Species Documentation

STATE OF VERMONT
ENDANGERED SPECIES COMMITTEE

1. Scientific Name: *Bombus affinis* (Cresson, 1863)	7. Surrounding State & Provincial Status:
2. Common Name: Rusty-patched Bumble Bee	Maine: SH
3. Species Code *(Department use only)*:	New Hampshire: SH
4. Current Vermont Status: S1	Massachusetts: SH
5. Recommended Vermont Status: Endangered	New York: SH
6. Federal Status: ESA petition filed Jan. 2013 with USFWS, Global Status- G1 (critically imperiled), Canada Status- endangered, listed as imperiled on the Xerces Society's *Red List of Pollinator Insects of North America* (Shepherd *et al.* 2005).	Quebec: not ranked Ontario: Endangered

POPULATION STATUS

8. Global, North American, and Vermont Ranges:

Until recently, the Rusty-patched Bumble Bee (*Bombus affinis*) was broadly distributed across the eastern U.S. and Upper Midwest, north to Maine and southern Quebec and Ontario in Canada, south to the northeast corner of Georgia, reaching west to the eastern edges of North and South Dakota at elevations from sea level to approximately 6,000 feet (Fig. 1). Since 2000, *B. affinis* has been observed or collected in Connecticut (Litchfield County), Illinois (Champaign, Cook, DeWitt, Dupage, McHenry, Ogle, Peoria, and Winnebago Counties), Indiana (Jasper, Marion, Montgomery, Newton, and Starke Counties), Iowa (unknown county), Maryland (Anne Arundel and Prince George Counties), Massachusetts (Barnstable County), Minnesota (Cass, Hennepin, Itasca, Ramsey, and Washington Counties), Ontario (Lambton and Norfolk Counties), Tennessee (Blount/Swain County), and Wisconsin (Dane and Iowa Counties) (Jepsen et al. 2013).

Three recent studies indicated a recent and rapid decline of *B. affinis* populations and range contraction (Cameron et al. 2011a, Colla et al. 2012, Colla and Packer 2008). Cameron et al. (2011a) conducted a field survey that collected more than 16,000 bumble bee specimens from throughout the U.S. during 2007-2009 and compared this to a database of more than 73,000 historical bumble bee specimens. Their analysis revealed that the range of *B. affinis* had contracted by an estimated 87%. This same study concluded that the relative abundance of *B. affinis* declined by 95%, with B. affinis only detected in low numbers at three Illinois locations and one Indiana location during the recent survey. A separate analysis of nearly 45,000 eastern *Bombus* records, from museum collections and contemporary surveys in Canada and the U.S., concluded that *B. affinis* has suffered greater than a 70% range contraction (Colla et al. 2012). The authors classified *B. affinis* as "Endangered" using modified criteria from the International Union for the Conservation of Nature. The relative abundance of *B. affinis* from 1991-2009 was 87% less than its relative abundance in collections from <1931-2000 (derived from data presented in Figure 1, Colla *et al.* 2012). A 2004-2006 study collected approximately 9,000 bumble bees from 28 sites where *B. affinis* historically occurred, as well as 15 additional sites within the bee's historic range in eastern North American, and found only a single specimen in southern Ontario, despite numerous reports that the species was once common (Colla and Packer 2008). In addition to these three studies, there are multiple local examples of extirpations and decreases in the relative abundance of *B. affinis*, summarized in Evans *et al.* (2008) and presented below.

Midwestern U.S. - A study comparing records from a contemporary survey to previous records of *B. affinis* in Illinois revealed that the distribution of this species has decreased by nearly one-third in that state since 2000, with only 67% of its pre-2000 distribution remaining (Grixti *et al.* 2009). A multi-year survey in northern Indiana found 25 *B. affinis* specimens out of 217 *Bombus* (12%) in 2001, two out of 451 (0.004%) in 2002, and 0 out of 553 in 2003 (R. Jean & P. E. Scott pers. comm. with E. Evans, Xerces Soc., September 2007 from Jepsen *et al.* 2013). A survey from 1994-1995 of 464 bumble bees at Long Lake Regional Park in New Brighton, Minnesota found 98 *B. affinis* individuals (Reed 1995; C. Reed, pers. comm. with E. Evans, Xerces Soc., June 2007 from Jepsen *et al.* 2013). A survey during the summers of 2007 and 2008 of 593 bumble bees at the same park found no *B. affinis* (E. Evans, Xerces Soc., personal obs., July 2008 from Jepsen *et al*. 2013).

Mid-Atlantic U.S. - In a sample of nearly 1,000 bumble bees on the Patuxent National Wildlife Refuge in Maryland from 2002 to 2007, a single *B. affinis* specimen was collected in 2002 and none have been collected since (S. Droege, pers. comm. with E. Evans, Xerces Soc., Feb. 2008 from Jepsen *et al.* 2013). The same researcher reports that *B. affinis* was numerous in collections in the 1980s in areas near Patuxent National Wildlife Refuge north of Baltimore, Maryland and in northern Delaware. Since 2000, *B. affinis* has not been seen in the Great Smoky Mountains National Park in North Carolina and Tennessee, where it was once abundant (A. J. Mayor, pers. comm. with E. Evans, Xerces Soc., Sept. 2007 from Jepsen *et al.* 2013). Surveys of spring queens in North Carolina consistently found *B. affinis* from 1995 to 2001, yet between 2002 and 2007, no queens were found while other *Bombus* species were present (R. Jacobson, pers. comm. with E. Evans, Xerces Soc., Sept. 2007 from Jepsen *et al.* 2013).

Northeastern U.S. and Southeastern Canada - A 2003 survey including over 1,261 bumble bees in New York, where *B. affinis* was previously considered to be "moderately abundant in the eastern to southern parts of the state..." (Leonard 1928, referenced in Giles and Ascher 2006), failed to find any *B. affinis* (Giles and Ascher 2006). The authors noted that *B. affinis* was well represented in historical collections from the northeastern U.S. A study by Colla and Packer (2008) of two sites in southern Ontario comparing a collection of nearly 1,200 bumble bees from 2004-2006 to a historical collection (Macfarlane 1974) of >3,600 bumble bees from the same locations in 1971-1973, revealed that *B. affinis* had been extirpated from those sites, despite the fact that it comprised approximately 14% of the 1970s collection. P. Williams reported that *B. affinis* was formerly abundant in Toronto, Ontario in 1983 but was not seen during regular surveys in the Toronto area from 2003 to 2008 (pers. comm. with E. Evans, Xerces Soc., July 2008 from Jepsen *et al*. 2013).

Vermont – Previously, *B. affinis* appears to have been a common component of the Vermont bee fauna. Regional data suggest that it was probably found throughout the entire state (Figs. 1 and 2). The Vermont Bumble Bee Survey assembled over 2,500 *Bombus* records from 1915 to 2011 from over 25 collections (Vermont Center for Ecostudies unpub. data). *B. affinis* represented about 8.5% of the records (n=211), the 6th most common species of 17 known species in Vermont, and were found in 38 Vermont towns scattered across the state. About 13% of the specimens from 1915-1987 were *B. affinis* representing 36 towns (n=1,412 Bombus, n=183 *B. affinis*), while only 2% of the total specimens from 1989-2011 were *B. affinis* from 10 towns (n=1,072 Bombus, n=24 *B. affinis*). The earliest known record for Vermont was a specimen in the UVM Zadock Thompson Invertebrate Collection from September 13, 1928 in Bolton. Collection data show that entomology students at UVM, assigned to assemble a general insect collect each year, regularly collected the species from the 1960s through the 1990s (Figs. 2 and 3).

The last known *B. affinis* record for Vermont was a drone collected on August 31, 1999 in the Intervale in Burlington. Despite surveys by students and biologists during the 2000s, no *B. affinis* have been observed in the state (L. Richardson unpub. data). In 2012 and 2013 the Vermont Bumble Bee Survey visited over 1,500 sites throughout the state, many of these multiple times, yielding over 10,000 *Bombus* records (Fig. 2; Vermont Center for Ecostudies, unpub. data). No *B. affinis* were found. One of the formerly most common bumble bees of fields, farms, and gardens declined drastically across Vermont in the span of a decade or less.

9. Vermont's Position within Global Range: _X_ Central ___ Peripheral ___ Disjunct

10. Historic Occurrences in Vermont More Than 25 Years Ago *(Type, Number, General Location, Regularity of Use, Confidence in Records, etc.)*:

There are 178 historic records confirmed from collections representing 36 towns scattered across Vermont ranging from as far back as 1928 (see Section 24.4). These collections are likely spatially biased to areas near institutions such as UVM, Dartmouth College, and Middlebury College (Figure 2). The entire state was widely believed to be part of *B. affinis* range before the sudden decline in the 1990s.

11. Historic Abundance More Than 25 Years Ago *(Number of Breeding Individuals or Size of Area Occupied, Confidence in Records, etc.)*:

Although there are no survey data to determine accurate abundance or density estimates for this species, *B. affinis* was regularly present in student collections at UVM until the 1990s (Fig. 3, section 24.4).

12. Current Occurrences in Vermont *(Type, Number, General Location, Regularity of Use, Confidence in Records, Extent to which the Species has been Inventoried, etc.):*

Despite surveys by students and biologists during the 2000s and intensive surveys across the state by the Vermont Bumble Bee Survey during 2012 and 2013 (Fig. 2), no *B. affinis* has been observed in the state since 1999 (Vermont Center for Ecostudies, unpub. data). *B. affinis* once represented about 8% of *Bombus* collections to an absence in recent, more intense and targeted surveys conducted across the state.

13. Current Abundance *(Number of Breeding Individuals or Size of Area Occupied, Confidence in Records, Problems in Estimating Abundance, etc.):*

Intensive targeted surveys across the state in 2012 and 2013 encountered no individuals (Fig. 2).

14. Population Trend:

_x_ Declining
___ Stable
___ Increasing
___ Unknown

Estimate Based on:

_x_ Surveys
___ Counts
_x_ Observations
___ Other *(explain)*

Documentation & Comments:

HABITAT IN VERMONT

15. General Description:

B. affinis has been found in a variety of places including: woodlands, marshes, agricultural landscapes, and residential parks and gardens (Colla and Packer 2008; Colla and Dumesh 2010; Xerces Society 2012). This species, relative to other sympatric bumble bees, is cold tolerant, allowing it to occur at high elevations and emerge earlier in the year (Colla and Dumesh 2010). It usually nests in old rodent burrows, hollow tree stumps and fallen dead wood (Macfarlane 1974). All members of this subgenus typically nest underground (Macfarlane 1974, Laverty and Harder 1988). There are no data on the overwintering requirements of *B. affinis*, although they are assumed to be similar to those of other species in the *Bombus* genus, including sites suitable for underground burrows in loose soil or the presence of fallen dead wood (MacFarlane 1974). Foraging habitat for *B. affinis* typically contains an abundance of wild flowers in the forest understory or in open fields. It has been recorded feeding from a wide variety of plant genera for pollen and nectar.

Bumble bees are generalist foragers, meaning that they gather pollen and nectar from a wide variety of flowering plants. To meet its nutritional needs, *B. affinis* requires a constant supply of flowers that bloom throughout the duration of the colony life cycle, which is from approximately April to September (Plath 1922; Mitchell 1962; Milliron 1971; Macfarlane *et al.* 1994). Nectar provides bumble bees with carbohydrates and pollen provides them with protein. The amount of pollen available to bumble bee colonies directly affects the number of queens that can be produced (Burns

2004). Since queens are the only bumble bees capable of forming new colonies, pollen availability directly impacts future bumble bee population levels.

B. affinis probably needs floral resources to be located in relative close proximity to its nest sites, as studies of other bumble bee species indicate that they routinely forage within less than one kilometer from their nests (Knight *et al*. 2005; Wolf and Moritz 2008; Dramstad 1996; Osborne *et al*. 1999), although in some cases nearly two kilometers (Walther- Hellwig & Frankl 2000). Colla and Dumesh (2010) suggest that *B. affinis* is likely dependent upon woodland spring ephemeral flowers, since this bumble bee emerges early in the year and is associated with woodland habitats.

This is a short-tongued species (Medler 1962) and thus is not able to easily access the nectar in flowers with deep corollas. Short-tongued bees are better suited for pollination of open flowers and those with short corollas, including cranberry (Patten *et al.* 1993). During collection of pollen and nectar from flowers, bumble bees also transport pollen between flowers, facilitating seed and fruit production. Bumble bees have many qualities that contribute to their suitability as agricultural pollinators. They are able to fly in cooler temperatures and lower light levels than many other bees, which extends their workday and improves the pollination of crops during inclement weather (Corbet *et al.* 1993). To release pollen, bumble bees are able to grab onto a flower and move their flight muscles rapidly, causing the anthers to vibrate, dislodging pollen. This resonant vibration is called "buzz pollination" (Buchmann 1983). Some plants, including tomatoes and peppers, benefit from buzz pollination. *B. affinis* has been shown to be an excellent pollinator of cranberry (Cane and Schiffauer 2003) and other important food crops such as plum and apple (Medler and Carney 1963; Mitchell 1962), alfalfa (Holm 1966), and onion for seed production (Caron *et al.* 1975).

In addition to commercially important crops, *B. affinis* also plays a vital role as a generalist pollinator of native flowering plants, and its loss may have far ranging ecological impacts. An examination of the theoretical effect of removing specialist and generalist pollinators on the extinction of plant species concluded that the loss of generalist pollinators, especially bumble bees, caused the greatest number of plant extinctions (Memmott *et al.* 2004). In Britain and the Netherlands, where multiple pollinators have declined, there is evidence of a parallel decline in the abundance of insect pollinated plants (Biesmeijer *et al.* 2006).

16. Habitat Losses in Past *(Amount and Location)*:

Like most North American bumble bee species, *B. affinis* faces general threats from habitat alterations that can interfere with its primary habitat requirements, including: access to sufficient food (nectar and pollen from flowers), nesting sites (such as underground abandoned rodent cavities or above ground in clumps of grasses), and overwintering sites for hibernating queens. Like many other *Bombus*, it has historically occupied open areas and grasslands of the Upper Midwest and Northeast. Some of these areas in Vermont have certainly been lost or fragmented by agriculture, urban development, and conversion back to forest. Although it has certainly occurred, quantifying past habitat loss would be difficult for this species.

Bombus species richness, abundance, and genetic diversity are influenced by the quality of habitat on a landscape level. Isolated patches of habitat may not be sufficient to support *Bombus* populations (Hatfield and LeBuhn 2007; Öckinger and Smith 2007), and populations existing in fragmented habitats can also face problems with inbreeding depression (Darvill *et al.* 2006 and 2012; Ellis *et al.* 2006). Darvill *et al.* (2012) found that *Bombus* populations limited to less than 15 km2 of habitat were more likely to show signs of inbreeding. Goulson (2010, p.193) suggests that a viable population probably requires 3.3-10 km2 of suitable habitat.

17. Probable Habitat Losses in Future *(Amount, Location, and Type)*:

Unknown.

18. Current Protected Status of Habitat: ___ Unknown Whether Any Protected ___ Believed To Be None Protected ___ At Least One Protected Occurrence ___ Several Protected Occurrences ___ Many Protected Occurrences _X__ Other *(explain)*	*Comments:* Some known areas of occurrence are likely protected within state, federal and private conservation lands (wildlife management areas, UVM natural areas, TNC properties, Green Mountains National Forest, etc). To our knowledge, no protected areas have any specific management practices in place regarding *Bombus* populations such as specific mowing regimes or providing nectar and pollen sources (Hatfield et al. 2012).

POPULATION BIOLOGY

19. Population Threats *(Contaminants, Predation, Competition, Disease, Human Disturbance from Recreation, Collection, Harvest, etc.)*:

Degree of Threat:

_x_ Very Threatened, Species Directly Exploited or Threatened by Natural or Man-caused Forces
___ Moderately Threatened, Habitat Lends Itself to Alternate Use but is not Currently in Jeopardy
___ Little Threat, Self-protecting by Unsuitability for Other Uses
___ Unknown

Documentation & Comments:

Pathogens and Parasites

Pathogens and parasites pose a substantial threat to the continued survival of *B. affinis*. Worldwide, reported pathogens and parasites of bumble bees include: viruses, bacteria, fungi, protozoa, nematodes, hymenopteran and dipteran parasitoids, one lepidopteran parasite, and mites (Acari) (summarized in Schmid-Hempel 2001). Pathogen prevalence and fitness effects in wild North American bumble bees are generally not well understood. The microparasites and macroparasites that have been identified as pathogens of concern to wild North American bumble bees (Cameron *et al.* 2011b, page 16) are discussed below.

Nosema bombi is a microsporidian parasite that infects bumble bees primarily in the malpighian tubules, but also in fat bodies, nerve cells, and sometimes the tracheae (Macfarlane *et al*. 1995). Colonies can appear to be healthy but still carry *N. bombi* (Larsson 2007) and transmit it to other colonies. *N. bombi* can reduce colony fitness, as well as reduce individual reproduction rate and life span in bumble bees (Schmid-Hempel & Loosli 1998; Schmid-Hempel 2001; Colla *et al*. 2006; Otti & Schmid-Hempel 2007, 2008; van der Steen 2008; Rutrecht & Brown 2009). This parasite has been observed recently in wild bumble bees throughout North America (Colla *et al.* 2006; Gillespie *et al.* 2010; Kissinger *et al.* 2011; Cameron *et al.* 2011; Cordes *et al.* 2012).

Cameron *et al.* (2011) found a significantly higher prevalence of *N. bombi* in declining North American bumble bee species (*B. occidentalis* and *B. pensylvanicus*). *B. affinis* was tested, but the sample size was so low that the data were excluded from the statistical analyses. However, the authors note that the available data show that this species followed the same infection trend of the other declining species with infected individuals collected at 4 of 5 sites, and infections detected in 7 of the 14 individuals collected. *N. bombi* infection was significantly lower in species that have not exhibited recent declines in range and relative abundance.

Crithidia bombi is a trypanosome protozoan that can dramatically reduce bumble bee longevity and colony fitness (Brown *et al.* 2003; Otterstatter & Whidden 2004), interfere with learning among bumble bee foragers (Otterstatter *et al.* 2005), increase ovary development in workers (Shykoff and Schmid-Hempel 1991), and decrease pollen loads carried by workers (Shykoff and Schmid-Hempel 1991). In the UK, researchers found a higher prevalence of the pathogen *C. bombi* in bumble bee populations with reduced genetic diversity, suggesting that as populations become smaller and lose heterozygosity, the impact of this parasite will increase (Whitehorn *et al.* 2010), pushing already at-risk populations closer to extinction.

Apicystis bombi is a neogregarine protozoa that has been shown to infect 2.5% of *B. affinis* queens in Ontario, Canada (Macfarlane *et al.* 1995). This parasite is associated with rapid death of infected bumble bee queens early in the season

(Macfarlane *et al.* 1995; Rutrecht & Brown 2008). It has also been shown to inhibit ovary development and reduce queen longevity (Rutrecht & Brown 2008). More research is needed to understand causal effects that this parasite has on bumble bees and how this parasite is transmitted. This parasite has been found in commercial bumble bee colonies (Meeus *et al.* 2011), and researchers suggest that this pathogen may have been introduced from Europe to NW Patagonia, Argentina on commercial bumble bees, potentially causing an observed population collapse in a native bumble bee species (Arbetman *et al.* 2012). *Apicyistis bombi* poses a serious potential threat to *B. affinis*.

RNA viruses that have historically been considered to be specific to honey bees (*Apis mellifera*), including Israeli acute paralysis virus, black queen cell virus, sacbrood virus, deformed wing virus, and Kashmir bee virus, have been recently detected in wild North American bumble bees foraging near apiaries (Singh *et al.* 2010). Deformed wing virus, which is associated with severe winter losses in honey bees (Highfield *et al.* 2009), was also detected in bumble bees in Germany, and the infected bumble bees displayed the same deformities that are typical of infected honey bees (Genersch *et al.* 2006). To understand the extent of the threat to *B. affinis*, the prevalence of these viruses in wild populations of bumble bees, as well as their effects on bumble bee fitness, are in urgent need of further study.

Bumble bees are infected by mites, including *Locustacarus buchneri*, a species that parasitizes the trachea of bumble bees (Husband and Shina 1970). *Locustacarus buchneri* is associated with reduced foraging and lethargic behavior (Husband and Shina 1970) and a significantly reduced lifespan in male bumble bees (Otterstatter and Whidden 2004). Otterstatter and Whidden (2004) reported that this mite was most prevalent in bumble bees of the subgenus *Bombus sensu stricto* (*B. occidentalis, B. moderatus, B. terricola*) in a study in southwestern Alberta. Although *B. affinis* was not present at these study sites, it belongs to the same subgenus as the species listed above that were heavily parasitized by *L. buchneri*, and thus may also be particularly susceptible to this parasite.

Sphaerularia bombi is an entomopathogenic nematode that infects hibernating bumble bee queens and sterilizes them (Schmid-Hempel 2001). In a literature review, Macfarlane *et al.* (1995) notes that bumble bee queens infected with this parasite in New Zealand colonized new areas at a rate of less than 1% of that of healthy queens. This parasite has been detected in *B. affinis* (Macfarlane *et al.* 1995) and may pose a threat to the long-term survival of the species.

In summary, a variety of microparasites (*Nosema bombi*, *Crithidia bombi*, *Apicystis bombi*, and RNA viruses) and macroparasites (*Locustacarus buchneri* and *Sphaerularia bombi*) can cause harm to bumble bees and pose a threat to *B. affinis*.

Pathogen Spillover

The spread of pathogens to *B. affinis* from the domesticated common eastern bumble bee (*Bombus impatiens*) and other species of *Bombus* that are currently being developed for commercial use, threatens *B. affinis* with extinction. In addition, RNA viruses from the domesticated honey bee (*Apis mellifera*) can be transmitted to bumble bees at shared flowers (Singh *et al.* 2010), and pose a novel threat to *B. affinis*.

The spillover of RNA viruses from honey bees to bumble bees is a recently identified threat to wild bumble bees, including *B. affinis*. A number of RNA viruses that were formerly thought to be specific to honey bees have now been reported to infect bumble bees (Genersch *et al.* 2006; Meeus *et al.* 2010; Singh *et al.* 2010; Morkeski and Averill 2012). The virulence of many of these RNA viruses in bumble bees has not yet been evaluated. RNA viruses can be transmitted from honey bees to wild bumble bees when they interact at shared flowers (Singh *et al.* 2010), where infected pollen grains left by honey bees are collected by bumble bees and brought back to the nest. Bumble bees may also be infected by RNA viruses when commercial bumble bee producers use honey bee pollen to rear bumble bee colonies (if the pollen is not treated with radiation). Morkeski & Averill (2012) found what appear to be deformed wing virus and black queen cell virus in colonies of bumble bees from two North American commercial production facilities and Singh *et al.* (2010) found Israeli acute paralysis virus in colonies from one North American commercial bumble bee production facility.

Commercial bumble bees are used primarily to pollinate greenhouse tomatoes, and increasingly to pollinate a wide variety of other greenhouse and open field vegetable and fruit crops in the U.S. and worldwide (Velthius & van Doorn 2006). The commercial bumble bee industry has grown dramatically in the past two decades (Velthius & van Doorn

2006), coincident with the growth of the greenhouse tomato industry. From 1985-2005, there was a 30% increase in fresh tomato consumption in the U.S., with more than one-third of the fresh tomatoes in stores coming from greenhouses (Calvin & Cook 2005). Commercial bumble bees often escape greenhouses to forage on nearby plants (Whittington *et al*. 2004; Morandin *et al*. 2001), where they interact with wild bumble bees and have the opportunity to transmit pathogens at shared flowers. Commercially raised bumble bees frequently harbor high pathogen loads (Goka *et al*. 2000; Niwa *et al*. 2004; Colla *et al*. 2006) and the spillover of pathogens from commercial bumble bees in greenhouses to wild, native bumble bees foraging near greenhouses has been documented (Colla *et al*. 2006; Goka *et al.* 2006; Otterstatter & Thomson 2008).

Meeus *et al.* (2011) reviewed the effects of invasive parasites on bumble bee declines. They report that the commercial production of bumble bees has the potential to lead to bumble bee declines in three ways: commercial colonies may have high parasite loads, which could then infect wild bumble bee populations; commercial production may allow higher parasite virulence to evolve, leading to the introduction of parasites that are potentially more harmful to wild bumble bees than naturally occurring parasites; and the global transport of commercial bumble bees can introduce novel parasites to which resident, native bumble bees have not adapted. Pathogens reported from commercial bumble bee colonies worldwide include: *Apicystis bombi*, *Crithidia bombi*, *Locustacarus buchneri*, *Nosema bombi*, black queen cell virus, deformed wing virus, Israeli acute paralysis virus, and Kashmir bee virus (Meeus *et al.* 2011). Commercial bumble bee colonies in North America have tested positive for *Crithidia bombi*, *Nosema bombi*, *Locustacarus buchneri*, deformed wing virus, black queen cell virus, sacbrood virus (Morkeski & Averill 2012), and Israeli acute paralysis virus (Singh *et al.* 2010).

The spillover of the microsporidian parasite *Nosema bombi* from commercial to wild bumble bees has been hypothesized as a cause of the sudden, rapid decline of *B. affinis* and three other closely related North American bumble bees – Franklin's bumble bee (*Bombus franklini*), the western bumble bee (*Bombus occidentalis*) and the yellow-banded bumble bee (*Bombus terricola*) (Thorp and Shepherd 2005; Evans *et al*. 2008). This hypothesis is supported by the timing, speed, and severity of the population declines of *B. affinis* and its close relatives.

In the early 1990s, commercial bumble bee producers brought *B. occidentalis* queens from western North America to European bee rearing facilities, where those bees may have come into contact with pathogens of the commercially produced European buff-tailed bumble bee (*Bombus terrestris*). From 1992-1994, the USDA-APHIS allowed commercial colonies of B. *occidentalis* and the Common Eastern Bumble Bee (*Bombus impatiens*) to return from European facilities to the U.S. (Flanders *et al*. 2003). In 1997, bumble bee producers reported an outbreak of *Nosema bombi* in laboratory populations of *B. occidentalis,* and eventually had to stop producing this species commercially (Flanders *et al.* 2003; Velthuis & van Doorn 2006). Coincident with the crash in commercial colonies of *B. occidentalis,* researchers noticed that *B. occidentalis, B. affinis* and their relatives began disappearing from the wild in the late 1990s (Thorp & Shepherd 2005; Evans *et al*. 2008; Thorp *et al.* 2010).

This hypothesis is currently under investigation by Dr. Cameron at the University of Illinois. Her research team has already determined that declining bumble bee species harbor higher levels of *N. bombi* than stable species. They initially determined that *N. bombi* was genetically identical to *N. bombi* found in European bumble bees (Cameron *et al.* 2011), but a more recent, in-depth analysis by Cordes *et al.* (2012) revealed that North American bumble bees harbor a unique strain of *N. bombi*. The research that has been done to date, however, has been insufficient to determine whether or not a European strain of *N. bombi* was released in the U.S., and if so, whether it led to the decline of *B. affinis* (Cordes *et al*. 2012).

A recent analysis by Szabo *et al.* (2012) found a significant correlation between vegetable greenhouse density, which was used as a proxy for commercial bumble bee use, and the decline of the *B. terricola* and *B. pensylvanicus,* but found no significant correlation between vegetable greenhouse density and the decline of *B. affinis*. However, this analysis did not address the possibility of an acute pathogen spillover event in which a rapid disease spread through wild populations. Furthermore, the analysis did not include areas where bumble bees are used in open field settings.

In Canada, higher levels of the protozoan parasite *Crithidia bombi* were detected in wild bumble bees foraging near greenhouses that used commercial bumble bees (Colla *et al.* 2006; Otterstatter & Thomson 2008), and it was suggested that this pathogen may be implicated in the sudden, widespread decline observed in North American bumble bees in the subgenus *Bombus sensu stricto*, including *B. affinis* (Otterstatter & Thomson 2008). However, a more recent analysis of pathogen prevalence in wild bumble bees, including *B. affinis*, did not find evidence that *Crithidia* infections are involved in the decline of U.S. bumble bee species (Cordes *et al*. 2012).

Pesticides

Pesticides are used widely in agricultural, urban, and even natural areas and can exert both lethal and sublethal toxic effects on bumble bees. Foraging bumble bees can be poisoned by pesticides when they absorb toxins directly through their exoskeleton, drink contaminated nectar, gather contaminated pollen, or when larvae consume contaminated pollen. Because bumble bees nest in the ground, they may be uniquely susceptible to pesticides used on lawns or turf (National Research Council 2007). Pesticides applied in the spring, when bumble bee queens are foraging and colonies are small, are likely to be most detrimental to bumble bee populations (Goulson *et al.* 2008). Since males and queens are produced at the end of the colony cycle, sublethal doses of pesticides applied at any time during the bumble bee lifecycle can have substantial adverse effects on subsequent generations. Any application of pesticides can threaten bumble bees, but pesticide drift from aerial spraying can be particularly harmful. In Europe, the recent declines in bumble bees have been partially attributed to the use of pesticides (Williams 1986; Thompson and Hunt 1999).

B. affinis is threatened by the widespread use of pesticides across its range. Insecticides are designed to kill insects directly and herbicides can indirectly affect bumble bees by removing floral resources. There are very few data available concerning the effect of fungicides on bumble bees, although a literature review suggests that most active ingredients in fungicides are compatible with commercial bumble bees (Mommaerts & Smagghe 2011).

Insecticides- Neonicotinoids are a relatively new class of systemic insecticides that are used widely to combat insect pests of agricultural crops, turfgrass, gardens, and pets (Cox 2001). Colla & Packer (2008) suggested that neonicotinoids may be one of the factors responsible for the decline of *B. affinis* since the use of this class of insecticides began in the U.S. in the early 1990s, shortly before the decline of this species was noticed.

A recent study exposing bumble bees to field-realistic levels of the neonicotinoid imidacloprid found an 85% reduction in the production of new queens and significantly reduced colony growth rates compared to control colonies (Whitehorn *et al.* 2012). The authors suggest that neonicotinoids "may be having a considerable negative impact on wild bumble bee populations across the developed world" (Whitehorn *et al.* 2012). Another study of bumble bees exposed to varying levels of imidacloprid found a dose-dependent decline in fecundity and documented that levels of this pesticide realistically found in the field were capable of reducing brood production by one-third (Laycock *et al.* 2012). The authors speculate that this decline in fecundity is a result of individual bumble bees failing to feed, which raises concerns about the impact of this pesticide on wild bumble bees (Laycock *et al.* 2012). Other toxicity studies have demonstrated that contact exposure of imidacloprid and clothianidin to bumble bees can be very harmful (Marletto *et al.* 2003; Gradish *et al.* 2009; Scott-Dupree *et al.* 2009), and an acute oral dose of imidacloprid is highly toxic to bumble bees (Marletto *et al.* 2003, Hopwood *et al.* 2012). Mommaerts *et al.* (2010) found that chronic exposure of three neonicotinoids to bumble bees was dose dependent, and another study by Incerti *et al.* (2003) found that one third of bumble bees in a flight cage exposed to blooming cucumbers treated with a "field dose" of imidacloprid died within 48 hours. A study by Gill *et al.* (2012) examining the effects of the combined exposure of bumble bees to field realistic levels of two pesticides – an imidicloprid and a pyrethroid – found that foraging behavior was impaired, worker mortality increased, and both brood development and colony success were significantly reduced. Other studies have also documented sublethal effects of neonicotinoids on bumble bees, including: reduced foraging ability (Morandin & Winston 2003); reduced drone production and longer foraging times (Mommaerts *et al.* 2010); reduced foraging activity, reduced food storage, and reduced adult survival (Al-Jabr 1999); and lower worker survival and reduced brood production (Tasei *et al.* 2000 In Hopwood *et al.* 2012).

Neonicotinoids are widely used on agricultural crops that are attractive to pollinators, as well as on horticultural plants and lawns in urban and suburban areas. Thus, this class of insecticide is likely to negatively affect *B. affinis*. Of particular concern is a finding in a recent review of the impact of neonicotinoid pesticides on pollinating insects which found that products approved for home and garden use may be applied to ornamental and landscape plants and turf grass at significantly higher concentrations (potentially 32 times higher) than the allowable concentration of the same products applied on agricultural crops (Hopwood *et al.* 2012).

In forested areas, insecticides have been used to control defoliators such as tussock moth, gypsy moth, and spruce budworm. In New Brunswick, Canada, bumble bee populations declined drastically when exposed to fenitrothion (reviewed in Kevan and Plowright 1995) resulting in reduced pollination of nearby commercial blueberries and other plants such as orchids and clovers (Kevan 1975; Plowright *et al.* 1978, 1980). Organophosphate, carbamate, and pyrethroid insecticides have been associated with bee poisonings in food crops (Johansen 1977; Kearns *et al.* 1998). Bumble bee deaths have been reported after application of a pyrethroid insecticide to oilseed rape (Thompson 2001). The use of Spinosad, a commonly used insect neurotoxin, has resulted in reduced worker foraging efficiency when bumble bee larvae are fed with pollen containing this pesticide (Morandin *et al*. 2005). Skyrm (2011) observed significant queen mortality when exposed to low doses of Spinosad. In an examination of the effect of chitin synthesis inhibitors on *Bombus*, Mommaerts *et al.* (2006) found that even at very low concentrations, diflubenzuron and teflubenzuron increased egg mortality.

Transgenic Plants- Increasing numbers of insecticidal transgenic plants are being used to control pest species, and the effect of most of these transgenic plants on bumble bees is not known (Malone & Pham Delègue 2001). However, there is evidence of negative effects on bumble bees from two compounds that are produced in transgenic plants; the soybean trypsin inhibitor (a protease inhibitor) and *Galanthus nivalis* agglutinin (a lectin) have been shown to reduce bumble bee longevity and reproduction when administered experimentally (Babendreier *et al*. 2008). However, the amount of transgene product expressed in pollen and nectar is still unknown, so it is difficult to determine the impact of these products on bumble bees in the wild.

Herbicides- These can be a valuable tool for the control of invasive weed species. However, the use of broad-spectrum herbicides to control weeds can indirectly harm pollinators by decreasing their habitat quality through removal of flowers that provide pollen and nectar for existing populations (Williams 1986; Shepherd *et al*. 2003, Pleasants & Oberhauser 2012).

Just as pollinators can influence the plant community, changes in vegetation can have an impact on pollinators (Kearns & Inouye 1997). The broadcast application of a non-selective herbicide can indiscriminately reduce floral resources, host plants, and nesting habitat (Smallidge and Leopold 1997). Bumble bees require consistent sources of nectar, pollen, and nesting material during times adults are active. The reduction in resources caused by non-selective herbicide use could cause a decline in bumble bee reproductive success and/or survival rates. Kevan (1999) found that herbicides reduced Asteraceae and Lamiaceae flowers in France, contributing to a decline in bumble bee populations. Kevan (1999) also found that herbicide applications have reduced the reproductive success of blueberry pollinators by limiting alternative food sources that can sustain the insects when the blueberries are not in bloom. Kearns *et al.* (1998) state "herbicide use affects pollinators by reducing the availability of nectar plants. In some circumstances, herbicides appear to have a greater effect than insecticides on wild bee populations... Some of these bee populations show massive declines due to the lack of suitable nesting sites and alternative food plants."

The use of the herbicide glyphosate has dramatically increased with the widespread planting of genetically modified glyphosate-tolerant corn and soybeans, which were introduced in 1998 and 1996, respectively (Pleasants & Oberhauser 2012). Increased use of glyphosate in agricultural areas has likely led to the reduced availability of wildflowers in field margins – which otherwise would have been an important resource for *B. affinis*. Pleasants and Oberhauser (2012) estimate a 58% reduction in milkweed, an important nectar plant for bumble bees, in the Midwestern U.S. from 1999-2010, and suggest that this decline is due to the increased use of glyphosate in corn and soybean fields.

Habitat Change and Loss

Like many North American bumble bees, *B. affinis* faces general threats from habitat alterations that may interfere with its primary habitat requirements, including: access to sufficient food (nectar and pollen from flowers), nesting sites (such as underground abandoned rodent cavities or above ground in clumps of grasses), and overwintering sites for hibernating queens. It may have historically occupied open areas, which have largely been lost or fragmented by agricultural conversion and urban development or transformed by forest succession. Bumble bee species richness, abundance, and genetic diversity are influenced by the quality of habitat on a landscape level. Isolated patches of habitat may not be sufficient to support bumble bee populations (Hatfield and LeBuhn 2007; Öckinger and Smith

2007), and populations of bumble bees existing in fragmented habitats can also face problems with inbreeding depression (Darvill *et al.* 2006 and 2012; Ellis *et al.* 2006). Darvill *et al.* (2012) found that bumble bee populations limited to less than 15 km2 of habitat were more likely to show signs of inbreeding.

Agricultural intensification is primarily blamed for the decline of bumble bees in Europe (Williams 1986; Carvell *et al.* 2006; Diekotter *et al.* 2006; Fitzpatrick *et al*. 2007; Kosior *et al.* 2007; Goulson *et al.* 2008), and may also pose a significant threat to bumble bees in the U.S. Increases in farm size and changes in technology and operating efficiency have led to many practices that are detrimental to bumble bees, including loss of hedgerows, weed cover, and legume pastures. Although *B. affinis* generally nests one to four feet below ground, reports exist of this species nesting above ground, such as "in an open mowing place on the surface of the ground" (Plath 1922). Bumble bee nests may be at risk of being destroyed by farm machinery (Goulson 2003).

The conversion of the landscape to urban and suburban uses continues to transform and fragment habitat, which has likely had a negative effect on populations of many bumble bee species in some areas. Roads and railroads fragment plant populations and thus restrict the movement of bumble bees (Bhattacharya *et al.* 2003). Recent research in northern California found that the overall area of the landscape covered by pavement had a negative effect on the density of bumble bee nests (Jha and Kremen 2012). *B. affinis* has been found in some natural areas within urban environments, such as parks, restored prairies, and other natural areas within the urban centers of Philadelphia, PA, Minneapolis, MN, and Madison, WI as well as historically in Vermont. Some residential gardens and urban parks can provide valuable floral, and in some cases, nesting and overwintering resources and may serve as important habitat refuges for bumble bees (Frankie *et al*. 2005; McFrederick and LeBuhn 2006, Goulson *et al.* 2010), even though they may not support the species richness that was found historically (McFrederick and LeBuhn 2006).

20. Tolerance To Human Activity:

___ Fragile
___ Fairly Resistant
___ Tough
_X_ Unknown

Documentation & Comments:

21. Reproduction Parameters *(Age to Sexual Maturity, Annual Production of Offspring, Reproductive Life, or Other Factors that Warrant Consideration)*:

B. affinis, like other bumble bee species, lives in colonies consisting of a queen (foundress) and her offspring, the workers, and near the end of the season the reproductive members of the colony, the males and new queens. There is a division of labor among these three types of bees. The foundress is responsible for initiating colonies and laying eggs. Workers are responsible for most food collection, colony defense, and feeding of the young. Males leave the nest once they reach maturity and their sole function is to mate with queens. New queens remain with the nest until the end of the season when they leave to mate and find hibernacula.

Colonies are annual, progressing from colony initiation by solitary queens in spring, to production of workers, and finally to production of queens and males. *B. affinis* queens are one of the earliest species to emerge, with observations as early as March and April in some areas (Plath 1922; Mitchell 1962; Milliron 1971; Colla and Dumesh 2010). The foundress begins searching for suitable nesting sites and collects nectar and pollen from flowers to support the production of her eggs, which are fertilized by sperm she has stored since mating the previous fall. In the early stages of colony development, the queen is responsible for all food collection and care of the young. As the colony grows, workers take over the duties of food collection, colony defense, and care of the young. The foundress then remains within the nest and spends most of her time laying eggs. Colonies of *B. affinis* are considered large compared to other species of bumble bees, producing up to 1,000 workers throughout the season (Macfarlane *et al.* 1994). New queens and males are produced during the later stages of colony development, which is generally from mid-July to September (Plath 1922; Milliron 1971; Macfarlane *et al.* 1994). This species may be particularly susceptible to stressors because it emerges so early, but does not produce the next generation until late in the summer. The new queens mate before entering diapause. At the end of the season, the foundress dies.

Occasionally, nests of *B. affinis* have been observed above ground. However, nests are usually one to four feet below ground in abandoned rodent nests or other cavities (Plath 1922; Macfarlane *et al.* 1994). Thus, nesting sites may be limited by the abundance of rodents. Although little is known about the overwintering habits of *B. affinis* queens, queens of other species frequently dig a few centimeters into soft, disturbed soil and form an oval shaped chamber in which they will spend the duration of the winter. Compost in gardens or mole hills may provide suitable sites for queens to overwinter (Goulson 2010).

Bumble bees are particularly vulnerable to extinction due to their complementary sex determination system and haplodiploid life history (Zayed and Packer 2005). Loss of genetic diversity, which is frequently the result of inbreeding or random drift, can pose significant threats to small, isolated populations of bumble bees (Whitehorn *et al.* 2009). A loss of genetic diversity limits the ability of a population to adapt and reproduce when the environment changes and can lead to an increased susceptibility to pathogens (Altizer *et al.* 2003).

Bumble bees have a single locus complementary sex determination system, meaning that the gender of an individual bee is determined by the number of unique alleles at the sex-determining locus (van Wilgenburg 2006). Normally this gender determination comes through a haplodiploid genetic structure in which female bees are diploids and are produced from fertilized eggs with two different copies of an allele at the sex-determining locus. Most male bees are haploid, and they are produced from unfertilized eggs (with only a single copy of an allele at the sex-determining locus). However, when closely related bumble bees mate, the offspring can have two copies of the exact same allele (or be homozygous) at the sex-determining locus, which causes a diploid male to be produced instead of a diploid female. These diploid males may have reduced viability or may be sterile (van Wilgenburg 2006). When diploid males are able to mate, they produce sterile triploid offspring, which has been found to be negatively correlated with surrogates of bumble bee population size (Darvill *et al.* 2012). Diploid males are produced at the expense of female workers and new queens, and the production of diploid males can reduce colony fitness (including slower growth rates, lower survival, and colonies that produce fewer offspring) in bumble bees (Whitehorn *et al.* 2009). It has been suggested that diploid male production in inbred populations substantially increases the risk of extinction in bumble bee populations compared to other animal taxa (Zayed and Packer 2005).

Inbreeding and loss of genetic diversity can increase parasite prevalence in populations and parasite susceptibility in individuals (Frankham *et al.* 2010 *in* Whitehorn *et al.* 2010). Populations of bumble bees with low genetic diversity have been found to have a higher prevalence of pathogens (Whitehorn *et al.* 2010; Cameron *et al.* 2011), suggesting that as populations lose genetic diversity, the impact of parasitism will increase and threatened populations will become more prone to extinction.

22. Reproductive Status: *Documentation & Comments:*

_x_ Reproduces in Vermont

- ___ Confirmed In Last 2 Years
- ___ Confirmed In Last 10 Years
- _x_ Confirmed In Last 25 Years
- _x_ Confirmed Prior to 25 Years Ago
- ___ Unconfirmed

___ Does Not Breed or is Migratory

23. Additional Study or Documentation Needed:

Continued and additional surveys throughout Vermont during the growing season aimed at detecting any overlooked populations, refugia, or future recolonization. A better understanding of the role of each identified threat (parasites, pesticides, land use, etc.) to Vermont *Bombus* populations in the past and present is needed.

24. Attachments:
24.1 Narrative Summary. 24.2 Acknowledgments. 24.3 Literature Cited. 24.4 Historic records from Vermont.

25. Scientific Subcommittee Chairman: Kent P. McFarland Date: 11/15/13

24.1 Narrative Summary

Bumble bees are essential pollinators of crops and wildflowers in agricultural, urban, and natural ecosystems. They play an important role in the reproduction of tomato, blueberry, pepper, cranberry, clover, apple, and many other crops. Although the Rusty-patched Bumble Bee (*Bombus affinis*) was historically distributed throughout the Upper Midwest, Northeast and Eastern Seaboard, recent range-wide studies have estimated that *B. affinis* is no longer found in 70-87% of its historic range. Where it does still occur, its relative abundance has declined by 87-95% (Colla *et al.* 2012; Cameron *et al.* 2011).

Historically, *B. affinis* appeared to be a common component of the Vermont bee fauna. Regional data suggest that it was probably found throughout the entire state. Collection data show that entomology students at UVM regularly collected the species from the 1960s through the 1990s. Despite surveys by students and biologists during the 2000s and intensive surveys across the state by the Vermont Bumble Bee Survey during 2012 and 2013, no *B. affinis* has been observed in the state since 1999 (Vermont Center for Ecostudies, unpub. data). It is currently ranked as an S1 (critically imperiled) species in Vermont.

Research has indicated that declines in *B. affinis* and other North American bumble bees have been associated with increased levels of pathogens and reduced genetic diversity (Cameron *et al.* 2011). Also habitat loss or degradation, pesticides, climate change, and competition with honey bees may have exacerbated the range wide decline of this species. When considered individually, each of these factors pose a significant threat to *B. affinis*. However, when considered together, they present a daunting case for the recovery of this animal.

B. affinis is not alone. At least five other species of North American *Bombus* have undergone severe range reductions, including three species found in Vermont- Yellow-banded Bumble Bee (*B. terricola*), Ashton Cuckoo Bumble Bee (*B. ashtoni*), and American Bumble Bee (*B. pensylvancus*); and two species in the West- Franklin's Bumble Bee (*B. franklin*) and Western Bumble Bee (*B. occidentalis*). Unfortunately, as more data are gathered, more *Bombus* appear to be in decline, such as the Yellow Bumble Bee (*B. fervidus*) in Vermont. Like *B. affinis*, many of these species apparently declined drastically in the 1990s.

Although the exact causes and mechanisms of decline are not yet fully understood, they are likely to be the same culprits for much of the *Bombus* fauna: introduced pathogens and parasites, habitat change or loss, pesticides, and climate change. Existing management and regulations are inadequate to protect *B. affinis* and other *Bombus* from these threats. Any management or regulations aimed at the protection and recovery of *B. affinis* will undoubtedly help the conservation of the entire *Bombus* fauna. Some of these might include examination and changes of neonicotinoid pesticide use and regulation, the spread of pests and diseases by the commercial bumble bee industry, land management specific to bumble bee conservation, or perhaps one day discovering populations that are resistant to pathogens followed by reintroductions to their former range.

24.2. Acknowledgments

We would like to thank Sarina Jepsen and Rich Hatfield of the Xerces Society for Invertebrate Conservation for permission to draw directly from their petition to list *B. affinis* as an endangered species under the U.S. Endangered Species Act (Jepsen et al. 2013) for this report. Thank you to Leif Richardson for sharing rangewide data and consultations. The historical data and recent surveys across the state were completed by the Vermont Bumble Bee Survey, a project of the Vermont Atlas of Life at the Vermont Center for Ecostudies (VCE). Funding for the survey was provided by: the Binnacle Foundation, the Riverledge Foundation, the State Wildlife Program at the Vermont Fish and Wildlife Department, and generous individual supporters of VCE. Extensive surveys would not have been possible without the dedication of volunteer citizen scientists for whom we are grateful. We thank the many private and institutional collections for kindly allowing us access to their specimens.

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24.4 Known *Bombus affini*s records from Vermont. All specimens determined by Leif Richardson.

Date	Collection	Caste	County	Town	Locality Name
9/13/28	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Bolton	Bolton
5/12/29	Yale Peabody	queen	Chittenden	Burlington	Burlington
9/10/36	UVM Zadock Thompson Invertebrate Collection	drone	Bennington	Dorset	East Dorset
9/10/37	UVM Zadock Thompson Invertebrate Collection	drone	Bennington	Dorset	East Dorset
9/29/37	Yale Peabody	drone	Windsor	Norwich	Norwich
10/6/37	Yale Peabody	worker	Windsor	Norwich	Norwich
6/17/38	UVM Zadock Thompson Invertebrate Collection	queen	Bennington	Dorset	East Dorset
10/12/38	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods
5/12/49	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
6/19/58	UVM Zadock Thompson Invertebrate Collection	queen	Bennington	Dorset	East Dorset
6/19/58	UVM Zadock Thompson Invertebrate Collection	queen	Bennington	Dorset	East Dorset
8/16/60	Middlebury College	drone	Addison	Middlebury	East Middlebury
8/21/60	RUAC	worker	Chittenden	Underhill	Underhill
9/15/60	UVM Zadock Thompson Invertebrate Collection	drone	Rutland	Wallingford	Wallingford
9/20/60	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
9/25/60	UVM Zadock Thompson Invertebrate Collection	drone	Rutland	Wallingford	Wallingford
9/27/61	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Shelburne	Shelburne
9/29/62	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Milton	Milton
9/19/63	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/22/63	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Red Rock Acres
9/25/63	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/28/63	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
10/5/63	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
10/15/64	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	South Burlington	South Burlington
7/27/65	Lyman Entomological Collection--McGill University	worker	Chittenden	Burlington	Burlington
9/6/65	Ohio State University		Rutland	Castleton	Castleton
9/6/65	Ohio State University		Rutland	Castleton	Castleton
9/6/65	Ohio State University		Rutland	Castleton	Castleton
9/6/65	Ohio State University		Rutland	Castleton	Castleton
9/6/65	Ohio State University		Rutland	Castleton	Castleton
9/12/65	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Colchester	N.W.Colchester
9/12/65	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Colchester	N.W.Colchester
9/17/65	UVM Zadock Thompson Invertebrate Collection	worker	Chittenden	Shelburne	Shelburne Pond
9/22/65	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Red Rock Acres
9/26/65	UVM Zadock Thompson Invertebrate Collection	drone	Addison	Middlebury	Middlebury
10/10/65	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/14/66	UVM Zadock Thompson Invertebrate Collection	drone	Washington	Waterbury	Waterbury
9/25/66	UVM Zadock Thompson Invertebrate Collection	drone	Rutland	West Rutland	West Rutland
10/15/66	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Shelburne	Shelburne
9/16/67	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	North Beach

Date	Collection	Caste	County	Town	Locality Name
7/27/68	Middlebury College	worker	Addison	Middlebury	East Middlebury
8/5/68	Middlebury College	worker	Addison	Middlebury	East Middlebury
8/28/68	Middlebury College	worker	Addison	Hancock	Snow Bowl
9/18/68	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Westford	Westford
10/9/68	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Williston	Williston
7/30/69	UVM Zadock Thompson Invertebrate Collection	drone	Washington	Barre	Graniteville
9/7/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Hinesburg	Hinesburg
9/10/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/11/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/13/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	South Burlington	South Burlington
9/15/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/15/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/15/69	UVM Zadock Thompson Invertebrate Collection	drone	Grand Isle	Grand Isle	Grand Isle
9/17/69	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
9/17/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/18/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/19/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/19/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/19/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/21/69	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
9/22/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/24/69	UVM Zadock Thompson Invertebrate Collection	drone	Addison	Addison	Addison
9/28/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Shelburne	Shelburne
10/5/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Richmond	Richmond
10/5/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Shelburne	Shelburne
10/5/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Westford	Westford
10/6/69	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
10/7/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
10/7/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Ethan Allen Cemetary
10/8/69	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
2/2/70	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Essex	Essex
5/10/70	UVM Zadock Thompson Invertebrate Collection	queen	Washington	Barre	Barre
5/13/70	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
5/19/70	UVM Zadock Thompson Invertebrate Collection	queen	Washington	Barre	Barre
6/7/70	UVM Zadock Thompson Invertebrate Collection	queen	Addison	Vergennes	Vergennes
7/15/70	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Milton	7 miles sw Milton
8/16/70	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	South Burlington	South Burlington
9/5/70	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Underhill	Underhill
9/5/70	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Underhill	Underhill Flats
9/6/70	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Shelburne	Shelburne
9/14/70	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
9/16/70	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods

Date	Collection	Caste	County	Town	Locality Name
9/19/70	Middlebury College	drone	Addison	Middlebury	Middlebury
9/20/70	UVM Zadock Thompson Invertebrate Collection	worker	Chittenden	Shelburne	Shelburne
10/1970	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden		
10/2/70	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Essex	Essex
10/2/70	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Essex	Essex
10/3/70	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Essex	Essex
10/3/70	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Essex	Essex
10/3/70	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Essex	Essex
10/6/70	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
5/13/71	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
5/14/71	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
5/17/71	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
5/29/71	UVM Zadock Thompson Invertebrate Collection	queen	Bennington	Manchester	Manchester
8/14/71	UVM Zadock Thompson Invertebrate Collection	drone	Washington	Barre	Barre
9/6/71	UVM Zadock Thompson Invertebrate Collection	drone	Orange	Williamstown	Williamstown
9/7/71	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/11/71	UVM Zadock Thompson Invertebrate Collection	drone	Washington	Cambridge	Smuggler's Notch
9/20/71	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/23/71	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Shelburne	Shelburne
9/25/71	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Huntington	Huntington
10/10/71	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Colchester	Colchester
10/15/71	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Colchester	Colchester
5/25/72	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Bolton	Camel's Hump
10/1/72	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
7/27/73	Yale Peabody	worker	Addison	Middlebury	Middlebury
9/9/73	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
10/10/73	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	UVM Natural Area
7/24/74	UVM Zadock Thompson Invertebrate Collection	drone	Windham	Brattleboro	82 Clark St.
9/13/74	Middlebury College	worker	Addison	Middlebury	Middlebury
9/29/74	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Jericho	Jericho
10/15/74	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/15/75	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Shelburne	Shelburne Pond
9/20/75	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Shelburne	Shelburne Pond
9/21/75	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	South Burlington	South Burlington
10/1/75	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Lake Champlain
5/28/76	UVM Zadock Thompson Invertebrate Collection	queen	Addison	Weybridge	Snake Mt.
6/8/76	UVM Zadock Thompson Invertebrate Collection	queen	Addison	Weybridge	Snake Mt
7/12/76	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Bayview St.
9/12/76	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/12/76	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/12/76	Middlebury College	drone	Addison	Middlebury	Middlebury
9/14/76	Middlebury College	drone	Addison	Middlebury	Middlebury

Date	Collection	Caste	County	Town	Locality Name
9/26/76	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
9/26/76	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
10/4/77	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Colchester	Munson Flat
10/23/77	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods
9/8/78	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Hinesburg	Hinesburg
9/9/78	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/14/78	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/19/78	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	UVM Natural Area
Oct-78	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	UVM Natural Area
9/13/79	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
9/22/79	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
10/4/79	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods
9/4/80	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods
9/10/80	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Winooski	Winooski
9/26/80	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Shelburne	Shelburne
9/29/80	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/30/80	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	South Burlington	Centennial Woods
10/1/80	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
7/23/81	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Shelburne	Shelburne
9/21/81	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
10/1/81	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Shelburne	Shelburne Pond
10/11/81	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods
9/26/82	UVM Zadock Thompson Invertebrate Collection	drone	Addison	Ferrisburg	Fuller Mtn.Rd.
9/28/82	UVM Zadock Thompson Invertebrate Collection	drone	Washington	Cabot	Cabot
8/3/83	Jeff Freeman personal collection	worker	Rutland	Castleton	
8/3/83	Jeff Freeman personal collection	drone	Rutland	Castleton	
9/9/83	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Colchester	Colchester
9/12/83	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/21/83	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
9/21/83	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
9/25/83	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Burlington
10/13/83	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods
5/1984	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
5/1984	UVM Zadock Thompson Invertebrate Collection	queen	Chittenden	Burlington	Burlington
9/19/84	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods
9/20/84	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Williston	Williston
9/21/84	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods
9/21/84	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods
9/21/84	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods
9/24/84	UVM Zadock Thompson Invertebrate Collection	worker	Chittenden	Williston	N. Williston
9/29/84	UVM Zadock Thompson Invertebrate Collection	drone	Rutland	Shrewsbury	Shrewsbury
9/4/85	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	South Burlington	South Burlington

Date	Collection	Caste	County	Town	Locality Name
9/18/85	UVM Zadock Thompson Invertebrate Collection	drone	Grand Isle	Grand Isle	Grand Isle
9/23/85	UVM Zadock Thompson Invertebrate Collection	drone	Franklin	Fairfax	Goose Pond Rd.
9/23/85	UVM Zadock Thompson Invertebrate Collection	drone	Franklin	Fairfax	Goose Pond Rd.
9/6/86	UVM Zadock Thompson Invertebrate Collection	drone	Addison	New Haven	Town Hill Rd.
9/9/86	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods
9/20/86	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods
10/8/86	UVM Zadock Thompson Invertebrate Collection	drone	Addison	New Haven	Town Hill Rd.
11/6/86	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Huntington	GMAC field
9/2/87	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods
9/16/87	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods
9/16/87	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	UVM Natural Area
9/16/87	UVM Zadock Thompson Invertebrate Collection	drone	Chittenden	Burlington	Centennial Woods
9/13/89	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Burlington	Centennial Field
10/3/89	Middlebury College	worker	Addison	Middlebury	Middlebury
9/20/90	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Burlington	Burlington
9/27/90	UVM Zadock Thompson Invertebrates Collection	drone	Essex	Canaan	Canaan
9/9/91	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Shelburne	Shelburne Bay
9/22/92	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	South Burlington	South Burlington
9/2/93	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Burlington	UVM Natural Area
9/14/93	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Burlington	Ethan Allen Homestead
9/16/94	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Shelburne	Mouth of Laplatte River
9/18/94	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Colchester	Mallett's Bay,north shore
9/10/96	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Burlington	Ethan Allen Home
9/10/96	UVM Zadock Thompson Invertebrates Collection	worker	Chittenden	Essex	Essex Junction
9/24/96	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Essex	Essex Junction, Cascade Street
9/30/96	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	South Burlington	South Burlington
9/11/97	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Colchester	Half Moon Cove
9/10/98	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Burlington	North Shore
9/13/98	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Burlington	Burlington
9/29/98	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Shelburne	Shelburne Pond
10/12/98	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Burlington	225 East Ave.
5/?/1999	Jeff Collins personal collection	queen	Chittenden	Jericho	Ethan Allen Firing Range
7/5/99	Leif Richardson research collection	worker	Chittenden	Huntington	Handy Road
8/9/99	Helen Young Research Collection	worker	Addison	Bristol	Bristol Pond
8/10/99	Helen Young Research Collection	worker	Addison	Middlebury	Middlebury
8/31/99	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Burlington	Intervale,Floodplain forest
199?	UVM Zadock Thompson Invertebrates Collection	drone	Chittenden	Burlington	Ethan Allen Farm

Figure 1. The known historic (1881-1988) and contemporary (1989-2012) locations of *Bombus affinis* records are depicted by circles (Richardson 2013). A maximum entropy method (Maxent) for modeling species geographic distributions, a general-purpose machine learning method, was used with these presence-only data to estimate the former range (Richardson 2013). It should be noted that *Bombus* search effort has dramatically increased since 2000 relative to the entire 20th century (see Figure 1 in Colla *et al*. 2012, which uses this same dataset), and many observers have specifically targeted *B. affinis* in recent years.

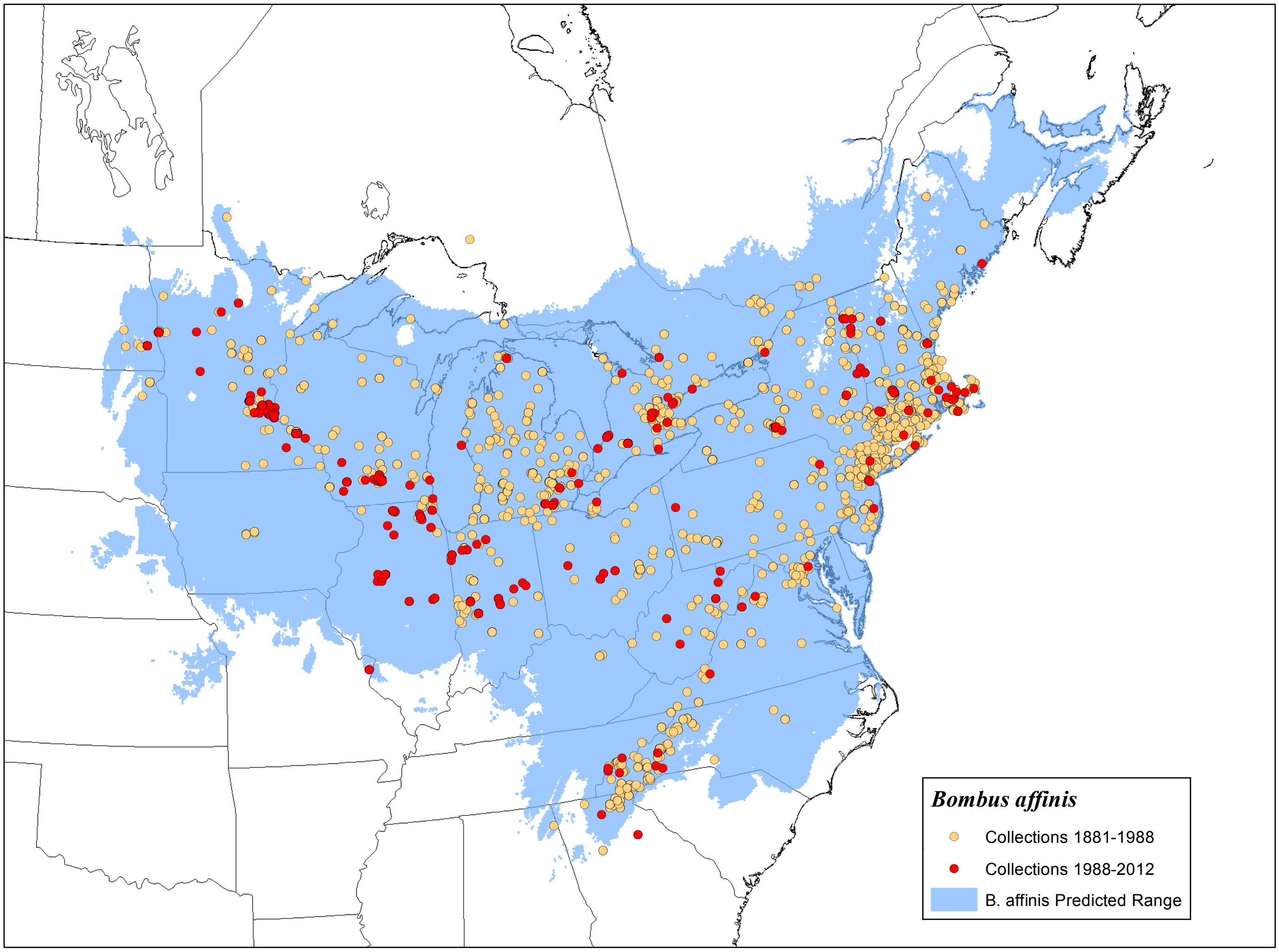

Figure 2. Confirmed records of *Bombus affinis* mapped by town (green) compiled from private and public natural history collections. Most specimen labels contained location information only to town, as depicted here, rather than exact geographic location. Historic records are those more than 25 years old (n=183 specimens), while recent records represent those collected after 1988 (n=24 specimens). The last verified record of *B. affinis* was in 1999. There were none found during the Vermont Bumble Bee Survey in 2012 and 2013 at over 1,500 visited locations and ~10,000 *Bombus* collected. Previously, the range of *B. affinis* was thought to cover the entire state until the sudden decline in the 1990s (Fig. 1).

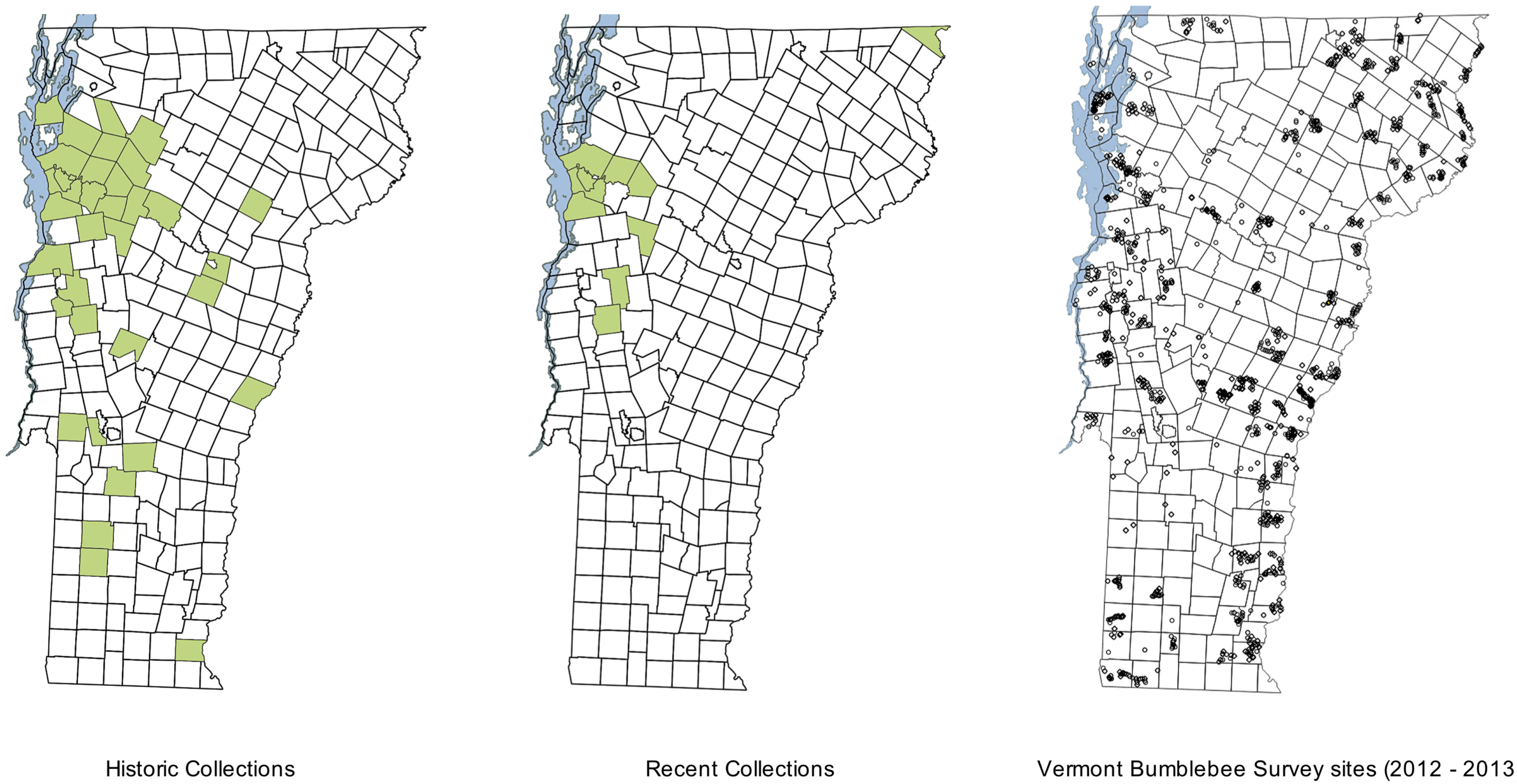

Figure 3. Number of *Bombus affinis* specimens (n=83) by decade from Burlington, Vermont during the past 50 years.