A demographic approach to evaluating the impact of stressors on bumble bee colonies

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Abstract. 1. Natural and anthropogenic stressors threaten the sustainability of bumble bees and evaluating their impact is essential to the stewardship of these valuable pollinators. Demographic modelling provides a framework for testing hypotheses about the impacts of stressors, but it has not previously been applied to bumble bees.

2. I therefore formulated a demographic model for a bumble bee colony and then quantified the impact of two stressors, pesticide exposure and spider predation, by perturbing it with their known effects.

3. By simulating a laboratory exposure of *Bombus terrestris* L. to dietary imidacloprid (a neonicotinoid insecticide), I tested whether the observed colony decline was explained solely by a toxic effect on the fecundity of the foundress queen. By simulating field observations of *B. terricola* Kirby, I tested whether predation by crab spiders reduced colony fitness sufficiently to provide an adaptive explanation for avoidance behaviours seen when bumble bees encounter spiders.

4. In *B. terrestris*, a dose-appropriate decrease in fecundity predicted the observed colony decline, which implicates this as a principal mechanism of toxicity. In *B. terricola*, doubling the rate of spider predation reduced a colony's production of new queens by 11%, which implies that spider avoidance is highly adaptive.

5. These analyses illustrate the utility of demographic modelling for quantifying the impacts of stressors on bumble bees. In the future, models of this type could be used to investigate a wider range of stressors and to produce thereby knowledge and tools useful for safeguarding bumble bees and the pollination services that they provide.

Key words. Demography, ecotoxicology, imidacloprid, neonicotinoid, population decline.

Introduction

Bumble bees are important pollinators of crops and wild plants, and their decline is a serious concern (Williams & Osborne, 2009). Bumble bees are affected by both anthropogenic and natural stressors (Potts *et al.*, 2010) whose importance is not yet fully resolved (Roulston & Goodell, 2011). Experimental investigations to establish the impacts of stressors on bumble bees can be costly and laborious, and so a theoretical approach is valuable for differentiating among them provisionally and for generalising predictively to new or changing circumstances. Ecologists have developed theoretical approaches to investigate the causes of population decline and to evaluate the role of various stressors (Dirzo *et al.*, 2014). Among these, demographic matrix models

(Caswell, 2001) can assist both in disentangling threats to population viability (Akçakaya *et al.*, 2008; Bakker & Doak, 2009) and in detecting the causes of decline retrospectively (Landis, 2008).

In bumble bees, a matrix model cannot yet achieve the conventional goal of evaluating long-term population sustainability because projecting over successive years is hampered by a lack of information about a crucial life stage. Specifically, wild bumble bees in seasonal climates have an annual cycle in which only the dormant queen overwinters, the probability of which is poorly known (but see Crone & Williams, 2016). However, a matrix model can instead be used to simulate an individual colony, whose growth is projected based on the fecundity and mortality rates of its constituent individuals. Modelling the impact of stressors on a bumble bee colony can nevertheless offer insight into population sustainability because the colony is the reproductive unit for this social insect. Additionally, the necessary matrix parameters are obtainable from published studies

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because experimentalists often report the effects of stressors on the mortality or fecundity of individuals, which can be readily harnessed as perturbations of colony-level demographic rates. For example, experimentalists have measured the relationships between mortality rates and a variety of stressors including: exposure to agrochemicals (Barbosa *et al.*, 2015); pollen starvation (Smeets & Duchateau, 2003); and infection by pathogens (Brown *et al.*, 2000).

Whereas ecologists already use sophisticated demographic simulations to evaluate the impact of stressors on honey bee colonies (Becher *et al.*, 2013), previous treatments of bumble bees have lacked a demographic structure (Schmid-Hempel & Heeb, 1991; Bryden *et al.*, 2013; Crone & Williams, 2016). Here, I formulate a demographic matrix model for simulating the growth of a bumble bee colony and I illustrate its utility by using it to test hypotheses about the potential impacts of two contrasting stressors, namely: (i) exposure to a dietary insecticide, an anthropogenic stressor that affects the fecundity rate; and (ii) predation by crab spiders, a natural stressor that affects the mortality rate.

How do dietary neonicotinoids affect bumblebees?

Bees are exposed to dietary insecticides by foraging from systemically treated mass-flowering crops because the toxicant appears in the plants' nectar and pollen, which bees collect and consume (Rortais *et al.*, 2005). For example, fields of canola and sunflowers are systemically protected by neurotoxic neonicotinoids (Nauen & Jeschke, 2011) such as imidacloprid, clothianidin, and thiamethoxam, which have been used worldwide. Dietary intake of neonicotinoid residues causes various harmful sublethal effects in bumble bees (Desneux *et al.*, 2007), such as reductions in foraging activity (Gill & Raine, 2014), colony growth (Gill *et al.*, 2012), and reproduction (Whitehorn *et al.*, 2012). In honey bees, a principal mechanism by which the neurotoxic neonicotinoids cause harm is by disrupting memory, learning and cognition. For example, a dietary neonicotinoid can jeopardise honey bee colonies by causing high mortality among foragers as homing failure (Henry *et al.*, 2012), which occurs because neonicotinoidal intoxication impairs navigational memory (Fischer *et al.*, 2014). Similarly, dietary neonicotinoids may threaten bumble bee colonies because the intoxication of adult workers disrupts colony functions that depend on collective coordination, such as foraging, thermoregulation or social hygiene (Bryden *et al.*, 2013).

To test whether collective narcosis of a colony's adult workers could explain bumble bee colony decline, Bryden *et al.* (2013) used a mathematical model (termed the 'sublethal stress' model, or SLS). The SLS successfully explained the trajectory of colony decline observed when enclosed laboratory colonies (*B. terrestris*) were fed neonicotinoid-laced syrup (imidacloprid at a concentration of 10 parts per billion, or ppb). To fit the SLS model, however, two postulates were necessary: (i) that the narcotic effect of the neonicotinoid was so powerful that each exposed worker was rendered almost completely ineffective; and (ii) that the collective ineffectiveness of workers so severely disrupted colony function that it rapidly increased the *per capita* death rate of adult workers. From this basis, the SLS model provided a statistically successful explanation for the observations (R-squared = 73%), but its biological postulates are questionable for two reasons. First, the levels of dietary imidacloprid used in the focal experiment (10 ppb) do not cause severe narcosis in individual bumble bees (Cresswell *et al.*, 2012). Second, it is unclear which collective function could fail so severely as to cause the required increase in individual death rates. In the focal experiment, colonies were fed directly from an attached hopper that precluded foraging excursions so that the neonicotinoid could not cause mortality by homing failure. Other collective colony functions in bumble bees, such as thermoregulation or hygienic behaviours, have not yet been shown to be disrupted by dietary neonicotinoids. As the biological postulates of the SLS are therefore contingent, an alternative explanation for the observed population decline can be sought.

Besides an increased death rate, population decline can also be driven by a decreased birth rate, i.e. by reduced fecundity. Dietary exposure to imidacloprid, a neonicotinoid insecticide, represses egg laying by both queens (Laycock & Cresswell, 2013) and workers (Laycock *et al.*, 2012, 2014). Furthermore, this effect occurs at low dietary concentrations in the laboratory; when fecundity is the focal attribute, the concentration of imidacloprid that halves performance relative to an undosed control (i.e. the EC_{50}) is approximately 1 ppb (Laycock & Cresswell, 2013), which is within the environmentally relevant range (Godfray *et al.*, 2014). However, it has been unclear whether this demographically potent mechanism could be responsible for the reported impacts of dietary imidacloprid on colony growth and reproduction in bumblebees (e.g. Whitehorn *et al.*, 2012). Therefore, the first aim of the present investigation was to test the hypothesis that repressed fecundity owing to a dietary neonicotinoid can cause colony decline.

How strongly do crab spiders affect fitness in bumble bees?

Crab spiders are ambush predators that occupy flowers and capture flower-visiting insects, including bumblebees. For bumble bees, the likelihood of capture appears to be generally low. Even in areas where crab spiders are common, field observations suggest that a foraging bumblebee will be attacked by a spider only every 4–6 days and the expected period until capture exceeds the bee's foraging lifespan (Morse, 1986). On this basis, the impact of crab spiders on bumble bees has been interpreted as less important than other mortality factors such as parasitism and nest predation (Sladen, 1912; Alford, 1975). In contrast, an important ecological role for spider predation is suggested by evidence that the selective removal of spiders by a pesticide coincided with greatly improved survivorship among worker bumble bees (Plowright & Rodd, 1980). Additionally, if the impact of spiders is indeed minimal, it is less easy to adaptively explain why bumblebees avoid spiders (Dukas & Morse, 2003; Ings & Chittka, 2008). However, the hypothesis that bumble bee responses to crab spiders are adaptive could be better evaluated if the impact of spider predation on colony performance and fitness were quantified, but no previous study has

done this. The present study, therefore, aimed to undertake this quantification.

Materials and methods

Matrix model of colony growth in bumblebees

Bumble bee colonies are monogynous, and the foundress queen produces eggs steadily once her first adult worker emerges, or ecloses. However, colony size (the number of adult workers) increases first linearly then geometrically under benign laboratory conditions where the rate of pre-senescence mortality is low (Duchateau & Velthuis, 1988). The geometric phase indicates that the daily eclosion rate increases with colony size, probably because of improving brood husbandry. To model this scenario, assume that the queen lays L eggs per day and let the number of adult workers eclosing on day t be denoted E_t. The initial pre-geometric phase of colony growth lasting α days (i.e. from $t = 0$ to $t < \alpha$) is described by:

$$E_t = L \tag{1}$$

Let W_t denote the number of adult workers in the colony on day t. Incorporate the onset of geometric colony growth (for all $t \geq \alpha$) as:

$$E_t = L W_{t-\alpha}^{\beta} \tag{2}$$

Under eqn 2, the colony size-dependent impact of worker husbandry on eclosion rate is controlled by the value of parameter β and its onset is controlled by α. In effect, α imposes a time lag that makes worker husbandry retrospective because its impact on the adult eclosion rate at any given time in the geometric phase ($t \geq \alpha$) depends on the earlier number of adult workers, $W_{t-\alpha}$, that provided husbandry to these emerging individuals as larvae.

Using demographic matrix projection, colony growth is modelled by:

$$n_{t+1} = A n_t \tag{3}$$

Let w_x denote the number of adult workers in a colony that are aged x days post-eclosion and let m_x denote their *per capita* daily mortality rate. The product of a matrix A and the population state vector n_t is then given by:

$$A n_t = \begin{bmatrix} 0 & 0 & 0 & \ldots & 0 & E_t \\ (1-m_1) & 0 & 0 & \ldots & 0 & 0 \\ 0 & (1-m_2) & 0 & \ldots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \ldots & (1-m_i) & 0 \\ 0 & 0 & 0 & \ldots & 0 & 1 \end{bmatrix} \begin{bmatrix} w_1 \\ w_2 \\ w_3 \\ \vdots \\ w_i \\ Q \end{bmatrix} \tag{4}$$

The lowest element of n_t is the number of foundress queens, Q, which is here set to $Q = 1$ for all analyses, and the total number of adult workers in the colony at any time is $W_t = \sum_x w_x$.

In effect, eqn 4 describes age-based mortality in a population that each day recruits adults by eclosion based on a number of eggs previously laid by one or more individuals of single stage class, namely queens. The model (eqn 4) is intended to simulate only the early stages of colony growth and it does not cover the colony's production of new sexual individuals (female gynes and males). Where required in the following analyses, the production of sexuals is estimated by separate colony size-reproduction relationships. The matrix models analysed in the present study are deterministic, and no stochastic variants were developed.

To enable a stressor (e.g. dietary insecticide) to reduce the queen's fecundity by a proportion a, write the perturbed daily rate as:

$$L^* = (1-a) L \tag{5}$$

To enable a stressor (e.g. increased the frequency of crab spiders) to increase mortality rates, assume that adult workers that normally survive each day are exposed to an additional daily age-independent risk of death with probability δ, which yields the perturbed schedule, m^*_x:

$$m^*_x = m_x + (1-m_x) \delta \tag{6}$$

These perturbations (eqns 5 and 6) can be construed as a projection matrix, A^*, by substituting m^*_x and L^* in the model (eqns 1–4) where appropriate.

Repressed fecundity and colony growth

The repressed fecundity hypothesis, denoted RFH, was evaluated by making a dose-appropriate reduction to fecundity (eqn 5) after first calibrating the demographic matrix model to simulate an unexposed control colony. Specifically, I tested whether this perturbed model predicted the decline observed in the experimentally exposed colonies. This approach was applied to a laboratory experiment that investigated the effect of dietary imidacloprid (a neonicotinoid) in feeder syrup (10 ppb) on the growth of bumble bee colonies (*B. terrestris*) over a period of 42 days (Bryden *et al.*, 2013).

To calibrate the model initially, I parameterized A from the undosed controls as follows. Because the control colonies grew only linearly during the experiment, I set $\alpha = \infty$ so that $E_t = L$ throughout. As the bees in the experimental colonies were not newly eclosed when the experiment began, I populated m_x with the mortality schedule of a representatively mixed-age cohort of *B. terrestris* workers in laboratory conditions feeding on control syrup, which took values as follows. For adult workers aged between 0 and 21 days post-eclosion $m_x = 0$, whereas for adults aged 22 days post-eclosion or older, the values of m_x are respectively: 0.050, 0.053, 0.056, 0.059, 0.0, 0.063, 0.067, 0.0, 0.0, 0.071, 0.0, 0.0, 0.154, 0.0, 0.0, 0.182, 0.111, 0.125, 0.0, 0.0, 0.0 (Fig. 1). The experimental colonies initially comprised a mean of 8.25 adult workers and a queen, so I employed a starting vector of $n_0 = [8.25, 0, 0, \ldots, 0, 1]$. The daily eclosion rate in the experimental colonies was not reported by the investigators, so I adjusted the value of L so that projection by eqn 4 over the duration of the experiment (i.e. 42 successive projections each representing a 1-day time step) generated the observed mean colony size of $W_{42} = 42.7$, which required $L = 1.23$. To
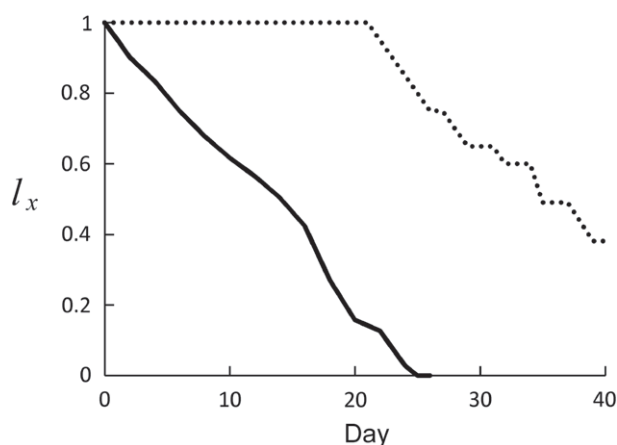
Fig. 1. Survivorship schedules used for demographic modelling. The dotted line indicates the survivorship schedule of *Bombus terrestris* under laboratory conditions (representative mixed-age cohort collected at *Day* = 0, *n* = 20; Cresswell *et al.*, 2012) and the solid line indicates survivorship in *B. terricola* (cohort of workers eclosing at *Day* = 0, *n* = 274) under field conditions in Doakland, Canada (Rodd *et al.*, 1980). Schedules are l_x = proportion of original cohort surviving for day = 0–40.

introduce the impact of 10 ppb dietary imidacloprid on foundress fecundity, I used Laycock and Cresswell's (2013) dose-response relationship, which predicted an 84% reduction in foundress fecundity (i.e. $a = 0.84$). Bryden *et al.* (2013) dosed colonies that contained a pre-existing brood so that the eclosion rate was initially maintained by the emergence of workers who had completed their larval development before the neonicotinoid exposure. I therefore delayed the pesticide effect to best fit the observed trajectory, i.e. $a = 0.0$ until $t = 5$ days and $a = 0.84$ thereafter.

Spider predation and bumble bee fitness

The crab spider *Misumena vatia* Clerck is a habitual ambush predator whose range in North America coincides with that of the yellow-banded bumblebee, *B. terricola* (Morse, 1981). Both species have been the focus of meticulous studies that provide comprehensive demographic descriptions of colony growth in *B. terricola* (Owen *et al.*, 1980; Rodd *et al.*, 1980) and details of the frequency of spider-bumble bee interactions (Morse, 1981, 1986). The demographic data on *B. terricola* also related colony size to reproductive success (i.e. outputs of sexual offspring, daughter queens and drones), which made it possible to evaluate the impact of spider predation on colony fitness.

Rates of predation by crab spiders were obtained from a study of *B. terricola* foraging on milkweed (*Asclepias syriaca* L.) umbels conducted during July and August of 1981 and 1982 in Lincoln Co., Maine, USA (Morse, 1986). In this survey, crab spiders successfully captured two workers of *B. terricola* during 531 bee visits to umbels that harboured crab spiders. The estimated mean of the number of captures per sample is statistically uncertain because these were so rarely observed, so I also used the confidence limit on this datum, which was derived

as follows. Assuming that attacks are Poisson-distributed, the largest mean number of kills per survey that could reasonably generate two or fewer attacks ($P \geq 0.05$) is $\mu = 6.3$ and the smallest is $\mu = 0.4$, which yields a 95% confidence interval of 0.71×10^{-5} to 1.18×10^{-2} kills per encounter on an estimated mean of 0.34×10^{-2}. In the calculations below, the limits of this confidence interval are reported parenthetically. In the study area, a survey of 1912 umbels yielded nine spiders or 4.7×10^{-3} spiders per umbel. Combining the prevalence of spiders with their attack success, the probability that a bee visits an umbel with a spider and is killed is estimated to be 1.8×10^{-5} (3.3×10^{-6}, 5.6×10^{-5}). If *B. terricola* workers visit an umbel every 27.3 s over 4 h of foraging each day (Morse, 1986), they visit 527 umbels per day. Consequently, the probability of surviving each day is $[1 - (1.8 \times 10^{-5})]^{527} = 0.991$ (0.998, 0.971) and the expected daily mortality rate is therefore 1% (0.2%, 3%). The voraciousness of the crab spiders in Lincoln County is probably representative because similar capture rates were observed in Bern, Switzerland [Doakland: 0.37% vs. Bern: 0.24%, or two captures in 821 encounters (Brechbühl *et al.*, 2011)].

To simulate colonies of *B. terricola*, I assumed that the values of α and β are intrinsic to the species and estimated them from published laboratory data (Laverty & Plowright, 1985) as follows. After estimating L from the linear phase of worker production ($L = 1.27$ workers per day, $r^2 = 0.99$, $n = 6$; Fig. 2), I fitted the matrix model to the subsequent geometric phase by assuming unperturbed colony growth ($a = 0, \delta = 0$) from a single worker ($W_0 = 1$) by least-squares regression thereby obtaining: $\alpha = 25.1$ and $\beta = 0.542$ ($r^2 = 0.98$, $n = 8$; Fig. 2). For fitting the model, a mortality schedule for laboratory *B. terricola* was not available, so I assumed that it followed the schedule of laboratory *B. terrestris* (Fig. 1).

Using these data, I simulated a population comprising 32 colonies that were studied in the 1970s under field conditions near Doaktown (Southeastern Canada) by Owen *et al.* (1980). I assumed that each colony initially comprised a single adult worker and a queen by employing a starting vector of $\boldsymbol{n}_o = [1, 0, 0, \ldots, 0, 1]$ in eqn 4 with m_x taking the mortality schedule at Doaktown (Fig. 1). The daily eclosion rate in each colony was not reported by the investigators, so I estimated these parameter values as follows. For each colony, I used eqn 4 to project colony growth over the duration of the experiment (i.e. 40 1-day time steps) with no perturbation ($a = \delta = 0$) and adjusted the value of L for each simulated colony to generate its observed eventual colony size, W_{40}. For the collection of 32 colonies, the median value of L used in the simulations was 0.93 (SD = 0.71). To generate the reproductive output of each colony, I used least-squares regression to describe the relationships between W_{40} and the number of gynes (daughter queens) and males produced by the Doaktown colonies (*males* = $4.34W_{40}e^{-0.0166W_{40}} - 2.00$, $r^2 = 0.84$; *gynes* = $0.334W_{40} - 4.665$, $r^2 = 0.66$; Fig. 3). Before perturbation, the model population closely simulated the collective reproduction at Doaktown as required (gynes: 359 simulated, 351 observed; males 2131 vs. 2143).

To investigate the impact of spider predation on fitness, I assumed that the observed daily mortality rate as a result of
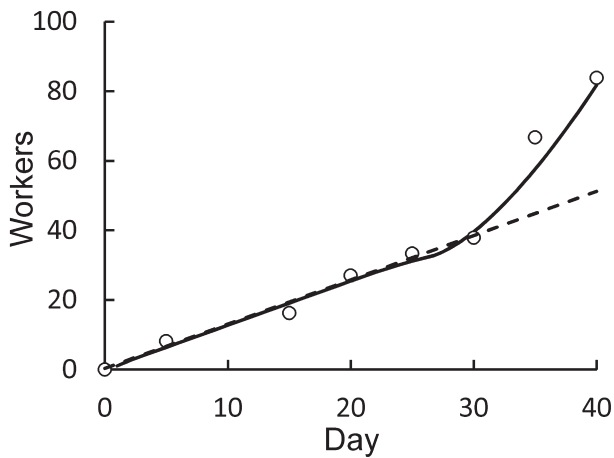
Fig. 2. Estimating the parameters for colony growth in *Bombus terricola* under benign laboratory conditions. The relationships are between the mean number of adult workers (*y*-axis) and time (*x*-axis, days). Open symbols depict the observed mean number of workers in five laboratory colonies over 40 days (Laverty & Plowright, 1985); the dashed line indicates a linear relationship fitted to the observations made over the first 30 days ($W_t = \text{days} \times 1.27$) and the solid line indicates the best-fitting model described by eqns 1–4 (see text) using $L = 1.27$ and m_x appropriate to bumble bee survivorship under laboratory conditions (Fig. 1), which yielded $\alpha = 25.1$ and $\beta = 0.542$ ($r^2 = 0.98$).

spider predation of $\gamma = 1\%$ (0.2%, 3%) applied to *B. terricola* workers at Doaktown. Therefore, to estimate the total mortality due to spiders at Doaktown, denoted S, including where appropriate an enhanced rate of spider predation (δ), I calculated:

$$S = \sum_t \left(\frac{w_t}{1 - (\gamma + \delta)} - w_t \right) \qquad (7)$$

Assuming that hypothetical bees that were maladapted to spider predation would be up to twice as vulnerable to spiders, I investigated the effect on colony growth and reproduction of perturbations in the region of $0 < \delta \leq 0.03$.

Results

A demographic simulation based on the reduced fecundity hypothesis (RFH) accurately predicted the observed colony decline owing to dietary imidacloprid in laboratory colonies of *B. terrestris* (*R*-squared = 81%; Fig. 4).

Bumble bee fitness depended strongly on the extent to which adult workers avoided capture by ambush spiders (Fig. 5). When adult workers of *B. terricola* were twice as vulnerable to spider predation, the average gyne production per colony decreased by 11% (3%, 26%) and male production decreased by 1% (0.3%, 5%).

Spiders had a major impact on mortalities among adult bumble bee workers. For an average colony simulated under ambient levels of spider predation ($\delta = 0$), almost half of the workers eventually died through predation by spiders (Table 1). If workers were twice as likely to be attacked and killed by spiders, a further 10% suffered this fate (Table 1).
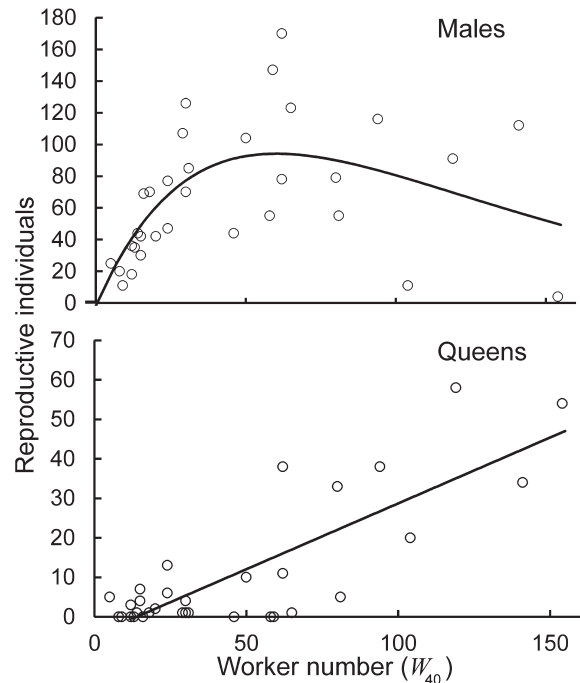

Fig. 3. Relationships between attained colony size (*x*-axis: number of adult workers 40 days after the emergence of brood begins) and the number of sexual adults produced by colonies of *Bombus terricola* (*y*-axes: upper panel, number of males, or drones; lower panel, number of daughter queens, or gynes). Open symbols depict the observed number of males (upper panel) or queens (lower panel) produced by 32 bumble bee colonies at Doaktown, Canada (Owen *et al.*, 1980) in relation to the number of workers present. The solid lines indicate best-fitting regression relationships: $males = 4.34W_4 e^{-0.0166W_{40}} - 2.00$, $r^2 = 0.84$; $queens = 0.334W_{40} - 4.665$, $r^2 = 0.66$.

Discussion

The first analysis presented here tested the 'repressed fecundity hypothesis' (RFH), which proposes that a dietary neonicotinoid, imidacloprid, reduces colony growth in laboratory bumble bees principally by repressing the fecundity of the colony's foundress queen. If fecundity is sufficiently repressed, colony decline eventually occurs because the rate of worker mortality owing to senescence exceeds the replacement rate. When compared to experimental observations of an imidacloprid-induced colony decline, a demographic simulation based on the RFH achieved statistical correspondence equivalent to that of the SLS (sublethal stress hypothesis), which instead posits an increased mortality rate among individual worker bees because of their collective narcosis (Bryden *et al.*, 2013). Consequently, there is no statistical basis for differentiating between them. However, to further discriminate between these two hypotheses, two additional criteria can be considered besides statistical correspondence (Hill, 1965). First, the SLS required statistical regression against the observed decline to infer the magnitude of the posited narcotic effect, whereas the magnitude of the RFH effect was independently measured by the experiment and introduced into
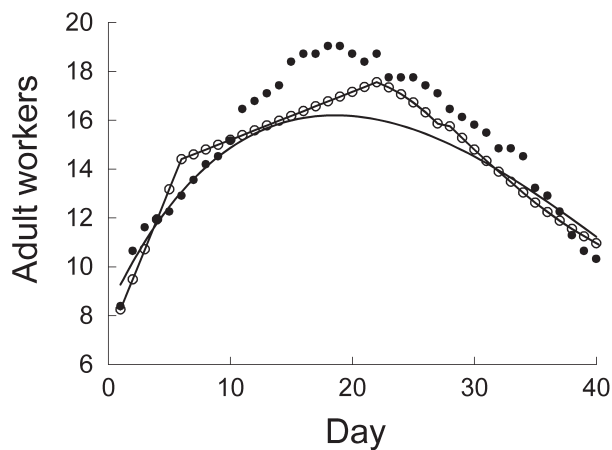
Fig. 4. Observed and simulated patterns of colony growth in laboratory colonies of *Bombus terrestris* during exposure to dietary imidacloprid. Filled circles indicate the mean number of workers (*y*-axis) in eight colonies that fed on dietary imidacloprid at a concentration of 10 ppb in feeder syrup over a period of 40 days (*x*-axis) as observed by Bryden *et al.* (2013). The interpolated open circles indicate colony growth as simulated by matrix projection (eqns 1–6; *R*-squared = 81%). The solid line indicates colony growth as simulated by the Sublethal Stress (SLS) model (Bryden *et al.*, 2013) with best-fitting parameter values (*R*-squared = 73%).
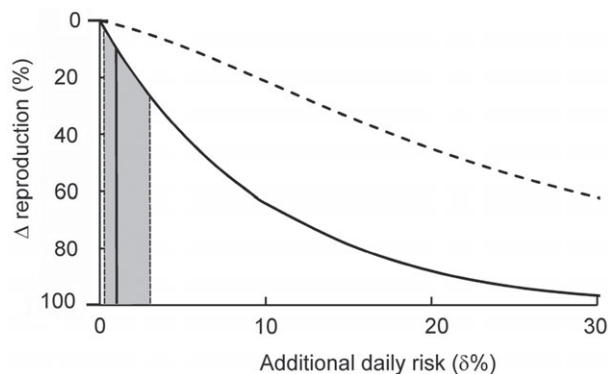

Fig. 5. Effect of increased mortality of adult workers on reproductive output in an average colony of *Bombus terricola*. The *x*-axis shows the perturbation (increase) in the daily rate of mortality among adult workers (δ%); the *y*-axis denotes the change (% relative to an unperturbed colony) of drones (dashed line) and gynes (solid line). A realistic addition to daily risk due to heightened predation by crab spiders is indicated by a vertical line for the estimated mean increase, $\delta = 1$%, and its 95% confidence interval ($\delta = 0.2$–3%) is indicated by a grey area bordered by vertical dashed lines.

the model. In this respect, only the RFH is coherent with independently derived experimental data. Second, the collective narcosis required by the SLS has not yet been measured empirically, which reduces its plausibility compared to the RFH. In summary, the RFH can be favoured over the SLS because it is based on an empirically demonstrated mechanism that predicts the observed impact of dietary exposure on colony dynamics when invoked at a biologically appropriate strength.

Table 1. Performance indicators of a simulated average colony of *Bombus terricola* at Doakland under either ambient or heightened levels of spider predation.

Mortality schedule	E_{40}	W_{40}	*Prey (S)*
$\delta = 0.00$	60	30	14 (47%)
$\delta = 0.002$	60	30	15 (50%)
$\delta = 0.01$	59	28	18 (58%)
$\delta = 0.03$	58	24	24 (70%)

The simulated colony's queen has the median fecundity of those observed at Doakland, i.e. $L = 0.93$, and under ambient conditions the workers in the colony experience the mortality schedule observed at Doakland (m_x). A heightened level of spider predation was simulated by replacing the mortality schedule with m^*, which set either $\delta = 0.01$ or its confidence interval ($\delta = 0.002, 0.03$). E_{40} denotes the number of adult workers produced (eclosed) by the end of 40 days after the eclosion of the first worker; W_{40}: the number of adult workers alive 40 days after the first eclosion; *Prey*: the number of adult workers killed by spiders as estimated by eqn 7 (% indicates the proportion of all deaths of adult workers caused by spiders, i.e. $100 \times Prey/[E_{40} - W_{40}]$). For biological relevance, all data are rounded to the nearest integer.

The corroboration of the RFH has implications for assessing the sustainability of farmland bumble bees that are exposed to agricultural neonicotinoids. Among the responses whose dose-dependence under neonicotinoid exposure has been studied, repression of fecundity by dietary imidacloprid is the most potent (Fig. 6) because its EC_{50} is approximately 1 ppb, which is environmentally realistic for residues found in nectar and pollen from treated crops (Godfray *et al.*, 2014). Consequently, the magnitude of the colony-level effects predicted by the present study raises the concern that agricultural imidacloprid may threaten the sustainability of farmland populations of bumble bees. However, the severity and generality of this threat remain uncertain for three reasons.

First, neonicotinoids have the potential to harm bumblebees in various ways besides repressing fecundity. For example, unlike laboratory colonies, wild bumble bees depend on foraging excursions and may, therefore, also suffer impact from mortality owing to homing failure caused by neonicotinoid intoxication (Gill & Raine, 2014). Consequently, the present study may underestimate the harm due to dietary imidacloprid. Second, the capacity of bumble bees to recuperate from intoxication by imidacloprid (Laycock & Cresswell, 2013) may enable colonies to survive short-term exposures caused by the transient blooming of treated mass-flowering crops, which means that the impact of imidacloprid on wild bees may be overestimated by the sustained laboratory exposure analysed here. Finally, the potency of imidacloprid as a repressor of fecundity in the environmentally realistic range (<10 ppb) probably does not represent the effect of the neonicotinoids currently in widespread agricultural use, namely thiamethoxam and its derivative clothianidin. For example, the dose-dependent effect of thiamethoxam on fecundity in bumble bees has a higher EC_{50} (Laycock *et al.*, 2014) than imidacloprid's (Fig. 6), which means that thiamethoxam and its toxic metabolite, clothianidin, may have a smaller impact on wild bees than imidacloprid at environmentally relevant levels. In summary, these three uncertainties mean that further research
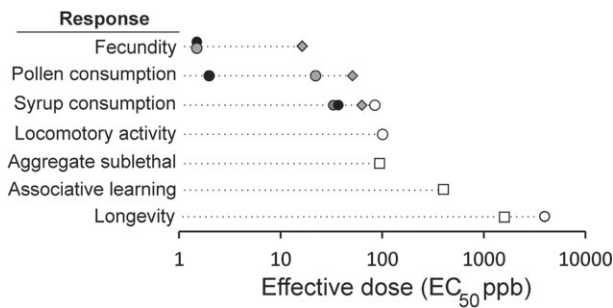
Fig. 6. The relative sensitivity of various responses in bees to the neonicotinoid dose, or the dietary concentration in units of parts per billion (ppb) by mass. Response variables are identified in the leftmost column and symbols in the corresponding row show each endpoint's sensitivity by its EC_{50}, or the 'effective concentration' that reduces performance in that attribute by 50% relative to an undosed control. For example, a dose of approximately 100 ppb reduced locomotory activity by 50% in individual bumble bees. Circular symbols denote bumble bees exposed to imidacloprid; diamond symbols - bumble bees exposed to thiamethoxam; and squares - honey bees exposed to imidacloprid. Black-filled symbols denote attributes of colonies with foundress queens, grey fill denotes queenless (orphaned) microcolonies and open symbols are attributes of individual bees. 'Aggregate sublethal' refers to the average of a collection of behavioural and other performance variables measured on colonies and individuals. Data extracted from published sources (Decourtye *et al.*, 2003; DEFRA, 2007; Cresswell, 2011; Cresswell *et al.*, 2012; Laycock & Cresswell, 2013; Laycock *et al*, 2014) except bumble bee longevity – Philippa Holder and James Cresswell, unpublished.

is required to fully evaluate the overall severity of the impact of agricultural neonicotinoids on bumblebees.

The second analysis presented here suggests that fitness in bumble bees depends strongly on the ability of adult workers to avoid spider predation while foraging. Specifically, each percentage increase in the daily mortality rate of workers is predicted to reduce queen production by approximately 10% in an average colony. In effect, the adaptive landscape imposes a steep selection gradient against worker vulnerability. Consequently, the extant behaviours that promote avoidance of spiders are likely to be highly adaptive in bumble bees.

The analysis also predicts that spider predation can have a major impact on both the mortality of individual adult workers and reproduction by colonies. When the demographic model simulated a population of 32 colonies of *B. terricola* at Doaktown, it estimated that almost half of the adult workers succumbed to spiders (over 400 individual deaths) even without an enhanced predation rate (i.e. $\delta = 0$). Large fluctuations are common in the populations of terrestrial invertebrates that inhabit plants (Root & Cappuccino, 1992) and the perturbation considered here (a doubling of spider numbers, i.e. $\delta = 1\%$) is small by comparison, but it is realistic for crab spiders because in a 5-year survey (Dukas & Morse, 2003) the highest prevalence of crab spiders was twice the mean. In a season when spider numbers double, the model predicted that almost 60% of Doakland workers are killed by spiders (over 500 individual deaths), and the bumble bee population is predicted collectively to produce 40 fewer gynes, or daughter queens

(i.e. a reduction of 11%). These findings suggest that the low daily rate of predation incidents per bee, which is the variable most commonly reported by empiricists, does not convey the full severity of the impact that spider predation may have on bumble bees, which instead relates to the integral of the predation rate over the lifetimes of the adult forager workforce. An additional implication is that ambush spiders could in some circumstances be a determining factor (Varley *et al.*, 1973) of the within-season abundance of adult workers, as previously inferred by Plowright and Rodd (1980). However, the extent to which bumble bees encounter crab spiders consistently while visiting various types of flowers across spatially widespread foraging areas will modify the overall impact of this form of predation. More generally, the demographic analysis shows that a small increase in worker mortality irrespective of its source can have large impacts on colony reproductive output in some bumble bee species. Colony growth is less sensitive to worker losses in *B. terrestris* (Schmid-Hempel & Heeb, 1991) than in *B. terricola*, however, which suggests that bumble bee species differ in their demographic sensitivity to worker mortality.

The focal species for the present demographic investigation of spider predation, *B. terricola*, is now rare, having declined in North America since the 1970s (Colla & Packer, 2008). Coincidentally, the demographic model of the focal population at Doakland was based on information that was collected in the 1970s. Analysis of the model predicted that the Doakland population would have received a major impact from a commonplace environmental fluctuation such as annual variation in spider abundance. Hypothetically, therefore, *B. terricola*'s sustainability was already in jeopardy 50 years ago, and its subsequent decline was then caused by the sustained and widespread further impacts of various stressors (Potts *et al.*, 2010). It is unlikely that predatory spiders were the driver of population decline in *B. terricola*, however, because there is no published evidence for a sustained and widespread increase in spider numbers coincident with *B. terricola*'s collapse across its historic range (Cameron *et al.*, 2011).

Bumble bees are valuable pollinators whose decline is a serious concern. Like all wild populations, the sustainability of a bumble bee population depends on its capacity to recover from stochastic insults, such as harsh winters, by population increase. One implication of the present study is that two important questions about stewardship for sustainable populations of bumble bees could be further addressed within its demographic framework. First, to protect the capacity for population increase, what is the relative importance of managing the availability of floral resources, nesting sites and the impacts of stressors (e.g. pesticide exposure and natural enemies)? Specifically, it would be valuable to link each of these factors to rates of mortality and fecundity, i.e. the elements of the demographic projection matrix, so that their potential effects can be quantified. Second, what demographic indicators best identify a bumble bee species as susceptible to decline and, therefore, as a priority for conservation? In a demographic framework, this query is addressed by conducting a sensitivity analysis that investigates the effect on population growth of realistic perturbations to the projection matrix (Caswell, 2000). Further research will be necessary to obtain the information required to address the two preceding

questions demographically. Potentially, however, in conjunction with complementary models (Bryden *et al.*, 2013; Crone & Williams, 2016), the demographic framework established here will begin to enable future investigators to assemble a comprehensive mechanistic understanding of population dynamics in bumblebees that can inform both risk assessments and conservation strategies that focus on farmland bees.

Acknowledgements

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