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Shipping honey bee (*Apis mellifera*) queens across Canada after overwintering in bank colonies: Effects on queen quality and colony introduction success

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Abstract

Winter queen banking has emerged as a promising strategy for increasing the availability of domestically produced honey bee queens (*Apis mellifera* L.) (Hymenoptera: Apidae) early in spring. For this approach to be adopted commercially, banked queens must withstand long-distance shipment without compromising their quality or colony establishment. This study assessed the effects of shipping overwintered banked queens across Canada on reproductive quality, acceptance into nucleus colonies, and resumption of oviposition. Domestically reared queens were banked from August to April and shipped to three destinations across Canada. Temperature was monitored throughout shipment, and queen body weight, sperm viability, colony acceptance, and oviposition onset were assessed upon arrival. Queen survival during winter banking averaged 77.3 %. During spring shipping, queens exposed to moderate temperatures (12–27 °C) maintained high quality, with body weight and sperm viability comparable to unshipped queens. In contrast, queens exposed to prolonged cold temperatures exhibited a ~ 15 % reduction in sperm viability. Despite this, acceptance rates remained high across all groups (> 90 %), and queens resumed oviposition rapidly. These findings indicate that banked queens can be shipped successfully over long distances under appropriate transport conditions, supporting queen banking as a practical tool for reducing reliance on imported queens in northern beekeeping regions.

Introduction

In Canada, approximately 30% of honey bee (*Apis mellifera* L.) (Hymenoptera: Apidae) colonies are lost every winter because of multiple interacting factors, including *Varroa destructor* infestations, food shortages, extreme weather conditions, and poor queen health [1]. Because environmental conditions limit local queen rearing until late spring, beekeepers rely on imported queens and packaged bees in early spring to replace winter losses [2–5]. This dependence on imported bees is risky, as it may facilitate the introduction and spread of pathogens, parasites, and treatment-resistant strains [6–11], as well as undesirable genotypes or genotypes that are poorly adapted to local environmental conditions [11–15]. Bee imports may also be restricted following outbreaks of honey bee pathogens or parasites, or due to socio-economic considerations [16, 17]. Consequently, developing reliable methods to supply of domestically produced queens early in the spring is essential to improving the resilience, biosecurity, and long-term sustainability of the Canadian beekeeping industry.

Queen banking has repeatedly been shown to be an effective method for overwintering queens [18–22]. By rearing queens in late summer and storing them individually caged within strong colonies throughout the winter, beekeepers can gain access to Canadian queens early in the spring, when newly mated domestic queens are unavailable. This approach not only reduces reliance on imported bees, but also promotes the development of Canadian honey bee stocks selected for desirable traits, such as winter survival.

Reported survival rates of overwintered banked queens vary considerably among studies, ranging from 16 to 85 % depending on storage conditions [18–23]. However, several studies have improved queen

survival by optimizing key management practices, including the use of queenless colonies [19], sufficient populations of young worker bees [18], and a storage temperature of 16 °C [20]. Recent work has reported particularly promising results, with survival rates ranging from 74 to 85 % [20, 21, 24]. Despite its potential, queen banking has not been widely adopted by commercial queen producers, likely due to concerns that prolonged storage and subsequent shipment may compromise queen quality or performance.

Encouragingly, several studies suggest that long-term storage does not affect queen quality [21, 22, 25]. Levesque et al. [21] demonstrated that queen fertility remained intact after eight months of banking, and that even though queen abdominal length and body weight were reduced in the spring, these effects were temporary and reversed once queens resumed egg laying. Studies have shown that banked queens perform comparably to both conventionally overwintered [21, 22] and newly mated queens [25] at the colony level, exhibiting similar brood populations, colony strength, honey production, and winter survival. The only reported effect is a delay in the onset of egg laying following queen introduction [21, 22]. Collectively, these studies indicate that queen banking is a promising strategy for ensuring a reliable source of high-quality Canadian-adapted queens early in the spring.

To date, however, no study has evaluated whether banked queens can be shipped successfully without compromising their quality or performance. Because commercial adoption depends on transporting queens from queen producers to beekeepers, banked queens must be sufficiently resilient to withstand temperature fluctuations encountered during shipment. During transport, queens may be exposed to substantial temperature fluctuations, ranging from 5 °C to above 40 °C [26, 27], conditions that have been associated with reduced reproductive quality. Several studies have shown that exposure to either low (4 °C) or high (> 40 °C) temperatures for two hours or more can adversely affect queen fertility [27, 28]. Consistent with these findings, McAfee et al. [29] further demonstrated that exposure to temperature below 15 °C or above 38 °C for two hours or more reduces sperm viability beyond the threshold they identified as being associated with queen failure (> 11.5 % loss).

Since the queen is typically the sole reproductive female in the colony, egg production, and consequently brood population and overall colony productivity, depend directly on her reproductive potential [27, 30, 31]. It is essential to determine whether banked queens can withstand long-distance transport while maintaining their quality and colony performance. The objective of this study was therefore to determine whether queens banked throughout the winter can be successfully shipped across Canada without compromising their quality or acceptance by new colonies. To achieve this, queens overwintered in bank colonies in Quebec were shipped to three honey bee research centres in Western Canada (one in British Columbia and two in Alberta), where their quality and acceptance into colonies were assessed.

Results

Queen survival in bank colonies

Survival rates were calculated for two periods: the winter period (October 4 to April 17) and the total banking period (August 28 to April 17). The number of surviving queens in each colony at each assessment, as well as the survival rate for each banking period, is reported in Table 1. Mean survival rates (± SD) for the winter period and the total banking period were 77.3 ± 18.1 % and 63.0 ± 22.6 %, respectively.

Table 1
Survival of queens in bank colonies (N = 10 banks, 40 queens per bank colony) calculated from establishment (August 28, 2024), or the start of winter (October 4, 2024), until spring (April 17, 2025).

	N live queens			Survival rate (%)	
Bank colony #	**Aug 28**	**Oct 4**	**Apr 17**	**Winter survival (Oct-Apr)**	**Total survival (Aug-Apr)**
1	40	35	21	60.0	52.5
2	40	40	28	70.0	70.0
3	40	37	35	94.6	87.5
4	40	37	37	92.5	92.5
5	40	39	34	87.2	85.0
6	40	11	11	100.0	27.5
7	40	27	15	55.6	37.5
8	40	35	30	85.7	75.0
9	40	30	17	56.7	42.5
10	40	38	24	63.2	60

Temperature variations during shipping

Temperature variations inside the shipping cages, recorded every five minutes during transport from Québec City to the three destinations (Beaverlodge, Lethbridge and Vancouver), are shown in Fig. 1.

For the route from Québec City to Beaverlodge, the total shipping duration was 32 h, including 21 h 25 min in airports, 4 h 55 min in flight, and 5 h 40 min by automobile to the apiary. Temperatures within the shipping cages remained relatively stable at the Québec City and Montréal airports (20.9–23.4 °C), except for a cooler period prior to the Montréal-Edmonton flight, when temperature declined to 14.3 °C. During the Québec City-Montréal flight, temperature increased to the highest value recorded along the route (26.9 °C), whereas during the Montréal-Edmonton flight it initially rose to 21.7 °C before decreasing to the lowest value observed (12.0 °C). Upon arrival, temperature gradually increased to 21.8 °C and then

stabilized around 18.5 °C during ground transport to the apiary. The mean temperature over the entire itinerary was 19.7 ± 3.2°C.

For the route to Lethbridge, the total shipping duration was 29 h 30 min, including 21 h 10 min spent in airports, 5 h 25 min in flight, and 2 h 55 min by automobile to the apiary. Temperature variations followed a very similar pattern to that observed for the Beaverlodge route. At the Québec City and Montréal airports, temperatures generally ranged between 19.2 and 22.4 °C, before decreasing to 14.8 °C prior to the Montreal-Calgary flight. During the Montreal-Calgary flight, temperature increased to 18.9 °C and then declined to the lowest value recorded along the route (14.0 °C). Upon arrival at Calgary airport, temperature gradually increased and continued to rise during ground transport to the apiary, where it stabilized between 22.7 and 24.6 °C. The mean temperature over the entire itinerary was 20.1 ± 2.5 °C.

For the route to Vancouver, the total shipping duration was 25 h 30 min, including 16 h 35 min spent in airports, 5 h 55 min in flight, and 3 h by automobile to the apiary. Following the Québec City-Montréal flight, temperature patterns differed markedly from those observed for the other two routes. At Montréal airport, temperatures ranged from 22.4 to 26.8 °C. Prior to the Montréal-Vancouver, temperature decreased to 20.1 °C and then rose to 23.2 °C at the beginning of the flight. During the Montréal-Vancouver flight, temperature declined substantially, reaching the lowest value recorded among the three routes (-0.9 °C). At Vancouver airport, the package was likely mistakenly stored in a refrigerated unit at approximately 7.7 °C for 6 h 20 min. The queens were in a chill-coma upon pickup, but the temperature increased immediately after retrieval and stabilized around 19 °C. The mean temperature over the entire itinerary was 14.3 ± 8.4 °C.

Queen weight and sperm viability

Queen body weight (Fig. 2a), measured on May 6, 2025, did not differ significantly among groups ($F = 0.9585$, $p = 0.4265$). Estimated mean body weights were 191 ± 6.28 mg, 191 ± 6.43 mg, 194 ± 6.40 mg and 181 ± 6.34 mg for the CRSAD, Beaverlodge, Lethbridge and Vancouver groups, respectively.

For the sperm viability analyses, the Lethbridge group was excluded because technical issues encountered during fluorescence imaging biased estimates toward higher viability. Queen sperm viability (Fig. 2b) differed significantly among groups ($F = 17.7538$, $p = 0001$), except between the CRSAD and Beaverlodge groups, with estimated mean viabilities of 81.6 ± 1.6 % and 78.9 ± 1.8 %, respectively (t-ratio = 1.119, p = 0.5152). The Vancouver group exhibited the lowest sperm viability (64.9 ± 2.5 %), which was significantly lower than that of the CRSAD (t-ratio = 5.639, $p < .0001$) and Beaverlodge (t-ratio = 4.524, $p = 0.0007$).

Queen acceptance into nucleus colonies

On May 6, 20 queens were introduced into nucleus colonies for each of the four treatment groups. However, one queen from the Vancouver group was excluded because the nucleus colony was

considered too weak to be viable (< 2 frames of bees). All introduced queens were successfully released from their cages three days later, and no signs of worker aggression were observed. Queen acceptance 10 days after introduction was high across all groups, ranging from 90 to 100% (Table 2). Although a small number of queens were rejected, acceptance rates did not differ significantly among groups (χ^2 = 2.06, p = 0.560). Furthermore, neither queen provenance (z = -0.574, p = 0.566) nor nucleus colony strength (z = 0.032, p = 0.974) significantly influenced queen acceptance.

Oviposition was observed in all accepted queens from the CRSAD and Vancouver groups. In the Vancouver group, assessments were conducted five days (May 14) after queen release rather than seven because of adverse weather forecasts. At that time, 17 of the 19 accepted queens had initiated oviposition, and the remaining two queens were confirmed to be laying eggs upon reassessment four days later (May 18). In the Beaverlodge and Lethbridge groups, one accepted queen in each group had not initiated oviposition seven days after release. Oviposition success did not differ significantly among groups (χ^2 = 5.072, p = 0.167).

Table 2
Introduction, release and acceptance of banked queens into nucleus colonies for the control group (unshipped queens; CRSAD) and the three experimental groups (queens shipped to Beaverlodge, Lethbridge and Vancouver).

Group	N queens			
	Introduced – Day 0	Released - Day 3	Accepted - Day 10[1]	Laying - Day 10[1]
CRSAD	20	20	20	20
Beaverlodge	20	20	19	18
Lethbridge	20	20	18	17
Vancouver	19	19	19	19

[1] Acceptance of queens was assessed on day 8, and egg presence was assessed on day 8 and on day 12 for the Vancouver group due to the weather.

Onset of oviposition

The onset of oviposition was estimated based on the age of the oldest larvae present in nucleus colonies seven days after queen release. The Vancouver group was excluded from this analysis because colonies were assessed two days earlier due to adverse weather conditions. At that time, only eggs were present, indicating that queens had initiated oviposition between three and five days after release; however, the precise onset of oviposition could not be determined.

Queens from the CRSAD, Beaverlodge, and Lethbridge groups initiated oviposition 3.2 ± 0.3, 2.6 ± 0.3, and 3.0 ± 0.4 days after release, respectively. The onset of oviposition did not differ significantly among groups ($F = 1.2258$, $p = 0.3036$). In contrast, queen provenance significantly affected the onset of oviposition ($F = 4.3137$, $p = 0.0438$). Queens from *Breeder A* initiated oviposition earlier (2.6 ± 0.3 days) than queens from *Breeder B* (3.3 ± 0.3 days).

Discussion

By banking queens throughout the winter and shipping them to three destinations across Canada, our study demonstrates that overwintered banked queens can be transported via air cargo successfully over long distances without compromising their quality. Queens exposed to moderate temperature fluctuations during shipment maintained high levels of fertility, whereas fertility declined in the group exposed to unusually low temperatures for an extended period, as would be expected regardless of whether the queens had been banked. Banked queens were also accepted into nucleus colonies as successfully as unshipped queens, with acceptance rates ranging from 90 to 100 % across all experimental groups. In addition, this study provides a more precise estimate of the time required for banked queens to resume oviposition following their introduction into colonies. To our knowledge, this is the first study to evaluate the effects of shipping on queens that have been banked throughout the winter, and it represents an important step toward the commercialization of this management practice. If adopted on a larger scale, queen banking could provide Canadian beekeepers with access to domestically produced queens early in the beekeeping season.

Queen survival during winter banking (October to April) was high, averaging 77.3 % and exceeding 90 % in some bank colonies. These survival rates are comparable to those reported in previous studies conducted at CRSAD using the same banking method [20, 21, 44], further supporting its effectiveness. The consistency of survival rates observed over multiple years suggests that this banking technique is both reliable and robust. These findings indicate that winter queen banking could be successfully implemented by beekeepers on a larger scale, provided that appropriate storage conditions are maintained throughout the overwintering period.

Queen shipments followed the planned routes and were successfully retrieved at each destination. However, our results confirm that shipping can expose queens and their attendant workers to substantial temperature fluctuations, as previously reported by other studies [26–28]. Temperature conditions experienced by the Beaverlodge and Lethbridge groups remained relatively moderate, ranging from 12 to 27 °C throughout transport. In contrast, queens shipped to Vancouver were exposed to much more extreme conditions. During the Montreal–Vancouver flight, temperatures remained below 10 °C for several hours and dropped to as low as − 0.9 °C. Upon arrival, air cargo workers likely mistakenly placed the shipment in a refrigerated storage room maintained at approximately 7.7 °C for more than six hours before being retrieved. At the time of collection, the package was still cold to the touch and all bees were immobile. Normal bee activity resumed only several hours later, after the shipment had warmed to room

temperature. Such adverse conditions are an inherent risk present for all bee shipments, both domestic and international.

Honey bees can alternate between an endothermic state, in which they actively regulate their body temperature, and an ectothermic state, in which body temperature largely depends on ambient conditions [45–49]. At the colony level, several collective mechanisms contribute to maintaining an internal temperature of approximately 35 °C, including cluster formation and heat production through the activation of flight muscles, as well as fanning behavior used to cool the hive when necessary [47, 49–51]. These thermoregulatory abilities enable colonies to tolerate low temperatures during winter and allow beekeepers to maintain them in refrigerated buildings as an overwintering strategy. In contrast, small groups of bees and isolated individuals have a much more limited capacity for thermoregulation and are therefore more susceptible to ambient temperature fluctuations [52–55]. A temperature of approximately 30 °C appears to be optimal for groups of bees maintained outside the colony environment [28]. When temperatures fall below approximately 10 °C, bees may enter a state of chill-coma, during which they become immobile and cease generating metabolic heat [52, 56]. Although chill-coma can be reversible if cold exposure is of limited duration, prolonged exposure to low temperatures may ultimately result in mortality.

The queens and attendants in the Vancouver group were likely in a state of chill-coma when the shipment was retrieved and regained normal activity only after being warmed to room temperature. Although bees can survive episodes of chill-coma, exposure to low temperatures may nonetheless result in irreversible physiological damage [27–29, 57]. One of the primary objectives of this study was therefore to determine whether long-distance shipment, and the temperature fluctuations associated with it, could affect the quality of overwintered banked queens.

Shipping had no effect on queen body weight, which was similar among all research centres. Although temperature stress during queen development can influence queen weight at emergence [58–60], no effects of shipping-related temperature exposure on the body weight of mature queens have been reported to date. Banked queens are lighter following winter storage but gradually regain their normal weight once oviposition resumes [21]. Queen fertility also remained high in groups exposed to moderate temperature fluctuations during shipment (12–27 °C), with sperm viability averaging approximately 80 % in the CRSAD and Beaverlodge. Overall, these results indicate that banked queens can be transported successfully across Canada without compromising their reproductive quality, if temperatures remain within a moderate range.

In contrast, queens from the Vancouver group, which were exposed to temperatures low enough to induce chill-coma for several hours, exhibited sperm viability values approximately 15% lower than those of unshipped queens and the queens shipped to Beaverlodge. These findings are consistent with previous studies showing that exposure to extreme temperatures during shipment, whether low (~ 5 °C) or high (~ 40 °C), for as little as two hours can reduce queen fertility by 12 to 50 % [27, 28]. McAfee et al. [29] further demonstrated that exposure to temperatures below 15 °C or above 38 °C can decrease

sperm viability beyond the 11.5 % threshold associated with queen failure under field conditions. Reduced queen fertility can have important consequences for colony performance, including poor brood patterns, reduced colony population growth, lower honey production, decreased resistance to pathogens and parasites, and reduced winter survival [27, 61–62].

These observations highlight the importance of providing clear guidelines for the handling of honey bee shipments during transport. There appears to be some confusion regarding appropriate storage conditions for live bees, as temperature requirements differ among shipment types, including full colonies, packages of bees, and queen shipments accompanied by attendant workers. Prominent warning labels indicating that queen shipments must not be exposed to low or high temperatures could help prevent handling errors such as those encountered in the present study and reduce the risk of temperature-related damage during shipping. Methods for transporting queens over long distances should be further investigated to minimize the effects of temperature fluctuations, which can be substantial and difficult to predict. Rousseau et al. [28] demonstrated that the presence of free attendant workers within queen shipments improved thermoregulation during prolonged exposure to 6 °C. When 250 or 500 loose workers were included in the shipment, temperatures inside the package remained above 25 °C despite several hours of cold exposure. The authors also showed that packaging design influenced the ability of bees to retain heat. In the present study, queens were shipped in individual cages containing only five attendant workers, which likely limited their capacity for thermoregulation. Although the inclusion of loose bees is not permitted in all shipping carriers, it should be considered whenever possible.

Despite the temperature fluctuations experienced during shipment, queen acceptance remained high across all groups, exceeding 90 % in every case. Interestingly, all queens in the Vancouver group, having experienced chill-coma for several hours, were successfully accepted into nucleus colonies. Overall, only three queens were rejected, and two additional queens had not yet initiated oviposition seven days after release. These findings indicate that shipment did not affect queen acceptance and confirm that queens banked throughout the winter are resilient to stress and can be successfully introduced into new colonies in spring. Similar acceptance rates have been reported for banked queens compared with conventionally overwintered queens [21] and newly mated queens [22, 25]. Although monitoring colony performance throughout an entire season would have provided valuable insight into the long-term effects of shipping, our results suggest that banked queens exposed only to moderate temperature fluctuations during transport are likely to perform well following introduction. Previous studies have shown that colonies headed by banked queens achieve brood populations, colony strength, honey production, and overwinter survival comparable to those headed by newly mated queens [22, 25]. Holmes et al. [25] even reported that banked queens exhibited higher seasonal retention rates than imported queens, highlighting an additional advantage of this management approach. However, when queen fertility is compromised by exposure to extreme temperatures during shipment, colony productivity, queen longevity, and overall colony health may be adversely affected [27, 62], whether the queen source is domestic or imported.

Another objective of this study was to determine how quickly banked queens resume oviposition after being introduced into colonies in spring following nearly eight months of individual confinement in cages. Previous work conducted at CRSAD showed that non-shipped banked queens exhibited reduced egg-laying activity during the first 12 days after their introduction into colonies compared with queens overwintered conventionally [21]. Similarly, Lamothe-Boudreau and Giovenazzo [22] reported that colonies headed by banked queens had smaller brood populations and were weaker than colonies headed by newly mated queens during the first month following introduction, although these differences disappeared later in the season. One possible explanation for these observations is that banked queens require a period of recovery before resuming normal oviposition. When queens cease laying eggs, as occurs during winter or while in storage, their ovaries regress [64] and gradually regain their size and mass once oviposition resumes. Consequently, banked queens may require additional time to initiate egg laying compared with queens that have continued laying eggs from mating onward.

By estimating the age of the oldest larvae present in colonies seven days after queen release, we determined that banked queens initiated oviposition approximately three days after being released into nucleus colonies. To our knowledge, data describing the time required for introduced mated queens to resume oviposition are scarce, although it is generally assumed to range from one day to one week following introduction [65, 66]. This suggests that the reduced brood population observed in previous studies in colonies headed by banked queens during the first weeks after introduction is more likely attributable to a lower egg-laying rate than delayed oviposition. Indeed, it has been shown that banked queens have not yet fully regained their pre-storage body weight after two weeks in a colony [21], suggesting that ovarian recovery may still be ongoing during this period and could temporarily limit egg laying rates. Despite this, banked queens resumed oviposition rapidly after introduction, indicating that prolonged confinement does not prevent queens from quickly recovering their reproductive function. This finding is encouraging for beekeepers seeking to rebuild colony populations early in the spring.

Overall, our study demonstrates that queens banked throughout the winter can be successfully shipped over long distances across Canada without compromising their reproductive quality or acceptance into colonies, provided that they are not exposed to extreme temperatures during transport, a risk inherent to all queen shipments. Exposure to prolonged cold temperatures resulting in chill-coma was associated with a substantial reduction in sperm viability, highlighting the importance of maintaining appropriate thermal conditions throughout shipment. Further work is needed to refine shipping methods in order to minimize the risk of temperature-related damage to banked queens. Additional studies should also evaluate the long-term performance of shipped banked queens, including colony productivity, queen longevity, and subsequent winter survival. Together, these findings support the potential of queen banking as a practical means of providing domestically produced queens early in the beekeeping season. By reducing reliance on imported queens, this management practice could facilitate the development and distribution of locally adapted genetic stocks better suited to regional environmental conditions and the specific needs of the Canadian beekeeping industry.

Methods

Queen rearing and bank colonies

From August 10 to 27, 2024, a total of 415 honey bee queens were reared using the Doolittle method, grafting larvae into prepared cups, and were subsequently mated in nucleus colonies [32, 33]. Queens were produced by three queen breeders in the province of Quebec: 65 from the Centre de Recherche en Sciences Animales de Deschambault (CRSAD; http://crsad.qc.ca, QC, Canada; 46°40'28.4" N, 71°54'52.6" W), and 175 each from two commercial queen breeders, hereafter referred to as breeder A and breeder B to preserve confidentiality. Young laying queens were harvested and individually isolated in cages (California Mini Queen Cages, Mann Lake Ltd., Hackensack Minnesota USA #HD-398). Queens from commercial breeder A and B were transported by car to CRSAD in cardboard boxes with attendant workers, sugar candy, and water, and were received on August 26 and 27, respectively. Upon arrival, they were kept in a dark room at 25 °C until their transfer into bank colonies.

On August 27, ten bank colonies were prepared at CRSAD. For this purpose, twenty Langstroth double brood chambered colonies were selected. These colonies had a high brood population, estimated visually by measuring the brood area with a staggered grid [34], and a low Varroa infestation rate (0–1 %), ensuring a strong nurse bee population to care for the banked queens during winter. All colonies were treated with Apivar® according to label instructions on August 2 (2 strips per brood chamber, 4 strips per bank colony). The bank colonies were made queenless on August 26, and the following day, pairs of colonies were merged. This was achieved by stacking one colony above the other and separating them with a perforated sheet of newspaper, a beekeeping technique which allows for gradual merging and reduces aggressive behavior between workers from different colonies. The resulting bank colonies contained approximately 59,000 ± 2,430 brood cells (eggs, larvae and pupae).

Queen banking and winter preparation

On August 28, 2024, 400 queens were distributed among the ten bank colonies. In each bank colony, 40 individually caged queens were randomly placed within a modified frame. Each frame contained an equal number of queens from each queen breeder (7 from CRSAD, 17 from Breeder A and 17 from Breeder B), and queens were given a number based on their position in the frame for future identification purposes. Each frame was randomly introduced into a bank colony and positioned at the centre of the second brood chamber (Fig. 3). A HOBO data logger (Onset MX2303A, Hoskin Scientific Ltée, Saint-Laurent, QC, Canada) was installed near the queen frame to record temperature and relative humidity throughout the banking period.

On September 3, the bank colonies were inspected to remove queen cells and to assess queen survival on the frames. Each colony was subsequently fed 32 L of sucrose syrup (2:1) with a Miller top feeder (FE-1100, Propolis etc.) in September. Queen survival was evaluated again on October 4, and the colonies were weighed on the same day.

Colony overwintering

On October 4, bank colonies were transferred into a temperature-controlled overwintering chamber. The chamber consisted of an insulated 20-foot container equipped with an individual opening for each colony, connected to a tube leading outdoors, allowing bees to exit the chamber during the fall and spring. These openings were closed for the winter period on November 20 and remained closed thereafter to prevent temperature fluctuations within bank colonies. The chamber was maintained at 15 ± 1 °C and 50–60 % relative humidity, with the selected temperature based on findings from our team's previous research [23]. A HOBO data logger was installed inside the chamber to monitor temperature and relative humidity throughout the overwintering period. The bank colonies remained in the chamber from October 4, 2024, through April 17, 2025, and no additional bees, brood or food were added during this time.

Queen survival and shipping

On April 17, 2025, queen survival was assessed by inspecting each queen frame in the ten bank colonies and recording the positions of live and dead queens. Bank colonies were also weighed on the same date. On May 2, 140 queens were randomly selected from the surviving queens. Only queens from two of the three breeding centres (Breeder A and Breeder B) were selected for shipment to reduce the influence of random factors, as fewer queens from CRSAD were available. Selected queens were transferred into JZ/BZ cages (#9150, Stanabbey Bee Supplies, St-Stanislas de Kostka, QC, Canada) with five attendant workers from their bank colony, and the cages were filled with a candy tube. They were then inserted on JZ/BZ shipping bars (#9190, Stanabbey Bee Supplies, St-Stanislas de Kostka, QC, Canada) and were returned to their respective bank colonies until shipment on May 5. The breeding centre and bank colony number were written on each cage for subsequent identification.

On May 5, 105 queens were randomly chosen from the selected group (54 from Breeder A and 51 from Breeder B) and removed from the bank colonies. They were distributed into three shipping boxes (BB-6100, Propolis etc.), with 35 queens per box and approximately equal representation from each breeding centre in each shipping box (18 queens from Breeder A and 17 queens from Breeder B per box). In each shipment, ten queens were reserved for subsequent quality assessment, 20 queens were introduced in nucleus colonies, and the remaining five queens were extras in case of mortality during shipment. A HOBO data logger was installed next to the queens to monitor temperature during shipping (Fig. 4).

Shipping boxes were dispatched on May 5 with AirCanada Cargo Animals to three destinations: Edmonton Airport (YEG), Calgary Airport (YYC) and Vancouver Airport (YVR). They arrived on May 6 at 11:30 (Mountain Daylight Time; MDT), 14:00 (MDT), and 07:15 (Pacific Daylight Time), respectively. Upon arrival, shipments were collected at the airports and transported via automobile to their respective research centres (Beaverlodge Research Farm, AB, Canada, 55°12'11.9"N, 119°23'45.8"W; University of Lethbridge, AB, Canada, 49°41'26.4"N, 112°44'42.5"W; University of British Columbia, BC, Canada, 49°03'03.8"N, 123°02'48.7"W). Queens were kept in a dark room at room temperature until quality assessment and distribution in nucleus colonies.

Preparation of nucleus colonies and queen introduction

On May 5, in parallel with queen shipment, 20 nucleus colonies were prepared at each research centre (CRSAD, Beaverlodge, Lethbridge, and Vancouver). Each colony was assembled in a Langstroth brood box, and consisted of three brood frames, one honey frame, one frame feeder filled with 1 L of sucrose syrup (1:1), ½ pound of pollen patty (Global Patties, 15 % pollen, Airdrie, Alberta, Canada), and four empty drawn comb frames. Hive entrances were closed with mesh screens until queen introduction the following day to ensure worker retention within their respective colonies.

On May 6, for the three groups of queens that had been shipped (Beaverlodge, Lethbridge and Vancouver), 20 were randomly selected from each shipment (10 queens from each breeding centre) and introduced into their new colonies. Worker bees were removed from the queen cages before introduction to minimize aggressive behavior from workers of the new colony. Queen cages were placed in the centre of the bee cluster, between two brood frames, with the candy tube oriented horizontally. Three days after introduction, queens were manually released from their cages to ensure equal acclimation periods across sites. Of the unshipped queens at CRSAD, 20 were randomly selected from the remaining queens in JZ/BZ cages within the bank colonies (10 queens from each queen breeder). The candy tubes of the JZ/BZ cages were sealed with plastic caps until manual release of the queens three days after introduction. One queen was randomly introduced into each nucleus colony by placing the cage horizontally between the two central frames. The mesh screens closing the hive entrances were subsequently removed.

Queen quality

On the same day (May 6), two metrics of queen quality were assessed on ten random queens from each shipment, as well as on ten random queens from the unshipped group (CRSAD): queen body weight (mg) and sperm viability within the spermatheca (%). Queen weight was assessed by immobilizing each queen in a small tube and weighing the tube using a precision scale (CRSAD: KHA 203, Kilotech Inc, Kitchener, ON, Canada; Beaverlodge; CPA224S, Sartorius, Göttingen, Germany; Lethbridge: AZ124, Sartorius, Göttingen, Germany; Vancouver: XS105DU, Mettler Toledo, Columbus, OH, USA).

To assess sperm viability, the spermatheca was removed from each queens' abdomen and placed in a 1 mL Eppendorf tube containing 500 µL of modified Kiev Buffer [35] (0.3 g D + Glucose, 0.41 g potassium chloride, 0.21 g sodium bicarbonate, 2.43 g sodium citrate 2 hydrate in 100 mL of deionized water). The spermatheca was delicately opened with fine forceps to release the sperm. Two fluorescent dyes, SYBR-14 and propidium iodide (live/dead Sperm Viability Kit, L-7011; Life Technology Inc., Carlsbad, CA, USA), were added to the solution (2.5 µL of propidium iodide and 5 µL of 1:50 diluted SYBR-14). The mixture was incubated at room temperature for 15 minutes and gently vortexed. Sperm viability was assessed using a fluorescence microscope (CRSAD: BA410 Elite, Motic, Richmond, BC, Canada; Beaverlodge: Fluoview FV10i, Olympus, Tokyo, Japan; Lethbridge: FV10C-W3, Olympus, Tokyo, Japan; Vancouver: Cellometer X2, Revvity, Waltham, MA, USA) at 400 x magnification. For each queen, four slides were prepared with 10 µL of the sperm solution. In Vancouver, 3 µl of stained sperm solution was loaded into two counting chambers (25 µm depth), with images from four fields of view acquired per chamber.

Images of the slides were captured and analyzed later, with 200 sperm counted per slide. The mean value across the four slides was then calculated for each queen.

Queen acceptance and oviposition

Three days after their introduction into the nucleus colonies, on May 9, queens were manually released from their cages. Worker bee behavior was observed to ensure that queens were not attacked upon release. One week later, on May 16, queen acceptance was confirmed by the presence of a laying queen, except in Vancouver, where inclement weather necessitated acceptance inspections on May 14. At the same time, photographs of the oldest larvae in each nucleus colony were taken to determine the onset of oviposition. Larval age was estimated based on size [36, 37] (Fig. 5). Colony population was also assessed by estimating the number of frames covered by bees, with a precision of half frames.

Statistical analyses

Statistical analyses were carried out with R software [38] (version 4.1.2) and the results were interpreted with a significance level of 0.05.

Mixed linear models (*nlme::lme*) (v3.1-155) [39] were used to assess differences among shipment groups in queen quality traits (body weight and sperm viability) and oviposition onset in nucleus colonies. To meet model assumptions of normality and homoscedasticity, sperm viability data were logit-transformed according to:

Equation (1)

$$\text{logit}(p) = \ln\left(p/(1-p)\right)$$

where p is the proportion of viable sperm cells. Oviposition onset data were square-root transformed to improve the normality of model residuals. Queen provenance (*Miel d'Anicet* or *Breeder A*) was included as a fixed effect, whereas source bank colony was included as a random effect (9 levels). Nucleus colony strength was also included in the oviposition onset model.

Each fixed effect significance was determined using analysis of variance tables with the *emmeans::joint_tests* (v1.7.2) [40] function. When a significant difference was present, pairwise comparisons were made using Tukey-adjusted tests (functions *emmeans::emmeans* and *emmeans::pairs*).

The normality of the scaled residuals across the models was validated by visual inspection of histograms, a Shapiro-Wilk test (stats::*shapiro.test*) (v4.1.2) [38], and the values of skewness and kurtosis (functions *moments::skewness* and *moments::kurtosis*) (v0.14.1) [41]. Homoscedasticity was validated with a residual versus predicted values plot.

Queen acceptance and egg presence in nucleus colonies seven days after release were analyzed using a bias-reduced logistic regression model (*brglm2::brglm*) (v.0.7.2) [42] with a binomial error distribution

and logit link function. Bias-reduced estimation was used to account for the very high acceptance rates observed and the presence of complete or near-complete separation among groups. Queen provenance and nucleus colony strength were included as fixed effects. The overall effect of the research centre was assessed using likelihood ratio tests based on model deviance comparisons.

The results were graphically represented using the ggplot2 package (v3.5.2) [43]. For visualization purposes, model-estimated sperm viability and onset of oviposition values were back-transformed to their original scale.

Declarations

Additional Information

Competing interests

The authors declare no competing interests.

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Author Contribution

M.L and P.G. conceptualized the study and developed the methodology with contributions from all other authors. L.J.F. secured funding, and L.J.F. and A.M. supervised the project, and contributed to project administration. M.L. and A.R. led the laboratory fieldwork activities conducted at CRSAD, and S.F.P, S.E.H., A.M., L.P.O. and A.I. led the laboratory and field activities for the Beaverlodge, Lethbridge and Vancouver groups. M.L conducted data processing and statistical analyses and wrote the original draft of the manuscript. All authors reviewed, edited, and approved the final manuscript.

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Data Availability

The data that supports the findings of this study are available from the corresponding author, P.G., upon reasonable request.

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Figures



Figure 1

Average temperature (°C) inside shipping cages, recorded every five minutes during air transport from Québec City Airport on May 5 to the different apiaries (Beaverlodge, Lethbridge, and Vancouver) on May 6. Vertical lines indicate the different stages of the route and are labelled with the corresponding date and time. The horizontal dotted line represents the average temperature recorded inside bank colonies at

CRSAD during the travel period of shipped queens. The abbreviations EDT, MDT and PDT stand for Eastern Daylight Time, Mountain Daylight Time, and Pacific Daylight Time, respectively.

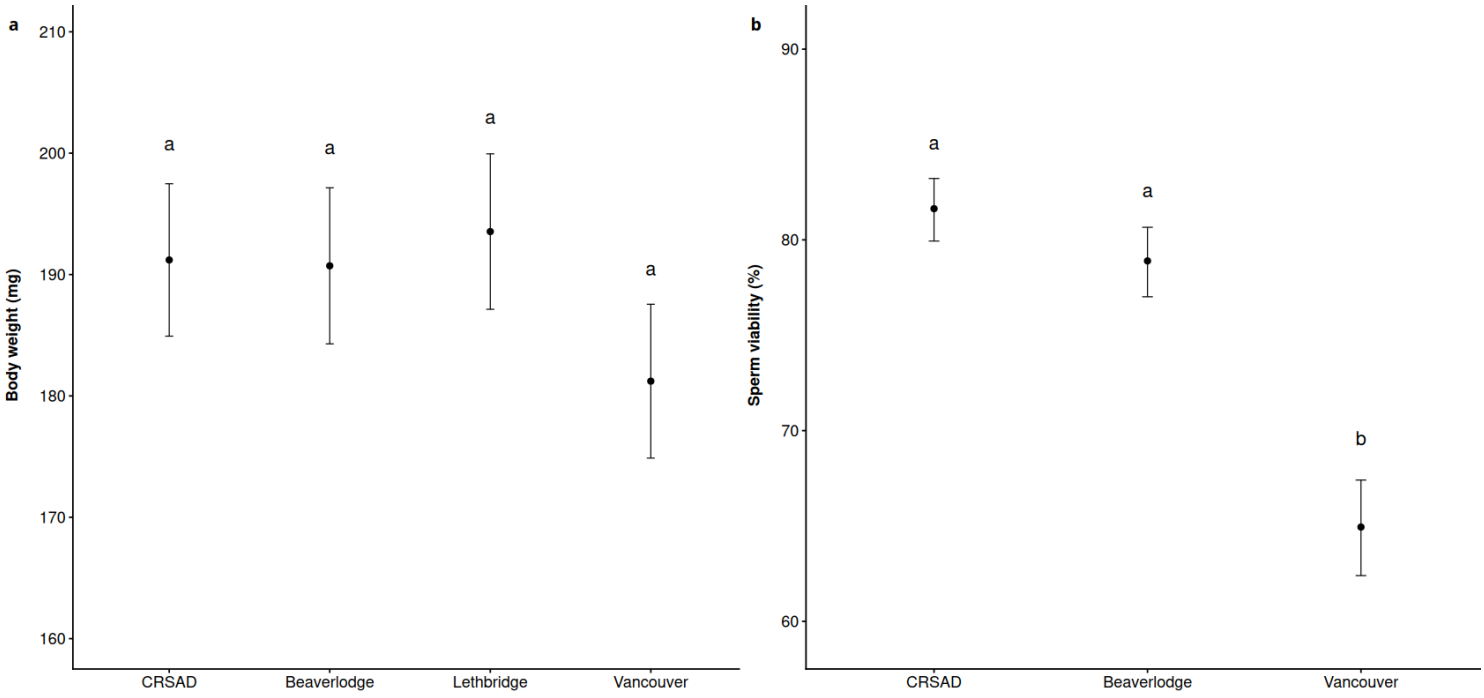


Figure 2

Body weight (mg) (a) and sperm viability (%) (b) (mean ± SE) of banked queens from the control group (unshipped queens; CRSAD) and the three experimental groups (queens shipped to Beaverlodge, Lethbridge and Vancouver). The Lethbridge group is not shown in panel (b) because it was excluded from the sperm viability analyses due to methodological issues. Measurements were taken on May 6, 2025. The y-axis scales of both panels do not start at zero to better illustrate the values and their standard errors. Sperm viability values were back-transformed to their original scale for visualization purposes. Statistically significant differences among groups are indicated by different letters above the values ($p \leq 0.05$).

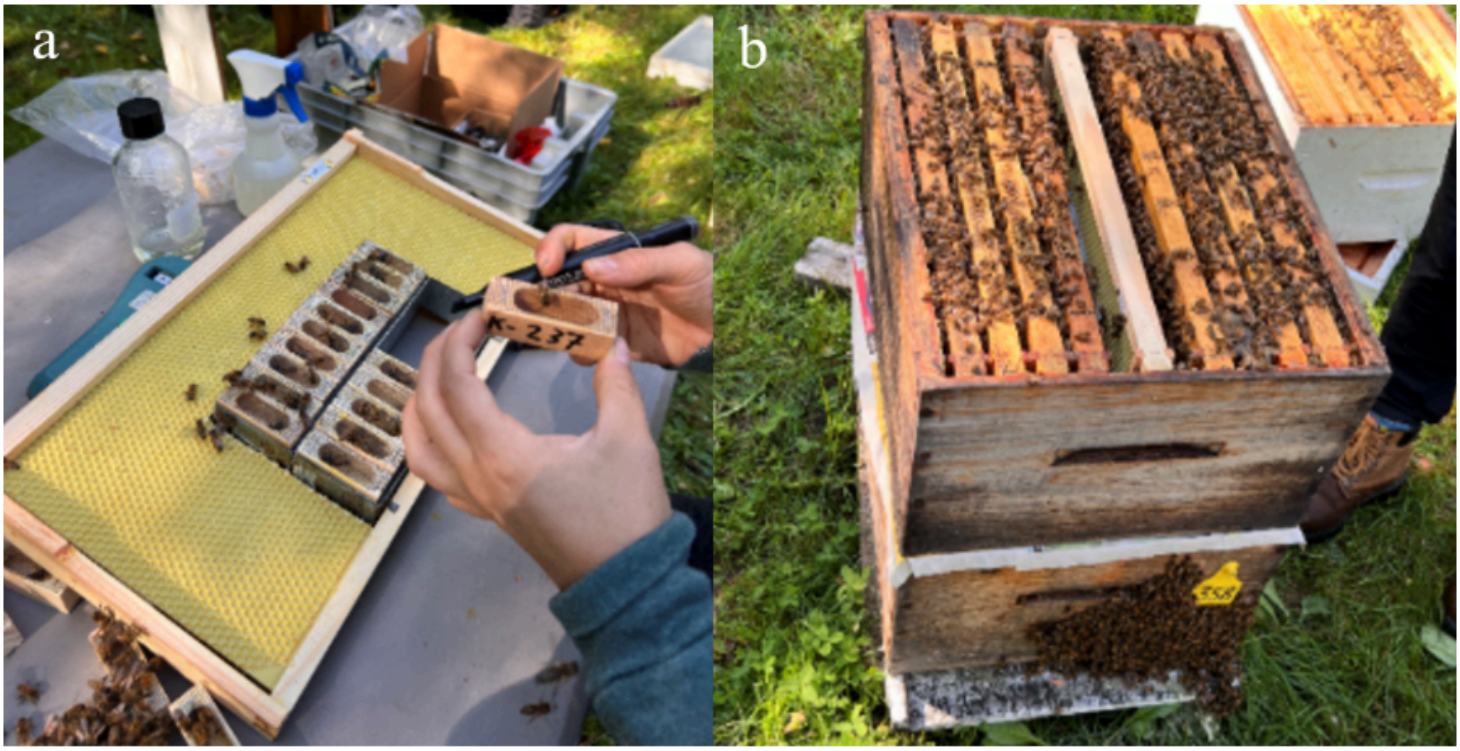


Figure 3

Preparation of a modified banking frame in which 40 queens (20 on each side), individually isolated in California Mini Cages, were placed at the centre of the frame (a), and introduction of the frame into the centre of the upper brood chamber of a queenless bank colony (b).



Figure 4

Shipping box with 35 JZ/BZ cages, each containing a banked queen and five attendant workers from their bank colony. A HOBO data logger was attached inside the box, with the temperature sensor positioned at the centre.

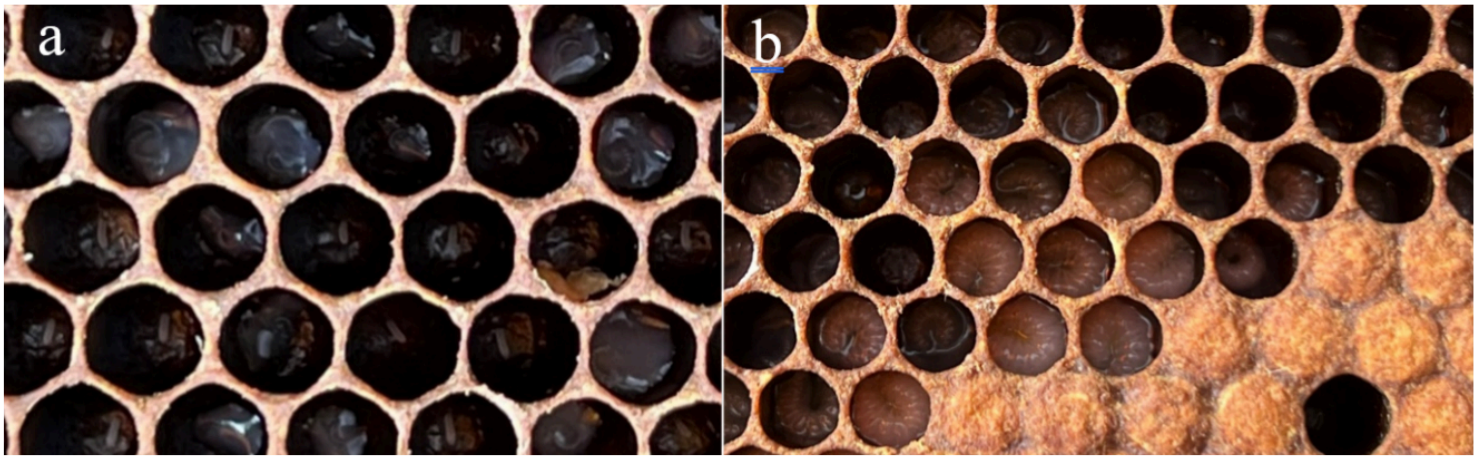


Figure 5

Sample images of larvae, taken seven days after queen release. The largest larvae in panels (a) and (b) were classified as 1-day-old larvae and 4-day-old larvae, respectively.