Chapter 9

Rearing Bumble Bees for Research and Profit: Practical and Ethical Considerations

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Additional information is available at the end of the chapter

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Abstract

The commercial production of bumble bee colonies is a multi-million dollar business worldwide. The pollination of greenhouse tomatoes is largely dependent on this industry. However, microparasites are prevalent in many of these colonies and can spread to wild populations of bumble bees. Academic researchers now commonly purchase colonies for their work. I believe that this raises some questions: (a) What is the danger of exacerbating the problem of spread of parasites and pathogens to wild population of bumble bees from field studies using purchased colonies? (b) How representative studies are done on only a few species, for example, *B. terrestris, B. impatiens*? (c) Does the purchase and use of these colonies give tacit approval to the industry, which may be having a detrimental effect on the native populations of bumble bees? This is an ethical issue. (d) Loss of "feeling for the organism" by researchers and particularly graduate students. These issues were discussed, and the classical method of bumble bee rearing which avoids these problems was described.

Keywords: *Bombus*, rearing methods, parasites, bumble bees, pathogens

1. Introduction

The mass rearing of bumble bee colonies for commercial purposes started in the mid-1980s and since then has expanded into a worldwide industry worth millions of euros [1]. In the mid-1980s bumble bees (*Bombus* spp.) were found to be particularly effective and economical for the pollination of greenhouse tomatoes and have replaced labour intensive mechanical methods of pollination or hormonal treatments [1]. There are now over a million colonies produced per year and exported to and from countries in Europe, North and South America, and Asia [1]. In addition

to the tomato, there are 19 other commercially important crops pollinated by bumble bees and the use of bumble bees for pollination on this scale has been an enormous benefit for the production of essential food crops giving higher yields, and better fruit quality with lower costs [1].

There are more than 30 commercial producers worldwide; however, the market is dominated by three companies: Koppert Biological Systems and Bunting Brinkman Bees (BBB) both headquartered in The Netherlands and Biobest in Belgium [1]. The production of large numbers of colonies requires the development of rearing methods that can be carried out on an industrial scale. These methods involve manipulating aspects of the bumble bee life cycle to be able to produce colonies at any time of the year and in numbers as needed. The exact methods used by each company are proprietary secrets, although the general methods have been published [1, 2].

In spite of the benefits this domestication of bumble bees has brought, it also has unwittingly engendered detrimental effects on wild populations of bumble bees, two of which have been well documented, to wit: the escape of non-native bees from greenhouses and their establishment in the wild, and associated with this, the spread of parasites from these bees to the wild populations of native bees.

However, I also believe that there have been some insidious effects on the way in which academic research on bumble bees is being conducted. In this paper I will discuss some concerns I have regarding the use of commercially reared colonies of *Bombus* species for fundamental (i.e., "pure") academic research. It has become more and more common for academics at universities to purchase colonies from the various commercial bumble bee rearing companies for use in their research and for their graduate students' research. This is particularly the case in North America and in Europe. The issues fall into two categories, the first two being tangible and the second pair being intangible.

a. What is the danger of exacerbating the problem of spread of parasites and pathogens to wild population of bumble bees from field studies using purchased colonies?

b. How representative are studies done on only a few species, for example *B. terrestris, B. impatiens*?

c. Does the purchase and use of these colonies give tacit approval to the industry, which may be having a detrimental effect on the native populations of bumble bees? This is an ethical issue.

d. Loss of "feeling for the organism" by researchers and particularly graduate students.

I believe that these are some important issues which should be discussed by the entomological and conservation community. In this paper, I will first review the biology and the life cycle of bumble bees, then briefly discuss some aspects of the commercial colony rearing industry, and review the potential for the spread of parasites from infected colonies to bees in greenhouses and in the wild. Next, I will discuss the use of commercially reared colonies for 'pure' research and the issues surrounding this practice. Finally, I will summarize the 'classical' methods for rearing bumble bee colonies and discuss the advantages of using these for research work.

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2. Bumble bee biology and life cycle

Bumble bees all belong to one genus, *Bombus*, and there are only about 250 species worldwide [3]. The species are generally confined to the northern temperate regions, but also occur naturally in South America. A few species have been introduced to New Zealand and Tasmania where they are non-native.

Bumble bees generally have an annual life cycle. Reproductive individuals, young queens and males, are produced by colonies towards the end of summer, and mate after they leave their natal nests [4]. The new queens then enter hibernation with the sperm from the males stored in a sac, called the spermatheca. In most species, queens only mate once [5]. The queens emerge from hibernation in the spring, and spend their time foraging for nectar and searching for a suitable nest site. Bumble bees nest, where there is some pre-existing nesting material, for instance underground in an abandoned rodent (e.g., mouse) nest, or on the surface in a ball of dried grass, or around human habitation (e.g., in the insulation of houses) [4]. Once a queen has chosen her nest site, she then forages for pollen to eat to develop her ovaries, and she also collects pollen and deposits it on the floor of her nesting cavity. On this she makes cells out of wax extruded from glands between the segmented plates on her abdomen. She then lays an initial brood of 6–10 eggs. The queen also builds a honey pot in which she stores honey. The first 3–4 weeks comprises the solitary phase of the life-cycle, in which the queen alone forages and tends to her first brood.

Bumble bees like all Hymenoptera have a haplodiploid genetic system, in which fertilized eggs develop into females, while unfertilized eggs develop into (haploid) males. This allows a queen to control the sex of her offspring, either by releasing or by withholding sperm from the spermatheca. For the first part of the season, the eggs laid by the queen will be fertilized and will develop into workers. Once the first brood of workers emerge, the queen remains in the nest and the workers take over the tasks of foraging, defence, and colony maintenance [4]. The colony grows rapidly for 2–3 months, and when the worker force is large enough to accumulate sufficient resources, young queens are produced [4]. A successful colony can produce large numbers (>50) queens [6]. The foundress queen also switches to laying unfertilized eggs to produce males. At the end of the summer, the old queen, the workers, and the males die, leaving the inseminated new queens to repeat the cycle the following spring.

Bumble bee (*Bombus*) species, with their varying tongue-lengths, ability to forage at lower temperatures, and capacity to buzz pollinate, are one of the most effective pollinators of wild plants and crops [4].

3. The commercial rearing industry

3.1. Economics of the industry

In 2006 when Velthuis and van Doorn [1] reviewed the state of the industry, the growth in commercial sales of bumble bee colonies had reached around one million in 2004 from its beginnings in 1988. It has certainly continued to expand since then, and the supply of bumble

bee colonies is essential for global tomato production. In 2004, 99,000 acres of greenhouse tomato production were pollinated worldwide by bumble bees, with an estimated value of ~ $15 billion [1]. Exact revenues from bumble bee colony sales are hard to estimate because the companies are private. However, since, for example costs of bumble bee colonies sold by Green Methods.com (https://greenmethods.com/) run from US $109.95–$252.95. The industry as a whole must be worth hundreds of millions of dollars.

3.2. Species used

Until recently only species of two subgenera, *Bombus sensu stricto* and *Pyrobombus* [3], have been used for commercial rearing [1]. These are listed in **Table 1**. These are all pollen-storing species, which store pollen in wax cylinders near to the brood clumps, as opposed to the pocket-making species which pack pollen into pockets next to the developing larvae which feed directly. Workers of pollen-storing species feed larvae with a regurgitated mixture of pollen and honey. Pollen-storing species can be fed additional pollen which aids in their domestication. Of the species which have been reared commercially, two species are used predominately: *B. terrestris* in Europe and *B. impatiens* in North America (**Table 1**). Biobest has just started to supply colonies of *B. atratus* for use in South America. This is a pocket-maker, which has large colonies and is a vigorous and aggressive species. *B. occidentalis* is no longer produced commercially as the cultures were severely infected by *Nosema bombi* in 1996, which probably came from wild-caught queens [1].

Species	Subspecies	Origin	Used in	Source (Company[1])
Subgenus *Bombus*				
B. terrestris L.				
	B. t. audax (Harris 1780)	Belgium	U.K. only	Biobest
	B. t. canariensis Pérez	Canary Islands	Canary Islands	Biobest
	B. t. dalmatinus Dalla Torre	south-eastern Europe, Turkey	Europe	Koppert
	B. t. sassaricus Tournier	Sardinia		Koppert
	B. t. terrestris L.	Europe, Turkey, North Africa, China	Europe, North Africa, Asia, Australasia and Chile	Biobest, Koppert

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Species	Subspecies	Origin	Used in	Source (Company[1])
B. lucorum L.		Europe, Asia	East Asia	
B. ignatius Smith		Belgium	Japan only	Biobest
B. occidentalis Greene[2]		Western North America	Western North America	
Subgenus *Pyrobombus*				
B. impatiens Cresson		Canada, Mexico	North, Central and South America	Biobest, Koppert, Green Methods
Subgenus *Fervidobombus*				
B. atratus Franklin		Argentina	South America	Biobest

[1] Example company.
[2] No longer in production.

Table 1. Species and subspecies of bumble bees (*Bombus*) which have been or are currently used for commercial rearing of colonies.

4. Dangers from commercially reared colonies

The use of commercial reared colonies for the greenhouse tomato industry alone has become essential and a reduction in the pollination services provided would have serious economic consequences for the growers. However, the widespread use of these colonies has introduced some dangers for populations of wild bumble bees, which should not be underestimated.

4.1. Escape of non-native species

Species of bumble bees have been intentionally introduced to countries where bumble bees are non-native. For example four species were introduced to New Zealand in 1885 and 1906 for the pollination of red clover [7]. One of these, *B. ruderatus*, was later introduced to Chile in 1982, where there is one species of native bumble bee, *B. dahlbomi* [8]. However, there have also been instances where species have possibly been introduced accidentally. In 1992 *B. t. audax*, most likely from New Zealand, arrived in Tasmania where it has spread at a mean rate of 25 km/year [9]. In Chile, *B. terrestris* colonies were imported in 1998 from Israel and Belgium for use in greenhouses and later used for the pollination of field crops [8]. It is undoubtedly spreading and is likely to become established in the wild. In 2001, I collected a number of *B. terrestris* males at high elevations south of Santiago. In Japan, *B. terrestris* has been imported since 1992 and colonies have established in the wild [1]. More worrying is that hybridization has been recorded between *B. terrestris* and *B. ignitus* in the wild [10]. It is worth noting also that mating between some subspecies of *B. terrestris* in captivity occurs quite readily [11]. In North America, the eastern species *B. impatiens* is being used in unsecured greenhouses in

Alberta and British Columbia. For example, Ratti and Colla [12] collected a queen and five workers in pan traps in fields, a minimum of two km from the nearest greenhouse. Also, *B. impatiens* workers have been collected while foraging on trees next to a commercial greenhouse in Sylvan Lake, Alberta (Beaudin and Owen, unpublished).

4.2. Common parasitic diseases

The escape and establishment of species in areas where they are non-native is a real danger as it is simply not possible to ensure that bees will not escape from greenhouses. Moreover, even in areas where greenhouse bees are the same species as the native bees, escaped bees can carry and spread diseases to native populations of bees. Although commercial operations strive to keep their colonies free of microparasites, a large proportion of colonies are probably infected [13]. Graystock *et al*. [13] assessed levels of nine parasites in colonies produced in 2011 and 2012 from the three main producers of colonies. Using molecular methods, they screened for the three main bumble bee microparasites, all of which are faecal-orally transmitted parasites of adult bees: (1) the trypanosome *Crithidia bombi*, (2) the microsporidian *Nosema bombi*, and (3) the neogregarine *Apicystis bombi*. They also screened for six widespread honeybee parasites: (4) *Nosema apis*, (5) *N. ceranae*, (6) the orally infecting foulbrood bacteria *Melissococcus plutonius*, (7) *Paenibacillus* larvae of bee larvae, (8) deformed wing virus (DWV), which is a common parasite in honeybees and bumblebees, and (9) the orally infecting fungal parasite *Ascosphaera* of bee larvae. They also screened the pollen provided to feed the colonies for the same pathogens. They examined 48 colonies of *B. terrestris* purchased from the three main suppliers in Europe, all of which were imported into the United Kingdom on the producers claim that they were disease-free; however, 37 of the 48 colonies (77%) were infected, and in these 5 parasites were present in 13–56% (depending on parasite) of the colonies [13]. Similarly 24 of 25 pollen samples were contaminated with parasites [13]. Also, when bumble bee workers were fed infected pollen or faeces from the commercially produced colonies they would then become infected, and this reduced their survival. The parasites tested were *Crithidia bombi, Apicystis bombi, Nosema bombi,* and *N. ceranae* [13]. It is necessary to test for honeybee diseases as there has been a spread of some of these from honeybees to wild populations of bumble bees [14]. For example Graystock *et al*. [13] found deformed wing virus (DWV) in about 15% of the *B. terrestris* colonies and in 10% of the pollen samples. The recent spread of DWV has been well documented [14, 15]. DWV is endemic in the European honeybee, *Apis mellifera* [15]; however, it is currently remerging as a global epidemic of honeybees. This resulted from the spread of its vector, the mite *Varroa destructor*, from the Asian honeybee *Apis cerana*, which is its normal host [15]. This leap occurred in the middle of the twentieth century and now *V. destructor* is distributed worldwide. The mites are particularly infective because they bear a heavy load of the virus, as it may replicate within the mite or accumulate in the gut; moreover they also inject the virus directly into the hemolymph of the bee [15]. Both the mite and DWV have been implicated as one possible cause of Colony Collapse Disorder in honeybees [15]. Global movement of honeybee colonies brought the Asian and European honeybees into contact and allowed the spread of the host and the virus [15], and the introduction of infected bees into Hawaii, previously free of *Varroa*, led to an increase in virulence of DWV [15]. Where infected honeybees and bumble bees are sympatric, the latter have higher prevalence of DWV

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than in other locations, and they have lower survival rates than uninfected bees [16]. Thus, potential danger of spillover of pathogens from domesticated bees must be taken seriously.

4.3. Spillover of diseases to natural populations: models and data

One factor implicated in the decline of wild bumble bees is the possible spread or 'spillover' of pathogens from greenhouse populations to the wild bees [17–19]. This has been modelled by Otterstatter and Thomson [19]. An initial question is, if a single infected colony is introduced into a greenhouse along with other non-infected colonies, how will the infection spread through this closed population? Here we are ignoring the loss of bees to the outside. This can be analysed with a deterministic model of pathogen spread. Here we will consider *C. bombi* which is an intestinal protozoan which spreads both within and between colonies. Once ingested, the parasites attach to wall of the gut using their flagellum. Here they multiply and in a few days, infective cells are shed in the host's faeces. There is no direct transmission from bee to bee, but the infection spreads within a colony when a new host comes into contact with cells on substrates in the nest [20]. In the field, *C. bombi* spreads when bumble bee workers pick up infective cells deposited on flowers by infected bees [17, 19]. The cells are either shed from the body surface of the bee or deposited when the bee defecates [19]. Infection by *C. bombi* can have multiple effects including severely reducing the colony-founding success of queens, the growth rate of established colonies, and worker survival and foraging efficiency [19, and references therein].

4.3.1. Spread in a greenhouse population

If we consider a closed population of bees in a greenhouse, then a basic SIR epidemiological model can easily be constructed. *S, I,* and *P* are the densities of susceptible bees, infected bees, and infective pathogen particles in the environment respectively. Let a = the birth rate of the susceptible population, β = the transmission rate of pathogen particles, α = the mortality rate of infected bees, λ = the rate at which infected bees produce and deposit pathogen particles in the environment, and μ is the rate at which pathogen particles breakdown in the environment and are no longer infective (see **Table 2**). It is assumed that (1) the parameters (a, b, α, λ, μ, γ) are constant, (2) there is no vertical transmission (i.e., no within colony transmission), (3) no bumble bee may be infected more than once, (4) the disease does not spread directly from bumble bee-to-bumble bee. Additionally the duration of the epidemic is set to be roughly 90 days (during June to August) while colony growth is occurring and involves only workers.

Parameter	Symbol	Value
Birth rate of the susceptible population	a	0.220 d^{-1}
Natural (non-disease) mortality rate	b	0.183 d^{-1}
Disease-induced mortality	α	0.102 d^{-1}
Pathogen production rate	λ	4.23×10^4 d^{-1}
Pathogen decay rate	μ	12.98 d^{-1}

Parameter	Symbol	Value
Transmission rate	β	1.08×10^{-4} m^2 d^{-1}
Initial host population density	S_0	0.08 m^{-1}
Diffusion coefficient	D	800 m^2 d^{-1}

From: Otterstatter MC and Thomson JD (2008) Does pathogen spillover from commercially reared bumble bees threaten wild pollinators? *PLoS ONE* 3(7): e2771. doi:10.1371/journal.pone.0002771

Table 2. Parameter estimates used by Otterstatter and Thomson (2008) for their model of *Crithidia bombi* spillover to wild bumble bees near greenhouses.

Therefore,

$$\frac{dS}{dt} = (a-b)S + aI - \beta SP$$

$$\frac{dI}{dt} = \beta SP - (\alpha + b)I$$

$$\frac{dP}{dt} = \lambda I - \mu P$$

Thus $S(t)$ = Number (or density) of *susceptible bumblebee*s at time t, $I(t)$ = Number (or density) of *infective bumblebees* at time t, and $P(t)$ = Number (or density) of pathogens present in the environment at time t. As an example, if we start with a population of 100 bees with five of these infected, that is, the non-negative initial conditions are $S(0) = S_0 = 100$, $I(0) = I_0 = 5$, and $P(0) \geq 0$. The parameter estimates are those used by Otterstatter and Thomson [19] and are

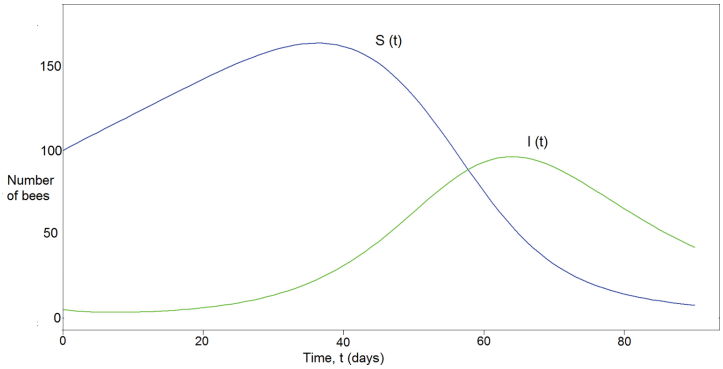

Figure 1. Theoretical course of an infection of *Crithidia bombi* in a closed greenhouse.

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given in **Table 2**. As shown in **Figure 1** the infection sweeps through the population in about 80 days leaving the majority of the bees infected. Clearly in closed populations infections are likely to spread easily.

4.3.2. Spatial effects: spillover to wild populations

In the field, horizontal transmission between workers occurs indirectly when infected and susceptible bumble bees share flowers. *C. bombi*, and other pathogens, can spread to wild populations of bees when infected commercially reared bees that escaped from a greenhouse and deposit short-lived pathogen particles on flowers near the greenhouse [19]. Susceptible wild bees foraging near the greenhouse then acquire infection from these particles when foraging for nectar or pollen, subsequently, they become infectious themselves and can introduce this pathogen into their natal colony and also deposit them on more flowers.

Otterstatter and Thomson [19] modelled this by modifying the equations given above to track spread of pathogens not only in time (t), but also in space (x, displacement from starting point).

$$\frac{\delta S}{\delta t} = (a-b)S - \beta SP + D\frac{\delta^2 S}{\delta x^2}$$

$$\frac{\delta I}{\delta t} = \beta SP - (\alpha + b)I + D\frac{\delta^2 I}{\delta x^2}$$

$$\frac{\delta P}{\delta t} = \lambda I - \mu P + D\frac{\delta^2 P}{\delta x^2}$$

Here, the parameters are as defined earlier (**Table 2**) with the addition of D, the dispersal rate of hosts and pathogen particles [19]. Otterstatter and Thomson [19] assumed that wild bees and pathogen particles, which can be picked up and carried on bees' bodies, move about the environment *via* simple diffusion. The model did not include vertical transmission within colonies. Prevalence curves were generated through numerical simulation of the diffusion model using the parameter estimates given in **Table 2**, to predict the long-term dynamics of *C. bombi* spillover [19]. Initially (t= 0–13 wks), pathogen spillover into wild populations is localized around the source; the prevalence of *C. bombi* is about 20% next to greenhouses, and declines to 0% at a distance of roughly 2 km. However in the weeks following, a large wave of infection develops and by 15 weeks, peak prevalence of *C. bombi* near greenhouses has increased to ~ 75%. By 18 weeks, peak prevalence has reached 100%. The wave spreads through the wild bumble bee population at a rate of 2 km/wk [19].

Otterstatter and Thomson [19] tested the predictions of their model by sampling bumble bee workers from wild populations adjacent to greenhouse populations at two locations in southern Ontario, Canada. Given the parameter estimates used, the model gave a good fit to the pathogen prevalence observed in the field. The model predicted the sharp decline in

pathogen prevalence observed near greenhouses and matched well with the prevalence observed over several kilometres [19].

5. Using bumbles bees for research

Bumble bees have become important and widely used organisms for research. Their study illuminates many areas of biology not only of practical importance, such as crop pollination, but also of theoretical interest, such as the evolution of eusociality and optimal foraging. Since the widespread availability of commercially produced colonies, many research workers have simply purchased colonies for their graduate students' research. The advantages are obvious; no time or effort is required to rear colonies and colonies are available at any time of year. This is particularly useful since research can be carried out in the winter months in the temperate regions. However, I believe that there are potential and actual detrimental aspects to this approach.

Commercially produced colonies have been used for research in the following areas: colony development, worker behaviour, foraging behaviour, estimating mortality rates of workers, and transplantation experiments to assess the pollination efficiency of different subspecies (of *B. terrestris*).

I will now discuss the issues I listed earlier in more detail. The issues fall into two categories, the first two being intangible and the second pair being tangible:

a. What is the danger of exacerbating the problem of spread of parasites and pathogens to wild population of bumble bees from field studies using purchased colonies?

As discussed earlier, the major microparasites of bumble bees are very infectious and liable to spread very rapidly among greenhouse and wild bees. There is a risk of spreading infections from commercially reared colonies to wild populations of bumble bees and this should be avoided.

b. How representative are studies done on only a few species, for example *B. terrestris, B. impatiens*?

There is considerable variation among bumble bee species and subgenera in morphology, behaviour, etc. Concentration on only one or two species for detailed study would seem to be inadvisable.

c. Does the purchase and use of these colonies give tacit approval to the industry, which may be having a detrimental effect on the native populations of bumble bees?

The same scientists may also be decrying the spreading of parasites, etc., and supporting petitions to limit the importation and movement of bumble bee colonies while they are using purchased colonies for their research.

d. Loss of 'a feeling for the organism' [21] by researchers and particularly graduate students.

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The phrase 'a feeling for the organism' was how the late Nobel-prize winning geneticist Barbara McClintock described the almost intuitive understanding that a biologist can develop after deep study of a particular species [21]. It is often based on years of observation and work on this organism, and can lead to insights that superficial study cannot provide [21]. I believe that rearing bumble bee colonies goes a long way in giving one a feeling for the organism.

6. The art and science of rearing bumble bee colonies

One advantage of working with bumble bees is that it is possible and very easy to get to know the organism as a whole. The best way to do this is to rear bumble bee colonies using the methods developed years ago, which essentially provide the conditions under which queens will initiate colonies reasonably and naturally in captivity. It is labour intensive and involves work every day of the spring and summer, typically from mid-April/early May until mid-August in northern latitudes. This involves collecting queens and installing them daily, daily inspection of the nest boxes, feeding the queens, and later on feeding and monitoring the developing colonies. If done with sufficient numbers of bees and species, as in **Table 3**, then one gains invaluable knowledge of the nuances of each species and the 'individuality' of each queen.

The method described here is based on the one developed by Plowright and Jay [22] as modified by Owen [23]. It is designed to be of use to students and researchers who want to rear a fair number (up to100 or so in a season) of colonies, without undue effort and under reasonably natural conditions. The idea is to provide a queen with a simulation of what she would encounter in nature, after emerging from hibernation in the spring, that is, a nesting site with pre-existing nesting material and a supply of pollen and nectar.

Species	Year	Dates collected	Median	*N*	Days to B1E	Mean	*N*	Success rate
B. nevadensis	1985	May 7–June 7	14 May	23	5 to 17	9.70	12	12/23 = 52%
	1986	May 2–June 14	19 May	23	5 to 24	12.70	11	11/17 = 65%
B. occidentalis	1985	April 30–May 16	08 May	56	3 to 11	6.50	30	30/55 = 54%
	1986	April 19–May 28	12 May	70	3 to 19	7.40	47	47/67 = 70%
B. terricola	1985	May 2–May 16	07 May	27	4 to 12	7.14	14	14/25 = 56%
	1986	May 2–May 28	09 May	30	3 to 13	7.25	16	16/21 = 76%
B. californicus	1985	June 5–June 18	11 Jun	5	4 to 11	7.50	2	2/5 = 40%
B. bifarius	1985	May 2–June 7	07 May	24	3 to 10	6.10	15	15/24 = 62%
	1986	May 2–May 28	09 May	12	4 to 12	7.70	7	7/10 = 70%
B. frigidus	1985	May 2–May 16	06 May	42	2 to 8	4.20	21	21/42 = 50%
	1986	April 19–May 28	22 May	30	2 to 13	4.40	16	16/20 = 80%

Species	Year	Dates collected	Median	*N*	Days to B1E	Mean	*N*	Success rate
B. huntii	1985	May 2–June 7	13 May	25	3 to 11	6.80	10	10/13 = 77%
	1986	May 2–May 28	12 May	22	3 to 9	5.80	11	11/18 = 61%
B. perplexus	1985	May 3–May 15	08 May	20	3 to 17	7.10	14	14/19 = 74%
	1986	May 3–May 20	09 May	14	3 to 6	4.80	10	10/14 = 71%
B. ternarius	1985	May 2–June 4	07 May	31	3 to 10	6.50	6	6/27 = 22%
Total				454			242	242/400 = 60%

Fifty four of the queens died after installation (12% mortality) therefore the success rate is calculated for the surviving queens. N, the total number of queens collected for each species; n, the number of queens successfully initiating colonies.

Table 3. Dates that queens of nine *Bombus* species were collected in the vicinity of Calgary, Alberta in 1985 and 1986, and the number of days until brood one eggs (B1E) lay.

6.1. Queen collection

It is best to collect queens which have been newly emerged from hibernation; the exact timing depends on the phenology of the species (**Table 3**). These queens are in prime condition and using them gives optimal starting rates and more vigorous colony development. Queens which are gathering pollen have already started their nests and should not be collected. In the early spring the queens forage on pussy-willow (*Salix* spp.) and are easy to find and catch. The bees should be put into 5 dram vials with air holes punched in the lid. They need to be kept cool on a freezer pack or on bags of ice (covered with a 'J-cloth') in a small cooler for a maximum of 4–5 hours before they are transported to the laboratory. Ideally the queens should be installed immediately upon return to the laboratory; however, they can be kept in their vials (with no food) in the fridge (~ 4°C) overnight if necessary. At this stage, bees can be wet-weighed [24], and a data sheet started for each queen. Queens should be inspected for mites; some queens are heavily infested with the mites completely covering their thorax, and these bees should not be installed. However, if only a few mites are present then they can be picked off using forceps. It is best to always transfer queens by using a vial, and it is rarely (if ever) necessary to anaesthetize them. If bees must be picked up, then use broad-tipped forceps and grab the bee by one of its middle legs.

Table 3 gives the dates that queens of nine *Bombus* species were collected in 1985 and 1986. These are two of the years in the 1980s when I was doing intensive collecting of all bumble bee species in Calgary and nearby in southern Alberta. Valuable comparative data can be collected this way, such as the dates of emergence and the number of days until brood one eggs (B1E) laid. Interestingly these earlier records later revealed some important trends in the declining abundance of *B. occidentalis* [25]. The starting or success rate under the laboratory conditions for the different species is also given in **Table 3**. If one assumes that all species establish their colonies equally well in the wild, then clearly some species do better in the lab than others. However, the average starting rate can be counted upon to be about 60%. A queen that does not start a colony within two weeks is very unlikely to do so and can be preserved (frozen or

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in ethanol) for genetic studies, and her wings can be removed for morphometrics [26, 27]. Likewise, queens heading colonies can also be preserved until the end of their life.

6.2. Nest boxes and queen installation

The rearing system is very simple and consists of two wooden boxes: a larger box for foraging and defecating (the 'front' box) and a smaller nesting box (the 'back' box). Only the dimensions of the nesting box are critical, and if constructed from half-inch plywood, its exterior dimensions should be 4"x4"x2". It can be lined with upholsterer's cotton for nesting material (see **Figure 2**). The boxes should be placed on, but not attached to, a ½" plywood board and be provided with glass lids. Bees can be kept at room temperature (~20°C) and ambient humidity, although moist filter paper or a piece of paper towel can be placed in the nesting box if so desired. Light condition or dark/light cycle does not seem to matter. I have found that this works very well in Alberta where the humidity is generally low. However, where the ambient humidity in the spring and summer is higher, as in eastern North America, the nesting boxes can be kept in a room with high heat (~30°C) and humidity as was done in Chris Plowright's lab. However, I am not convinced that this is always necessary given my experience with rearing colonies in Alberta. Honey solution (1:1) or sugar solution (60:40 water: sugar v/v) is supplied in the foraging box. Plexiglass bars with 1 cm deep holes are ideal. A pollen lump is provided in the nesting box and this should be ~1 cm in diameter and ~0.5 cm in height. Pollen lumps of uniform size can be made by using a cork borer and a scalpel. The pollen dough is made by grinding up fresh, clean pollen (which can be stored frozen), with honey or sugar solution, in a mortar. The resulting paste must be of just the 'right' consistency, that is, neither too sticky nor too dry. Pollen can be obtained from honeybee colonies and can be purchased

Figure 2. A queen with her first brood clump and a well constructed honey pot. Note also the honey squirted by the queen on the cotton.

from honeybee breeders. Given the findings of Graystock *et al*. [13] discussed earlier, it is of crucial importance to ensure a disease-free source of pollen, which may prove difficult today.

6.3. Inspection and feeding

Bees should be inspected on alternate days before they have started laying eggs. The plexiglass feeding should also be replaced at this time, and dirty bars should be thoroughly washed in hot water. On the intervening days, the bar can just be topped-up using a squeeze bottle of sugar solution. If no eggs have been laid, the pollen lump is replaced; however, it is often also necessary to rearrange the upholsterer's cotton. If eggs have been laid, or are about to be laid, then small sausage-shaped lumps of the pollen dough are placed next to the incipient brood mass. Once a queen has 'started' then she must be inspected and fed every day, as she will continue to eat pollen herself and, of course, feed it to the developing larvae. If the queen is in the back box incubating her brood, then a gentle tap on this will usually bring the now agitated bee into the front box. The back box can then be move over a cm or two to block her return. Alternatively, the glass lid on the front box can be moved back a fraction followed by blowing on the entrance hole to bring the queen out. Pollen should be provided in a number of small lumps placed around the brood, and the total amount given should be equivalent to about one quarter the size of the brood clump. This is a rule of thumb that works quite well and avoids over-feeding. Any old, dried-up pieces of pollen are removed. If there are any wax pockets in the brood clump, as will be the case with the pocket-making species such as *B. atratus*, then pollen should be pushed into each pocket. This is essential if the species is a pocket-maker, because if this is not done, then the bees will not feed their larvae. With aggressive bees such as *B. atratus*, the feeding room can be kept in the dark and a red light used to feed and manipulate the bees as they cannot see this end of the light spectrum. Interestingly even some pollen-storer species will sometimes make pockets in their first-brood larval clumps, and so pollen should be provided in these pockets if present.

There are a number of signs that a queen may be about to lay eggs, or is starting to develop her ovaries; two important ones are that the pollen lump has been nibbled and that there is pollen in faeces. The latter can clearly be seen on the floor of the front box. Additionally a 'cavity' is often formed in the cotton in the back box, and this should not be disturbed if at all possible. Sometimes the pollen lump is covered with cotton, and then it should be left and a new one added rather than disturb the cavity. Sometimes wax will be deposited on the pollen lump, and also egg cell cups are also formed. In this case, the pollen lump should NOT be replaced, and only pollen sausages should be added around the wax. Construction of a honey pot will usually be started at the same times as or shortly after the queen has laid eggs. Some species, for example, *B. nevadensis*, start their honey pots *before* they actually lay any eggs. In nature, the storage of sufficient honey is crucial for the survival of the queen if there are a few cool, wet days in a row, so in addition to the honey pot, she will sometimes squirt honey on the cotton (**Figure 2**). This is often done at, or just before, egg laying. One drawback of upholsterer's cotton is that it tends to become very matted and sticky, and when this occurs, these patches should be removed. Finally the queen may remove the pollen lump and make

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egg cells directly on the cotton. In this case just add pollen bits around the cells and they will be incorporated into the growing brood clump by the queen.

6.4. Abnormal development

Sometimes a broody queen, or even one that has already started, will show abnormal behaviour. One particularly bothersome one is front box incubating (FBI) when she will incubate on the floor of the foraging box. If this is caught early on, it can be cured by putting wire screen (window screen with a fine mesh) down over the floor. This will usually induce the queen to move to the nesting box and lay her first brood eggs, or will resume their incubation if they are already laid. On the other hand, the condition can continue to worsen and the brood and colony is lost. Occasionally, a queen will deposit wax directly on the floor of the front box and lay her eggs there. In this case it is best to put some cotton around the brood clump and let it develop *in situ*, rather than try to move it into the nesting box. After this, perfectly normal development usually results.

6.5. Colony transfer

About three weeks after the first eggs have been laid, the first brood workers enclose, and the comb must soon thereafter be transferred to a larger nesting box for the remainder of the colony development. It is best to wait a few days until most, or all of the first brood workers have enclosed before moving the comb. Almost any type of larger nesting box will do, for instance a front box can be used. The comb is placed in the middle, and the rest of the space filled with upholsterer's cotton. This is best for colonies that are to be put outside and allowed to free-forage, but is not so convenient for colonies that are to be kept in the laboratory for observation and manipulation. In this case it is better to move the colony to a Porous Concrete Hive (a 'perlite' hive) as described by Pomeroy and Plowright [28]. The advantage of this type of hive is that no nesting material is required, and so these hives are ideal for laboratory based observation and manipulation. The hive can be lined with a cone of cardboard placed on the floor of the hive. This makes final removal of the comb and cleaning of the hive easier.

7. Conclusions

The commercial rearing and use of bumble bee colonies is essential for the production of many food crops. However, there are some serious drawbacks with their use which should be acknowledged.

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References

[1] Velthuis, H.H.W. and van Doorn, A. (2006) A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie* 37: 421–451.

[2] Hughes, M.J. (1996) Commercial rearing of bumble bees. In: Matheson A. (Ed.), *Bumble bees for pleasure and profit*. IBRA, Cardiff, pp. 40–47.

[3] Williams, P.H., Cameron, S.A., Hynes H.M., Cederberg, B., and Rasmont, P. (2008) A simplified subgeneric classification of the bumblebees (genus *Bombus*). *Apidologie*, 39: 46–74.

[4] Alford, D.V. (1975). *Bumblebees*. Davis-Poynter, London.

[5] Owen, R.E. and Whidden, T. L. (2013) Monandry and polyandry in three species of North American bumble bees (Hymenoptera: Apidae, *Bombus)* determined using microsatellite DNA markers. *Canadian Journal of Zoology*, 91: 523–528.

[6] Owen, R.E., Rodd, F.H., and Plowright, R.C. (1980) Sex ratios in bumble bee colonies: Complications due to orphaning? *Behavioral Ecology and Sociobiology*, 7: 287–291.

[7] Gurr, L. (1972) The introduction of bumblebees into North Island, New Zealand. *New Zealand Journal of Agricultural Research*, 15: 635–638.

[8] Ruz, L. (2002) Bee pollinators introduced to Chile: a review. In: Kevan, P. and Imperatriz Fonseca, V.L. (Eds.), *Pollinating Bees - The Conservation Link Between Agriculture and Nature*, Ministry of Environment, Brasília. pp. 155–167.

[9] Hingston, A.B., Marsden-Smedley, J., Driscoll, D.A., Corbett, S., Fenton, J., Anderson, R., Plowman, C., Mowling, F., Jenkin, M., Matsui, K., Bonham, K.J., Ilowski, M., McQuillan, P.B., Yaxley, B., Reid, T., Storey, D., Poole, L., Mallick, S.A., Fitzgerald, N., Kirkpatrick, J.B., Febey, J., Harwood, A.G., Michaels, K.F., Russell, M.J., Black, P.G., Emerson, L., Visoiu, M., Morgan, J., Breen, S., Gates, S., Bantich, M.N., and Desmarchelier, J.M. (2002) Extent of invasion of Tasmanian native vegetation by the exotic bumblebee *Bombus terrestris* (Apoidea: Apidae). *Austral Ecology* 27: 162–172.

[10] Yoon, H.J., Kim, S.Y., Lee, K.Y., Lee, S.B., Park, I.G., and Kim, I. (2009) Interspecific hybridization of the bumblebees *Bombus ignitus* and *B. terrestris. International Journal of Industrial Entomology*, 18: 41–48.

[11] Ings, T.C., Raine, N.E., and Chitka., L. (2005A) Mating preference in the commercially imported bumble bee species *Bombus terrestris* in Britain (Hymenoptera: Apidae). *Entomologia Generalis*, 28: 233–238.

[12] Ratti, C.M. and Colla, S.R. (2010) Discussion of the presence of an eastern bumble bee species (*Bombus impatiens* Cresson) in western Canada. *The Pan-Pacific Entomologist* 86: 29–31.

[13] Graystock, P., Yates, K., Evison, S.E.F., Darvill, B., Goulson, D., and Hughes, W.O.H. (2013) The Trojan hives: pollinator pathogens, imported and distributed in bumblebee colonies. *Journal of Applied Ecology* 50: 1207–1215.

[14] Villalobos, E.M. (2016) The mite that jumped, the bee that traveled, the disease that followed. *Science* 351: 554–556.

[15] Wilfert, L., Long, G., Leggett, H.C., Schmid-Hempel, P., Butlin, R., Martin, S.J.M., and Boots, M. (2016) Deformed wing virus is a recent global epidemic in honeybees driven by *Varroa* mites. *Science* 351: 594–597.

[16] Fürst, M.A., McMahon, D.P., Osborne, J.L., Paxton, R.J., and Brown, M.J.F. (2014) Disease associations between honeybees and bumblebees as a threat to wild pollinators. *Nature* 506: 364–366.

[17] Colla, S.R., Otterstatter, M.C., Gegear, R.J., and Thomson, J.D. (2006) Plight of the bumble bee: pathogen spillover from commercial to wild populations. *Biological Conservation* 129: 461–467.

[18] Szabo, N.D., Colla, S.R., Wagner, D.L., Gall, L.F, and Kerr, J.T. (2012) Do pathogen spillover, pesticide use, or habitat loss explain recent North American bumblebee declines? *Conservation Letters*, 5: 232–239. doi: 10.1111/j.1755-263X.2012.00234.x

[19] Otterstatter, M.C. and Thomson, J.D. (2008) Does pathogen spillover from commercially reared bumble bees threaten wild pollinators? *PLoS ONE* 3(7): e2771. doi:10.1371/journal.pone.0002771

[20] Schmid-Hempel, P. (2001) On the evolutionary ecology of host-parasite interactions: addressing the question with regard to bumblebees and their parasites *Naturwissenschafen* 88: 147–158.

[21] Keller, E.F. (1983) *A feeling for the organism*. W.H. Freeman & Co., New York.

[22] Plowright, R.C. and Jay, S.C. (1966) Rearing bumble bee colonies in captivity. *Journal of Apicultural Research*, 5, 155–165.

[23] Owen, R.E. (2001) Rearing bumble bee colonies. *Hivelights* 14: 15, 22–23.

[24] Owen, R.E. (1988) Body size variation and optimal body size of bumble bee queens (Hymenoptera: Apidae). *Canadian Entomologist*, 120: 19–27.

[25] Owen, R.E, Otterstatter, M.C., Cartar, R.V., Farmer, A., Colla, S.R., and O'Toole, N. (2012) Significant expansion of the distribution of the bumble bee *Bombus moderatus* (Hymenoptera: Apidae) in Alberta over twenty years. *Canadian Journal of Zoology*, 90: 133–138.

[26] Owen, R.E., Whidden, T.L., and Plowright, R.C. (2010) Genetic and morphometric evidence for the conspecific status of the bumble bees *Bombus melanopygus* and *B. edwardsii* (Hymenoptera, Apidae). *Journal of Insect Science* 10: 109.

[27] Owen, R.E. and Whidden, T.L. (2013) Discrimination of the bumble bee species *Bombus occidentalis* Greene and *B. terricola* Kirby by morphometric, colour and RAPD variation *Zootaxa*, 3608 (5): 328–344.

[28] Pomeroy, N. and Plowright, R.C. (1980) Maintenance of bumble bee colonies in observation hives (Hymenoptera: Apidae). *The Canadian Entomologist*, 112: 321–326.