

Do pathogen spillover, pesticide use, or habitat loss explain recent North American bumblebee declines?

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Abstract

Several North American bumblebee species have recently undergone dramatic declines. The use of managed, pathogen-carrying bumblebees for pollination of greenhouse crops began shortly before these declines, and wild bumblebees near greenhouses now have high pathogen loads. This has led to speculation that pathogen spillover from commercial bumblebees caused declines of these species. We test this hypothesis using a large dataset of bumblebee occurrence records and agricultural census data. We find support for the pathogen spillover hypothesis for two species but no evidence that pathogen spillover caused the near disappearance of the previously widespread *Bombus affinis*. Furthermore, we show that pesticide use and habitat loss are unlikely to be major causes of decline for any of the *Bombus* species examined. Collectively, our analyses underscore that there remains an urgent need to identify causes of pollinator population losses.

Introduction

Several bumblebee (*Bombus* Latreille) species have recently undergone dramatic declines in North America. In the late 1990s, species in subgenus *Bombus* Latreille began declining precipitously (Thorp & Shepherd 2005). Losses of species in this subgenus and in subgenus *Thoracobombus* Dalla Torre have since been documented regionally (Colla & Packer 2008; Gixti *et al.* 2009; Colla & Ratti 2010) and across the United States (Cameron *et al.* 2011). Bumblebees are major pollinators of both wild and agricultural plants in temperate regions, so widespread declines have implications for biodiversity and food production (Goulson *et al.* 2008).

Pathogen spillover could explain widespread bumblebee declines. In the early 1990s, shortly before declines of species in subgenus *Bombus* became apparent, bumblebees began to be commercially supplied to North American greenhouses to pollinate tomatoes (Velthuis & van Doorn 2006) and sweet peppers (Shipp *et al.* 1994).

Commercial bumblebees tend to have high pathogen loads (Whittington & Winston 2003; Otterstatter & Thomson 2008), and because bumblebees often escape from greenhouses (Morandin *et al.* 2001; Whittington *et al.* 2004), commercial bees may spread disease to wild populations. Indeed, pathogen loads of wild bumblebees have been shown to be higher near greenhouses (Colla *et al.* 2006; Otterstatter & Thomson 2008), indicating that pathogen spillover from greenhouses occurs. Furthermore, declining species have higher pathogen levels than stable species (Cameron *et al.* 2011), consistent with the hypothesis that certain bumblebee species are declining because they are more susceptible to these diseases. Consequently, there has been speculation that pathogen spillover may contribute substantially to declines of species in subgenus *Bombus* (see e.g., Berenbaum *et al.* 2007; Williams & Osborne 2009).

Here, we evaluate the contribution of pathogen spillover from commercial bumblebees to declines of wild bumblebees in North America. Assessing the role

of pathogen spillover is critical to developing appropriate policies for protecting native species and regulating the commercial bumblebee industry. The temporal association between bumblebee declines and the onset of commercial bumblebee use makes pathogen spillover a compelling hypothesis, but it is premature to conclude this is the main cause for declines (Meeus *et al.* 2011). We evaluate the hypothesis that chronic pathogen spillover from commercial bumblebees, as demonstrated in areas surrounding greenhouses (Colla *et al.* 2006; Otterstatter & Thomson 2008), is responsible for wild bumblebee declines by testing whether spatial patterns of decline are associated with commercial bumblebee use. Recent surveys show that pathogen loads in wild bumblebees decline rapidly with distance from greenhouses using commercial bumblebees (Otterstatter & Thomson 2008), indicating that, if chronic pathogen spillover is the main cause of decline, range contractions should generally have occurred in areas with high use of commercial bumblebees, whereas species should have persisted in areas with less commercial bumblebee use. It should be noted, however, that our analysis does not address the possibility that declines were caused by an acute spillover event and rapid disease spread through wild populations (see Cordes *et al.* 2012 for evidence of high pathogen loads in some bumblebee populations far from greenhouses).

We also consider pesticide use and habitat loss, other factors that have been proposed as potential causes of North American bumblebee declines (Colla & Packer 2008). If either were causing declines, we predict that species should generally have been lost from areas with high pesticide use or habitat loss, while persisting in areas with low pesticide use or habitat loss.

Our study is the first to test whether pathogen spillover is directly linked to declines of North American bumblebee populations. Our results substantiate calls for policies to reduce pathogen spread from commercial bumblebees and highlight the need for more research to identify threats to bumblebees.

Methods

Bumblebee range contractions

We compiled a database with 65,866 North American bumblebee occurrence records, mostly consisting of specimen records with some expert observations, from 11 museum or natural history collections and 3 private collections. The three largest contributing datasets were the Canadian National Collection (14,393 records), American Museum of Natural History (13,786 records), and Peabody Museum of Natural History (10,239 records; see supporting information for complete list of sources).

To this database we added records from Cameron *et al.* (2011).

Using this database, we examined declines of *Bombus affinis* Cresson, *Bombus terricola* Kirby, and *Bombus pensylvanicus* DeGeer. *B. affinis* and *B. terricola* are both in the subgenus *Bombus*, and may be declining because of pathogen spillover (Berenbaum *et al.* 2007; Otterstatter & Thomson 2008). Three western species, *Bombus occidentalis* Greene, *Bombus franklini* Frison, and *Bombus moderatus* Cresson (part of the *Bombus lucorum* Linnaeus complex), also members of this subgenus, were omitted from analyses because our dataset was dominated by eastern collections (Figure 1). *B. pensylvanicus* is in the subgenus *Thoracobombus* and persists at higher numbers than the other species, but was analyzed because it also declined sharply in the past 20 years and has high pathogen loads compared to stable species (Cameron *et al.* 2011).

For each species, we compared occurrence records from 1980–1990, before the start of rapid declines (Cameron *et al.* 2011), with those from 2000–2010, after rapid declines had begun (Thorp & Shepherd 2005), to evaluate how ranges contracted after several years of commercial bumblebee use, which began in the early 1990s (Velthuis & van Doorn 2006). This was done per county (USA) or census division (Canada), using county and census division boundary files (USDA 2004; Statistics Canada 2002). A species was considered to have persisted in a county/division if it had at least one occurrence record in that county/division in 2000–2010. A species was considered to have been lost (where a loss was loosely defined as the loss of detectable numbers of the species) from a county/division if the following criteria were met: (1) the species had at least one occurrence record for the county/division in 1980–1990; (2) the species had no occurrence records for the county/division in 2000–2010; and (3) there was at least one occurrence record for the county/division in 2000–2010 for any species in the database (this criterion prevented areas without recent collections from being falsely identified as losses).

Predictors

The first predictor variable was vegetable greenhouse density, used as a proxy for the density of commercial bumblebee use. The area of vegetable greenhouses per county/division was obtained from the 2001 and 2002 Canadian and American Agricultural Censuses (Statistics Canada 2001; USDA 2002) and converted into densities by dividing by county/division land areas. About 95% of global commercial bumblebee sales are for use in tomato greenhouses and, in countries where this practice

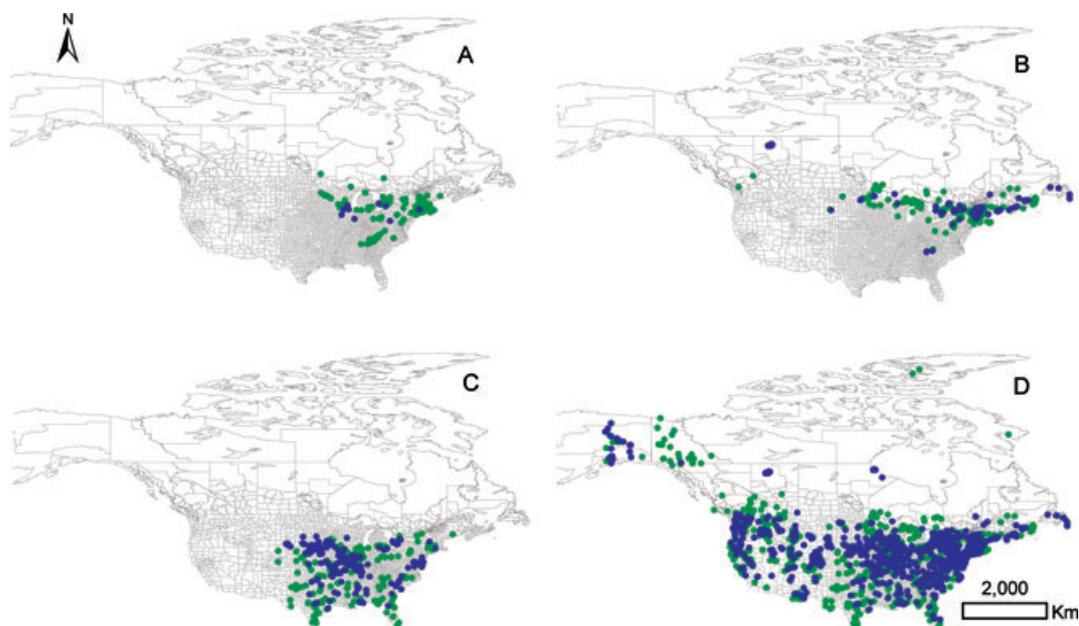


Figure 1 Occurrence records for *B. affinis* (A), *B. terricola* (B), *B. pennsylvanicus* (C), and all bumblebee species (D). Blue points representing records from 2000 to 2010 are overlaid on green points representing

records from 1980 to 1990. The background shows the outline of U.S. counties and Canadian census divisions.

has been adopted, a large majority of greenhouse tomato growers use commercial bumblebees (Velthuis & van Doorn 2006) indicating that tomato greenhouse density is a good proxy for commercial bumblebee use. We instead used vegetable greenhouse density because tomato greenhouse data were not available for the time period and entire region of interest but tomato and vegetable greenhouse densities from the 2007 American Agricultural Census are tightly correlated ($r = 0.94$, see supporting information), indicating that vegetable greenhouse density is also a reasonable proxy. We used data from the 2001 and 2002 agricultural censuses because, although census data is also available for other years, the area of tomato greenhouses (which increased during the 1990s) had plateaued in Canada and the U.S. by 2000 (Cook & Calvin 2005) indicating that data from the 2001–2002 censuses should reasonably represent the entire second time period.

Because counties and census divisions are arbitrary boundaries, and greenhouses may cause pathogen spillover into surrounding counties/divisions, we adjusted all vegetable greenhouse density values to reflect the average greenhouse density in the surrounding area. We made a grid map of Canada and the U.S. where each 1 km² cell was assigned the greenhouse density value of the county/division, using county and census divi-

sion boundary files (Statistics Canada 2002; USDA 2004). Cell values were then replaced by average values for all cells within 100 km of the target cell. Finally, averages of these new density values were calculated for each county/division. These adjusted values are more representative of pathogen spillover, which is not limited by political boundaries, and therefore results reported here use adjusted values; however, use of raw density values does not substantially alter our results (see supporting information).

We also examined pesticide use as a predictor of decline of our target *Bombus* species. The application area of herbicides and insecticides was obtained per county/division from the 2001 and 2002 Canadian and American Agricultural Censuses (Statistics Canada 2001; USDA 2002) and converted into densities by dividing by land area. Although it would have been ideal to employ a measure of pesticide use that incorporates varying pesticide toxicities and application rates, these data were unavailable across our entire study area and pesticide application area has been shown to be a strong predictor of species losses (Gibbs *et al.* 2009).

As a proxy for habitat loss, we used the change in human population density between 1990 and 2000 (the interval between the periods we considered for our bumblebee data). Population change was calculated using

American census counts (US Census Bureau 2002) and Canadian population estimates based on census counts (Statistics Canada 2007) and converted into densities by dividing by land area. This variable was used to represent habitat loss, even though it may not capture habitat loss in all contexts (e.g., agricultural expansion involving little population change), because, to our knowledge, a dataset more directly measuring habitat loss (e.g., a map of land use or land cover change) was not available at the required temporal and spatial scales.

Spatial analyses used ArcGIS/ArcInfo 9.2 and 10 (ESRI, Redlands, CA, USA).

Analysis

We used logistic regression to evaluate the relationships between bumblebee losses and the predictor variables. We evaluated losses per county or census division for each species, as described above, and then evaluated the relationships between losses and vegetable greenhouse density, herbicide use density, insecticide use density, and change in human population density. Individual logistic regressions were carried out for each predictor and then stepwise regression was used to identify multivariate models that better explain losses than single variable models. Although our analysis involved spatial data, we used standard nonspatial logistic regression because analysis of residuals from our nonspatial models indicated that spatial autocorrelation in the residuals was weak and limited to short distances (see supporting information) and, even in the presence of spatial autocorrelation, coefficient estimates from current spatial logistic regression methods are more biased, or at best no better than estimates from nonspatial logistic regression models (Dormann 2007). Predictor variables were log-transformed (with constants added to bring values above zero before transformation) to help satisfy the assumption of logit linearity (Hosmer & Lemeshow 2000). We used Nagelkerke R^2 , a likelihood-based measure that can be interpreted similarly to OLS R^2 (Nagelkerke 1991), as a measure of model fit. Statistical analyses were conducted using R 2.11.1 (R Development Core Team 2010) with use of the Design package (Harrell 2009).

Results

There were significant, although weak, relationships between losses of *B. terricola* and *B. pensylvanicus* and vegetable greenhouse density, with losses more frequently occurring in counties/divisions with higher vegetable greenhouse density (Figure 2; $P = 0.0048$, Nagelkerke $R^2 = 0.17$ for *B. terricola*; $P = 0.0034$, Nagelkerke

$R^2 = 0.08$ for *B. pensylvanicus*). We did not detect a significant relationship between losses of *B. affinis* and vegetable greenhouse density (Figure 2; $P = 0.14$, Nagelkerke $R^2 = 0.07$).

We did not find significant relationships between losses and pesticide use or the relationships were in the opposite direction than predicted (Table 1). We also did not detect significant relationships between losses and change in human population density for *B. affinis* or *B. pensylvanicus* (Table 1). For *B. terricola*, there was a significant relationship with change in human population density ($P = 0.0070$). However, change in human population density is correlated with vegetable greenhouse density ($r = 0.50$), and is not significant after controlling for vegetable greenhouse density ($P = 0.10$).

Stepwise regressions carried out with all predictors for each species did not reveal any superior multivariate models. The final model always only had one significant predictor (herbicide density in the opposite direction than predicted for *B. affinis* and vegetable greenhouse density for *B. terricola* and *B. pensylvanicus*).

Discussion

Here, we find the first evidence linking pathogen spillover directly to declines of North American bumblebees. Consistent with the hypothesis that pathogen spillover is causing widespread bumblebee declines, populations of *B. terricola* and *B. pensylvanicus* are more likely to have been lost from areas with high densities of commercial bumblebee use. This underlines the increasingly recognized threat of emergent diseases to biodiversity (Daszak et al. 2000) and has policy implications for bumblebee conservation. Given the threat of pathogen spillover to *B. terricola* and *B. pensylvanicus* and potentially other bumblebee species, we urge actions that reduce or eliminate pathogen spread from commercial bumblebee operations, such as rigorous pathogen screening in rearing facilities, implementing controls to prevent bumblebees from leaving greenhouses, and restricting use of commercial bumblebees in open fields (see Meeus et al. 2011 for a review). An alternative explanation for the patterns we found could be competition with escaped managed bees. We find this a less likely explanation given that the managed species is native in the ranges of our study species. Regardless, either explanation justifies increased control of managed bumblebee use. One example of a legislative approach is the controlled use of exotic *Bombus terrestris* Linnaeus in Japan, which was developed using studies of the ecological impacts of this species and strives to balance threats to biodiversity and benefits to agriculture (Goka 2010).

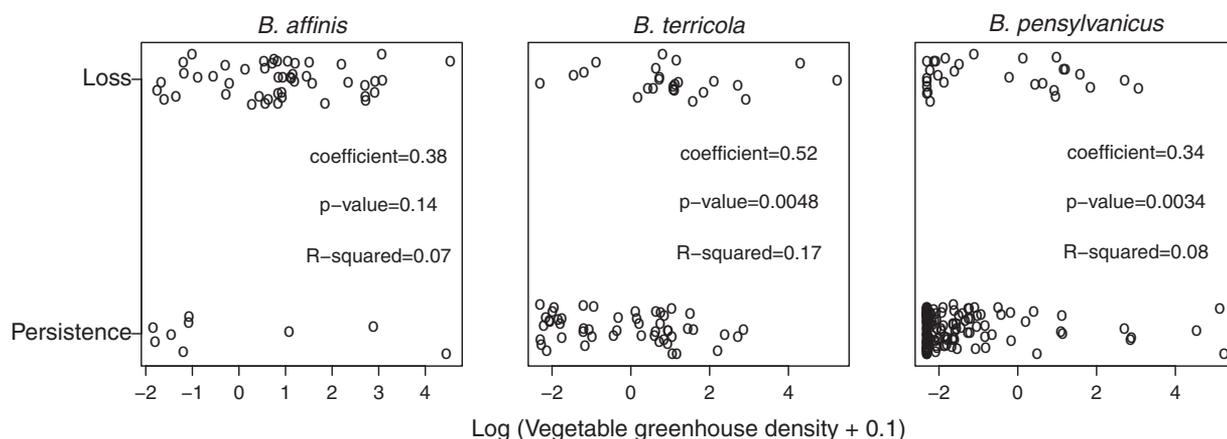


Figure 2 Scatterplots and logistic regression results of losses of *B. affinis*, *B. terricola*, and *B. pensylvanicus* in American counties or Canada census divisions against vegetable greenhouse density. Vegetable greenhouse density is in units of m² of greenhouse per km² land area. Coefficients, *P*-values, and Nagelkerke *R*² values from logistic regressions are shown. *N* = 57, 74, and 168 for *B. affinis*, *B. terricola*, and *B. pensylvanicus*, respectively.

Table 1 Results of logistic regression of losses of *B. affinis*, *B. terricola*, and *B. pensylvanicus* in American counties or Canada census divisions against herbicide use density, insecticide use density, and change in human population density. Herbicide and insecticide use density are in units of km² area of use per km² land area and change in human population density is in units of people per km² land area. All predictor have been log transformed with constants added to allow for the transformation. Sample size varies within species because of undisclosed census data.

| Species | Predictor | <i>N</i> | Coefficient | <i>P</i> value | Nagelkerke <i>R</i> ² |
|-------------------------|------------------------------------|----------|-------------|----------------|----------------------------------|
| <i>B. affinis</i> | Herbicide density | 56 | -1.59 | 0.0079 | 0.22 |
| | Insecticide density | 43 | -1.43 | 0.43 | 0.02 |
| | Change in human population density | 57 | 0.38 | 0.18 | 0.06 |
| <i>B. terricola</i> | Herbicide density | 73 | 0.45 | 0.34 | 0.02 |
| | Insecticide density | 59 | 0.01 | 0.99 | <0.001 |
| | Change in human population density | 74 | 0.75 | 0.0070 | 0.16 |
| <i>B. pensylvanicus</i> | Herbicide density | 167 | -0.60 | 0.066 | 0.04 |
| | Insecticide density | 128 | -0.33 | 0.84 | 0.001 |
| | Change in human population density | 168 | 4.81 | 0.52 | 0.004 |

Although our analysis of the relationships between declines of particular bumblebee species and density of greenhouses is consistent with and supports a role for pathogen spillover, it also suggests that this hypothesis cannot completely explain observed declines. Most importantly, we did not find any evidence of a relationship between declines of *B. affinis* and vegetable greenhouse density. Small sample size could partly account for this, but the low pseudo-*R*² and the fact that sample size was not too small to preclude detecting other statistically significant relationships for *B. affinis* indicate that if a relationship does exist between declines of *B. affinis* and greenhouse density, it is at most a weak one. Furthermore, although we did detect significant effects of vegetable greenhouse density on losses of *B. terricola* and *B. pensylvanicus*, the relationships were weak and patterns of persistence and loss remain substantially unexplained. All species persisted in some areas with high vegetable greenhouse density while being lost from other areas with low

greenhouse density, indicating that there are other important threats.

Although habitat loss and pesticides have well-established negative effects on bumblebees (Goulson et al. 2008; Williams & Osborne 2009), they are unlikely to be main drivers of recent North American declines. We did not find significant relationships between patterns of bumblebee losses and pesticide use, or the relationships were in the opposite direction than predicted. Although pesticides have negative impacts on bumblebees at the individual or colony level (e.g., Morandin et al. 2005), our results suggest that pesticides are not a main contributor to declines of these species when their entire ranges are considered. Our analysis also suggests that habitat loss is not a major driver of widespread declines. Furthermore, the species we examined were recently common in both rural and urbanized regions (Colla & Packer 2008; Cameron et al. 2011) and had therefore already persisted through extreme habitat loss. These

observations suggest that habitat loss is not responsible for recent precipitous declines unless acting through threshold effects (Groffman *et al.* 2006). We consider it unlikely that such effects could have simultaneously triggered population declines among multiple species living in different areas, including areas that still retain extensive potential habitat.

Therefore, there remains an urgent need to identify other causes of North American bumblebee declines. The role of diseases in declines deserves further study. There are two main ways pathogen spillover can drive disease dynamics: chronic transmission from a reservoir population to a nonreservoir population where transmission rate is too low to sustain an epizootic; or transmission from a reservoir population followed by an epizootic in the nonreservoir population (Power & Mitchell 2004). The first type of pathogen spillover has been demonstrated around greenhouses (Colla *et al.* 2006; Otterstatter & Thomson 2008) and here we show that pathogen spillover in this form cannot fully account for declines. However, although evidence of the second type of pathogen spillover is lacking, an epizootic could have occurred following transmission of a virulent strain of pathogen at one or a few greenhouses and our analyses could not detect the effects of such an epizootic. It is also possible that diseases from other sources are involved, e.g. some viruses can be transmitted from honeybees (*Apis mellifera* Linnaeus) to bumblebees (Singh *et al.* 2010). Climate change is another potential factor that deserves investigation. Climate change is a major threat to biodiversity in general (Maclean & Wilson 2011) and has been suggested as a threat to bumblebees (Williams & Osborne 2009) but there has been little research specific to bumblebees. It is also possible that multiple stressors are acting together and that no single factor accounts for the declines of all species. Honeybee populations have also suffered great losses in recent decades, and, despite extensive research, no single explanatory factor has emerged; it is now thought that multiple factors are most likely responsible (Potts *et al.* 2011).

Developing strategies to effectively protect North American bumblebees will require further research. There is ample evidence of North American bumblebee declines (Colla & Packer 2008; Grixti *et al.* 2009; Colla & Ratti 2010; Cameron *et al.* 2011), and some movement has been made toward protecting them. In Canada, *B. affinis* is listed as an endangered species (COSEWIC 2010), and *B. terricola* is listed as a high priority species for assessment (COSEWIC 2011). Until the causes of decline are better known, however, designing and implementing recovery strategies will be difficult. Our results indicate

that, at least for *B. terricola* and *B. pensylvanicus*, chronic pathogen spillover from commercial bumblebees should be one consideration for policies aimed at protecting these species; however, effective policies will need to take into account additional threats.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1: Scatterplot of log transformed tomato greenhouse area against vegetable greenhouse area in American counties from the 2007 American Agricultural Census. Greenhouse area is in m². Spearman's rho is shown. N=2114 (data for some counties was undisclosed)

Figure S2: Scatterplots and logistic regression results of losses of *B. affinis*, *B. terricola*, and *B. pensylvanicus* in American counties or Canada census divisions against vegetable greenhouse density. Vegetable greenhouse density is in units of m² of greenhouse per km² land area. Coefficients, P-values, and Nagelkerke R² values from logistic regressions are shown. N=38, 46, and 116 for *B. affinis*, *B. terricola*, and *B. pensylvanicus*, respectively

Table S1: Summary of bumblebee occurrence record data sources.

Table S2: Moran's I values of residuals from logistic regressions of losses of *B. affinis*, *B. terricola*, and

B. pennsylvanicus against vegetable greenhouse density, change in human population density, herbicide use density, and insecticide use density. Moran's I values and associated P-values based on permutation tests are given for the first distance class and any subsequent distance class with significant Moran's I values after sequential Bonferroni correction. Distance classes are in increments of 200 km.

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References

- Berenbaum, M., Bernhardt E., Buchmann S. et al. (2007) *Status of pollinators in North America*. The National Academy Press, Washington.
- Cameron, S.A., Lozier J.D., Strange J.P. et al. (2011) Patterns of widespread decline in North American bumble bees. *P Natl Acad Sci USA*, **108**, 662–667.
- Colla, S.R., Otterstatter M.C., Gegear R.J., Thomson J.D. (2006) Plight of the bumble bee: pathogen spillover from commercial to wild populations. *Biol Conserv* **129**, 461–467.
- Colla, S.R., Packer L. (2008) Evidence for decline in eastern North American bumblebees (Hymenoptera: Apidae), with special focus on *Bombus affinis* Cresson. *Biodivers Conserv* **17**, 1379–1391.
- Colla, S.R., Ratti C.M. (2010) Evidence for the decline of the western bumble bee (*Bombus occidentalis* Greene) in British Columbia. *Pan-Pac Entomol* **86**, 32–34.
- Committee on the Status of Endangered Wildlife in Canada (COSEWIC). (2010) *Canadian wildlife species at risk*. The Canadian Wildlife Service, Ottawa, ON.
- Committee on the Status of Endangered Wildlife in Canada (COSEWIC). (2011) Candidate wildlife species. Available from: http://www.cosewic.gc.ca/eng/sct3/index_e.cfm. Accessed 6 July 2011.
- Cook, R., Calvin, L. (2005). Greenhouse tomatoes change the dynamics of the North American fresh tomato industry. USDA Economic Research Report Number 2. Available from: <http://www.ers.usda.gov/publications/err2/err2.pdf>. Accessed 11 January 2011.
- Cordes N., Huang W.-F., Strange J.P. et al. (2012) Interspecific geographic distribution and variation of the pathogens *Nosema bombi* and *Crithidia* species in United States bumble bee populations. *J Invertebr Pathol* **109**, 209–216.
- Daszak, P., Cunningham A.A., Hyatt A.D. (2000) Wildlife ecology—emerging infectious diseases of wildlife—threats to biodiversity and human health. *Science* **287**, 443–449.
- Dormann, C.F. (2007) Assessing the validity of autologistic regression. *Ecol Model* **207**, 234–242.
- Gibbs, K.E., Mackey R.L., Currie D.J. (2009) Human land use, agriculture, pesticides and losses of imperiled species. *Divers Distrib* **15**, 242–253.
- Goka K. (2010) Introduction to the special feature for ecological risk assessment of introduced bumblebees: status of the European bumblebee, *Bombus terrestris*, in Japan as a beneficial pollinator and an invasive alien species. *Appl Entomol Zoolog* **45**, 1–6.
- Goulson, D., Lye G.C., Darvill B. (2008) Decline and conservation of bumble bees. *Annu Rev Entomol* **53**, 191–208.
- Gixti, J.C., Wong L.T., Cameron S.A., Favret C. (2009) Decline of bumble bees (*Bombus*) in the North American Midwest. *Biol Conserv* **142**, 75–84.
- Groffman, P., Baron J., Blett T. et al. (2006) Ecological thresholds: the key to successful environmental management or an important concept with no practical application? *Ecosystems* **9**, 1–13.
- Harrell, F.E. (2009) Design: Design Package. R-package version 2.3–0.
- Hosmer, D.W., Lemeshow S. (2000) *Applied logistic regression*. John Wiley & Sons Inc., Toronto.
- Macleán, I.M.D., Wilson R.J. (2011) Recent ecological responses to climate change support predictions of high extinction risk. *P Natl Acad Sci USA* **108**, 12337–12342.
- Meeus, I., Brown M.J.F., De Graaf D.C., Smagghe G.U.Y. (2011) Effects of invasive parasites on bumble bee declines. *Conserv Biol* **25**, 662–671.
- Morandin, L.A., Laverty T.M., Kevan P.G., Khosla S., Shipp L. (2001) Bumble bee (Hymenoptera: Apidae) activity and loss in commercial tomato greenhouses. *Can Entomol* **133**, 883–893.
- Morandin, L.A., Winston M.L., Franklin M.T., Abbott V.A. (2005) Lethal and sub-lethal effects of spinosad on bumble bees (*Bombus impatiens* Cresson). *Pest Manag Sci* **61**, 619–626.
- Nagelkerke, N.J.D. (1991) A note on a general definition of the coefficient of determination. *Biometrika* **78**, 691–692.
- Otterstatter, M.C., Thomson J.D. (2008) Does pathogen spillover from commercially reared bumble bees threaten wild pollinators? *PLoS One* **3**, e2771. doi:10.1371/journal.pone.0002771.
- Potts, S.G., Biesmeijer J.C., Kremen C., Neumann P., Schweiger O., Kunin W.E. (2011) Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evol* **25**, 345–353.
- Power A.G., Mitchell C.E. (2004) Pathogen spillover in disease epidemics. *Am Nat* **164**, S79–S89.
- R Development Core Team. (2010) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Shipp, J.L., Whitfield G.H., Papadopoulos A.P. (1994) Effectiveness of the bumble bee, *Bombus impatiens* Cr (Hymenoptera, Apidae), as a pollinator of greenhouse sweet-pepper. *Sci Hortic-Amsterdam* **57**, 29–39.

- Singh R., Levitt A.L., Rajotte E.G. *et al.* (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.
- Statistics Canada. (2001) 2001 Census. Available from: <http://www.statcan.gc.ca/estat/licence-eng.htm>. Accessed 18 January 2011.
- Statistics Canada. (2002) *Population ecumene census division boundary file*. Statistics Canada, Ottawa, Canada.
- Statistics Canada. (2007) Table 051–0034. Total population, census divisions and census metropolitan areas, 2001 Census boundaries, annual (persons). Available from: <http://www.statcan.gc.ca/estat/licence-eng.htm>. Accessed 17 May 2011.
- Thorp, R.W., Shepherd M.D. (2005) *Profile: subgenus Bombus*. The Xerces Society for Invertebrate Conservation, Portland, OR.
- U.S. Census Bureau. (2002) Intercensal estimates. Available from: <http://www.aiea.ualr.edu/population-estimates-a-projections.html>. Accessed 9 June 2011.
- U.S. Department of Agriculture (USDA). (2002) 2002 census of agriculture. Available from: <http://quickstats.nass.usda.gov/>. Accessed 26 January 2011.
- U.S. Department of Agriculture (USDA). 2004. 2002 Census of Agriculture County Level Boundaries. Available from: <http://www.agcensus.usda.gov/Publications/2002/Ag-Atlas-Maps/index.asp>. Accessed 20 August 2010.
- Velthuis, H.H.W., van Doorn A. (2006) A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie* **37**, 421–451.
- Whittington, R., Winston M.L. (2003) Effects of *Nosema bombi* and its treatment fumagillin on bumble bee (*Bombus occidentalis*) colonies. *J Invertebr Pathol* **84**, 54–58.
- Whittington, R., Winston M.L., Tucker C., Parachnowitsch A.L. (2004) Plant-species identity of pollen collected by bumblebees placed in greenhouses for tomato pollination. *Can J Plant Sci* **84**, 599–602.
- Williams, P.H., Osborne J.L. (2009) Bumblebee vulnerability and conservation world-wide. *Apidologie* **40**, 367–387.