Rusty Patched Bumble Bee (*Bombus affinis*) *Ex Situ* Assessment and Planning Workshop

FINAL REPORT

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MINNESOTA ZOO

IUCN SSC Species Survival Commission

CONSERVATION PLANNING SPECIALIST GROUP Changing the Future for Wildlife

IUCN SSC BUMBLEBEE SPECIALIST GROUP

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Rusty Patched Bumble Bee (*Bombus affinis*) *Ex Situ* Assessment and Planning Workshop

February 25-27, 2020
Minnesota Zoo

FINAL REPORT

2020 Workshop Participants

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Executive Summary

Rusty patched bumble bees (*Bombus affinis*) once occupied a variety of habitats across 29 states in the Upper Midwest and Northeast United States and 2 provinces in southeastern Canada (Macior 1968; USFWS Species Status Assessment 2016). The cause of the widespread precipitous decline of this species is unknown but may be due to a number of interacting stressors, including pathogens, pesticides and fungicides, habitat loss and degradation, and the effects of climate change and small population biology. A draft recovery plan for the rusty patched bumble bee (RPBB) was developed in September 2019 by the US Fish and Wildlife Service (USFWS 2019).

To support the goals and actions outlined in the recovery plan, the IUCN SSC Conservation Planning Specialist Group (CPSG) was requested to convene a multi-stakeholder workshop to assess the potential role(s) that *ex situ* management might play in contributing to the recovery of this species. Experts from governmental agencies, universities and the *ex situ* community met on February 25-27, 2020 at the Minnesota Zoo to conduct an *ex situ* conservation assessment for the rusty patched bumble bee.

Prior to the workshop, the following information was compiled: 1) status of and threats to wild *B. affinis* populations; 2) existing *B. affinis* draft recovery plan; and 3) existing expertise in *ex situ* management and reintroduction for *Bombus*. These were shared as briefing materials and/or as introductory presentations at the start of the workshop during plenary presentations. Participants discussed the impact of threats across the stages of the species' annual life cycle and identified important knowledge gaps in species biology, threats and their impacts, and population management.

Participants identified nine potential conservation roles for *ex situ* activities that might address conservation challenges and/or priority knowledge gaps for *B. affinis*. Four concurrent working groups discussed the relative conservation benefit(s) of these potential nine roles, as follows: 1) preventing population/species extinction (*Insurance Population; Rescue* roles); 2) reinforcing existing populations (*Population Reinforcement; Demographic Manipulation* roles); 3) establishing new populations (*Reintroduction; Assisted Colonization* roles); and 4) addressing knowledge gaps or behavior (*Research, Training, Conservation Education* roles). Each of these strategies involves either short-term or long-term care of at least one life stage in the annual cycle of this species. Feasibility and related issues were discussed in relation to acquisition, short- and long-term care, and release (where appropriate).

After plenary review of all considerations, four overall *ex situ* strategies were recommended for development and implementation. Working groups were convened around these four strategies for detailed discussions of the relative benefits, challenges and feasibility of different options for implementation. Important data gaps were also identified in each group to facilitate the development of appropriate research questions and investigations within various *ex situ* activities.

Recommended *ex situ*-related conservation activities for the Rusty Patched Bumble Bee:

1. Demographic supplementation of current *in situ* populations
2. Reintroduction to establish new *in situ* populations
3. Genetic supplementation of *in situ* populations
4. *Ex situ* insurance population (with potential associated education opportunities)

Based on these evaluations, working groups developed objectives and recommended action steps to begin implementation of these four *ex situ* strategies. These objectives and actions were presented and revised during final plenary discussion and are given in this report.

Species Status and Challenges

The rusty patched bumble bee, *Bombus affinis*, is listed as Endangered by the US Fish and Wildlife Service (USFWS) and by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). This species has declined by 87% in the last 20 years and is likely to be present in only 0.1% of its historical range (USFWS 2019). The cause of the widespread precipitous decline of this species is unknown but may be due to a number of interacting stressors. These may include pathogens and parasites, pesticides and fungicides, habitat loss and degradation, managed bees, and the effects of climate change and small population biology.

While most workshop participants were familiar with the *in situ* status of the rusty patched bumble bee, Elaine Evans (University of Minnesota) and Tamara Smith (USFWS) gave a plenary presentation to bring all workshop participants up to speed on the current distribution of and pressures on the wild population. Additional presentations were given by Ben Sadd (Illinois State University) on pathogens and parasites and by James Strange (The Ohio State University) on genetics, both in relation to collection, *ex situ* rearing and release. Insights into *ex situ* management were provided by Genevieve Rowe (Wildlife Preservation Canada). A case study of a reintroduction attempt of *Bombus subterraneous* in the United Kingdom was presented remotely by Nikki Gammans (Bumble Bee Conservation Trust). Summaries of these presentations follow or can be found in Appendices III and IV of this report.

Bombus affinis population pressures, status and distribution

Provided by Tamara Smith (status and plan) and Elaine Evans (life history)

Status

Historically, the rusty patched bumble bee *B. affinis* was distributed across the eastern United States and Upper Midwest and north to southern Quebec and Ontario in Canada (USFWS 2016). *B. affinis* has undergone a widespread and rapid decline since the late 1990s (USFWS 2016), and the USFWS listed *B. affinis* as endangered on January 11, 2017 (82 FR 3186). In Canada, the species was federally listed as endangered under the Species At Risk Act (SARA) on June 20, 2020; however it is believed to be extirpated as it has not been observed in the country since 2009 despite annual surveys of historic locations since 2013 (Wildlife Preservation Canada, unpublished data).

Draft Recovery Plan

The Service drafted a recovery plan for *B. affinis* (USFWS 2019). The recovery strategy focuses on a sequence of first halting declines, then reversing declines, and ultimately securing the long-term viability of the species across a specified range. To achieve long-term viability, the species must endure the pressures of: 1) environmental stochasticity; 2) stressors; 3) catastrophes; and 4) novel changes in its environment, which requires multiple, healthy populations widely distributed across the breadth of adaptive diversity.

The draft plan includes three downlisting criteria. The first criterion gives a minimum number of populations[1] and their distribution across five geographically defined Conservation Units needed to preserve the breadth of genetic and ecological diversity, thereby maintaining the species' ability to adapt to a changing environment. Of those populations, the draft plan recommends that a minimum number of populations are documented to be healthy (Criterion 2). Criterion 2 gives some insights on how to measure healthy populations, but the methods to measure and assess population health will be refined throughout the Recovery Implementation Strategy process. Generally, healthy populations may be documented through consistent detection, genetics, and pesticide (and fungicides) and pathogen loads. Criterion 3 gives guidance regarding the spatial arrangement of populations. Population clusters are needed within each Conservation Unit to foster gene flow between populations, which in turn, facilitates demographic rescue and ensures genetic health and adaptability of populations.

Several broad actions are identified in the draft recovery plan (USFWS 2019). One of those actions is specific to population management – increasing the number and distribution of populations, increasing effective population size, implementing captive rearing, and conducting research on aspects of populations to improve their health and numbers. In addition to population management and other actions, investigating key uncertainties will be vital to a successful recovery program and some of these uncertainties may be answered through *ex situ* programs.

Published Recovery Strategies for this species exist in Canada at both the federal and provincial (Ontario) level. The US Recovery Plan also includes Canada.

Stressors

We are currently aware of six primary stressors for remaining *Bombus affinis* populations: 1) habitat loss; 2) pesticides and fungicides (agrochemicals); 3) pathogens and parasite;, 4) climate change; 5) managed bees; and 6) small population size. These stressors do not act alone but have many combined effects. Habitat loss and small population size exacerbate the effects of most other stressors due to strains on immune function and detoxification capacity, limitation of movement, and concentration of populations in smaller areas of appropriate habitat. In general, we don't know which stressors have the strongest impact on bumble bee populations overall or *B. affinis* populations in particular.

To determine population impacts, an examination of impacted life stages can be useful in determining if there is a life stage where most mortality occurs and what causes that mortality. Bumble bees have four life stages: egg, larva, pupa, and adult. In general, the egg and pupal stage have higher survival rates, and most mortality occurs with larvae and adults. However, in looking at population impacts, individual survival does not connect directly with population effects as the entire colony is the reproductive unit. Bumble bee populations depend on the success of individual colonies rather than individual bees. Distinct points in colony development to examine for impacts on populations are 1) overwintering queen survival; 2) successful nest founding by individual queens; 3) colony growth; 4) reproductive production; and 5) reproductive dispersal and mating. The following is an examination of the impact of the above-mentioned stressors on these colony stages.

Overwintering queen survival

[1] A population is a collection of tens to hundreds of colonies. For monitoring the number of populations over time, a population is a single 10 x 10 kilometer (km) grid. Population grids were delineated by overlaying 10 x 10 km grids across the range of rusty patched bumble bee and assigning a unique numerical identifier to each 10 x 10 km grid (for further explanation see USFWS 2016, p. 11).

There is very little documentation of baseline overwinter survival rates. It is possible that climate change and pesticides and fungicides in the soil could negatively impact the survival rates of overwintering queens (Anderson & Harmon-Threatt 2019; Bale & Hayward 2010. The nutritional status and the pathogen load of queens entering diapause will impact their survival (Woodard *et al*. 2019). While we have documentation of habitat preference of overwintering for some bumble bees (Alford 1969), there are only a few documented overwintering *B. affinis* and not enough samples to indicate overall habitat preference. There is some anecdotal evidence of overwintering in forested habitats, for example an overwintering *B. affinis* queen, discovered in a maple oak-woodland in Wisconsin in 2016, was found under a few centimeters of leaf litter and loose soil (Herrick 2016, pers. comm.).

Nest searching and founding

We have some indication that *Bombus* queens found nest sites 600 m to 5 km from their natal nests (e.g., Lepais *et al*. 2010; Makinson *et al*. 2019; Mola *et al*. n.d.; Mola & Williams 2019; Webb 1961), but are lacking specifics for spring queen dispersal for *Bombus affinis*. Lack of floral resources or the contamination of those resources with pesticides and fungicides could be significant stressors for nest searching queens. There is not a great deal of documentation of *Bombus affinis* preferred nesting habitat (but see Hobbs 1968; Laverty & Harder 1988; Plath 1922), but there is an assumed loss of preferred habitat due to agricultural intensification and urbanization. Decreased availability of preferred nesting sites and insufficient floral resources due to habitat loss will decrease nest founding success. There is evidence of nest site limitation for bumble bees generally through multiple gynes attempting to use the same nest, but the importance of that nest site limitation to bumble bee population structure is unknown. Queen-specific nematode parasites, including *Sphaerulia bombi,* are well documented across many bumble bee species and can greatly reduce the success of infected nest founding queens (Lundberg & Svenson 1975). The prevalence of parasites and pathogens in nest founding *B. affinis* are undocumented. Climate change may impact early spring foraging *B. affinis* queens through aberrant and severe weather events.

Colony growth

Colony development is well studied and has been clearly tied to the availability of floral resources, with hampered growth leading to a loss of reproductive output (Carvell *et al.* 2008; Williams *et al.* 2012). Lack of access to sufficient floral resources through habitat loss or competition from managed bees could hamper colony growth. Pesticides also can negatively affect colony development (Bernauer *et al.* 2015). Some parasites and pathogens are known to hamper colony growth and are associated with declining bumble bee species (Cameron *et al*. 2011), but specific impacts in *B. affinis* colonies is undocumented. Managed bees can also impact colony growth through pathogen spillover through shared flower use (Alger *et al*. 2018; Durrer & Schmid-Hempel 1994), which tends to be more prominent later in the season.

Reproductive production

Reproductive castes are typically produced at the end of the annual colony lifecycle, with *B. affinis* typically producing males and new queens in late July through early September. Colonies are only able to produce queens when they have access to sufficient pollen resources and have a large enough work force to bring enough resources back to the nest (Pelletier & McNeil 2003; Schmid-Hempel & Schmid-Hempel 1998). Reproductive production can be affected by all the above-mentioned stressors, as any of them can result in reductions of the workforce available to bring pollen back to the nest. Stress to earlier stages of colony development can have the greatest impact on future reproductive success (Malfi *et al*. 2018).

Reproductive dispersal and mating
There is little information on the dispersal distance of males before mating (but see Kraus *et al*. 2009) and of newly produced gynes before overwintering, with the exception of species documented hibernating close to their natal nest. Small populations are the stressor of highest concern for this stage due to of the potential of insufficient genetic diversity in the population, increased difficulty in finding a mate, and the possible production of sterile/inviable diploid males homozygous at the sex determination allele. Bumble bees as well as other bees, wasps, and ants, have a single-locus haplodiploid sex determination mechanism. Males, which are usually produced from unfertilized, haploid eggs, can also be produced from fertilized, diploid eggs when there is homozygosity at the sex-determining locus. These diploid males are sterile and can increase the risk of a rapid extinction vortex (Zayed & Packer 2005).

Considering pathogens and parasites in collecting and rearing bumble bees

Provided by Ben Sadd, Illinois State University

Pathogens and parasites may be impossible to eliminate during rearing of individuals and populations sourced from the wild, but it is essential that careful consideration be given to the threats they pose and the minimization of these. For North American bumble bee species there is an association between decline in status and the prevalence of the pathogen *Nosema bombi* (Cameron *et al.* 2011). Although not analyzed in the study, due to a low sample size, this pattern appeared to be consistent in *Bombus affinis* with 50% infection of collected samples. Currently, we do not know that the relationship between *Nosema* and decline is causal; however, we lack information to assume safety for any pathogen or parasite. We know infection outcomes are determined by host identity and status, pathogen or parasite, and the environment. Infection outcomes can be altered by pesticide exposure, nutrition, and other factors (see examples in Cameron & Sadd 2020). Additionally, caution needs to be exercised when extrapolating results across host species. Most studies in bumble bees have been performed in common non-declining species, *B. impatiens* and *B. terrestris*, and findings may not be universally applicable to other species (Cameron & Sadd 2020).

Bumble bees are host to an array of pathogens and parasites, most of which are transmitted between individuals in social colonies, and between bumble bees and other bee community members at flowers (Koch *et al.* 2017). They include the trypanosome *Crithidia spp.*, microsporidian *Nosema bombi*, neogregarine *Apicystis bombi*, nematode *Sphaerularia bombi*, and a suite of RNA viruses traditionally considered honey bee viruses. *Crithidia* is widespread but patchy in its occurrence and is considered to have context dependent virulence (Sadd & Barribeau 2013). *Nosema bombi*, a spore producing, predominantly larval-infecting pathogen, has been linked with declines, as described above. It can have severe consequences for bee health (Otti & Schmid-Hempel 2007, 2008), but, importantly for detection and containment, hidden infections occur (Blaker *et al.* 2014). *Apicystis bombi* is an infrequent but widespread pathogen, and likely a generalist bee pathogen (Plischuk *et al*. 2011). Studies have been limited, but it appears its virulence is variable, but can be very high, and early death of infected queens may severely compromise successful colony foundation (Mullins *et al.* 2019; Rutrecht & Brown 2008). Similar negative effects on colony founding result from *S. bombi* infection, with infected queens emerging from over-wintering, foraging, but then returning to an over-wintering site where they eventually die. This makes this parasite essentially a queen castrator (Rutrecht & Brown 2008). Of recent concern is the spillover of RNA viruses from managed honey and other bees, into wild species (e.g., Alger *et al.* 2019; Singh *et al.* 2010). The effects of these viruses on bumble bee health can be substantial (Piot *et al.* 2016), but remain largely unexplored.

Of note for captive rearing are prior findings from commercial colonies and the collapse of commercial captive rearing of *B. occidentalis* in the 1990s associated with pathogen infection. Poor rearing and maintenance practices can enhance disease transmission, and it has been documented that commercially available bumble bee colonies carry many common pathogens (Graystock *et al.* 2013). Pathogens and parasites present various challenges to maintenance and rearing of bumble bees:

1) Infection may affect efficiency of rearing by reducing queen colony initiation, worker production and survival, and ultimately male and new queen numbers and quality.

2) Amplification of infections under intense rearing may occur from increased transmission and reduced resistance under stressful conditions, resulting in increased prevalence and intensity of infections, and associated consequences.

3) Evolution of increased virulence could result from ease of between-host transmission (see 2) relaxing constraints on the evolution of pathogen virulence.

4) Spillover or other ecological consequences to wild populations resulting from accidental/intentional releases of infected individuals.

5) Determining risk associated with different pathogens for points 1-4 above needs to be addressed, as it may not be possible to clear infections entirely.

The above challenges can be mitigated by following certain best practices:

Ensuring that starting individuals are as pathogen and parasite free as possible. If spring queens are collected, they should be collected early and before substantial opportunities for pathogen exposure have occurred post-overwintering.

Diagnostic monitoring should take place on initial source populations and individuals, continually through maintenance, before any release, and in sink populations (where relevant). Dead bees should be screened but also apparently healthy individuals. Non-destructive sampling can be achieved through feces for many common pathogens and parasites with varying accuracy, using either microscopy or molecular approaches.

Reducing contamination and transmission between bees and colonies. During rearing, aseptic technique should be used, with sterilization of materials and tools between isolated bees and colonies. Waste material should be autoclaved and disposed or incinerated. Reusable supplies and surfaces should be autoclaved where possible or disinfected with bleach. Measures could include sacrifice of colonies or bees considered high risk. Additionally, where it doesn't compromise conservation goal success severely, quarantine is recommended.

Pathogen risk should be classified through carefully maintained records, results of past research, and continuing research. Classification should be on a low to high risk scale relating to the challenges above, with the precautionary principle being employed throughout.

Genetic considerations for captive rearing and release

Provided by James Strange, The Ohio State University

Captive rearing and release of individuals requires both genetic and demographic considerations when sourcing, propagating, and, ultimately, releasing individuals back into wild populations. Further complicating decision making are issues of population augmentation versus population translocation (Lozier *et al.* 2015). Herein are outlined several key considerations when sourcing populations for captive rearing and potential issues related to release of captive bred individuals, but also recognize that there are many unknown factors that may arise in *ex situ* rearing operations.

Removing queens from wild populations to stock a captive population has the potential to impact both the source and the *ex situ* population. Prior to establishing a laboratory population, it is beneficial to understand both the diversity and structure of wild populations and the goals of the *ex situ* program. Various genetic characteristics of the source stock should be considered, such as existing genetic structure and gene flow, genetic diversity (within populations and globally), and potentially adaptive or deleterious genetic traits that can be selected through breeding (Beekman *et al*. 2000). With bees in general, one major consideration is obtaining a starting population that is significantly large enough and diverse enough to maintain sex allele diversity (Garofalo & Kerr 1975; Rinderer 2013) if captive breeding is considered. Ultimately, the diversity possible in *ex situ* populations is dependent on how much genetic diversity exists in the source populations (Lye *et al*. 2011). Finally, when conserving a species such as *B. affinis*, which has a broad historic range, it is necessary to also consider how many source populations to source stock from for the *ex situ* operation. Lozier *et al*. (2011) found variable levels of range wide population structure in several bumble bee species, but to date no such data exist for *B. affinis*.

Few studies in invertebrate systems have investigated the impact of removing individuals from wild populations. Theoretically, removing queens from wild populations for initiating rearing operations can have impacts on source populations. Specifically, the number of queens that are removed from a wild population could have impacts on the viability of the remaining individuals in that wild population, especially in cases where only a few wild individuals exist. Experience in rearing the closely related species, *Bombus occidentalis,* suggests that an expected nest initiation percent could be 50-75% in an experienced lab situation (Tripodi & Strange 2019) and thus to establish a captive population of 8-10 unrelated nests in the first year a minimum of 16 queens should be used to establish the breeding stock. However, in some species the rate that wild queens initiate nests is much lower (Strange 2010) and so those values should be viewed as a minimum to establish a year-round rearing scheme. Fewer colonies would be acceptable if the goal was to seasonally rear and release colonies. Macfarlane *et al*. (1994) found that five wild *B. affinis* nests produced an average of 181 new gynes annually, and this suggests that sourcing 16-20 individuals from a wild population that contained five or more nests would represent about 2% of the potential gynes in the population. Experience in lab rearing suggests that these should be viewed as minimum values and a larger initial number of gynes would increase the chances for success in establishing an *ex situ* population.

Key genetic and demographic considerations for *ex situ* rearing bumblebees

These considerations would benefit any *ex situ* rearing program, however gain particular importance if the goal is year round rearing and translocation of offspring colonies.

- **Colony estimation per population**. Prior to sourcing wild *B. affinis* queens to establish *ex situ* colonies, the potential source populations should be sampled and assessed for the number of colonies in those locations. Estimating the number of colonies that are providing queens to extant

populations will allow for sustainable removal of queens and establishment of diverse lab populations.

- **Historic assessment of genetic structure and diversity**. Determining historical range-wide population structure can inform the potential for translocation of extant populations throughout the historical range.
- C**urrent assessment of genetic structure and diversity**. Estimating current population structure and diversity will inform which populations to source stock for establishment of lab colonies.
- **Complementary sex determination locus diversity.** Understanding the current sex allele diversity in *B. affinis* will help to ensure *ex situ* populations can be maintained year-round.
- **Functional trait diversity**. Discovering locally adapted genetic traits would allow to target translocation to areas with a targeted approach, increasing the likelihood that reintroductions would be successful.
- **Dispersal range.** Quantifying the dispersal range of gynes and males would inform the release/reintroduction strategies that could be best applied to *B. affinis*.

Ex situ management

Provided by Genevieve Rowe, Wildlife Preservation Canada

Ex situ conservation tools are rare for invertebrates and have not been implemented for bumble bees in North America. Many *ex situ* rearing techniques have been trialed, but overall success rates remain low for most species when a complement to the natural annual bumble bee cycle is sought. Major challenges in the majority of *ex situ* bumble bee rearing programs lie in promoting broodiness and colony initiation in wild-caught queens and in the successful overwintering of gynes. The protocol document in Appendix III outlines some current methods for rearing bumble bees *ex situ*. These novel methods have helped minimize husbandry requirements and mitigate pathogen transfer in captive rearing programs, but they have not been trialed using *B. affinis* and success with surrogate species (e.g., *B. terricola*) has been variable.

A draft captive rearing protocol can be found in Appendix III of the expanded (full) version of this report, which is available at: www.cpsg.org

Attempted reintroduction of short-haired bumblebee, *Bombus subterraneous*, to the UK

Provided by Nikki Gammans, Bumble Bee Conservation Trust

Available in Appendix IV of the expanded (full) version of this report available at: www.cpsg.org

Ex Situ Assessment Process and Conservation Roles

Effective species conservation planning should consider all options when assessing actions to address the conservation pressures facing a particular species. In addition to actions directed at reducing or eliminating particular threats, such as habitat loss or illegal poaching, other management strategies may be needed to prevent severe decline or extinction, especially when wild populations are small and isolated. Addressing important knowledge gaps also can promote more effective conservation. *Ex situ* management is one possible option that can contribute to the conservation of threatened species. The range of *ex situ* scenarios and tools is diverse and can target different conservation needs and roles and, therefore, serve various purposes.

Ex situ conservation activities can support species conservation and prevent extinction in a variety of ways (Traylor-Holzer *et al*. 2019), by:

Offsetting the impact of threats. *Ex situ* activities can improve the demographic and/or genetic viability of a wild population by counteracting the impacts of primary or stochastic threats on the population, such as reduced survival, poor reproduction and genetic isolation – for example, through head-start programs that remove juveniles from the wild for *ex situ* care and return them once they are less vulnerable, or through releases to genetically augment isolated populations.

Addressing the causes of primary threats. *Ex situ* activities can help reduce primary threats such as habitat loss, exploitation, invasive species, or disease through specifically designed research, training or conservation education activities that directly and effectively impact the causes of these threats – for example, through *ex situ* research to detect, combat or treat disease.

Buying time. Establishment of a genetically diverse and sustainable *ex situ* rescue or insurance population may be critical in preventing species extinction when the wild population is declining and primary threats are not under control – for example, populations facing widespread disease epidemics or decimation by invasive species.

Restoring wild populations. Once the primary threats have been sufficiently addressed, *ex situ* populations can be used to re-establish wild populations.

This workshop focused on the assessment of *ex situ* activities for the rusty patched bumble bee (*Bombus affinis*) and the ability of such activities to contribute effectively to its conservation and recovery in the wild. This assessment was developed in concert with the strategies and actions outlined in the *USFWS Draft Recovery Plan for Rusty Patched Bumble Bee* (USFWS 2019) as part of a One Plan approach to conservation of this species (Traylor-Holzer *et al*. 2019). The workshop was structured around the IUCN SSC *Guidelines on the Use of Ex situ Management for Species Conservation*, which utilizes a five-step decision process to determine if and which ex situ activities might be appropriate to be included in the overall conservation strategy for the species (IUCN 2014; McGowan *et al*. 2017):

1) Conduct a thorough status assessment (of both *in situ* and any known *ex situ* populations) and threat analysis;
2) Identify potential roles that *ex situ* management can play in the overall conservation of the species;
3) Define the characteristics and dimensions of the program needed to fulfill the identified potential conservation role(s);

4) Define the resources and expertise needed for the *ex situ* management program to meet its role(s) and appraise the feasibility and risks; and
5) Make an informed and transparent decision as to which *ex situ* roles and activities (if any) to retain within the overall conservation strategy of the species.

This evaluative process was applied to the rusty patched bumble bee (*B. affinis*) through plenary and small group discussions. An essential element of this process is the involvement of both *in situ* and *ex situ* species experts in all stages of the evaluation to fully evaluate conservation needs and opportunities.

Workshop process for the rusty patched bumble bee

Prior to the workshop, the following information was compiled: 1) status of and threats to wild *B. affinis* populations; 2) existing *B. affinis* recovery plans; and 3) existing expertise in *ex situ* management and reintroduction for *Bombus* spp. These were shared as briefing materials and/or as introductory presentations at the start of the workshop during plenary presentations. Participants discussed the impact of threats across the stages of the species' annual life cycle and identified important knowledge gaps in species biology, threats and their impacts, and population management.

Participants reviewed the list of potential conservation roles for *ex situ* activities (see Appendix II for full list) to identify those that might address conservation challenges and/or priority knowledge gaps for *B. affinis*. Nine *ex situ* conservation roles were identified as potentially applicable for *B. affinis* and meriting further exploration. Four concurrent working groups discussed the relative conservation benefit(s) of these potential nine roles, as follows:

- Preventing population/species extinction: *Insurance Population; Rescue* roles
- Reinforcing existing populations: *Population Reinforcement; Demographic Manipulation* roles
- Establishing new populations: *Reintroduction; Assisted Colonization* roles
- Addressing knowledge gaps or behavior: *Research, Training, Conservation Education* roles

Each of these strategies involves either short-term or long-term care of at least one life stage in the annual cycle of this species. Feasibility and related issues were discussed in relation to acquisition, short- and long-term care, and release (where appropriate).

After plenary review of all considerations, four overall *ex situ* strategies were recommended for development and implementation. Working groups were convened around these four strategies for detailed discussions of the relative benefits, challenges and feasibility of different options for implementation. Important data gaps were also identified in each group to facilitate the development of appropriate research questions and investigations within various *ex situ* activities.

Recommended *ex situ*-related conservation activities for the Rusty Patched Bumble Bee:

1. Demographic supplementation of current *in situ* populations
2. Reintroduction to establish new *in situ* populations
3. Genetic supplementation of *in situ* populations
4. *Ex situ* insurance population (with potential associated education opportunities)

Based on these evaluations, working groups developed objectives and recommended action steps to begin implementation of these four *ex situ* strategies. These objectives and actions were presented and revised during final plenary discussion. Final recommendations are given in this report.

Potential *Ex Situ* Conservation Roles

Nine *ex situ* conservation roles were identified as potentially applicable for the rusty patched bumble bee. These can be grouped into four categories based on their conservation impact.

Prevent population / species extinction

Insurance Population: Maintenance of a long-term *ex situ* population to serve as a genetic backup to the wild population to prevent local, regional or global species extinction and preserve options for future conservation strategies.

Rescue: Collection of a colony or population that is under imminent threat of extinction. Conservation value may depend upon the uniqueness of the population based on its location, size, local adaptations, and genetic diversity. Rescued populations may be relocated to other habitat or retained to support an insurance population and/or other *ex situ* conservation roles.

Reinforce existing populations

Population Reinforcement: Source population to provide individuals to supplement an existing wild population to improve its demographic and/or genetic viability.

Demographic Manipulation: Similar to reinforcement, but releases are targeted to improve a vulnerable demographic rate (e.g., early survival) or status (e.g., adult sex ratio) in the wild.

Establish new populations

Reintroduction: Source population to provide individuals to re-establish the species to part of its former range to increase population viability, expand range, increase redundancy, and increase functional genetic diversity and restore adaptive capacity.

Assisted Colonization: Source population to provide individuals to establish the species in suitable areas outside of its historical range, in response to habitat shifts due to threats such as climate change. This provides similar conservation benefits as reintroduction.

Address knowledge gaps or change behavior

Research: Use of an *ex situ* population or activities for research that will directly benefit conservation of the species, or a similar species, in the wild (e.g., genetics, disease, sensitivity to threats).

Training: Use of an *ex situ* population or activities for training that will directly benefit conservation of the species, or a similar species, in the wild (e.g., handling, husbandry, monitoring, release).

Conservation Education: Use of an *ex situ* population or activities to support an education and awareness program that addresses specific threats or constraints to conservation of the species or its habitat (e.g., promote buy-in by land managers and other stakeholders).

Implementation of *Ex Situ* Conservation Roles

Nine *ex situ* conservation roles were identified as potentially applicable for the rusty patched bumble bee. These roles can provide conservation benefit to species in one of four ways:

Conservation Benefit	*Ex Situ* Roles
Preventing population/species extinction	*Insurance Population, Rescue*
Reinforcing existing populations	*Population Reinforcement, Demographic Manipulation*
Establishing new populations	*Reintroduction, Assisted Colonization*
Addressing knowledge gaps or behavior	*Research, Training, Conservation Education*

All of these *ex situ* conservation roles except for Conservation Education involve the acquisition of wild rusty patched bumble bees (and/or their biosamples) and the short- or long-term maintenance of bees and/or biosamples in *ex situ* conditions. In many cases, the role also includes the release of individuals to the wild. These implementation phases can be categorized as *Acquisition*, *Ex Situ Management* and *Release*, and may involve the same individual or may cross generations.

Figure 1 illustrates the general categories of source populations during *Acquisition*, two types of *Ex Situ Management*, and four categories of recipient populations or habitat during *Release*. These categories can be mapped to the potential *ex situ* conservation roles above. Population rescue is distinguished primarily by source population traits, insurance populations are defined primarily by length and type of *ex situ* management, and different types of population restoration are defined primarily by the traits of any existing populations and habitat (see Appendix II). Acquisition, *Ex Situ* Management and Release phases all provide opportunities for valuable research and training to address knowledge gaps, while *ex situ* populations provide educational opportunities to influence behavior change.

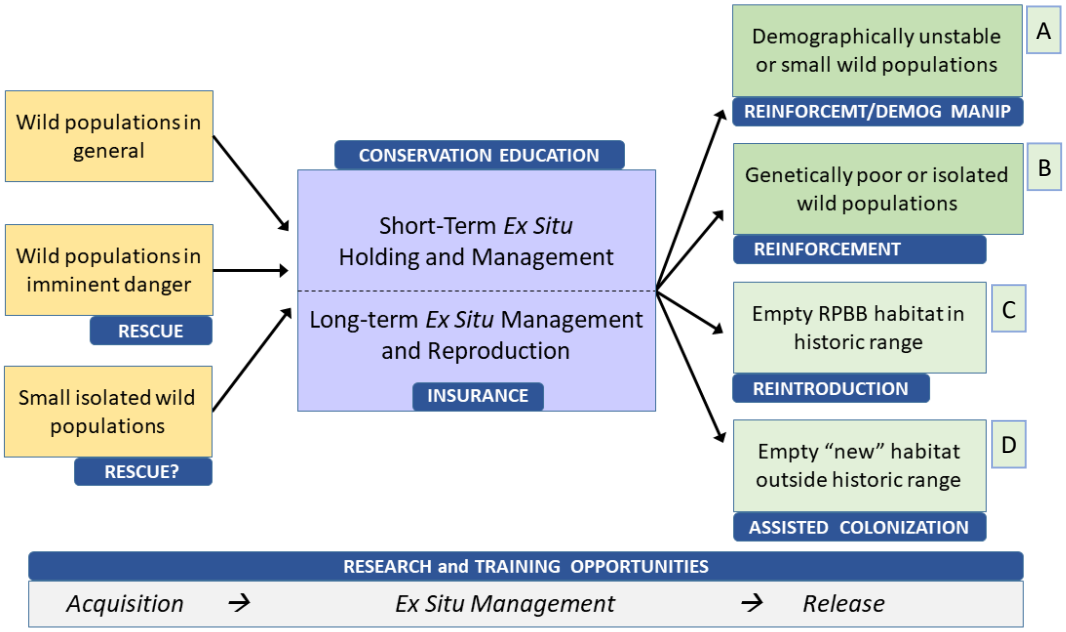

Figure 1. Diagram illustrating the three phases of *ex situ* conservation, with different source populations (yellow), *ex situ* management lengths (purple), and recipient populations (green) or habitats (light green). Associated *ex situ* conservation roles are indicated in dark blue.

A major consideration for all three phases of implementation is which life stage or stages should be considered. This question led to detailed discussions centered around the annual cycle of the species, as the colony cycles through its annual solitary (queen) and more social (colony) phases and forms (Figure 2). Workshop participants did not identify major differences in vulnerability to threats across this annual cycle, leaving other considerations such as feasibility and risks to drive the discussion.

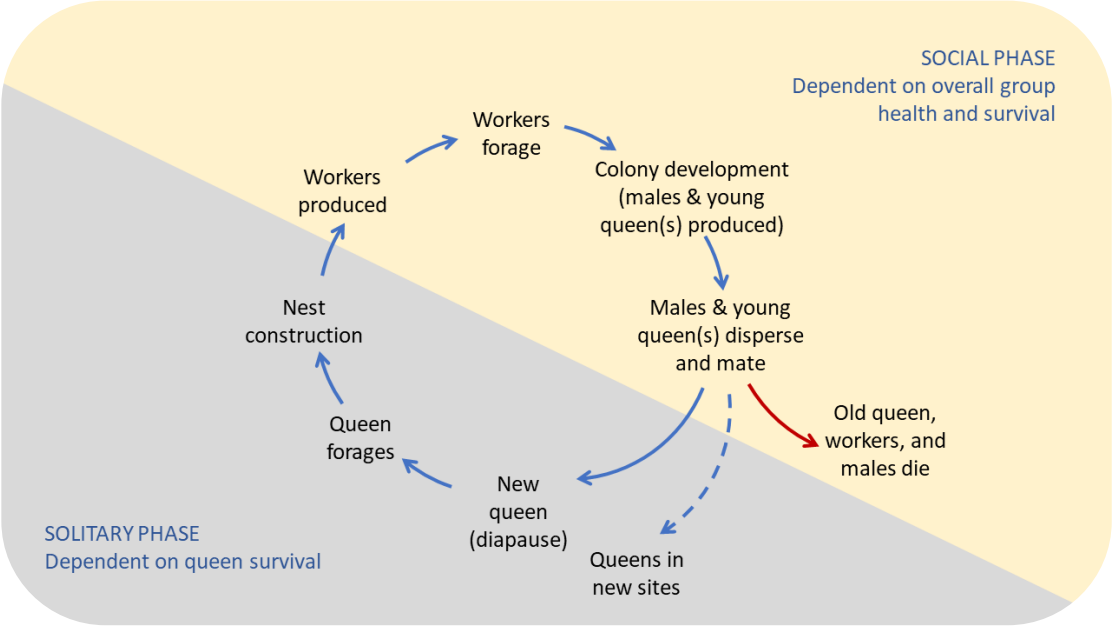

Figure 2. Diagram of annual cycle of rusty patched bumble bees discussed at the workshop, illustrating events during the primarily solitary and social phases and how these phases relate to colony survival.

To better evaluate the benefits, risks and feasibility of options, working groups discussed different scenarios with regard to which individuals to collect, how and how long to maintain them, and which individuals to release. It was recognized that in some cases, such as the emergency rescue of a doomed colony, there may be constraints. In most cases, however, a systematic approach is feasible and desired. Considerations for the Acquisition and Release phases can be found in the following section.

Most of the *ex situ* conservation strategies recommended by workshop participants (e.g., demographic supplementation, genetic supplementation, reintroduction) could be accomplished using either short-term or long-term *ex situ* care. The requirements for long-term care of bumble bees are not currently well understood and were explored by the insurance population working group (see *Role 4: Insurance Population*). A detailed analysis of short-term care options and feasibility was performed in small group work, including careful consideration of working with different life stages and number of individuals (see *Short-Term Holding and Ex Situ Management Strategies* section).

Workshop participants recognized that any individuals or biosamples held *ex situ* also may support research to better understand life history, genetic structure or the impact of threats to wild *B. affinis* populations as well as improve *ex situ* management practices.

Acquisition considerations

The capture of spring (mated) queens was considered to be the most feasible option in terms of management and conservation value. Anticipated challenges to this strategy are detection of wild spring queens and identification of appropriate source populations. Males also might be considered for genetic supplementation or if needed for demographic manipulation.

The number of bees to be removed depends upon the resulting impact on the source population, management feasibility, and the needs of the recipient population(s). The goal is to minimize risk to the wild source population while providing benefits to the recipient population. The impact of removing individuals is poorly understood. Important knowledge gaps to address before the Acquisition phase are:

- Source population size and genetic structure/variation
- Recipient population demographic and genetic needs (e.g., % diploid males)

Understanding the demographic status of wild *B. affinis* populations will support the identification of source populations and also recipient populations in need of, and likely to benefit from, supplementation.

Situations may occur in which bees are removed from source populations at risk. For example, consideration may be given to the removal of individuals from very small, threatened colonies that are unlikely to survive. An emergency plan could be developed to rescue colonies in the future that are under imminent threat.

Removed bees, either as rescued colonies or for population restoration, could be directly translocated or reintroduced (with little time spent *ex situ*), or they could be held *ex situ* for longer periods. Screening and quarantine protocols will help reduce risks (e.g., disease transmission).

Release considerations

Similar concerns regarding life stage and number of individuals were expressed with regard to releases. An important first step is to define what is considered to be a healthy population; this then will help determine which populations are in need of demographic or genetic supplementation and how best to accomplish this effectively. Local adaptations should be considered, which might entail using a source population of local provenance. Another approach would be to fill in genetic gaps between small isolated populations

Reintroduction is assumed to require more individuals to be released and representing more genetic variation than reinforcement, as this strategy is establishing a new population rather than “filling in the gaps” of an existing population. Establishing a new population between existing populations may promote connectivity and allow for eventual intermixing.

Reinforcement of existing populations may be the most logical approach if there are populations with low genetic diversity or number of individuals. Preference was expressed for releasing spring queens over fall queens, as this strategy offers the benefit of shorter *ex situ* holding requirements and requires fewer released queens as there is no need to account for overwinter losses. If spring (mated) queens are used, releases should occur prior to egg laying. If fall queens are used, they should be mated (caged mating) prior to release.

The option to relocate an entire colony was also discussed. An experimental framework could support better monitoring. Potential protection of relocated colonies (e.g., netting) was discussed.

Short-term holding and *ex situ* management strategies

Working group members: Rob Jean, Jonathan Koch, T'ai Roulston, Tamara Smith, Seth Stapleton, Jamie Strange

Definition of short-term ex situ holding and management strategy: The strategy of short-term holding and *ex situ* management of *B. affinis* is the process of holding and managing *B. affinis* individuals during **a portion** of the colony life cycle (involves less than all steps of the complete colony cycle). The ultimate goal of this strategy is to return reproductive individuals (queens and males) into the wild.

Strategy assumptions: We can safely acquire the bees, maintain them in **short-term** culture, and release individual bees (Scenarios 1 - 7) or colonies (Scenario 8) into the wild. Evaluation of these eight scenarios by the working group are given below. Estimated conservation impacts (A, B, C, D) for each scenario refer to Figure 1 and can be summarized as:

Conservation Impact A: Demographic supplementation of wild populations in need
Conservation Impact B: Genetic supplementation of wild population in need
Conservation Impact C: Reintroduction into empty bumble bee habitat (former range)
Conservation Impact D: Assisted colonization into empty habitat outside of indigenous range

Scenario 1: **Spring Queens -> Lab -> Reproductives Produced -> mated/unmated release in Fall**

In this scenario, spring queens are brought into a rearing facility/laboratory, where the queen is expected to develop a colony and produce reproductive individuals (females and males). The reproductive individuals would be released in the fall, either as mated or unmated individuals.

1. *Risks:* Immediate reduction of population reproductives from the wild, survivorship is less than x% (wild survivorship, estimate TBD).
2. *Feasibility:* Moderate. For genetic augmentation, success is simply adding alleles to the population; translocation success is any release that leads to establishment.
3. *Conservation Impact:* Increased numbers of bees; increased number of alleles, reestablishment or establishment of new site; captive colonies available for research needs (Conservation Impact A, B, C, D)
4. *Scope:* Local, regional or international; could have public display potential; # of facilities could vary, location is open question at this point and probably driven by purpose of project; requires a high level of husbandry experience
5. *Population:* depends on the outcomes desired, but probably need minimum of 10 spring queens to make this worth the effort (assuming 30% nest establishment rate); however, even one colony will provide a significant research benefit; length of the program is April to September annually.

Scenario 2: **Fall Queens + Fall Males (to increase mating success) -> Overwintering -> release Spring Queens**

In this scenario, fall queens are brought into a rearing facility. Males may also be brought into the facility and mating trials would be attempted. The queens would be kept in captivity over the winter and released in the spring.

1. *Risks:* Immediate reduction of population reproductives from the wild; death; husbandry limitation (limited ability to overwinter queens in captivity); more stress in lab; unsure of mating status or whether mating was successful
2. *Feasibility:* Low
3. *Conservation Impact:* same as scenario 1; ability to investigate overwintering survival and mortality (Conservation Impact A, B, C, D)
4. *Scope: not defined*
5. *Population:* Because of our uncertainty with survival, uncertain numbers needed; more research is needed before attempting (surrogate).

Note: Research need: Biomarker to determine if queen has mated (such as mating plug, hormones in feces).

Scenario 3: **Fall Queens (assume mated) ->Overwinter -> Spring Release**

In this scenario, fall queens are brought into a rearing facility. The queens would be kept in captivity over the winter and released in the spring. This scenario is like Scenario 2, except no males are brought into the facility to attempt mating. Instead, it is assumed that mating has already occurred in the wild.

1. *Risks:* unsuccessfully mated queens could be removed, similar to Scenario 2.
2. *Feasibility:* Low
3. *Conservation Impact:* similar to scenario 2 (Conservation Impact A, B, C, D).
4. *Scope / Population:* not defined

Scenario 4: Summer Workers -> micro-colonies -> males -> Fall males (can be established from Scenario 1 or long-term strategies; see long-term *ex situ* management section, below).

In this scenario, summer workers are brought into captivity and raised as queen-less micro-colonies[2]. Workers lay unfertilized eggs, which result in the production of males. Males are released in the fall to mate with wild females.

1. *Risks:* removal of workers can weaken wild "colonies"; genetic augmentation could lead to outbreeding depression/introduction of maladapted genes
2. *Feasibility:* High**; Husbandry expertise needed is fairly low/mod (depending on the source of workers, captive source more feasible)
3. *Conservation Impact:* probably highest for genetic augmentation (Conservation Impact B)
4. *Scope:* could be at local, regional levels
5. *Population:* For male releases we want both a high level of genetic diversity from the source population and lots of males to release. Need 5 workers for a micro-colony, would likely want 10 micro-colonies so that you could release 100+ males at the augmentation site. It would be useful to measure the genetic variability of the males (or the maternal workers) prior to release and the genetic diversity of the recipient population. This would allow for assessment of success at a later date. Requires a moderate level of husbandry skills, fewer human resources and costs than rearing full colonies.

*Note: this strategy could be triggered by monitoring for diploid males in the populations and having an action threshold for male introduction.

[2] From Klinger *et al.* 2019: "Micro-colonies are formed when a group of bumble bee workers are isolated in a queenless environment. Separation from the queen stimulates one of the workers (usually the largest one with the most developed ovaries) to establish dominance and begin laying eggs (Free 1955). These eggs are unfertilized and, due to the haplodiploid reproductive system in bees, result in the production of male offspring (i.e., drones)."

Scenario 5: Spring Queens ->Transport Screening -> Spring Queens to new locations

In this scenario, spring queens are temporarily held, screened for pathogens, and transported to new release sites.

1. *Risks:* Reduce demographics at source site; fail to establish at new site; failure/stress in transport.
2. *Feasibility:* Transport feasibility is low but could be improved; Likelihood of success is low
3. Conservation Impact: enhance demographics, increase number of populations; indirectly, the potential for outreach and stakeholder buy-in is high (Conservation Impact A, B, C, D)
4. *Scope:* regional or international
5. *Population:* low level of husbandry, high(ish) cost for pathogen screening, but a short time period. Lab of 2-3 people can likely screen 100 bees/week, with efficiency gains with experience.

Note: Research need: Try technique on surrogate species. Use of a surrogate species may be advisable for all of the scenarios presented here.

Scenario 6: Fall Queens ->Transport Screening -> Fall Queens to new locations

In this scenario, fall queens are temporarily held, screened for pathogens, and transported to new release sites.

1. *Risks:* Similar to Scenario 5; unsure of mating status of collected queens; pathogen spread; unknown rate of mortality in moving fall queens.
2. *Feasibility:* Similar to Scenario 5
3. *Conservation Impact:* Similar to Scenario 5 (Conservation Impact A, B, C, D)
4. *Scope / Population:* not defined

Scenario 7: Wild Males -> Transport Screening -> Wild Males to new locations

In this scenario, wild males are temporarily held, screened for pathogens, and transported to new release sites.

1. *Risks:* Reduce demographics at one site; reduce genetic diversity availability on origin site; pathogen spread; failure/stress in transport
2. *Feasibility:* High, straightforward and no need for rearing facility
3. *Conservation Impact:* genetic diversity augmentation (Conservation Impact B)
4. *Scope:* not defined
5. *Population:* Pathogen screening would likely be the limiting factor in the number of males transported due to time required to hold each bee to collect a fecal sample and then obtain microscopy and PCR screening results.

Note: Research Need: *B. affinis* male dispersal

Scenario 8: **Threatened colony rescue -> Lab/transport -> queens or same colony or males**

In this scenario, a colony in imminent danger is collected and transported to a new release site.

1. *Risks:* Low/no risk; bee stings
2. *Feasibility:* Moderate, depending on nest location (and time of year?)
3. *Conservation Impact:* Conserve colony unit and genetic diversity associated with it for population it serves, research opportunities? (Conservation Impact A, B, C, D).
4. *Scope:* not defined
5. *Population:* not defined

Note: This scenario may be an option for a colony(ies) in imminent danger (e.g., fire, flood, to be plowed over). Colonies rescued could be held for study or to build colony population before release.

Long-term *ex situ* management considerations

The maintenance of rusty patched bumble bees over the annual life cycle of a colony (multiple generations) requires additional considerations in husbandry, infrastructure and staffing. Some of these considerations discussed at the workshop include:

- permitting requirements
- recording keeping (and software)
- facilities (quarantine, holding), including locations and quantity
- overwintering conditions (substrate, temperature, humidity, duration)
- potential manipulation of colony cycle
- seasonal husbandry expertise and protocols (rearing, overwintering, breeding)
- nutritional requirements (for maintenance vs release)
- health and disease monitoring
- biosecurity and pest control
- population management (demographic and genetic)
- staff training in all areas
- program timeline
- financial support

Given the current state of *ex situ* husbandry expertise, it may be advisable to use a closely related surrogate species (e.g., *Bombus terricola*) to develop initial protocols.

An additional method for maintaining a genetic insurance population is to establish and maintain a biobank (genome resource bank). This might include sperm, larvae, cell lines or other biosamples, depending upon availability and desired purpose. Protocols for collection, storage and use would be needed as well as many of the considerations listed above (e.g., facilities, staff, funding).

ROLE 1: DEMOGRAPHIC REINFORCEMENT OF CURRENT POPULATIONS

Working group members: Elaine Evans, Jessica Petersen, Erik Runquist, Tamara Smith, Seth Stapleton, Jessica Steiner, Jamie Strange

Note that anticipated timelines subsequently have been affected by COVID-19 restrictions.

For the purpose of this discussion, ***demographic reinforcement*** is defined as an attempt to stabilize or increase the abundance of individuals in extant populations through short-term *ex situ* interventions. The goals of this strategy are to stabilize declining populations and increase the size of small populations. This will improve the resilience of these populations, increase connectivity among populations, and expand the geographic range of existing populations. All of these goals are aimed at improving the viability of *B. affinis* populations in the wild.

In the near term, wild populations would need to serve as the source of individuals for release after short-term periods of *ex situ* care. An additional outcome from short-term *ex situ* management and rearing associated with this role is the knowledge gained in the *ex situ* management of this species that helps to build capacity for long-term *ex situ* management options. If and when a long-term *ex situ* population is established, it may be able to serve as a source of individuals for reinforcement.

STRATEGY: Stabilize declining populations to prevent local extirpation and improve resilience of existing populations through periodic releases of individuals (queens) or captive-reared colonies into areas with declining populations.

Objective 1: Prioritize sites for reinforcement.

Data deficiencies for this objective: Disease transmission, genetic structure, current abundance at donor and recipient sites

Action 1: Analyze existing grid survey data (number of sites, number of *B. affinis* per grid, trends?).
- Responsible party: Tam Smith
- Timeline: April 25, 2020

Action 2: Develop criteria for prioritization for source and recipient populations (ideally informed by genetics, pathogen loads, abundance) and identify potential sites.
- Responsible parties: Elaine Evans, Jessica Petersen, Tam Smith
- Timeline: draft by September 30, 2020, with updating as new data become available

Action 3: Coordinate efforts to have abundance, genetics, pathogen information to pilot on a small geographic scale (e.g., 4-6 adjacent 10 km grids).
- Responsible parties: Tam Smith, Jamie Strange/Ben Sadd (pathogens), Elaine Evans (virus), John Mola, Jon Koch (genetics)
- Timeline: Ongoing

Action 4: Develop standardized protocols for surveys for spring and fall queens.
- Responsible parties: John Mola, Ian Pearse
- Timeline: March 15, 2020

Action 5: Devise tools to build survey capacity.

- Responsible parties: Elaine Evans, Minnesota Zoo staff
- Timeline: April 15, 2020

Action 6: Implement baseline surveys on population size and status during spring – fall.

- Responsible party: All
- Timeline: Beginning 2020; ongoing

Objective 2: Develop and implement protocols associated with collection of spring queens, rear in the laboratory, and release of fall queens.

General data deficiency for this objective: What are the demographic pinch points limiting local population growth?

Action 1: Review and refine rearing protocols and resource needs to establish rearing facilities (to include identifying current capacity and obtaining necessary permits).

- Responsible parties: E. Evans, J. Strange, B. Sadd, T. Smith
- Timeline: December 31, 2020
- Triggers for action: decision to actually start *ex situ* rearing
- Data deficiencies: Need best practices protocols
- Cost estimate to be developed

Action 2: Secure funding source for *ex situ* rearing.

- Responsible parties: T. Smith, J. Strange, Minnesota Zoo staff
- Timeline: Great Lakes Restoration Initiative 2022, AZA SAFE program, others; ongoing
- Triggers for action: RFP for GLRI, others
- Data deficiencies: need a budget by March 15, 2020

Action 3: Develop criteria for collection of spring queens (numbers, etc.).

- Responsible parties: T. Smith, J. Mola, I. Pearse, E. Evans
  - Timeline: TDB

Action 4: Establish colonies of *B. affinis* from spring queens for increase and release of individuals or colonies (single season)

- Trigger for action: criteria has been met, USFWS approves the permit
  - Responsible parties, Timeline: TBD

Action 5: Conduct post-release monitoring to evaluate effectiveness [continue spring surveys, use technology (video)]. Identify specific parameters to monitor.

  - Responsible parties, Timeline: TBD

Action 6: Devise and implement research to improve rearing / release protocols and prioritize and address information gaps.

  - Responsible parties, Timeline, Triggers for action: TBD

Objective 3: Explore and develop additional short-term release options through experimental design to stabilize or augment populations.

Data deficiencies for this objective: Demographic uncertainties, including overwintering survival in situ

Action 1: Identify appropriate surrogate species, perhaps a common species (*B. impatiens*).

Action 2: Fall collections of queens.

Action 3: Develop, implement and evaluate protocols for overwintering in an *ex situ* setting.

Action 4: Devise protocols for release of spring queens.

Action 5: Determine if there are other life history stages we should explore.

Responsible parties, timelines, and triggers for actions have not been identified for these actions.

Objective 4: Increase the population numbers of small populations. Expand the geographic range of the current populations.

Trigger for Objective 4: Objectives 1-3 are successful (or an alternate, better design is identified)

Action 1: Identify which populations need supplementation.

Action 2: Develop criteria for prioritization for source and recipient populations (ideally informed by genetics, pathogen loads, abundance) and identify potential sites.

Action 3: Implement best strategy and best practices identified above.

Responsible parties, timelines, and triggers for actions have not been identified for these actions.

ROLE 2: REINTRODUCTION TO ESTABLISH NEW POPULATIONS

Working group members: Sydney Cameron, Sheila Colla, Jeff Everett, Mark McCollough, Ian Pearse, Laura Ragan, T'ai Roulston, Genevieve Rowe, Logan Rowe, Hollis Woodard

Note that anticipated timelines subsequently have been affected by COVID-19 restrictions..

The goal of this strategy is to (re)establish *B. affinis* wild populations in areas of suitable habitat devoid of the species and free from significant threat. This will provide redundancy, restore ecological functions, increase connectivity among populations, and expand the geographic range of the species. All of these goals are aimed at improving the viability of *B. affinis* populations in the wild.

This working group developed recommendations for initiating this effort with the following *assumptions*:

- We have a source population sufficient to be able to do this.
- We have to believe we have appropriate habitat for reintroductions.
- We have to ensure we can rear *B. affinis* properly (unless translocating).
- We know what the threats are and/or take appropriate precautions to ensure the new populations are protected from them.
- We have a supportive on-the-ground entity and required funding.

There are many significant data gaps that hinder implementation of this strategy. Until these data gaps are addressed, it is inappropriate to develop specific recommendations for release sites and methodologies. Several research or field projects are currently in progress that will start to address relevant knowledge gaps for this strategy (key personnel):

- Development of spring survey protocols and spring searches (B. Sadd, J. Mola, I. Pearse)
- Study of the historical genetic diversity of *B. affinis* (J. Strange, J. Koch, J. Mola)
- Genome study of the pathogen *N. bombi* (S. Cameron)
- National native bee monitoring, improved coordinated framework (H. Woodard)
- Surrogate species *(B. terricola*) habitat and captive breeding (Wildlife Preservation Canada, S. Colla)

STRATEGY: Establish new *B. affinis* wild populations to provide redundancy, restore ecological function, and expand the geographic range with periodic releases of *B. affinis* into historic or new areas with suitable habitat.

The development and implementation of this role was divided into four phases over the next 10 years:

<u>Immediate actions</u>: initial data gathering to address priority knowledge gaps
<u>Medium-term actions</u>: data analysis, trial efforts, and preparations for implementation
<u>Reintroduction efforts</u>: implementation of reintroduction program
<u>Long-term actions</u>: program maintenance, monitoring and revision

These actions are detailed below.

Objective 1. Immediate actions to address data gaps.

Action 1: What do the places where *B. affinis* persist have in common? Explore suitability models, habitat descriptions/quantifications, identification/quantification of suitable habitat, habitat size, meta-population connectivity, and pathogen, pesticide and fungicide levels.

- Responsible parties: S. Colla for Canadian populations, Michelle Boone (UMN)?, possibly L. Richardson (UVT), J. Mola
- Timeline: ASAP

Action 2: Use remnant *B. affinis* populations to learn about possible resistance to pathogens.

- Responsible parties: TBD
- Timeline: ASAP

Action 3: Conduct surveys to identify which *B. affinis* populations have sufficient abundance to use as a source population (especially within each ecoregion).

- Responsible parties: TBD
- Timeline: ASAP, multi-year project

Action 4. Survey each ecoregion to identify possible sites for reintroduction, develop criteria, and perform a ranking activity to prioritize potential reintroduction locations.

- Responsible parties: TBD
- Timeline: ASAP, multi-year project

Action 5: Investigate the extent of commercially-produced bumble bee interregional movement across the country, across borders in the industry, and across the country. Obtain disease level and information about possible infectious disease outbreak emergency response protocols.

- Responsible parties, Timeline: TBD

Action 6: Develop city pollinator policies where at-risk species occur to ensure urban planning considers ecological requirements to conserve species where they currently occur.

- Responsible parties, Timeline: TBD

Action 7: Conduct translocations (without captive breeding) using a surrogate species (*B. terricola*) from central to southern Ontario to determine the feasibility.

- Responsible parties: Wildlife Preservation Canada, Colla's lab
- Timeline: 2-3 years
- Trigger for action: TBD

Action 8: Identify key threats to remnant *B. affinis* populations to ensure protection of future source populations.

- Responsible parties: TBD
- Timeline: ASAP, ongoing monitoring

Objective 2. Medium-term actions to analyze available data and prepare for implementation.

Action 1: Study baseline pathogen levels and pesticide and fungicide levels at possible reintroduction sites.

- Responsible parties: TBD
- Timeline: 3-5 years
- Trigger for action: Potential reintroduction sites identified

Action 2: Begin restoration and protection management programs at possible reintroduction sites (e.g. seed mixes).

- Responsible parties: TBD
- Timeline: 3-5 years
- Trigger for action: Reintroduction sites identified, stakeholders engaged

Action 3: Work on stakeholder engagement and relationship building at reintroduction sites.

- Responsible parties: TBD
- Timeline: 3-5 years
- Trigger for action: Reintroduction sites identified

Action 4: Map out possible corridors between isolated populations (but consider the role of parasite spillover implications (literature review?)).

- Responsible parties, Timeline, Triggers for action: TBD

Action 5: Conduct possible surrogate work to determine numbers of queens, colonies needed to reintroduce into an area successfully (e.g., City of Guelph and *B. terricola* since baseline data exists) and as a research system to understand stressors at various sites. Investigate release techniques.

- Responsible parties, Timeline: TBD
- Trigger for action: Successful establishment of a captive population of a surrogate species

Action 6: Create an organization with stakeholders (ENGOs, land managers, governments, researchers).

- Responsible parties, Timeline, Triggers for action: TBD

Action 7: Create a strategic plan for site reintroductions over a set timeline. Determine number of sites for each ecoregion. Prioritize locations where high-quality sites are located and other areas that could become high quality sites with some work.

- Responsible parties, Timeline, Triggers for action: TBD

Action 8: Develop a risk assessment for disease issues and impacts on source populations.

- Responsible parties, Triggers for action: TBD
- Timeline: 3-5 years

Action 9: Conduct a reintroduction protocol consultation process.

- Responsible parties: (connect with IUCN Conservation Translocation SG, Bumblebee SG)
- Timeline: TBD
- Trigger for action: Protocol developed

Objective 3. Reintroduction of *B. affinis* into historical and/or new sites.

Actions, responsible parties, and timelines to be determined based on progress for Objectives 1 and 2.

- Trigger for action: Sites identified, protocol for captive breeding or translocation developed, consultation completed

Objective 4. Long-term actions (after reintroduction) to continue, monitor and adapt efforts.

Action 1: Conduct reinforcements over multiple years.

Action 2: Develop multi-year survey protocols.

Action 3: Manage areas to minimize potential stressors.

Action 4: Continue ongoing habitat management.

Action 5: Continue relationship-building and stakeholder engagement.

Action 6: Conduct adaptive management planning.

Action 7: Develop protocols for other potential sites.

Action 8: Continue ongoing consultation process (scientific advisory board?).

Action 9: Continue ongoing analysis of threats, e.g. climate change and pathogens.

Responsible parties, timelines and triggers to be determined based on progress for Objectives 1-3.

ROLE 3: GENETIC SUPPLEMENTATION OF WILD POPULATIONS

Working group members: Amy Chabot, John Koch, John Mola, Ben Sadd

Note that anticipated timelines subsequently have been affected by COVID-19 restrictions.

Definition and overview

Genetic supplementation of *B. affinis* is the process of promoting heterozygosity and allelic diversity in extant populations that *improves* fitness and *approaches* pre-decline (prior to the late 1980s) patterns of population genetic diversity observed in congeneric taxa with similar habitat and life history characteristics. Genetic supplementation can be achieved using either wild or *ex situ* populations as the source, possibly involving a short- or long-term *ex situ* stage. Supplementation could be achieved through live release of queens, males or captive initiated colonies or supplementation of the local gene pool through artificial insemination. The different approaches have advantages and disadvantages and invariably depend on the development of other *ex situ* management strategies (e.g. acquisition and rearing) and closing of knowledge gaps, both of which dictate their feasibility.

Triggers for action

- Evidence for low effective population size (N_e) based on colony abundance estimates and individual counts (Cameron *et al.* 2011; Ellis et al. 2006).
- Evidence of diploid (2n) male frequency above a predetermined or historic threshold (Zayed *et al*. 2004). Threshold will need to be determined based on historical specimen data and surveys. Detecting males with first workers during the spring may be evidence for inbreeding (Gosterit 2016), and should trigger genetic analysis to determine ploidy levels in those males. An alternative method, if verified in *B. affinis*, would be to use a morphological marker *(*Gerard *et al.* 2015).
- Evidence of triploid females. Threshold of how many triploid females are in a population will need to be determined based on historical data and surveys (Darvill *et al.* 2012)
- Evidence of low allelic diversity at the complimentary sex determination locus (Zayed 2004).
- Evidence for low population genetic diversity (allelic richness and heterozygosity), based on historical levels and similar non-declining species (Cameron *et al.* 2011).

Knowledge Gaps (technical limits, methods, etc.)

Classification of knowledge gaps is presented on the axes of feasibility and urgency. Feasibility is defined as the state or degree of being easily or conveniently done, and urgency is defined as importance requiring swift action (Figure 3). The placement represents our informed opinions and is not based on a quantitative assessment. The highlighted text indicates knowledge gaps that are of high urgency and high feasibility to address.

1. Determine extant wild and historical population genetic diversity and structure. Historical population genetic diversity may be estimated with museum specimens (Lozier & Cameron 2009).
2. Identify genome and genome resequencing feasibility for contemporary and historical samples (Kent e*t al*. 2018).
3. Knowledge of local adaptation in functional genes (Kent *et al*. 2018).
4. Identity of and diversity at Sex Determining Locus (Zayed 2004).

5. Determine noninvasive techniques for genetic analysis. Specifically, investigate the potential for using frass for population genetic analysis with microsatellites or similar markers. Tarsal clipping, although effective for DNA extraction (Holehouse et al. 2003) has been shown to have an inconsistent negative effect on *B. vosnesenskii* queens and small workers (Mola *et al*. unpublished). Furthermore, tarsal clipping may exacerbate the negative effects of other environmental stressors such as pesticides, pathogens, and parasites. Wounding may allow for entry by opportunistic pathogens and the process of wound healing and its associated immune responses (Siva-Jothy *et al.* 2005) may be energetically costly (Sadd & Schmid-Hempel 2008).
6. Identify potential non-invasive biomarkers of mating (investigate heat differences (thermography), mating plug chemistry, hydrocarbons or other chemical changes) and develop artificial insemination techniques (Baer & Schmid-Hempel 2000).
7. Evaluate potential for cryopreservation of bumble bee semen (Hopkins *et al.* 2012).
8. Characterize mating behavior, specifically determine whether *B. affinis* queens are singly or multiply mated.
9. Develop a protocol for rearing microcolonies from wild caught workers to produce males for release or mating.
10. Evaluate the framework for monitoring success of genetic interventions through lineage reconstruction.
11. Establish the relationship between genetic diversity and fitness (possibly by using surrogates).

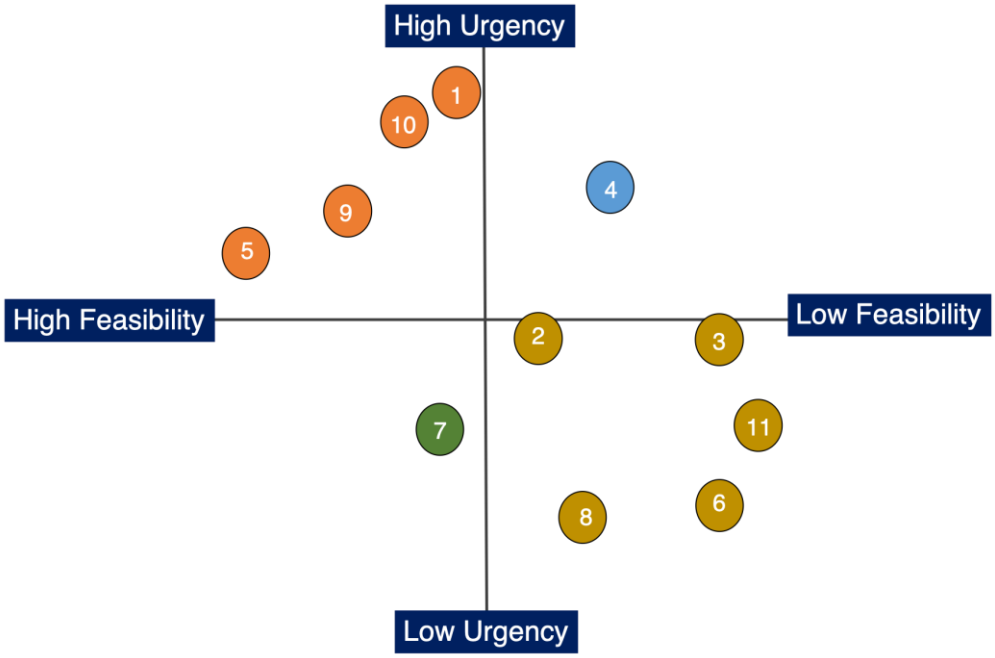

Figure 3. Prioritization of knowledge gaps based on feasibility and urgency. Orange dots indicate knowledge gaps that are of high urgency and high feasibility to address.

What are we trying to achieve?

The goal of genetic supplementation through *ex situ* management is to promote genetic diversity across *B. affinis* populations that *improves* fitness and *approaches* pre-decline (Colla & Packer 2008) population

genetic diversity and/or patterns observed in congeneric taxa. Ultimately, our aim is to achieve healthy sustainable populations with adaptation potential.

How will this improve *B. affinis* viability?
Genetic supplementation through *ex situ* management will improve *B. affinis* viability by avoiding the negative effects of small population sizes on population-wide genetic diversity and individual heterozygosity. Ensuring that the species has expected levels of standing genetic variation will facilitate population resilience to environmental pressures associated with extreme weather events, climate change, pathogens, parasites, disease, and other unknown stressors.

Strategies
The strategy for genetic supplementation through *ex situ* management will be informed by historic population structure data both within and between regions, and how that compares with extant populations. Should translocation occur (short *ex situ* period), individuals will be sourced from a captive population. Males may be sourced from micro-colonies or whole colonies, or queens, reared or collected with a short *ex situ* period, could be released. The infusion of genetic material into a population might also involve using fresh or cryopreserved semen for artificial insemination. The strategies below are considered urgent and feasible (Figure 3). They are a critical first step in promoting the conservation of *B. affinis*.

STRATEGY 1: Movement of reproductive individuals (queens, males) with a short *ex situ* period between existing sites / populations.

Objective: Increase the genetic diversity of extant *B. affinis* populations through the movement of reproductive individuals into the breeding pool.

Action 1: Determine historic genetic variation of *B. affinis* within conservation units (diversity metrics, inbreeding, etc.).*Also an action for all other strategies.

- Responsible parties: J. Mola, I. Pearse, J. Koch, J. Strange, B. Sadd
- Timeline: *Preliminary* results completed by December 2020; *update: action greatly delayed due to closure or museums due to COVID-19*.
- Trigger for action: Data deficiency on historic (pre-decline) population genetic diversity.

Action 2: Determine extant genetic variation of *B. affinis* within conservation units (diversity metrics, inbreeding, etc.).*Also an action for all other strategies.

- Responsible parties: J. Mola, I. Pearse, J. Koch, J. Strange
- Timeline: Sample collections 2020-2022; ongoing and ahead of schedule.
- Trigger for action: Data deficiency on extant *B. affinis* population genetic diversity.

Action 3: Movement of *B. affinis* reproductive individuals after criteria for movement and safety protocols are developed and the monitoring of success through tracking of lineages and/or changes in population diversity.

  - Responsible parties: TBD
  - Timeline: Initiated in 202X.
- Trigger for action: Evidence for low genetic diversity (CSDL, neutral GD), diploid males, triploid females in *B. affinis* populations.
- Limitations: Markers of population diversity.

STRATEGY 2: Release of males from worker-founded microcolonies.

Objective: Increase the genetic diversity of extant *B. affinis* populations through the movement of worker-produced males into the breeding pool.

Action 1: Determine historic genetic variation of *B. affinis* within conservation units (diversity metrics, inbreeding, etc.) as outlined in Strategy 1, Action 1 (see above).

Action 2: Determine extant genetic variation of *B. affinis* within conservation units (diversity metrics, inbreeding, etc.) as outlined in Strategy 1, Action 2 (see above)

Action 3: Determine feasibility of rearing males from microcolonies of surrogate species (e.g., *B. occidentalis*, *B. terricola*, *B. huntii*) and eventually wild caught or captive reared *B. affinis.* Note that unrelated workers exhibit low success for producing males (H. Woodard, pers. comm.).

- Responsible parties: J. Koch, J. Strange, B. Sadd
- Timeline: Completed by COB 2021
- Trigger for action: Evidence for low genetic diversity in wild *B. affinis* populations.

Action 4: Assess genetic diversity of worker founded microcolonies of *B. affinis* to evaluate ability and extent of genetic supplementation possible.

- Responsible parties: J. Koch will facilitate
- Timeline: Initiated in 2023
- Trigger for action: Evidence for low genetic diversity (CSDL, neutral GD), diploid males, triploid females in *B. affinis* populations.

Action 5: Movement of *B. affinis* reproductive individuals following safety criteria and monitoring (as outlined in Strategy 1).

- Responsible parties: TBD
- Timeline: Initiated in 2023
- Trigger for action: Evidence for low genetic diversity (CSDL, neutral GD), diploid males, triploid females in *B. affinis* populations.
- Limitations: Markers of population diversity

STRATEGY 3: Release of unmated or mated queens in Fall from captive rearing.

Objective: Increase the genetic diversity of extant *B. affinis* populations through the movement of unmated and/or mated Fall queens into the breeding pool.

Action 1: Determine historic genetic variation of *B. affinis* within conservation units (diversity metrics, inbreeding, etc.) as outlined in Strategy 1, Action 1 (see above).

Action 2: Determine extant genetic variation of *B. affinis* within conservation units (diversity metrics, inbreeding, etc.) as outlined in Strategy 1, Action 2 (see above)

Action 3: Evaluate lineage survival of unmated and mated queens in the wild using surrogate species (e.g., *B. occidentalis, B. huntii*).

- Responsible parties: J. Koch
- Timeline: Initiated in 2023
- Trigger for action: Evidence for low genetic diversity and detection of *B. affinis* populations.
- Limitations: Effective *ex situ* husbandry

Action 4: Movement of *B. affinis* reproductive individuals following safety criteria and monitoring (as outlined in Strategy 1).

  - Responsible parties: TBD
  - Timeline: Initiated in 202X
- Trigger for action: Successful captive rearing and identification of genetic stock need
- Limitations: Effective *ex situ* husbandry, markers of population diversity

STRATEGY 4: Release of mated and overwintered queens in Spring from captive rearing.

Objective: Increase the genetic diversity of extant *B. affinis* populations through the movement of mated and overwintered Spring queens into the breeding pool.

Action 1: Determine historic genetic variation of *B. affinis* within conservation units (diversity metrics, inbreeding, etc.) as outlined in Strategy 1, Action 1 (see above).

Action 2: Determine extant genetic variation of *B. affinis* within conservation units (diversity metrics, inbreeding, etc.) as outlined in Strategy 1, Action 2 (see above)

Action 3: Evaluate lineage survival and establishment of unmated and mated queens in the wild using surrogate species (e.g., *B. occidentalis, B. huntii*).

- Responsible parties: J. Koch, others
- Timeline: Initiated in 2023
- Trigger for action: Data deficiency on lineage survival
- Limitations: Effective *ex situ* husbandry of surrogate species

Action 4: Movement of *B. affinis* reproductive individuals following safety criteria and monitoring (as outlined in Strategy 1).

  - Responsible parties: TBD
  - Timeline: Initiated in 202X
- Trigger for action: Successful captive rearing and identified need
- Limitations: Effective *ex situ* husbandry, markers of population diversity

STRATEGY 5: Placement of initiated colonies from *ex situ* population or as part of a head-start program.

Objective: Increase the genetic diversity of extant *B. affinis* populations through the movement of captive colonies into the breeding pool.

Action 1: Determine historic genetic variation of *B. affinis* within conservation units (diversity metrics, inbreeding, etc.) as outlined in Strategy 1, Action 1 (see above).

Action 2: Determine extant genetic variation of *B. affinis* within conservation units (diversity metrics, inbreeding, etc.) as outlined in Strategy 1, Action 2 (see above)

Action 3: Determine feasibility of lineage survival and establishment from an *ex situ* population using a surrogate species.

- Responsible parties: TBD; J. Koch will facilitate
- Timeline: Initiated in 202X
- Trigger for action: Successful captive rearing, particularly colony initiation, and identified need
- Limitations: Effective *ex situ* husbandry of surrogate species

Action 4: Movement of *B. affinis* reproductive individuals following safety criteria and monitoring (as outlined in Strategy 1) using a surrogate species.

- Responsible parties: TBD
- Timeline: Initiated in 202X
- Trigger for action: Successful captive rearing and identified need
- Limitations: Effective *ex situ* husbandry of surrogate species, markers of population diversity

ROLE 4: LONG-TERM INSURANCE POPULATION

Working group members: Rob Jean, Ed Spevak

Note that anticipated timelines subsequently have been affected by COVID-19 restrictions.

Purpose: Reductions in rusty patched bumble bee populations in the past 20 years and reduction in known habitat have led to the need to establish a long-term *ex situ* insurance population to serve as a genetic reservoir for species recovery and support the preservation of genetic local adaptations.

Two strategies were identified for establishing an *ex situ* insurance population: establishing and managing a population of live bees, and establishing a biobank of gametes, tissues and whole specimens. These strategies are not mutually exclusive and can serve different functions and involve different challenges and actions. A living insurance population might provide the option to expand its function to a source population for reinforcement or reintroduction. It may be easier, however, to get a biobank off the ground immediately given the current knowledge of long-term *ex situ* management and breeding. Insurance populations may provide a source for research on genomics and other topics. Both strategies are recommended to be pursued.

Risks of a living insurance population include the risk of artificial selection under *ex situ* conditions and potential negative impacts on local populations if bees are removed. Strategies for collection and for *ex situ* management should be implemented to minimize these and any other risks.

STRATEGY: Establish a living *ex situ* insurance population.

Objective 1: Develop disease screening and quarantine protocol.

Action 1: Identify existing protocols (talk to Ben Sadd and Jamie Strange).

- Timeline: April 2020

Action 2: Develop screening, testing and quarantine protocols. Determine acceptable pathogen and parasite disease loads.

Action 3: Look into the efficacy of possible treatments (start with surrogate species, e.g., *B. impatiens*, *B. terricola*).

Action 4: Develop euthanasia policies and protocols.

Responsible parties, timelines, and triggers for actions have not been identified for these actions except where indicated.

Objective 2: Use a surrogate (e.g., *B. terricola*) to develop husbandry and management protocols and staff training (~3 year timeline).

Knowledge gap: Husbandry of bumble bees, especially overwintering and nutrition requirements.

Action 1: Develop successful husbandry protocols (talk to Jamie Strange, Sydney Cameron and Elaine Evans).

Action 2: Conduct staff training - e.g., husbandry, disease identification and treatment.

Action 3: Examine possible studbook/registry software to track individuals and colonies. Develop program if needed.

- Responsible Party: Ed Spevak

Action 4: Examine existing or develop genetic/demographic and population modeling software for bumble bee management and population supplementation and augmentation (e.g., males, spring queens, fall queens, or colonies).

- Responsible Party: Ed Spevak

Action 5: Establish surrogate population(s) of *B. terricola* to begin developing prior action items.

Action 6: Develop a biomarker/assay to determine mated/unmated queens.

Responsible parties, timelines, and triggers for actions have not been identified for these actions except where indicated.

Trigger to initiate the objective: Success with surrogate (i.e., B. terricola)

Objective 3: Build a facility (timeframe uncertain; permits can take a year).

Action 1: Conduct a cost analysis for a facility, general operations costs, and staffing in Saint Louis or another location.

- Responsible party: Ed Spevak
- Timeline: July/August 2020

Action 2: Explore the possibility and need for a seasonal/climate-exposed facility for release lineages.

Action 3: Determine possible locations and whether facility will be affiliated with a university, zoo/conservation organization, government agencies, etc.

Action 4: Investigate sources of funding and operation budget support. (e.g., grants, USFWS, NGOs, institutional, university).

Action 5: Develop animal welfare protocols.

Action 6: Look into permit issues for acquisition, maintenance, and movement of bumble bees. Is it possible to have a joint facility permit?

Responsible parties, timelines, and triggers for actions have not been identified for these actions except where indicated.
Trigger to initiate the objective: Acquisition of USFWS permits

Data needs that might be addressed:

Where is the sex locus? Nutritional requirements? Genetic diversity of historical populations? Genetic diversity of current populations? Is the species panmictic or are there unique alleles? Is there subpopulation structure? Rescue colonies in imminent threat may be good candidates for this or short-term strategies.

- Responsible parties: multiple labs in multiple locations, one per recovery unit
- Material needs: facilities, location, funding, staff

STRATEGY: Establish a gene/biobank of gametes, tissues, and whole specimens.

Objective 1: Determine location and cost of a facility in each conservation unit, leveraging existing biobanks.

Action 1: Compile a list of current biobank facilities (contact Ollie Ryder?).

Objective 2: Collect expertise.

Action 1: Contact Ollie Ryder, San Diego Zoo Global Institute for Conservation Research.
Responsible Party: Ed Spevak
Timeline: March 2020

Action 2: Contact known bee biobank experts. (e.g.: Anita Collins, honeybee gynecologist; Steve Sheppard, Brandon Hopkins and Sue Cobey havealso done work on honeybee sperm storage).

Objective 3: Acquire genetic material: gametes, tissues and potential cell lines, whole animals.

Action 1: Develop sampling, temporary storage and shipping protocols for use in field and lab

Action 2: Look into possibilities of blanket multi-institution/researcher sampling permit

Action 3: Coordinate with bumble bee researchers, museums, universities, etc. to acquire initial samples. (use Leif Richardson's database as starting point)

Action 4: Acquire samples from all known extant populations

Responsible parties, timelines, and triggers for actions have not been identified for actions for this strategy except where indicated.

Data needs: Inventory and genetic analysis of museum specimens; tarsal clippings of current populations; any accidental mortality specimens; genome of the rusty patched bumble bee; start with Leif Richardson's data. Note: xCell lines (*Mike Goblirsch created temporarily surviving *B. impatiens* cell line @ Minnesota, now at USDA-ARS MS)

SECONDARY ROLE 4a: CONSERVATION EDUCATION

It was noted that if an *ex situ* population were to be established, it may be able to serve an additional role in providing a focus for conservation education. The following potential components of a conservation education program were identified:

- Development of a guest/donor space for rearing facilities as well as developing Behind-the-Scenes Tours of facilities
- Live video feeds, blogs, tweets of ongoing work in facilities and at release sites
- Adopt-a-Colony Program
- Restoration volunteer event at release sites
- Rusty Patched Bumble Bee Species Survival Travel program. Guests pay to visit breeding facilities and release sites, survey for bumble bees, learn about bumble bee ecology and conservation, and assist with a release. All meals focused on pollinator dependent or enhanced foods. Tented safari at release sites

The following risks of conservation education activities such as those listed above were identified:

- Pathogen loads could increase
- Development of novel pathogens
- Reduction in wild population
- Program fails
- Unknown small population genetic and demographic problems, Captive population stochasticity
- Funding loss or loss of Institutional support

These opportunities can be considered as the development of an *ex situ* insurance population progresses.

Literature Cited

Alford, D. V. (1969). A Study of the hibernation of bumblebees (Hymenoptera: Bombidae) in Southern England. *Journal of Animal Ecology*, 38(1), 149–170.

Alger, S. A., Alexander Burnham, P., Boncristiani, H. F., & Brody, A. K. (2018). RNA virus spillover from managed honeybees (*Apis mellifera*) to wild bumblebees (*Bombus* spp.). *PLoS ONE*, 14(6), 1–13. https://doi.org/10.1371/journal.pone.0217822

Anderson, N. L. & Harmon-Threatt, A. N. (2019). Chronic contact with realistic soil concentrations of imidacloprid affects the mass, immature development speed, and adult longevity of solitary bees. *Scientific Reports*, 9, 3724. https://doi.org/10.1038/s41598-019-40031-9

Baer, B., and P. Schmid-Hempel. (2000). The artificial insemination of bumblebee queens. *Insectes Soc.* 47: 183–187.

Bale, J. S., & Hayward, S. A. L. (2010). Insect overwintering in a changing climate. *Journal of Experimental Biology*, *213*(6), 980–994. https://doi.org/10.1242/jeb.03791

Beekman, M., Van Stratum, P., & Lingeman, R. (2000). Artificial rearing of bumble bees (*Bombus terrestris*) selects against heavy queens. *Journal of Apicultural Research*, *39*(1-2), 61-65.

Bernauer, O. M., Gaines-Day, H. R., & Steffan, S. A. (2015). Colonies of bumble bees (*Bombus impatiens*) produce fewer workers, less bee biomass, and have smaller mother queens following fungicide exposure. *Insects*, *6*(2), 478–488. https://doi.org/10.3390/insects6020478

Blaker, E. A., Strange, J. P., James, R. R., Monroy, F. P., & Cobb, N. S. (2014). PCR reveals high prevalence of non/low sporulating *Nosema bombi* (microsporidia) infections in bumble bees (*Bombus*) in Northern Arizona. *Journal of Invertebrate Pathology*, 123, 25-33. https://doi.org/10.1016/j.jip.2014.09.001

Cameron, S. A., Lozier, J. D., Strange, J. P., Koch, J. B., Cordes, N., Solter, L. F., & Griswold, T. L. (2011). Patterns of widespread decline in North American bumble bees. *PNAS*, 108: 662-667. https://doi.org/10.1073/pnas.1014743108

Cameron, S. A., & Sadd, B. M. (2020). Global trends in bumble bee health. *Annual Review of Entomology*, *65*, 209-232. https://doi.org/10.1146/annurev-ento-011118-111847

Carvell, C., Rothery, P., Pywell, R. F., & Heard, M. S. (2008). Effects of resource availability and social parasite invasion on field colonies of Bombus terrestris. *Ecological Entomology*, *33*(3), 321–327. https://doi.org/10.1111/j.1365-2311.2007.00961.x

Colla, S. R., & L. Packer. (2008). Evidence for decline in eastern North American bumblebees (*Hymenoptera: Apidae*), with special focus on *Bombus affinis* Cresson. *Biodivers. Conserv*. 17: 1379–1391.

Darvill, B., O. Lepais, L. C. Woodall, & D. Goulson. (2012). Triploid bumblebees indicate a direct cost of inbreeding in fragmented populations. *Mol. Ecol.* 21: 3988–3995.

Durrer, S., & Schmid-Hempel, P. (1994). Shared use of flowers leads to horizontal pathogen transmission. *Proc Biol Sci*, *258*, 299–302.

Ellis, J. S., M. E. Knight, B. Darvill, & D. Goulson. (2006). Extremely low effective population sizes, genetic structuring and reduced genetic diversity in a threatened bumblebee species, *Bombus sylvarum* (*Hymenoptera: Apidae*). *Mol. Ecol.* 15: 4375–4386.

Free, J. B., (1955). The behaviour of egg-laying workers of bumblebee colonies. *Br. J. Anim. Behav*. 3, 147-153.

Garófalo, C. A., and Kerr, W. E. (1975). Sex determination in bees. I. Balance between femaleness and maleness genes in *Bombus atratus* franklin (Hymenoptera, Apidae). *Genetica*, 45(2), 203-209.

Gerard, M., D. Michez, D. Fournier, K. Maebe, G. Smagghe, J. C. Biesmeijer, & T. De Meulemeester. (2015). Discrimination of haploid and diploid males of *Bombus terrestris* (*Hymenoptera; Apidae*) based on wing shape. *Apidologie* . 1–10.

Gosterit, A. (2016). Adverse effects of inbreeding on colony foundation success in bumblebees, *Bombus terrestris* (*Hymenoptera: Apidae*). *Appl. Entomol. Zool*. . 51: 521–526.

Graystock, P., Yates, K., Evison, S. E., Darvill, B., Goulson, D., & Hughes, W. O. (2013). The Trojan hives: pollinator pathogens, imported and distributed in bumblebee colonies. *Journal of Applied Ecology*, 50, 1207-1215. https://doi.org/10.1111/1365-2664.12134

Hobbs, G. A. (1968). Ecology of species of *Bombus* (Hymenoptera: Apidae) in southern Alberta VII. Subgenus *Bombus*. *Canadian Entomologist*, *100*, 156–164.

Holehouse, K. A., R. L. Hammond, & A. F. G. Bourke. (2003). Non-lethal sampling of DNA from bumble bees for conservation genetics. *Insectes Soc.* 50: 277–285.

Hopkins, B. K., C. Herr, & W. S. Sheppard. (2012). Sequential generations of honey bee (*Apis mellifera*) queens produced using cryopreserved semen. *Reprod. Fertil. Dev.* 24: 1079–1083.

IUCN SSC. (2013). *Guidelines for Reintroductions and Other Conservation Translocations*. Version 1.0. Gland, Switzerland: IUCN Species Survival Commission.

IUCN SSC. (2014). *Guidelines on the Use of Ex situ Management for Species Conservation*. Version 2.0. Gland, Switzerland: IUCN Species Survival Commission.

Kent, C. F., A. Dey, H. Patel, N. Tsvetkov, & T. Tiwari. (2018). Conservation genomics of the declining North American bumblebee *Bombus terricola* reveals inbreeding and selection on immune genes. *Front. Genet*, 9: 316, https://doi.org/10.3389/fgene.2018.00316.

Klinger, E. G., Camp, A. A., Strange, J. P., Cox-Foster, D. & Lehmann, D. M. (2019). *Bombus* (Hymenoptera: Apidae) microcolonies as a tool for biological understanding and pesticide risk assessment. *Environmental Entomology*, 48(6), 1249-1259. https://doi.org/10.1093/ee/nvz117

Koch, H., Brown, M. J., & Stevenson, P. C. (2017). The role of disease in bee foraging ecology. *Current Opinion in Insect Science*, *21*, 60-67.https://doi.org/10.1016/j.cois.2017.05.008

Kraus, F. B., Wolf, S., & Moritz, R. F. A. (2009). Male flight distance and population substructure in the bumblebee Bombus terrestris. *Journal of Animal Ecology*, *2*, 247–252. https://doi.org/10.1111/j.1365-2656.2008.01479.x

Laverty, T. M., & Harder, L. D. (1988). The bumble bees of eastern Canada. *The Canadian Entomologist*, *120*, 965–987. https://doi.org/10.4039/Ent120965-11

Lepais, O., Darvill, B., O'Connor, S., Osborne, J. L., Sanderson, R. A., Cussans, J., Goffe, L., & Goulson, D. (2010). Estimation of bumblebee queen dispersal distances using sibship reconstruction method. *Molecular Ecology*, *19*(4), 819–831. https://doi.org/10.1111/j.1365-294X.2009.04500.x

Lozier, J. D., & Cameron, S. A. (2009). Comparative genetic analyses of historical and contemporary collections highlight contrasting demographic histories for the bumble bees *Bombus pensylvanicus* and *B. impatiens* in Illinois. *Mol. Ecol*. 18: 1875–1886.

Lozier, J. D., Cameron, S. A., Duennes, M. A., Strange, J. P., Williams, P. H., Goulson, D., ... & Jepsen, S. (2015). Relocation risky for bumblebee colonies. *Science (New York, NY)*, *350*(6258), 286-287.

Lundberg, H. & Svensson, B.G. (1975). Studies on the behaviour of *Bombus* Latr. species (Hym., Apidae) parasitized by *Sphaerularia bomb*i Dufour (Nematoda) in an alpine area. *Norwegian Journal of Entomology*, 22, 129–134.

Lye, G. C., Lepais, O., & Goulson, D. (2011). Reconstructing demographic events from population genetic data: the introduction of bumblebees to New Zealand. *Molecular Ecology*, *20*(14), 2888-2900.

Macfarlane, R. P., Patten, K. D., Royce, L. A., Wyatt, B. K. W., & Mayer, D. F. (1994). Management potential of sixteen North American bumble bee species. *Melanderi*a, 50, 1-12.

Macior, L. W. (1968). *Bombus* (Hymenoptera, Apidae) queen foraging in relation to vernal pollination in Wisconsin. *Ecology*, 49(1), 20-25.

Makinson, J. C., Woodgate, J. L., Reynolds, A., Capaldi, E. A., Perry, C. J., & Chittka, L. (2019). Harmonic radar tracking reveals random dispersal pattern of bumblebee (Bombus terrestris) queens after hibernation. *Scientific Reports*, *9*(1), 4651. https://doi.org/10.1038/s41598-019-40355-6.

Malfi, R. L., Crone, E., & Williams, N. (2019). Demographic benefits of early season resources for bumble bee (*B. vosnesenskii*) colonies. *Oecologia*, 0123456789. https://doi.org/10.1007/s00442-019-04472-3

McGowan, P.J., Traylor-Holzer, K. & Leus, K. (2017). IUCN guidelines for determining when and how *ex situ* management should be used in species conservation. *Conservation Letters, 10,* 361-366.

Mola, J. M., M. R. Miller, S. M. O'Rourke, and N. M. Williams. (n.d.). Wildfire reveals transient changes to individual traits and population responses of a native bumble bee *Bombus vosnesenskii*:12.

Mola, J. M., & N. M. Williams. 2019. A review of methods for the study of bumble bee movement. *Apidologie* 50:497–514.

Mullins, J. L., Strange, J. P., & Tripodi, A. D. (2020). Why are queens broodless? Failed nest initiation not linked to parasites, mating status, or ovary development in two bumble bee species of *Pyrobombus* (Hymenoptera: Apidae: *Bombus*). *Journal of Economic Entomology*, *113*, 575-581. https://doi.org/10.1093/jee/toz330

Otti, O., & Schmid-Hempel, P. (2007). *Nosema bombi*: a pollinator parasite with detrimental fitness effects. *Journal of Invertebrate Pathology*, *96*, 118-124. https://doi.org/10.1016/j.jip.2007.03.016

Otti, O., & Schmid-Hempel, P. (2008). A field experiment on the effect of *Nosema bombi* in colonies of the bumblebee Bombus terrestris. *Ecological Entomology*, *33*, 577-582. https://doi.org/10.1111/j.1365-2311.2008.00998.x

Pelletier, L. & McNeil, J. N. (2003). The effect of food supplementation on reproductive success in bumblebee field colonies. *Oikos*, *3*(3), 688–694. https://doi.org/10.1034/j.1600-0706.2003.12592.x

Piot, N., Snoeck, S., Vanlede, M., Smagghe, G., & Meeus, I. (2015). The effect of oral administration of dsRNA on viral replication and mortality in *Bombus terrestris*. Viruses, 7, 3172-3185. https://doi.org/10.3390/v7062765

Plath, O. E. (1922). Notes on the Nesting Habits of Some of the Less Common New England Bumble-Bees. *Psyche*, *280*, 122–128.

Plischuk, S., Meeus, I., Smagghe, G., & Lange, C. E. (2011). *Apicystis bombi* (apicomplexa: neogregarinorida) parasitizing *apis mellifera* and *bombus terrestris* (Hymenoptera: Apidae) in Argentina. *Environmental Microbiology Reports*, 3, 565-568. https://doi.org/10.1111/j.1758-2229.2011.00261.x

Rinderer, T. E. (Ed.). (2013). *Bee genetics and breeding*. Academic Press.

Rutrecht, S. T., & Brown, M. J. (2008). The life-history impact and implications of multiple parasites for bumble bee queens. *International Journal for Parasitology*, *38*, 799-808. https://doi.org/10.1016/j.ijpara.2007.11.004

Sadd, B. M., & Barribeau, S. M. (2013). Heterogeneity in infection outcome: lessons from a bumblebee-trypanosome system. *Parasite Immunology*, *35*, 339-349. https://doi.org/10.1111/pim.12043

Sadd, B. M., & P. Schmid-Hempel. (2008). Principles of ecological immunology. *Evol. Appl.* 2: 113–121.

Schmid-Hempel, R. & Schmid-Hempel, P. 1998. Colony performance and immunocompetence of a social insect, *Bombus terrestris*, in poor and variable environments. *Funct. Ecol*. 12: 22–30.

Singh, R., Levitt, A. L., Rajotte, E. G., Holmes, E. C., Ostiguy, N., Lipkin, W. I., ... & Cox-Foster, D. L. (2010). RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-Apis hymenopteran species. *PloS One*, *5*, e14357. https://doi.org/10.1371/journal.pone.0014357

Siva-Jothy, M. T., Y. Moret, & J. Rolff. (2005). Insect Immunity: An Evolutionary Ecology Perspective, pp. 1–48. *In* Simpson, S.J. (ed.), *Advances in Insect Physiology.* Academic Press.

Strange, J. P. (2010). Nest initiation in three North American bumble bees (*Bombus*): Gyne number and presence of honey bee workers influence establishment success and colony size. *Journal of Insect Science*, *10*(1), 130.

Traylor-Holzer, K., Leus, K., & Byers, O. (2019). *Ex situ* management for conservation. In: *Life on Land, Encyclopedia of the UN Sustainable Development Goals.* W. Leal Filho *et al*. (eds.). Springer Cham. https://doi.org/10.1007/978-3-319-71065-5_102-1

Tripodi, A. D., & Strange, J. P. (2019). No second chances for pollen-laden queens?. *Insectes Sociaux*, *66*(2), 299-304.

Webb, M. (1961). *The biology of the bumblebees of a limited area in eastern Nebraska*. http://digitalcommons.unl.edu/dissertations/AAI6302411/

Williams, N. M., Regetz, J., & Kremen, C. (2012). Landscape-scale resources promote colony growth but not reproductive performance of bumble bees. *Ecology*, *93*(5), 1049–1058. http://www.ncbi.nlm.nih.gov/pubmed/22764491

Woodard, S. H., Duennes, M. A., Watrous, K. M., & Jha, S. (2019). Diet and nutritional status during early adult life have immediate and persistent effects on queen bumble bees. *Conservation Physiology*, *7*(1), 1–10. https://doi.org/10.1093/conphys/coz048

U.S. Fish and Wildlife Service. 2016. Rusty patched bumble bee (*Bombus affinis*) species status assessment. 94pp.

U.S. Fish and Widllife Service. 2019. Draft recovery plan for the rusty patched bumble bee (*Bombus affinis*). Midwest Regional Office, Bloomington, MN. 10 pp.

Zayed, A. (2004). Effective population size in Hymenoptera with complementary sex determination. *Heredity,* 93: 627–630.

Zayed, A., D. W. Roubik, & L. Packer. (2004). Use of diploid male frequency data as an indicator of pollinator decline. *Proc. Biol. Sci.* 271 Suppl 3: S9–12.

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Appendix II: List of potential *ex situ* roles and descriptions

COMMON *EX SITU* AND POPULATION MANAGEMENT CONSERVATION ROLES

Based on a combination of the role descriptions in the IUCN SSC Guidelines on the Use of Ex Situ Management for Species Conservation, IUCN SSC Guidelines for Reintroductions and Other Conservation Translocations, and Appendix I of the Amphibian Ark Conservation Needs Assessment Process

In essence, *ex situ* management can support species conservation and prevent extinction by:

1) addressing primary threats and/or their causes;
2) counteracting the impacts of primary or stochastic threats on the population (such as reduced survival, poor reproduction and genetic isolation);
3) using *ex situ* populations for population restoration or conservation introduction; and/or
4) preventing extinction by gaining time in situations where threats are not under control or mitigation is not successful (enough).

This list of 10 potential conservation roles for *ex situ* (or other population management) activities are the most common roles that address these four functions.

Ark

Maintain a long-term *ex situ* population after extinction of all known wild populations and as a preparation for reintroduction or assisted colonization if and when feasible.

Insurance population

Maintain a long-term viable *ex situ* population of the species to prevent predicted local, regional or global species extinction and preserve options for future conservation strategies. These are typically species that are threatened and/or declining and for which it is unsure whether *in situ* threat mitigation will have the sufficient effect in a sufficient timeframe to prevent the extinction of the species or to prevent a dramatic decline in the numbers, populations and/or genetic diversity of the species. An *ex situ* population may be desired as an insurance population from which individuals can be taken for genetic and/or demographic supplementation or other conservation translocations as required, but these are not yet actively planned the foreseeable future.

Rescue (temporary or long term)

Establish an *ex situ* population for a species that is in imminent danger of extinction (locally or globally) and requires *ex situ* management, as part of an integrated program, to ensure its survival. The species may be in imminent danger because the threats cannot/will not be reversed in time to prevent likely species extinction, or the threats have no current remedy. The rescue may need to be long term or temporary (for example, to protect from catastrophes or predicted imminent threats that are limited in time, e.g. extreme weather, disease, oil spill).

Demographic manipulation

Improve a demographic rate (survival or reproduction) or status (e.g. skewed sex ratio) in the wild, often of a particular age, sex, or life stage. An example is a head-start program that removes individuals from the wild to reduce high mortality during a specific life stage and then subsequently returns them to the wild.

Population restoration: Reintroduction

Serve as a source of individuals for population restoration to re-establish the species to part of its former range from which it has been extirpated.

Population restoration: Reinforcement

Serve as a source of individuals for population restoration to supplement an existing population (e.g. for demographic, behavioral or genetic purposes).

Conservation introduction: Ecological replacement

Introduce the species outside of its indigenous range to re-establish a lost ecological function and/or modify habitats. This may involve species that are not themselves threatened but that contribute to the conservation of other taxa through their ecological role.

Conservation introduction: Assisted colonization

Introduce the species outside of its indigenous range to avoid extinction of populations of the species.

Ex situ research and/or training

Use an *ex situ* population for research and/or training that will directly benefit conservation of the species, or a similar species, in the wild (e.g. develop monitoring methods; address data gaps in disease transmission or treatment). The research or training must address specific questions essential for success of the overall conservation strategy for the species. This can include non-threatened species serving as a model for threatened species, or establishing *ex situ* populations of a threatened species to gain important species-specific husbandry and breeding expertise that is likely to be needed in the future to conserve the species.

Conservation education

Forms the basis for an education and awareness program that addresses specific threats or constraints to the conservation of the species or its habitat. Education should address specific human behavioral changes that are essential for the success, and an integral part of, the overall conservation strategy for the species. This primarily involves *ex situ* locations visited by the intended human audience.