89	226	243	226	243	No sample	No sample
#90	259	284	259	284	259	284
#91	207	350	207	350	207	350
#92	255	330	255	330	255	330
#93	260	335	260	335	260	335
#94	268	282	268	282	No sample	No sample
#96	207	335	207	335	207	335
#98	264	335	264	335	264	335
#99	204	284	204	284	No sample	No sample
#100	207	264	207	264	No sample	No sample

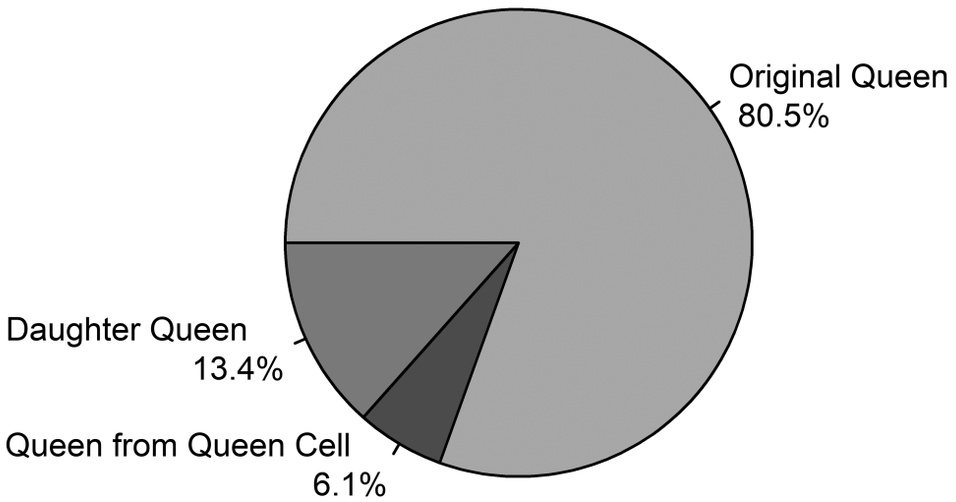

Fig. 1. Matriline allocation (percentage) of honey bee colonies located in canola pollination yards, north of Rainier, Alberta, Canada. Queenright colonies ($N = 82$) were sampled prior to and 6 wk after queen cell introductions during a honey flow to determine maternal sources of offspring (e.g., genotypes of original queen, new queen from introduced queen cell, or daughter of original queen). Genotypes of queens can be found in Table 1. Requeening queenright colonies with queen cells in honey supers was effective 6% of the time, less than the frequency of daughter supersedure (13%) or the retention of reigning queens (81%) ($\chi = 82.71$, df = 2, $P < 0.001$).

may be worthwhile to requeen queenright colonies with queen cells since one could requeen 5–10 times more colonies than requeening with mated queens if the colonies being requeened need a new queen. In addition, requeening from queen cells of local stocks may have better performance than commercial queens reared outside of the province, state, or country.

The use of microsatellite markers to determine the mothers of sampled offspring in our study allowed us to document an outcome of queen cell introductions that may not be on beekeepers' radar. Specifically, daughter queen, particularly if beekeepers introduce new queens and subsequently only check for queen status by the presence of eggs. We demonstrate that in the presence of an introduced

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queen cell, many colonies reared their own daughter queens instead of accepting the new queen cell or retaining their current queen. However, since we did not test queen cell introductions in queenless colonies, it is not clear if daughter queen supersedures after queen cell introductions could be predicted by the original queen's presence or absence (e.g., queenright or queenless). It is further possible that swarming events may have produced some of the daughter queen supersedures (13% of colonies); however, evidence of swarming (e.g., swarm cells or breaks in the brood cycle other than those which would be predicted by the timing of cell introduction) was not seen, and all colonies in our study had additional honey supers to accommodate large populations.

With the genetic profiles of the sampled brood, we can determine which queen is actively laying and the circumstances of supersedure (e.g., a new unrelated queen or daughter queen supersedure). The prevalence of daughter queen supersedures over new queen cell supersedures in our study requires further investigation. Szabo (1982) also saw a higher prevalence of daughter queen supersedures (24%) over new queen cell supersedures (13%), but to our knowledge, the possibility that the introduction of a queen cell may be signaling the rearing of daughter queens has not yet been explored. While we have seen much change in the environment and improved beekeeping management practices in the last 40 yr, the likelihood of queen supersedure when introducing queen cells to the honey supers of queenright colonies remains low. Importantly, commercial beekeeping has grown in the last 40 yr, where introducing new queens to queenless colonies is a laborious and expensive task. Thus, a commercial beekeeper may benefit from introducing queen cells to honey supers of queenright colonies if the desired outcome is a queenright colony, regardless of the queen's origin (e.g., original queen, new supersedure queen, or new daughter supersedure queen).

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Author Contributions

Leslie Holmes (Data curation-Equal, Formal analysis-Lead, Visualization-Lead, Writing – original draft-Lead, Writing – review & editing-Lead), Lynae Ovinge (Conceptualization-Lead, Data curation-Equal, Investigation-Equal, Methodology-Lead, Project administration-Equal, Resources-Supporting, Writing – original draft-Supporting, Writing – review & editing-Equal), Jeffery Kearns (Conceptualization-Supporting, Data curation-Equal, Investigation-Equal, Methodology-Supporting, Project administration-Equal, Resources-Supporting, Writing – review & editing-Equal), Patricia Wolf-Veiga (Resources-Supporting, Writing – review & editing-Equal), and Shelley Hoover (Conceptualization-Lead, Funding acquisition-Lead, Investigation-Lead, Methodology-Lead, Resources-Lead, Supervision-Lead, Writing – original draft-Supporting, Writing – review & editing-Equal)

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References

Amiri, E, Strand, MS Rueppell O, Tarpy DR. Queen quality and the impact of honey bee diseases on queen health: potential for interactions between two major threats to colony health. Insects. **2017**:8(2):48–66. https://doi.org/10.3390/insects8020048

Bixby M, Hoover SE, McCallum R, Ibrahim A, Ovinge L, Olmstead S, Pernal SF, Zayed A, Foster LJ, Guarna MM. Honey bee queen production: Canadian costing case study and profitability analysis. J Econ Entomol. **2020**:113(4):1618–1627. https://doi.org/10.1093/jee/toaa102

Boch R, Avitabile A. Requeening honeybee colonies without dequeening. J Apic Res. **1979**:18(1):47–51. https://doi.org/10.1080/00218839.1979.11099943

Currie RW, Jay SC. Drifting behaviour of drone honey bees (*Apis mellifera L.*) in commercial apiaries. J Apic Res. **1991**:30(2):61–68. https://doi.org/10.1080/00218839.1991.11101235

CAPA. Annual Colony Loss Reports, July 2021—statement on honey bee wintering losses in Canada 2021. Canadian Association of Professional Apiculturists (CAPA). **2021** [accessed 2022 Mar 19]. https://capabees.com/shared/CAPA-Statement-on-Colony-Losses-2020-2021.pdf.

Estoup A, Sollignac M, Cournuet JM. Precise assessment of the number of matings and of relatedness in honey bee colonies. Proc R Soc Lond B Biol Sci. **1994**:258:1–7. https://doi.org/10.1098/rspb.1994.0133

Goulson D, Nicholls E, Botías E, Rotheray EL. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science. **2015**:347(6229):1255957. https://doi.org/10.1126/science.1255957.

Jay SC. Requeening queenright honeybee colonies with queen cells or virgin queens. J Apic Res. **1981**:20(2): 79–83. https://doi.org/10.1080/00218839.1981.11100476

Seitz N, Traynor KS, Steinhauer N, Rennich K, Wilson ME, Ellis JD, Rose R, Tarpy DR, Sagili RR, Caron DM, *et al.* A national survey of managed honey bee 2014–2015 annual colony losses in the USA. J Apic Res. **2015**:54(4):292–304. https://doi.org/10.1080/00218839.2016.1153294

Szabo TI. Requeening honeybee colonies with queen cells. J Apic Res. **1982**:21(4):208–211. https://doi.org/10.1080/00218839.1982.11100543

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