LUC PELLETIER

FACTEURS AFFECTANT LE SUCCÈS REPRODUCTEUR DES BOURDONS EN MILIEU NATUREL

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Résumé long

Les facteurs limitants le succès reproducteur des colonies de bourdons en milieu naturel sont très peu connus. Grâce au suivi du développement de plus de 200 colonies de huit espèces de bourdons (*Bombus impatiens, B. fervidus, B. perplexus, B. terricola, B. bimaculatus, B. ternarius, B. rufocinctus* et *B. vagans vagans*) sur le terrain, j'ai exploré l'effet de la taille de la reine, de la disponibilité en nourriture et de certains parasites sur le succès reproducteur des colonies.

Les plus grandes reines avaient plus de chances de se reproduire et, parmi celles qui y sont parvenues, le nombre de sexués produits était positivement relié à la taille de la reine. Le meilleur succès des plus grandes reines est attribuable, du moins en partie, à la production de plus grosses colonies et, chez certaines espèces, à la capacité à mieux repousser les usurpations de *Psithyrus* (un sous-genre de bourdons parasites), mais n'est pas relié à la date de nidification ou à l'usurpation par d'autres reines *Bombus*.

Une expérience où j'ai ajouté de la nourriture dans la moitié des colonies tout au cours de la saison a démontré que la disponibilité en nourriture était aussi un facteur important. Les colonies avec ajout de nourriture sont devenues plus grosses (en nombre d'ouvrières) et ont eu un meilleur succès reproducteur que les colonies témoins, par 51% et 86% respectivement. Toutefois, malgré que l'ajout de nourriture a permis de produire des colonies plus grosses, cela n'a pas permis aux colonies de mieux se défendre contre les macroparasites *Psithyrus*, *Fannia canicularis*, *Brachicoma devia* et *Vitula edmandsae*.

Le suivi du taux d'activité de butinage de certaines de ces colonies démontre que les colonies nourries ont eu un taux d'activité de butinage par ouvrière 25% plus bas que les colonies témoins. Les ouvrières des colonies dont les réserves en nourriture sont abondantes semblent donc moins butiner sur une base journalière pour réduire les coûts associés à cette activité. Si les ouvrières peuvent en retirer une longévité plus longue, cela fournirait un mécanisme supplémentaire pour expliquer le meilleur succès reproducteur des colonies nourries.

Abstract

Factors that limit the reproductive success of bumblebee field colonies are poorly known. I explored the effect of the queen’s body size, food availability, and some parasites on reproductive success by following the development of more than 200 field colonies of eight species of bumblebees (*Bombus impatiens, B. fervidus, B. perplexus, B. terricola, B. bimaculatus, B. ternarius, B. rufocinctus* et *B. vagans vagans*).

Larger queens were more likely to reproduce, and, for queens that did so, there was a positive relationship between their body size and the number of sexuals produced. The higher success of larger queens is, at least in part, attributable to the production of larger colonies and, in some species, to the ability to prevent usurpations by *Psithyrus* (a parasitic subgenus of bumblebees). The higher success of larger queens was not related to the date of nest establishment or to usurpations by other *Bombus* queens.

A field experiment in which I added food to half of the colonies over the entire season showed that food availability was also an important factor. Colonies with increased food supplies reached larger sizes (in number of workers) and had a higher reproductive success than controls, by 51% and 86% respectively. In particular, food supplementation increased the number of males produced and the probability of producing gynes (young queens). However, despite some clear advantages of having larger food supplies such as the build-up of larger worker populations, food supplementation did not appear to help colonies defend themselves against macroparasites because experimental and control colonies experienced similar levels of parasitism by *Psithyrus*, *Fannia canicularis*, *Brachicoma devia*, and *Vitula edmandsae*.

By recording the foraging activity rate in some of these colonies, I showed that food supplementation reduced the foraging activity rate per worker by 25% relative to control colonies. Workers from colonies with abundant food supplies thus appear to forage less on a daily basis to reduce foraging risks and costs. If workers benefit from an increased longevity by reducing their activity, this would provide an additional mechanism to explain the increased reproductive success of colonies with increased food supplies.

Avant-Propos

Les chapitres 2, 3, 4 et 5, qui constituent le corps de ma thèse ont été rédigés en anglais en vue de leur publication dans des journaux scientifiques arbitrés. Le premier article, "Field techniques for use in bumblebee colonies", n'a pas encore été soumis à un journal scientifique. Le second, "The importance of the queen's body size on reproductive success in bumblebee field colonies", a été soumis pour publication dans la revue *Ecology*. Le troisième, "The effect of food supplementation on reproductive success in bumblebee field colonies" a été soumis et accepté pour publication dans la revue *Oikos*. Il est actuellement sous presse. Le quatrième, "Do bumblebees always forage as much as they could?" a été soumis pour publication dans la revue *Insectes Sociaux*. Ces quatres manuscrits ont été rédigés en collaboration avec mon directeur de thèse, le Dr Jeremy N. McNeil. Le premier manuscrit a aussi été rédigé en collaboration avec le Dr Ralph Cartar. Je suis l'auteur principal de chacun de ces articles. Les deux autres chapitres, l'introduction et la conclusion générales, ont été rédigés en français. Cette thèse est complétée en annexe par un article de vulgarisation scientifique, "Pourquoi le faire soi-même alors que d'autres peuvent le faire pour soi?", qui a été publié dans le *Bulletin de la Société d'entomologie du Québec*, et par un article scientifique, "Entomophily of the cloudberry (*Rubus chamaemorus*)", qui a été publié dans la revue *Entomologia experimentalis et applicata*.

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À mes parents que j’aime beaucoup

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Chapitre 1

Introduction générale

Les bourdons sont des pollinisateurs importants dans les écosystèmes naturels, agricoles et urbains. Leur importance en milieu agricole a surtout été démontrée pour les fleurs à corolle profonde, telles que la luzerne, le trèfle rouge et plusieurs petits fruits (Hobbs 1967; Morrissette et al. 1985; MacKenzie 1994; Willmer et al. 1994). Ceci, les bourdons le doivent entre autre à leur long proboscis qui leur permet d'accéder au nectar de ces fleurs (Harder 1983a,b, 1985). D'autres caractéristiques font des bourdons des pollinisateurs efficaces: leur capacité à thermoréguler qui leur permet de butiner même par temps froid (Heinrich 1979), leur capacité à libérer le pollen de certaines fleurs par vibration (buzz-pollination) (Thorp 2000) et leur pilosité qui aide à retenir et distribuer le pollen sur d'autres fleurs.

Les bourdons sont des abeilles sociales et ont un cycle de vie annuel dans les milieux tempérés. Vers la fin de l'été, les colonies de bourdons produisent des mâles et des gynes (les nouvelles reines). Suite à l'accouplement, les gynes s'enfouissent dans le sol pour y passer l'hiver, alors que tous les mâles et ouvrières meurent avant la venue de l'hiver. Au printemps, la gyne sort de sa torpeur et se met à chercher pour un site où nicher. Pour la plupart des espèces, cela signifie la recherche d'une cavité assez grande et isolée telle que d'anciens nids de souris ou l'entretoit des maisons. Au contraire, d'autres espèces, telles que *B. fervidus* et *B. borealis*, se contentent de nicher sur le sol. Pour débuter sa colonie, la gyne, devenue reine, façonne un pot de cire où elle entreposera du nectar et elle amasse un peu de pollen où elle pondra environ une dizaine d'oeufs. La reine se met alors à incuber ses oeufs, puis à nourrir les larves qui en éclosent. Après un peu plus de trois semaines, les premières ouvrières adultes commencent à émerger et à aider la reine dans ses tâches. Au cours des semaines subséquentes, d'autres couvées d'ouvrières arrivent à terme. Le nombre d'ouvrières dans la colonie augmente exponentiellement, jusqu'au point où la colonie se met à produire, non plus des ouvrières, mais exclusivement des sexués (gynes et mâles). Au cours des semaines suivant cette transition, le nombre d'ouvrières décline de plus en plus, jusqu'au point où la colonie s'éteint, faute d'ouvrières. De leur côté, les mâles quittent la colonie quelques jours après leur émergence, en quête de gynes. Les gynes, elles, peuvent rester un plus longtemps dans la colonie et s'enfouissent pour l'hiver après l'accouplement. Le cycle est alors complété et prêt à recommencer pour une nouvelle année (Alford 1975 et Kearns & Thomson 2001 pour les informations ci-haut).

Plusieurs étapes cruciales de ce cycle de vie peuvent limiter la croissance des populations de bourdons: la survie hivernale des reines, l'établissement de la colonie, le succès de la colonie à se développer et à produire des sexués, et le succès d'accouplement des sexués. De ces étapes, je me suis intéressé au développement des colonies. Plus particulièrement, j'ai visé à déterminer l'importance de certains facteurs (ou contraintes) naturels dans la production de sexués par les colonies en milieu naturel. Comme la stratégie reproductive des bourdons est de commencer par produire un grand nombre d'ouvrières et d'attendre vers la fin du développement des colonies avant de produire les sexuées, je dois inclure les facteurs qui peuvent affecter la persistance des colonies. Les facteurs affectant la croissance de la colonie (en nombre d'ouvrières) doivent aussi être pris en compte car le nombre de sexués produits dépend de la taille de la colonie (Owen et al. 1980; Müller & Schmid-Hempel 1992).

Les principaux facteurs limitant potentiellement le succès reproducteur des colonies de bourdons peuvent être regroupés en quatre grandes catégories: i) le climat, ii) les ennemis naturels, iii) la nourriture et iv) les facteurs parentaux. Pour chaque catégorie, je présente premièrement l'importance générale des facteurs dans la reproduction et la régulation de la taille des populations d'insectes, deuxièmement en quoi les insectes sociaux constituent un groupe méritant une attention particulière (en centralisant surtout sur les hyménoptères: fourmis, guêpes et abeilles), et finalement en quoi certains facteurs peuvent spécifiquement affecter le succès reproducteur des bourdons. Dans le cadre de cette introduction, le succès reproducteur d'un insecte est défini simplement comme étant le nombre total de ses petits qui parvient au stade adulte. Pour les insectes sociaux, cela se restreint au nombre de sexués produits (sauf chez ceux qui fondent de nouvelles colonies par fission de la colonie initiale).

1.1 Climat

L'importance du climat dans la régulation de la taille des populations d'insectes est connue depuis longtemps (Bodenheimer 1928; Uvarov 1931). La température et l'humidité en particuliers sont connus pour affecter directement la mortalité, le taux de ponte et le temps de développement de la progéniture (Chapman 1999), mais leur effet peut aussi être indirect en affectant la disponibilité en nourriture et l'abondance et/ou l'efficacité des ennemis naturels (Rosenheim 1998). En outre, l'effet des facteurs abiotiques sur la reproduction peut se faire à retardement. Ainsi, la photopériode et la température sous lesquelles vit la mère peuvent affecter chez sa progéniture le taux de croissance, le temps de développement, l'entrée en diapause, des traits physiques et le comportement de dispersion (Mousseau & Dingle 1991), autant de facteurs qui peuvent affecter la survie et le succès de la progéniture.

Les insectes sociaux, eux, minimisent normalement l'impact des conditions climatiques sur leur colonie en construisant un nid. Par sa structure, ce nid peut permettre de contrôler – parfois très étroitement comme dans le cas de certaines termitières – la température, l'humidité et la concentration de dioxide de carbone pour aider le développement du couvain (Wilson 1971). Les ouvrières peuvent également contribuer à réguler les conditions internes du nid. Par exemple, les abeilles y réussissent en ventilant le nid avec leurs ailes (Weidenmüller et al. 2002). Les fourmis, elles, profitent du gradient vertical de température et de dioxyde de carbone au sein du nid pour réguler le développement de la progéniture en la déplaçant dans le nid. Si le nid protège le couvain et les adultes des éléments, on peut toutefois s'attendre à ce que, hors de ce nid, les insectes sociaux soient aussi vulnérables que tout autre insecte aux caprices du climat.

Chez les bourdons, les conditions climatiques, et particulièrement la température, peuvent affecter les colonies d'une multitude de façons. Premièrement, un printemps tardif peut retarder la sortie d'hibernation des reines et l'établissement des colonies (Hobbs 1967), et donc réduire la durée potentielle du développement des colonies. Deuxièmement, un temps froid peut ralentir le développement des juvéniles, et donc le développement des colonies (Vogt 1986; Cartar & Dill 1991). Les ouvrières pourraient alors incuber le couvain pour lui permettre de conserver une vitesse de développement optimale, mais cela augmenterait d'autant les besoins en nectar de la colonie (Heinrich 1979). Troisièmement, un temps froid (même près du point de congélation) n'empêche pas les bourdons de butiner mais augmentera certainement les coûts pour le faire. En effet, le bourdon peut atteindre en produisant sa propre chaleur la température thoracique requise pour voler (au moins $30^{o}C$), mais le coût énergétique pour y réussir est grand (Heinrich 1979). Finalement, l'effet peut être indirect via un effet sur la phénologie et l'abondance des plantes, et donc la disponibilité en nourriture.

Les conditions climatiques étant très variables et difficilement contrôlables en milieu naturel, je n'ai pas poursuivi l'étude de leur importance sur le succès des colonies à se reproduire. Des dispositifs ingénieux seront probablement nécessaires pour répondre à cette question dans le futur.

1.2 Ennemis naturels

Les parasites, prédateurs et pathogènes peuvent contribuer à réguler la taille des populations d'insectes (régulation « top-down ») en éliminant une large fraction de la population. Les exemples font légion en lutte biologique où des populations d'insectes ravageurs sont réduites avec succès grâce à l'introduction massive de prédateurs, parasitoïdes et pathogènes dans l'environnement du ravageur visé (Rosenheim 1998; Symondson et al. 2002; Mason & Huber 2002). Les exemples les plus notoires, et qui sont maintenant commercialisés, incluent l'utilisation – surtout en serres – de prédateurs tels que les coccinelles, larves de chrysopes et mites prédatrices, et de parasitoïdes tels que les trichogrammes (Mason & Huber 2002). Le pathogène le plus couramment employé commercialement, et avec succès, pour le contrôle des insectes phytophages (lépidoptères, coléoptères et diptères) et nuisibles (ex. moustiques et mouches noires) est sans contredit le *Bacillus thuringiensis* (Mason & Huber 2002).

L'importance du contrôle « top-down » a également été démontrée suite à l'introduction d'ennemis exotiques contre lesquels l'organisme n'a pas évolué de défenses (ex. *Varroa destructor* chez l'abeille domestique en Amérique du Nord, Sammataro et al. 2000; Webster & Delaplane 2001), suite au retrait momentané d'un ennemi naturel (ce qui peut survenir après l'épandage d'insecticides non-sélectifs auxquels l'ennemi est plus vulnérable que le ravageur) ou encore suite à l'introduction de l'insecte dans un environnement où il n'a pas encore d'ennemis efficaces (ex. longicorne asiatique en Amérique du Nord). Notons que dans les deux derniers cas, c'est l'absence d'ennemis qui révèle leur importance en permettant aux populations d'insectes d'augmenter démesurément.

Des manipulations expérimentales de ce type, intentionnelles ou non, sont nécessaires pour démontrer l'importance des ennemis naturels comme régulateurs de la taille des populations d'insectes car i) il est difficile de distinguer les effets additifs des effets compensatoires (Wilson 1971; Krebs 1994) et ii) l'effet peut ne survenir qu'à certains moments (ex. lors d'un pic de population pour des espèces cycliques comme la tordeuse de bourgeon de l'épinette, Smith et al. 2002)

Les insectes sociaux ont une fois de plus leurs particularités en ce qui a trait aux ennemis naturels. Premièrement, les colonies sont sédentaires. En étant établi sur un même site pendant une période prolongée, le nid permet l'accumulation de parasites sociaux (ex. mites s'attaquant au couvain, larves de pyrales qui mangent la cire) et devient plus facilement repérable par les prédateurs. Deuxièmement, une colonie constitue un concentration élevée de ressources nutritives pour les prédateurs. En effet, une colonie peut regrouper jusqu'à des centaines de milliers d'individus, des milliers de larves et pupes sans moyens de défense propres, le tout complémenté de réserves alimentaires de choix (ex. des kilogrammes de miel chez les abeilles domestiques). Troisièmement, les insectes sociaux adultes ont souvent, contrairement aux immatures, des moyens de défence élaborés pour repousser les prédateurs. Dard, venim, jets d'acides formiques et mandibules puissantes ou acérées sont les fers de lance les plus connus de l'ouvrière pour défendre la colonie, mais ces armes deviennent d'autant plus efficaces lorsque les ouvrières se mettent à attaquer les prédateurs ensemble. Quatrièmement, tout comme les humains, les insectes sociaux sont peut-être leurs propres plus grands ennemis (Wilson 1971; Hölldobbler & Wilson 1990). En effet, tous les types de conflits suivants surviennent chez ou entre les fourmis, guêpes et abeilles: compétition territoriale ou pour les sites de nidification (par le biais de guerres et agressions physiques), compétition pour la nourriture, pillage d'autres colonies (surtout pour la nourriture mais également pour le couvain chez certaines espèces de fourmis esclavagistes), et usurpation de colonies (lorsqu'une reine prend le pouvoir d'une autre colonie, voir l’annexe A).

L'importance des ennemis n'est certainement pas négligeable pour les bourdons. Il sont entre autres la proie de plusieurs prédateurs (ex. pie-grièche, araignée crabe) et parasitoïdes (ex. mouches conopides), surtout les butineuses (Alford 1975; Schmid-Hempel et al. 1990; Shykoff & Schmid-Hempel 1991). La colonie elle-même peut être ravagée par le passage de prédateurs (ex. moufette, fourmis) ou suite à l'invasion de parasites de couvain (ex. pyrale de la cire) (Alford 1975). Des reines bourdons parasites (le sous-genre *Psithyrus* en particulier) tentent quant à elles d'usurper les reines fondatrices de colonies pour que les colonies élèvent leur progéniture (Richards 1978; Fisher 1987a,c; voir l’annexe A). Les usurpations de ce type sont communes et, lorsqu'elles réussissent, se font au détriment de la colonie hôte qui ne réussira pas ou peu à se reproduire (Richards 1978; Fisher 1987b). Les bourdons sont également la proie d'une multitude de pathogènes (bactéries, virus, moisissures, protozoaires, nématodes et mites, Macfarlane et al. 1995) qui peuvent nuire à leur reproduction. Par exemple, le nématode

Sphaerularia bombi stérilise les reines infectées (McCorquodale et al. 1998). L'évolution et le maintien d'un dard, de couleurs aposématiques, d'un comportement de défense de la colonie et d'un système immunitaire (Doums et al. 2002) démontre en quelque sorte l'importance qu'ont eu et qu'ont toujours ces ennemis sur le succès des bourdons à se reproduire.

Toutefois, l'étude de l'importance du parasitisme et de la prédation sur la reproduction des colonies de bourdons en est encore à ses premiers pas. Une approche simpliste pour une telle étude est de déterminer si le succès des colonies est associé de quelque façon que ce soit à l'occurence du phénomène (ex. Imhoof et Schmid-Hempel 1999 pour les infestations par *Nosema bombi*), mais cela ne permet pas de départager la part des effets additifs des effets compensatoires. Tel que mentionné précédemment, seule l'approche expérimentale (ex. par l'exclusion du parasite ou prédateur du système) peut clairement permettre d'établir la contribution de l'ennemi à la faillite des colonies en milieu naturel. Cette voie a jusqu'à présent été vouée à l'échec.

Par exemple, Imhoof et Schmid-Hempel (1999) ont tenté de comparer l'effet d'un parasite intestinal, *Crithidia bombi*, sur le succès des colonies en infestant intentionnellement une partie des colonies en laboratoire, mais toutes les colonies qui n'étaient pas déjà parasitées l'ont rapidement été suite à leur transfert en milieu naturel. Hobbs et al. (1962) ont quant à eux tenté d'exclure les *Psithyrus* des colonies en réduisant le diamètre de l'ouverture des nichoirs à bourdons. Une femelle *Psithyrus* a tout de même essayé d'entrer dans la colonie et est restée bloquée dans l'ouverture, empêchant la sortie des ouvrières et tuant la colonie. Hobbs (1967) a plus tard tenté de masquer l'odeur des colonies avec de l'eugénol (huile de trèfle) et de l'acide butyrique, mais les *Psithyrus* ont quand même trouvé et envahi les colonies.

Des protocoles expérimentaux ingénieux restent donc à trouver avant de pouvoir départager l'importance du parasitisme et de la prédation de celle des autres facteurs affectant le succès des colonies en milieu naturel. Toutefois, j'ai tout de même suivi la présence et l'abondance de certains parasites dans les colonies, en particulier les *Psithyrus*, et déterminé s'ils étaient associés au succès reproducteur et à certains autres facteurs pouvant affecter la colonie.

1.3 La nourriture

La nourriture fournit l'énergie et les matériaux nécessaires à la survie et à la reproduction des insectes (revue dans Chapman 1999). La disponibilité de la nourriture, tant par sa quantité que sa qualité, peut donc limiter la croissance des populations d'insectes (régulation « bottom-up »). Si la limitation peut être directe par la mort d'individus (ex. lors d'une famine), elle peut aussi se faire par réduction de leur performance reproductive.

En effet, il a été démontré que l'abondance et la qualité de la diète des femelles affecte généralement leur fécondité par régulation de l'oogénèse (Wheeler 1996; Awmack & Leather 2002), l'allocation de ressources aux oeufs, la taille des oeufs et leur résorption (Fox & Czesak 2000; Awmack & Leather 2002). Ces composantes reliées à la production des oeufs influencent tous la performance reproductive des femelles. Ainsi, une augmentation de la taille des oeufs est généralement favorable car les plus gros oeufs ont souvent une probabilité d'éclosion plus grande; une meilleure endurance aux stress environnementaux tels que la faim, la dessication, le froid et les toxines; et donnent des larves avec une meilleure survie, une taille plus grande ou un temps de développement plus court (Fox & Czesak 2000). Suite à l'éclosion, c'est la diète des larves elles-mêmes qui devient importante par son effet sur leur survie, temps de développement et taille corporelle adulte (ex. Sutcliffe & Plowright 1988, 1990; Hoddle et al. 1998).

Dû à l'importance de la diète, les femelles ont donc avantage à s'assurer d'avoir assez de nourriture de qualité pour elles et leur progéniture. Pour y réussir, elles sélectionnent leur nourriture (Waldbauer & Friedman 1991) et choisissent leurs sites de ponte et le moment pour se reproduire (Thompson & Pellmyr 1991; Fox & Czesak 2000; Awmack & Leather 2002). Une insuffisance en nourriture à un site donné peut ainsi pousser une femelle à retarder sa reproduction et à se déplacer/ migrer pour trouver plus loin ce dont sa progéniture a besoin pour son développement (ex. une plante hôte convenable pour un insecte phytophage, un organisme hôte pour un parasitoïde). Chez les espèces où le mâle donne un cadeau nuptial à la femelle lors de l'accouplement, les femelles tentent de s'accoupler avec le meilleur mâle possible pour augmenter l'apport nutritif du cadeau nuptial, et ainsi aider l'oogénèse (Vahed 1998).

En plus d'exercer des choix pour obtenir le plus de nourriture possible, les femelles peuvent également tenter d'optimiser l'utilisation de la nourriture disponible du moment. Par exemple, certaines guêpes parasitoïdes, dont la progéniture femelle requiert plus de ressources que la mâle pour se développer, vont jusqu'à choisir le sexe de l'oeuf qu'elle pondent dans un hôte en fonction de la taille de ce dernier, et pondent ainsi relativement plus d'oeufs femelles que de mâles dans les hôtes plus gros et l'inverse dans les hôtes plus petits (revue dans King 1987; voir aussi Cloutier et al. 2000). Somme toute, les insectes ont une certaine flexibilité dans leur stratégies pour tenter de maximiser leur succès reproducteur en fonction des ressources disponibles dans l'environnement.

Par rapport aux autres insectes, les insectes sociaux ont une relation particulière avec la nourriture, ce qui peut compliquer l'analyse de l'effet de la disponibilité de la nourriture sur leur reproduction. Premièrement, les insectes sociaux prodiguent des soins parentaux, contrairement à presque tous les insectes asociaux. La responsabilité de la reine et des ouvrières ne se borne donc pas à trouver un site de ponte convenable, mais aussi à assurer nourriture et protection à la progéniture tout au cours de son développement. En outre, les soins parentaux sont sans relâche car la colonie produit continuellement de nouveaux individus. Deuxièmement, les ouvrières vont quotidiennement chercher la nourriture sur de grandes distances (plusieurs kilomètres pour les abeilles) et l'obtiennent d'une multitude de sources. Il est donc difficile d'établir l'abondance de nourriture à leur disposition. Troisièmement, chez les fourmis et abeilles, la colonie accumule des réserves alimentaires. Ces réserves tamponnent et masquent l'effet de variations à court terme dans la disponibilité de la nourriture. Quatrièmement, il y a division du travail et des fonctions reproductives entre les castes, la reproduction étant normalement réservée à la reine et la quête alimentaire aux ouvrières. Cette spécialisation des rôles permet aux reines d'être très fécondes (les reines de certaines espèces de fourmis et termites pondent jusqu'à plusieurs dizaines de milliers d'oeufs par jour, Wheeler 1996) et aux ouvrières d'être des modèles d'efficacité pour la recherche de nourriture (Michener 2000; Hölldobbler & Wilson 1990). Tous ces facteurs doivent être pris en compte lors d'études sur l'effet de la disponibilité de la nourriture sur la reproduction des insectes sociaux.

Les bourdons, eux, dépendent de deux types de nourriture: le pollen et le nectar. Le pollen est principalement une source de protéines pour la croissance des larves, alors que le nectar est une source d'énergie (Heinrich 1979). On peut déjà soupçonner l'importance de la nourriture pour la reproduction des bourdons par les effets à court terme qu'un manque peut causer: oophagie et éjection de larves, ralentissement du développement larvaire et production d'adultes plus petits, et entrée en torpeur de la colonie par temps froid suite à un manque d'énergie pour thermoréguler (Sutcliffe & Plowright 1988, 1990; Cartar & Dill 1991).

Les bourdons doivent donc approvisionner suffisamment la colonie pour s'assurer qu'elle aie en tout temps assez de nourriture pour ses besoins.
L'approvisionnement de la colonie en nourriture dépend d'un côté du nombre de butineuses et de l'autre, de la disponibilité spatiale et temporelle de la nourriture. La disponibilité en nourriture a une composante temporelle due à la phénologie des plantes (les fleurs ne sont pas éternelles). La composante spatiale, elle, dépend du fait que la colonie est fixe dans l'espace. Les butineuses doivent donc constamment se déplacer entre la colonie et les fleurs pour l'approvisionner et,

plus loin sont les fleurs du nid, plus ces déplacements seront coûteux en énergie et en temps perdu pour un même butin.

En outre, cela se complique car lorsqu'une butineuse trouve des fleurs, cela ne l'assure pas nécessairement d'y trouver une quantité suffisante de nourriture. En effet, d'autres insectes (incluant d'autres bourdons) butinent généralement dans ces mêmes fleurs et y prennent ce qu'ils peuvent. Ce partage forcé des fleurs avec d'autres insectes réduit la quantité de nourriture disponible pour chacun. Cette compétition pour la nourriture est "exploitative" (aussi appelé "ressource competition" ou "scramble competition") et non d'interférence (voir Denno et al. 1995) car il n'y a normalement pas d'interactions directes entre les insectes butinant dans les fleurs, mais plutôt une nuisance mutuelle via l'exploitation des ressources par chacun (Morse 1977, 1978; Inouye 1978; Plowright et al. 1978; Bowers 1985). La quantité de pollen et nectar en un seul lot de fleurs n'est en effet pas suffisante pour justifier les coûts et risques de conflits directs entre pollinisateurs, et l'habileté à butiner le plus efficiemment possible dans un grand nombre de fleurs prime donc sur celle de défendre un lot de ressources contre d'autres exploitants. Pendant la période de l'année où il y a des fleurs, il y a donc presque toujours de la nourriture disponible dans l'environnement pour les bourdons, mais cela devient plutôt une question du coût nécessaire pour l'obtenir (qui peut parfois s'avérer trop grand pour les capacités des bourdons). En bout de ligne, toute augmentation du coût de butinage entraîne une réduction de la taille effective de la colonie car plus d'ouvrières doivent être allouées à cette fonction et/ou les butineuses doivent y allouer une portion plus importante de leur temps. Et lorsqu'il n'est plus possible pour une colonie d'acquérir toute la nourriture nécessaire pour son développement, les effets d'un manque de nourriture se font sentir.

Comme la nourriture est essentielle au développement des colonies et que les efforts consacrés par les ouvrières pour en obtenir sont très importants, on peut facilement suspecter que la disponibilité en nourriture limite la reproduction des bourdons en milieu naturel. J'ai donc étudié l'importance de ce facteur en observant les effets d'une augmentation des réserves de nourritures sur le développement des colonies.

1.4 Facteurs parentaux

Plusieurs traits d'un insecte peuvent affecter son succès reproducteur différemment selon la valeur qu'ils prennent. Ces traits peuvent être simples (ex. l'âge, la taille corporelle, la couleur corporelle) ou complexes (ex. le comportement et les stratégies de vie). Parmi les traits simples énumérés, l'âge (Mousseau & Dingle 1991; Fox & Czesak 2000) et la taille corporelle (Honek 1993) peuvent directement affecter la fécondité des femelles. Le morphe de la femelle peut également affecter sa fécondité (et ses stratégies de reproduction). Par exemple, les fulgores macroptères (longues ailes) ont une fécondité moindre que les individus brachyptères (ailes réduites), mais y gagnent en retour la capacité de migrer (Rankin & Burchsted 1992; Zera & Denno 1997). D'autres attributs physiques des femelles peuvent aussi affecter leur succès reproducteur, mais indirectement. Par exemple, chez plusieurs groupes d'araignées la couleur corporelle est variable et affecte leur habileté à se camoufler, et donc leur succès à capturer des proies et à se cacher de leurs propres prédateurs (Oxford & Gillespie 1998).

Le comportement et les stratégies de vie/reproduction de l'insecte doivent également être considérés comme facteurs parentaux lorsqu'ils varient d'un individu à l'autre face à une même situation (ex. une menace de prédation, aux conditions climatiques, à la disponibilité de la nourriture). Par exemple, les femelles d'une même population peuvent choisir différents moments et lieux pour se reproduire. Ainsi, certaines femelles peuvent se reproduire immédiatement sur place, alors qu'au contraire d'autres femelles se déplaceront ou iront même jusqu'à migrer avant de se reproduire pour trouver de meilleures conditions ailleurs pour leur progéniture. Toutefois, la migration se fait souvent au prix d'une fécondité moindre et de risques de mortalité plus élevés (Rankin & Burchsted 1992; Zera & Denno 1997).

Les insectes sociaux se distinguent une fois de plus de la majorité des autres insectes en tant que parents, et cela sur au moins deux plans fondamentaux. Premièrement, tel que mentionné précédemment, ils prodiguent des soins parentaux. Les parents ont donc un rôle actif même après la ponte. Deuxièmement, ils ont une organisation sociale basée sur la coopération et la division du travail et des fonctions reproductives. Cela implique qu'une colonie doit être considérée comme un tout et que ce ne sont pas seulement les traits de la reine (la reproductrice) qui comptent, mais aussi ceux des ouvrières. Toutefois, ce sont différent traits qui prennent une importance selon la caste, tels la fécondité chez la reine vs. la capacité à trouver de la nourriture chez une butineuse.

Les colonies d'insectes sociaux peuvent varier sur ces deux plans. Au niveau des soins parentaux par exemple, le comportement hygiénique des abeilles domestiques peut être plus ou moins développé (dépend surtout de la race d'abeilles), affectant ainsi l'habileté de la colonie à réduire les problèmes causés par le parasite du couvain *Varroa destructor* et affectant les chances de survie de la colonie. L'organisation sociale peut aussi varier, entre autre au niveau du degré de cohésion sociale (à quel niveau la reine domine les ouvrières et évite que ces dernières ne se mettent à pondre et détruire ses oeufs à elle) et dans la répartition du travail entre les ouvrières (ex. proportion d'ouvrières affectées à différentes tâches). La liste des facteurs parentaux pourrait donc être bien longue pour les insectes sociaux.

On connait encore mal quels traits spécifiques des bourdons peuvent influer sur leur succès reproducteur en nature. Un seul trait a été étudié formellement, la durée de la diapause chez la reine, mais cela a été fait en laboratoire (Beekman & van Stratum 2000). En nature, un trait qui pourrait avoir une certaine importance est la taille corporelle de la reine.

L'importance de la taille corporelle a souvent été étudiée chez les insectes. Plus souvent qu'autrement, la règle est que plus un insecte est grand par rapport à ses congénères de la même espèce, plus il aura de succès. Rares sont les cas démontrant le contraire (Blanckenhorn 2000). Au sein d'une population, les femelles plus grandes sont généralement plus fertiles (Honek 1993; del Castillo et al. 1999; Sokolovska et al. 2000) et pondent des oeufs plus gros (Fox & Czesak 2000). Les grands mâles eux ont souvent plus de succès à s'accoupler (Abell et al. 1999; del Castillo et al. 1999; Starks & Reeve 1999; Sokolovska et al. 2000; Andersen & McNeil 2001; Coelho & Holliday 2001; Jamieson 2002). Comme le choix d'un partenaire sexuel se fait souvent sur la base de la taille (del Castillo et al. 1999; Harari et al. 1999), les grands mâles se trouvent encore plus avantagés par rapport aux petits mâles en s'accouplant avec les grandes femelles, plus fertiles. Egalement, les grands individus survivent souvent plus longtemps, parfois parce que les prédateurs s'attaquent sélectivement aux petits (Rhainds et al. 1999; Van Dongen et al. 1999; Sokolovska et al. 2000).

Une reine bourdon pourrait quant à elle être avantagée par une plus grande taille pour butiner (Harder 1982, 1983a, 1986; Heinrich & Heinrich 1983) et thermoréguler (Heinrich 1979; Heinrich & Heinrich 1983; Bishop & Armbruster 1999). Une plus grande taille pourrait aussi accroître sa fécondité et l'aider à combattre les usurpatrices (Owen 1988; Sutcliffe & Plowright 1988) et dominer physiquement les ouvrières. Étant donné la variabilité de ce trait au sein des populations (Plowright & Jay 1968; Owen 1988; Sutcliffe & Plowright 1988) et la facilité de le mesurer même sur le terrain, j'ai décidé comme dernier volet de mon projet d'étudier l'importance de la taille corporelle des reines sur leur succès reproducteur.

1.5 Objectifs

Cette thèse vise à explorer l'importance de deux facteurs – la disponibilité en nourriture et la taille corporelle de la reine – pour le développement et le succès des colonies de bourdons en milieu naturel. L'importance des usurpatrices n'est présenté qu'en rapport aux deux facteurs précédents. Le choix de ces deux facteurs s'est fait sur une base pratique et non théorique: il était facile de mesurer la taille corporelle de la reine et d'ajouter de la nourriture aux colonies sans leur nuire. De plus, la taille corporelle de la reine est un des seuls facteurs statiques étudiables, ce qui permet des analyses relativement simples (la plupart des autres facteurs varient au cours du temps). La disponibilité en nourriture est un facteur dynamique et difficilement estimable sur le terrain, mais l'analyse ne vise qu'à établir dans quelle mesure un surplus alimentaire peut aider les colonies à produire plus de sexués (par une comparaison avec une situation où la nourriture est pratiquement fournie *ad libitum*).

Le premier chapitre en est un de méthodologie et présente les techniques utilisées pour obtenir des colonies de bourdons et travailler avec celles-ci. Le deuxième chapitre est une étude exploratoire qui vise à savoir si, chez plusieurs espèces de bourdons, le succès des colonies à produire des sexués peut dépendre de la taille corporelle de la reine fondatrice. Le troisième chapitre présente une expérience sur l'effet à long terme de la disponibilité de la nourriture sur le succès reproducteur des colonies. Le quatrième chapitre découle du troisième et vise à déterminer si les ouvrières modifient leur fréquence de butinage en fonction des réserves de nourriture dans la colonie. En annexes sont inclus un article de vulgarisation scientifique sur le parasitisme social chez les bourdons et un article sur la pollinisation de la chicouté, tous les deux réalisés au cours de mon doctorat.

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Chapitre 2

Field techniques for the study of bumblebee colonies

2.1 Résumé

Malgré le grand nombre d’études sur les bourdons, l’information sur comment obtenir et manipuler des colonies dans un but scientifique est incomplète et éparse dans la littérature. Dans cet article, nous faisons une revue et une comparaison des techniques pour obtenir des colonies de bourdons sur le terrain, et nous fournissons des conseils pour optimiser la collecte de données sur ces dernières tout en minimisant les effets négatifs causés par leur manipulation.

2.2 Abstract

Despite the large number of studies on bumblebees, information on how to establish and handle colonies for research purposes is incomplete. Furthermore, the published information is not easily accessible as it is scattered throughout the literature. In this paper, we review and compare techniques to obtain bumblebee colonies for research under field conditions, and provide advice on how to optimise data collection while minimising the detrimental effects of handling.

2.3 Introduction

Bumblebees are effective and important natural pollinators of many agricultural crops in temperate regions around the world, e.g., alfalfa, red clover, and small berries (Holm 1966; Mackenzie 1994; Kearns & Thomson 2001). In recent years, their importance has increased because of their use for the pollination of greenhouse crops, such as tomatoes (van den Eijnde et al. 1991; de Ruijter 1997). In North America, they may also grow in importance for the pollination of field crops, such as cranberries (Patten et al. 1994; Macfarlane & Patten 1997), as an alternative to honeybees whose populations have been decimated by the spread of the *Varroa* mite (Sammataro et al. 2000; Webster & Delaplane 2001). In this paper, we review and compare the techniques to obtain bumblebee colonies for research under field conditions, and provide advice on how to handle them to minimise detrimental effects.

2.4 Obtaining colonies

2.4.1 Finding natural colonies

Locating bumblebee colonies in their natural habitat is difficult, but can be achieved by tracking workers visually when they move in or out of places that are not evident foraging sites. The location of nests is highly variable and may include in or on the ground, holes of trees, or even the roof and wall insulation of human dwellings. Larger colonies become more conspicuous with the increased forager traffic they generate. Disturbing the vegetation around the location where the nest entrance is suspected will force foragers to search for its position and, thus, may help pinpoint the entrance faster (Free & Butler 1959). Nests in the grass, for some species such as *B. fervidus* and *B. borealis*, can sometimes be found by searching the grass thoroughly after hearing a buzzing noise originating from the ground. Other strategies to find natural colonies are to advertise in the local newspaper (Free & Butler 1959), and to ask a local exterminator to keep you in mind. Once a natural colony is found, it is usually necessary to tranfer it to a nest box (see next section) if one wishes to continue studying it (see Heinrich 1979; Kearns & Thomson 2001). However, the chances of finding natural colonies are low. Furthermore only well developed colonies, with high foraging activity, will normally be found. Consequenly, early colony development will be missed and this is an important phase that could be critical in many ecological studies.

2.4.2 Nest boxes

In areas where bumblebees are abundant, setting out nest boxes (domiciles) in the field to promote the establishment of bumblebee queens is the method that requires the least effort. The method has been used successfully in Canada and New Zealand (Hobbs et al. 1960, 1962; Palmer 1968; Clifford 1973; Donovan & Wier 1978; Richards 1978; Pomeroy 1981; Barron et al. 2000; Pelletier, unpubl. data).

2.4.2.1 Nest Box Design

There are many descriptions of how to build nest boxes (see Hobbs et al. 1960; Hobbs 1967; Richards 1978; Prys-Jones & Corbet 1991; Munn 1998; Kearns & Thomson 2001). A one-chambered nest box of internal dimensions 15 cm x 15 cm x 15 cm (Fig. 1) is usually sufficient as it accommodates a colony of up to 150 workers. Two-chambered boxes are useful if one has to shut the box for some time (e.g., when moved to another location); the larger chamber is the nest area, and the smaller chamber is a vestibule where food can be provided and bees can defecate. The box can be made out of any type of material (plywood, concrete, polystyrene, plastic) (see Macfarlane et al. 1984), but should be constructed to allow for the escape of water vapour as excessive humidity makes colonies susceptible to mould. Boxes made of plywood do not need to be painted if removed at the end of summer. If boxes are painted, paint should be applied only on the exterior, a few months before use as the smell may repel queens. The roof should be slanted and larger than the box to protect it from the rain. The roof may be covered with various waterproof materials, e.g., a piece of carpet underlay, a roofing tile or slate (Munn 1998), or a thin polypropylene sheet stapled to the corners. A hinged lid allows access to the colony and facilitates rapid closure. A piece of wood along the top of the inside back wall prevents bees from being killed when the lid is closed. The entrance should be in the center of the front wall and its diameter between 16 and 18 mm. When the entrance is larger, workers will have to close the gap with insulant and small rodents may enter the nest box (see Barron et al. 2000).

Line the inside surfaces with a 2-cm thick layer of insulation, such as water-repellent upholsterer’s cotton (Hobbs et al. 1960) or non-surgical white bleached cotton (e.g., Kendall® Lakeside® cotton). The bleached cotton has the advantage of allowing easy detection of nest parasites (e.g., mites, *Fannia*, wax moths). Cotton wool is not recommended, as bumblebees tangle their feet in it. The insulant will be fluffed up by the bumblebees once they have occupied the box.

This is a sure sign, along with the presence of yellowish faeces, that the nest box has been occupied (Fig. 2). A flat piece of rigid plastic (e.g., Coroplast®) can be put on the floor of domiciles before adding the insulant (Fig. 3). With the help of a wire frame, this false-bottom can later be pulled up to remove the comb out of the box. This is particularly useful in the detection of nest parasites hidden under the comb (Fig. 4).

2.4.2.2 When, where, and how to set up nest boxes

Nest boxes should be set out in the field as soon as possible in the spring, right after snow melt, before leaves appear on the trees and early flowers start blooming (e.g., willow), as some species (e.g., *B. bimaculatus* and *B. perplexus*) nest very early in the season. In areas that do not get snowfalls, boxes can be set out in late winter, or if well waterproofed, left out all year.

Nest boxes should be put close to abundant sources of spring food, as queens appear to search for cavities near rewarding habitats. Prairies (Hobbs et al. 1962), forests (Svensson et al. 2000), bogs, and margins of intensively managed fields (Barron et al. 2000) are generally poor sites. Meadows (Hobbs et al. 1962) and especially botanical gardens (where food is plentiful but natural nesting sites are limited) are good sites. As a rule of thumb, boxes perform best in habitats where bumblebees are typically common. It is also advisable to set boxes in sites where pesticide use and possible vandalism are minimal.

Each species of bumblebee usually has preferences for nest sites and set-up (Sladen 1912; Free & Butler 1959; Hobbs et al. 1960, 1962; Richards 1978; Macfarlane et al. 1984). Nest boxes can be placed underground, above-ground, and on trees (Hobbs 1967). The set-up is important because it attracts different species and affects occupancy. The difference is mainly observed between under- and above-ground boxes (Richards 1978). Underground boxes perform best if they are on slopes to allow for adequate drainage. A short, almost horizontal, tube (10 - 40 cm) of 20 mm diameter PVC pipe can then connect the box to the slope edge.

The tube entrance should be cleared of obstructing vegetation. A piece of sod should cover the tube, and a second larger piece should broadly cover the box top. When set out in this way, boxes are difficult for other people to detect and interfere with. Detailed notes on box location are essential, as vegetation grows quickly and makes the box difficult to find later in summer. An underground box can be faked by using a surface hive with a 30 cm long, attached tunnel, and a piece of sod covering the tunnel (the “false-underground” hives of Hobbs 1967). False-underground hives are easier to set out than underground hives and attract more queens of surface-nesting species, as well as underground species, than surface hives (Hobbs 1967). A drawback of underground hives is that they must be removed at the end of each summer to prevent deterioration.

Above-ground domiciles (Fig. 1) keep drier and are easier to set up and check than underground domiciles. Furthermore, if properly waterproofed they can remain in place year-round. However, care must be taken to keep above-ground boxes out of direct sun, particularly at mid-day, to prevent overheating which may melt the wax and damage the brood. Furthermore, above-ground boxes are more susceptible to vandalism, mammalian predation (e.g., skunks), and occupation by ants. To protect colonies, boxes must be fastened securely to a tree, post, or stakes, at least 10-15 cm above ground to ensure they do not get flooded after heavy rains. The lid must be shut securely. To prevent ants from getting in (particularly for boxes set in the sun), Tanglefoot® (a sticky paste) must be applied on the stakes and any vegetation around the box should be cleared away. To protect the colony from vertebrate predators, domiciles can then be wrapped with chicken wire mesh (3-cm diameter holes), making sure to leave a gap of about 15 cm between the entrance of the domicile and the mesh. Predators may also be deterred by putting boards with short nails or staples facing upward on the ground around the nest boxes. The mesh and boards are also effective against porcupines, for while these rodents are not direct enemies of bumblebees, they will chew on boxes made of plywood.

Domiciles accumulate parasites, diseases, and mould that may be transmitted to new occupants over years. To reduce these problems, boxes should be cleaned thoroughly after each season by scraping the inside surfaces of the box and replacing the nest insulant. They should be washed with a mild bleach solution to eliminate mould.

Once the first queens have finished hibernating and are looking for a nest site, boxes should be inspected every few weeks to detect occupancy. Routine inspection also permits one to detect and fix any problem boxes may have, e.g., water leaks, ants.

2.4.2.3 Success of colony establishment

Occupancy of domiciles is usually very low in the first year, but increases with successive years (see Frison 1926; Donovan & Wier 1978; Hobbs et al. 1962; Hobbs 1967; Macfarlane et al. 1984: from 7% the first year to 61% the fourth year; Barron et al. 2000 : from 0.3% the first year to 8.4% the fourth year; Pelletier, unpubl.: from 0% the first year to about 50% the third year). The increase in occupancy over years may be attributed, among other things, to the experience of the researcher in selecting more favourable sites, the weathering of treated timber, improved hive set-up, the return of overwintered queens to the site they come from, and the odour left by previous occupants (Frison 1926; Donovan & Wier 1978; Pomeroy 1981; Macfarlane et al. 1984; Barron et al. 2000). In particular, nesting boxes that have been colonized have a better success of occupancy the following year (Barron et al. 2000). The probability of occupation does not appear to be affected by previous occupation by mice or the smell of mice in the nest box (e.g., from mouse litter), the presence of pieces of honeybee comb with honey and pollen, or the compass direction a box faces (Hobbs et al. 1960; Barron et al. 2000; pers. obs.). Important factors for the establishment of queens are a dry and well-insulated nest (Donovan & Wier 1978) located near abundant spring food, in a site where bumblebees are commonly abundant.

2.4.3 Starting colonies in the laboratory

Another method to obtain colonies is to start them in the laboratory and move them out to the field when the first brood of workers has emerged (e.g., Doums & Schmid-Hempel 2000). There are numerous accounts about how to establish and rear year-round bumblebee colonies in the laboratory : Plowright & Jay 1966; Pomeroy & Plowright 1980; Ptácek 1991; Röseler 1985; Griffin et al. 1991; Pouvreau 1993; Tasei 1994; Tasei & Aupinel 1994; Delaplane 1995, 1996a,b,c; Gretenkord & Drescher 1997; Kearns & Thomson 2001. We refer you to these authors for a complete description of the laboratory methods. Delaplane's series of articles is a good starter as it is written with the layperson in mind. For scientific studies, we recommend using queens caught in the field early in spring. CO_2-narcosis of mated gynes makes it possible to rear bumblebees continuously by preventing the gynes from going into diapause (Röseler 1985). However, this approach should be avoided if colonies are required for behavioural and developmental studies because the narcosis has both dramatic immediate and delayed physiological and behavioral effects on insects (Pomeroy & Plowright 1979; Kukuk et al. 1997).

Starting colonies in the lab is useful to study species that do not readily establish in nest boxes and also to obtain a sufficient number of colonies of a specific species (Müller & Schmid-Hempel 1992; Imhoof & Schmid-Hempel 1999; Pelletier & McNeil, in press). However, the method is labour intensive with respect to the collection of a large number of nest site-searching queens (only a fraction of them will successfully found a colony) and the subsequent daily maintenance (e.g., feeding). In terms of material needed, obtaining good quality, freshly frozen pollen from honeybee keepers to feed the colonies is one point which is not stressed enough in the current literature.

2.4.4 Buying colonies

A last resort is to buy colonies. An industry (e.g., Koppert, Biobest) has built up to provide year-round bumblebee colonies for greenhouse crops, and scientists in this industry have perfected means of continuously breeding some species of bumblebees (particularly *B. terrestris* in Europe, and *B. impatiens* and *B. occidentalis* in North America). However, it should be noted that their methods (e.g., breaking diapause of queens and inducing their ovary development with CO_2- or N_2-narcosis, use of callow workers from other colonies to assist the queen in starting the colony, selection of queens for some traits such as the ability to produce large and long-lasting colonies) may significantly affect the natural behaviour of bees (e.g., queen-worker and worker-worker conflicts, division of labour and individual investment in labour) and colony dynamics (e.g., duration of colony development, number and type of sexuals produced). Moreover, since the exact rearing methods are secret and differ from the natural process, the use of commercial colonies could impede replicability, so we recommend against the use of commercial colonies for most scientific studies, especially long-term ecological ones. The cost of commercial colonies may also hinder their acquisition (a mature colony costs a few hundred dollars). If commercial colonies are used, they can be set in the field by attaching the cardboard box (in which they are usually sold) onto a wooden crate, with a thin sheet of plywood on top to protect them from the rain and sun.

2.5 Censusing colonies

There is very little information in the literature about handling colonies for census purposes, an essential component of long-term ecological studies. The whole procedure is cumbersome because one usually tries to obtain as much information on the colony as possible, while minimizing negative impacts and preventing bees from attacking during the numerous steps of the census. This trade-off makes censusing lengthy, particularly when dealing with large colonies. However, there are steps which can make a difference in the quality and efficiency of a census. The following section is based on our experience of censusing bumblebee colonies under field conditions.

2.5.1 General procedure

Removing individuals from the colony is easier at night or early morning when temperatures are below 15°C, as bees may then be torpid. Although CO_2 or N_2-narcosis of the colony occupants would make their removal easier, we recommend against this technique for the reasons stated above. It should also be noted that the honeybee keeper practice of smoking the bees to calm them down does not work with bumblebees.

Plug the nest entrance to ensure that workers currently in the nest do not escape. Insert a thin plastic sheet (e.g., overhead transparency) under the lid to detach bees standing on the inner surface. Open the box slightly and insert a thin sheet of clear plexiglass (20 cm x 20 cm) with a hole in the middle (diameter of 8 cm) (sheet 1). Cover sheet 1 with a second sheet of plexiglass (sheet 2) of the same size, but without any hole. Remove the transparency. Sliding sheet 2 on sheet 1 creates a slight gap through which bees will be collected; this gap can be rapidly closed whenever a bee tries to escape (Fig. 5). Remove bees individually, catching them by a leg with fine forceps (about 20 cm long) (Fig. 6), and place them in a clear jar. This jar should have a plastic lid with a V cut in it so that the lid pops back up to shut itself when a bee is inserted in the jar (Fig. 7). The neck diameter of this jar should match the diameter of the hole in sheet 1 to facilitate return of the bees to the colony at the end. The jar, like every vial and container used to keep bumblebees, should preferably be screened or perforated for ventilation. A small piece of the nest insulant put in the container will calm down workers.

Upon opening the nesting box, bumblebees will respond to vibrations by hissing, buzzing (Kirchner & Röschard 1999), climbing up the walls of the box, and trying to attack the intruder. Therefore, minimize vibrations because bees are harder to catch when they move about excitedly. When all of the bees on top of the canopy have been removed, shake the box a little bit and/or breathe on the brood as this stimulates more bees to come out. When this approach is no longer effective, open the canopy gently with forceps and scissors (Fig. 8) to remove the remaining bees on the brood. The bulk of bees collected so far will usually consist of workers. Some males and gynes (young queens) may come out, but most of them usually stay well hidden in the brood and nest insulation. Males are particularly timid in evading disturbances to the colony. To collect sexuals that are hiding, the comb must usually be taken out of the box (see section on nest box design) to reach them. Sexuals can be put with workers or in a separate container.

In the early stage of colony development, the foundress queen will be the only occupant and will come out of the nest quickly to attack the intruder. In contrast, later in colony development, she will hide in the brood or be one of the last to come out. The queen should always be put in a separate screened vial than workers to minimize any potential trauma to her. A clear plastic snap-cap pill vial (e.g., length 5 cm, diameter 4.7 cm), with aluminum insect screening hot glued at the end to allow ventilation, can be used.

When returning bees to their domicile, the queen must first be placed on the brood and the nest insulant rearranged to cover the brood. Failure to return the queen first sometimes results in workers attacking and killing her. Workers can then be returned by gently swirling the jar and dumping the content into the hole of sheet 1 in one fell swoop. The jar is then removed and sheet 2 repositioned over the hole. The box lid can then be shut immediately following the quick removal of the plexiglas sheets.

2.5.2 Timing

Field colonies can be censused at day or night. Field work is usually more convenient during daylight hours than in the dark. However, many foragers are absent from the colony during the day (leading to under-estimation of colony size) and daylight provides the opportunity for workers to see, fly to, and attack the observer. Therefore, greater care must be taken during the day to account for workers that escape from the box as well as returning foragers (see next section for precautions). Consequently, we recommend night censuses to people with little or no experience handling bumblebees, for large colonies (> 100 workers), or when a more accurate estimate of the colony size is needed. A red light should be used when working in the dark as bees do not react to this range of the light spectrum. About any red filter (e.g., Kodak® Wratten® gelatin red filter no. 29 CAT 149 7098) fitted on a head-mounted flashlight (to leave both hands free) should do for this purpose.

2.5.3 Particularities for day censuses

When hot and sunny, always keep bee containers in the shade as death from overheating can occur within a few minutes. To calm the bees (e.g., before transferring them back to the nest), the container can be chilled slightly by putting it on an ice pack (e.g., Coolpack®).

When bees are flying around, the best thing is to remain calm, avoid rapid movements, and net them one by one. Some species are known to be more aggressive than others (e.g., *B. fervidus*, Macfarlane et al. 1994; pers. obs.), i.e. they will attack upon slight disturbance of the nest. Within species, a general trend is that aggressivity grows with the size of the colony. If one looses control of the situation, it is wisest to leave the area and come back a few minutes later. Single attacks are not dramatic (assuming one is not allergic) since, in contrast to honeybees (and fortunately for the observer), attacks by bumblebee workers do not appear to promote additional attacks by other workers. We never used honeybee keeper clothes and veils, but one always has the option to use them to reduce risks of getting stung. Depending on the species, foragers may keep returning to the nest (e.g., *B. rufocinctus* and *B. fervidus*) or not (e.g., *B. impatiens* and *B. ternarius*) in the presence of an intruder. Returning foragers of aggressive species should be watched closely as they sometimes attack the intruder.

There are three different strategies to minimize the risk of being attacked by returning foragers. The first is to displace the nest box by at least 10 m during the census. Afterwards, when returning the nest to its original site, potentially dangerous foragers that are flying around are netted, the box is replaced and foragers then released in the nearby vegetation. The second strategy requires an assistant. The assistant nets all of the returning foragers while the observer conducts the census. The third strategy consists of preventing foragers from leaving the colony. A one-way "valve" can be fitted in the entrance about one hour before the census. The valve can be a 5-cm long clear plastic funnel that extends into the box, with a 16-mm diameter opening at the entrance and 8-mm opening at the inner end. This type of valve is used in some commercial bumblebee hives (e.g., Biobest®). Alternatively, a plastic flap that opens only inwardly (e.g., the celluloid doors of Hobbs et al. 1962) can be used as a valve system, but this requires a nest box with two entrances that had the system installed at the beginning of the field season. The normal entrance is blocked so that returning foragers must take the one way entrance. Since the bees are prevented from coming out for several hours, any valve system should be used with two-chambered domiciles to allow bees to defecate outside the main chamber. One advantage of using a valve compared with the first two strategies is that it provides more accurate estimates of colony size. On the other hand, it requires more equipment and labour.

2.5.4 Assessment of the degree of colony development

The mass or a photograph of the brood (Plowright & Jay 1968; Duchateau & Velthuis 1988) can be used to assess its development. Given that the nest box material and cotton insulant may absorb water, we recommend to weigh the brood separately from the box, with a pan balance (precision of ± 1 g). The number of bees of different sexes and castes can also be used to assess colony development through a regular census programme. Food levels cannot be easily estimated accurately without a destructive sample as i) reserves are often hidden under the brood, ii) honey/nectar and pollen pots are often of variable volumes, and iii) honey and pollen are hard to extract without damaging the brood.

2.5.5 Frequency of visits to the colonies

Colonies suffer in several ways from visits, so the number should be kept to a minimum, while ensuring that adequate data are obtained. First, bees may be stressed by repeated disturbances, and early in the season queens may even abandon their nest (Hobbs et al. 1962). Second, the wax canopy and brood (especially clumps of eggs and larvae on top) may be damaged during manipulation, which can then lead to ejection of eggs and larvae from damaged structures by workers (Pomeroy & Plowright 1979). This problem is exacerbated at high ambient temperature as the wax becomes extremely soft. We therefore recommend visiting nests only every 10 – 15 days, which is sufficient to get a good idea of the growth of the colony, and to compensate colonies by adding 1 ml of a 50:50 sucrose:water solution directly to honey pots.

For studies of within nest behaviour or to closely track the development of the brood, more frequent observations may be required. In such cases, a transparent plexiglass lid can be used to view the brood and nest activity at any times. Workers may rearrange nest insulation and thus impede observations, so for such studies nest boxes should not be insulated but rather have an internal heating system to keep the nest at 30^{o}C. Pomeroy and Plowright (1980) provide such a design that can be adapted to the field by using a marine battery as a power source. Alternatively, hives can be housed in small garden sheds, with the hive entrance connected to the outdoor to allow workers to forage freely. The use of a clear plexiglass tube as an entrance allows one to observe the individual foraging habits and contribution of marked bees, as they come and go from their colony.

2.5.6 Marking

Most studies with repeated observations of bumblebee colonies require marking at least some of the individuals, particularly the foundress queen. Among other things, marking permits one to track the behaviour and assess the longevity of individuals. Bees can be anaesthesized or chilled for immobilization, but both methods have negative effects (see section on rearing for negative effects of gas narcoses). Chilling can kill the smaller workers and tags are likely to come off because of condensation under the tag. Instead, bees can be held steady, either by hand (e.g., using a leather glove for females) or with a marking tube. Kwak (1987) recommended the marking tubes with foam-tipped plungers available commercially to mark honeybee queens, with a reduction of the gauze mesh size to 5 mm x 5 mm. Operlith® marking kits for honeybee queens which contain coloured plastic tags (numbered 1-99) and a resin are commonly used for marking bumblebees permanently, and such tags allow for accurate identification of individuals (Fig. 9). However, the procedure is time consuming for large number of bees. An alternative is to paint unique dots on a bee's dorsal thorax using a non-toxic water based paint or liquid correction fluid (e.g., Liquid Paper®). Painting is rapid and inexpensive, but paint wears off within a few weeks. Marking kits and paint can be purchased from honeybee equipment suppliers. In rare cases, the bees can play dead during marking, but normally recover well afterwards. Negative consequences of marking for the bees include disturbance of the insulative pile and interference with pollen collection on the thorax. While bees are immobilized for marking, it is a good opportunity to take some measurements (e.g.,, wing length, head width).

2.5.7 Identification of bees

One may want to identify the species, sex, and caste of each bee during a field census. Identification of bees can be done while holding them with the forceps or in more difficult cases after transferring them to a clear vial. Many species can readily be identified using the pile colour pattern (refer to charts like the one of Laverty &

Harder 1988), for others it may be necessary to examine bees for other characters under the binocular (e.g., genitalia, malar space).

The foundress queen can always be readily distinguished by marking her at the onset of colony development, when she is alone. In cases where she was not marked initially, she may still be distinguished from workers by her larger size and from gynes by the wear of her pile and wings. In species with a high size dimorphism between queens and workers, gynes can be identified based on their larger size. Males can be distinguished from females by the following features: males i) lack pollen corbiculae on the hind tibiae, ii) do not have a sting and the tip of their abdomen looks round or square, rather than pointed, iii) have claspers (part of the genitalia) which can be seen by pressing gently on the tip of the abdomen, iv) are usually more hairy on the sternites, v) hold their antennae straighter, vi) are timid and flee or hide when the nest is disturbed, and in some species, vii) sometimes have a different face and/or abdominal pile colouration, viii) have larger eyes and/or longer antennae (see Laverty & Harder 1988), and ix) usually appear only from the middle of summer on. Males sometimes mimick the defensive posture of workers (middle leg raised), so this behaviour should not be used to determine sex.

2.6 Conclusion

Overall, care should be taken in handling wild bumblebee colonies not to disturb them unduely and repeatedly. Night censuses of well-insulated colonies, provided with a small quantity of sucrose solution to compensate for stresses imposed, are probably the census technique of lowest impact and greatest accuracy for assessing colony size. Furthermore, despite their sturdy appearance bumblebees are fragile, and can easily lose portions of their tarsal segments, or leg function, so care must be taken when handling individuals.

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2.8 Legends of figures

Figure 2.1 An above-ground bumblebee nest box. Vegetation should be cleared beneath and around the box, and stakes should be coated on all sides with Tanglefoot® to prevent ants from entering.

Figure 2.2 The inside of a nest box occupied by a mature *Bombus fervidus* colony (view from above) where the cotton has been displaced by the queen and workers to create a canopy over the brood.

Figure 2.3 Coroplast® plastic board used as a removable bottom in nest boxes, with a wire frame to allow for easy handling.

Figure 2.4 A nest removed from the box for the easy detection of nest parasites below and around the brood. In this case, the colony is infested by wax moths.

Figure 2.5 The positioning of the two plexiglass sheets to allow the removal of workers from the nest box with forceps during a census.

Figure 2.6 The typical means of manipulating individuals with forceps. The bee is held by a leg.

Figure 2.7 The V-cut in the lid permits easy introduction of workers into the container during a census taking.

Figure 2.8 A view of a brood following the removal of the canopy constructed by the bumblebees with the insulating material. Note that the canopy is lined on the inside with wax, and this coating can be attached to the brood and nest structures (e.g., to egg clumps laid on top of the brood and to nectar pots in the periphery). Therefore, it may be very difficult to open the canopy without damaging these structures, especially in hot weather when the wax is softer, so considerable care must be taken.

Figure 2.9 *Bombus terricola* queen (left) and *Bombus (Psithyrus) ashtoni* usurper queen (right) marked with Operlith® discs on the thorax.

Figure 2.1

Figure 2.2

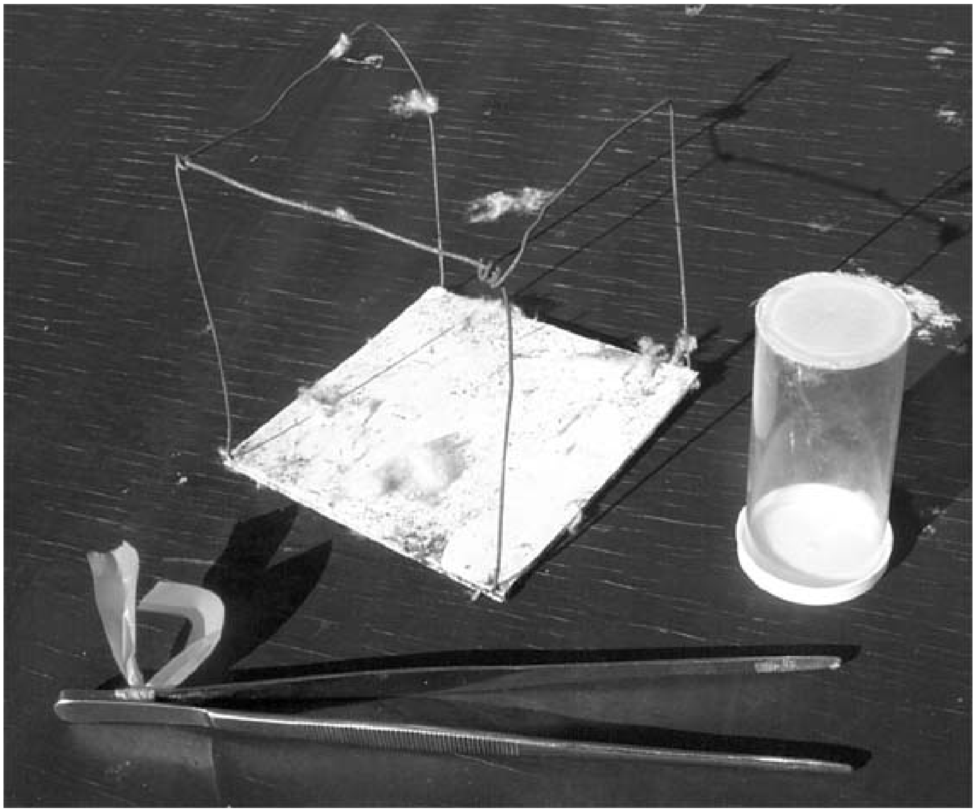

Figure 2.3

Figure 2.4

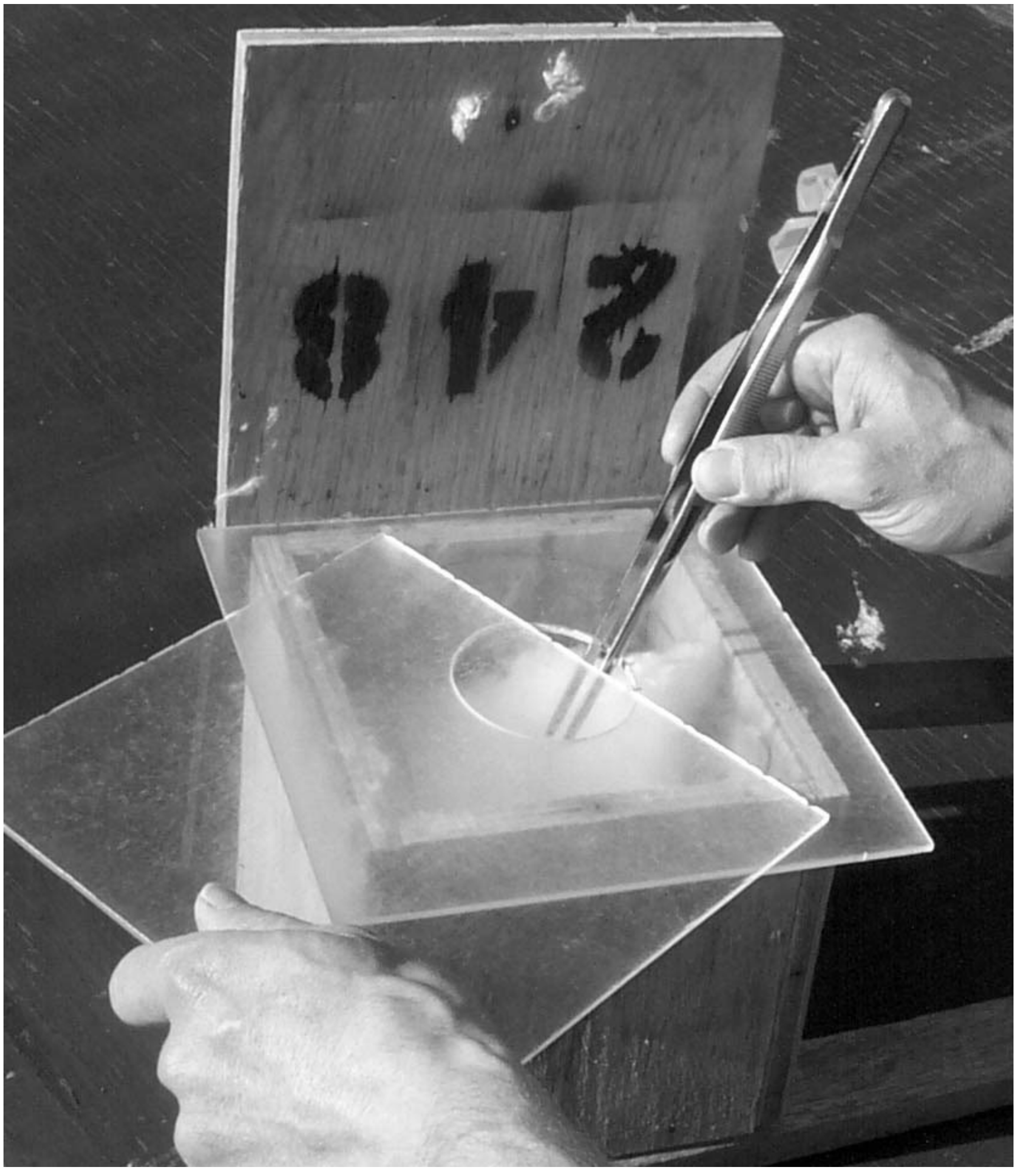

Figure 2.5

Figure 2.6

Figure 2.7

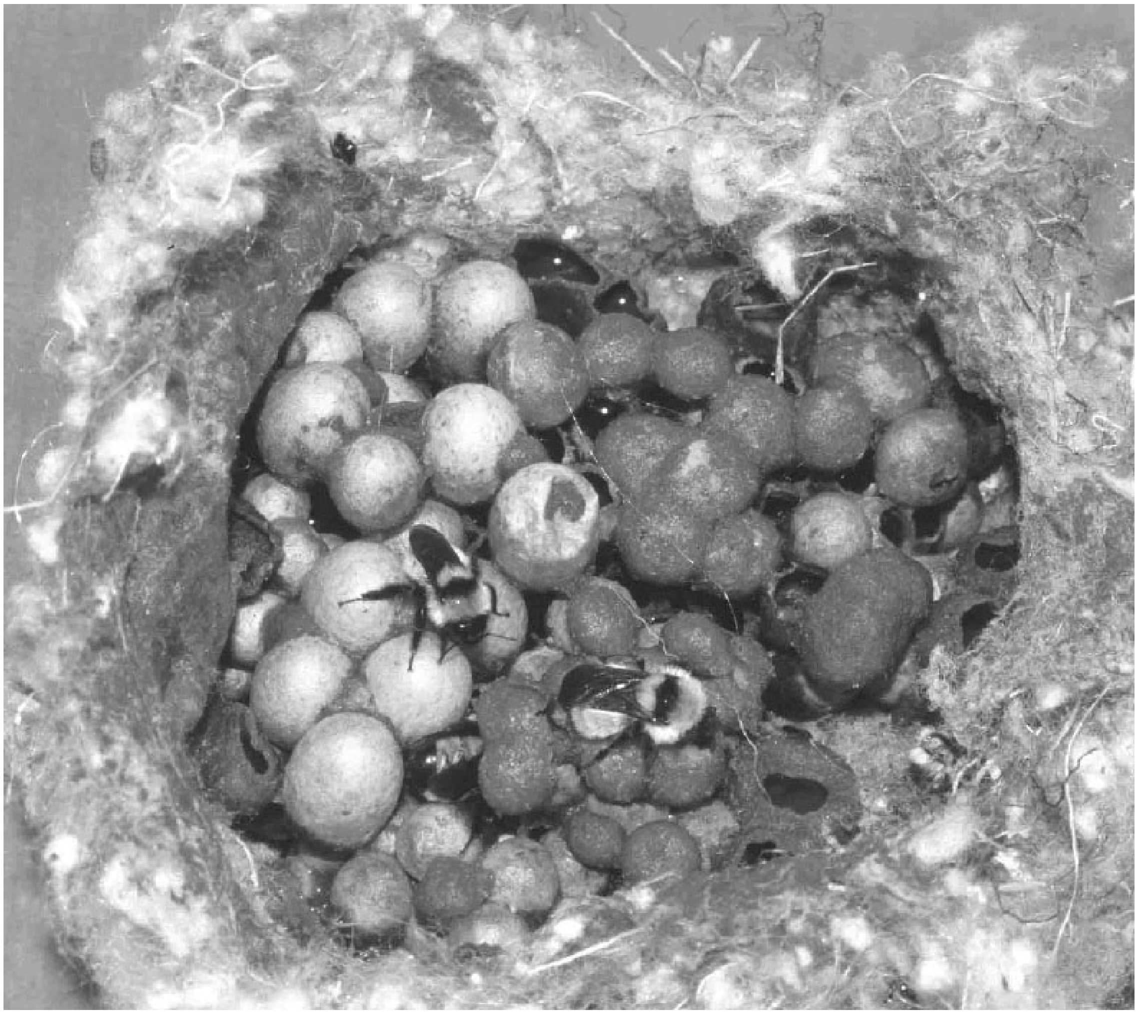

Figure 2.8

Figure 2.9

Chapitre 3

The importance of the queen's body size on reproductive success in bumblebee field colonies.

(Article scientifique soumis à la revue *Ecology*, actuellement en évaluation : Pelletier, L. et J. N. McNeil. The importance of the queen’s body size on reproductive success in bumblebee field colonies.)

3.1 Résumé

Les reines bourdons plus grandes que les autres peuvent possiblement être avantagées par une meilleure survie, fécondité, efficience de butinage, thermorégulation, dominance et capacité à repousser les usurpatrices. Nous avons donc testé l’hypothèse que les plus grandes reines devraient avoir un succès reproducteur plus élevé au sein de leur espèce, ceci en suivant le développement de 185 colonies de huit espèces de *Bombus* (*B. impatiens, B. fervidus, B. perplexus, B. terricola, B. bimaculatus, B. ternarius, B. rufocinctus* et *B. vagans vagans*) sur le terrain, dans la région de Québec, en 1999 et 2000. Nos résultats ont confirmé que les plus grandes reines avaient plus de chances de se reproduire et, parmi celles qui y sont parvenues, le nombre de sexués produits était positivement relié à la taille de la reine. Le meilleur succès des plus grandes reines est attribuable, du moins en partie, à la production de plus grosses colonies et, chez certaines espèces, à la capacité à mieux repousser les usurpations de *Psithyrus* (un sous-genre de bourdons parasites). Le meilleur succès des plus grandes reines n’était pas relié à la date de nidification ou à l’usurpation par d’autres reines *Bombus*, basé sur des données de colonies fondées naturellement par la reine en milieu naturel. Les reines ne gagnaient pas plus souvent les combats contre les usurpatrices *Bombus* en étant plus grandes que ces dernières que lorsqu’elles étaient plus petites. D’autres études seront nécessaires pour mieux comprendre les mécanismes sous-jacents, incluant l’effet de la taille de la reine sur le taux de croissance de la colonie et sur la dominance exercée sur les ouvrières.

3.2 Abstract

Bumblebee queens may benefit from being larger by increased survival, fecundity, foraging efficiency, and better thermoregulation, dominance, and ability to fight usurpers. We tested the hypothesis that, within a given species, larger queens should have a greater reproductive success by following the development of 185 field colonies of eight *Bombus* species (*B. impatiens, B. fervidus, B. perplexus, B. terricola, B. bimaculatus, B. ternarius, B. rufocinctus*, and *B. vagans vagans*) in the Quebec City area, in 1999 and 2000. We confirmed that larger foundress queens were more likely to reproduce, and, for queens that did so, there was a positive relationship between their body size and the number of sexuals produced. The higher success of larger queens is, at least in part, attributable to the production of larger colonies and, in some species, to the ability to prevent usurpations by *Psithyrus* (a parasitic subgenus of bumblebees). The higher success of larger queens was not related to the date of nest establishment or to usurpations by other *Bombus* queens, based on data from naturally established colonies. With regard to one-on-one fights with *Bombus* usurpers, size differences did not affect the outcome of fights from the resident queens' perspective. Additional research is required to obtain a clearer understanding of the mechanisms involved, including the effect of queen size on the growth rates of colonies and dominance over workers.

3.3 Introduction

Insect body size continues to be one of the most important morphological traits studied from an evolutionary perspective because of its strong association with many fitness characters such as fecundity, mating, and survival. More than often, studies show that a large body size is beneficial to the individual. Within a species, large females are generally more fecund (Honek 1993; del Castillo et al. 1999; Sokolovska et al. 2000) and large males often have an increased mating success (Abell et al. 1999; del Castillo et al. 1999; Starks & Reeve 1999; Sokolovska et al. 2000; Andersen & McNeil 2001; Coelho & Holliday 2001; Jamieson 2002). Furthermore, mating is often assortative with respect to body size (del Castillo et al. 1999; Harari et al. 1999), so large males can increase their fitness by mating with the larger, more fecund females. In addition, large individuals often have a higher survival, sometimes as a result of size-selected predation (Rhainds et al. 1999; Van Dongen et al. 1999; Sokolovska et al. 2000).

In social insects, there is usually an important size dimorphism between the female reproductives (queens) and non-reproductives (workers), with queens being the largest individual in a colony (Hölldobler and Wilson 1990; Michener 2000). Even among queens large size may confer reproductive advantages. In polygynous colonies of various social Hymenoptera, large size may help establish dominance (refs in Cameron & Jost 1998; Richards & Packer 1998; Keeping 2000) and increase survival (Bernasconi & Keller 1998). An extreme case of size advantage is found in some species of ants where two distinct sizes of queen exist, with the smaller queens (microgynes) having a markedly reduced reproductive capacity and dispersal ability compared with the larger ones (macrogynes) (Lachaud et al. 1999). However, evidence for a queen size advantage in social insects is still rare and relate mostly to polygynous situations.

Primitively eusocial bumblebees are monogynous in temperate regions, and size may affect the reproductive success of queens in several ways. Queens are usually produced at the end of the summer, mate, overwinter in the ground, and emerge the following spring to establish a colony. At this stage, larger queens are already advantaged by a higher incidence of winter survival (Owen 1988; Sutcliffe and Plowright 1988). They may also have greater fat reserves upon emergence in spring, and a superior physical condition at this time may help them acquire the best available nesting sites.

In the first weeks of colony development, the queen must forage and care for her initial brood. This is a critical period as she assumes all duties at a time of the year where conditions are often suboptimal. Extrapolating from general information on bumblebee foragers, one may expect larger queens to provide more food to the nest and/or more efficiently by (i) collecting larger loads and, therefore spend relatively more time actually foraging and less in transit to and from the nest (Heinrich & Heinrich 1983), (ii) having access to nectar from deeper flowers due to their longer proboscis (Medler 1962; Heinrich 1976, 1979; Harder 1982), (iii) ingesting nectar faster (Harder 1983, 1986), (iv) being able to gather food even during cool and windy weather, in part due to more effective thermoregulation (Heinrich 1979; Heinrich & Heinrich 1983; Bishop and Armbruster 1999 for bees in general), and (v) being able to competitively displace smaller bees at nectar- and pollen-rich flowers (Morse 1982 and refs therein). Increased food provisioning, and possibly increased brood incubation, should increase larval growth rates (see Plowright and Pendrel 1977), ensuring the earlier emergence of workers to assist the queen.

Early colony development is also a critical period as many other bumblebee queens may then try to usurp the foundress queen instead of founding their own colony. Usurpation by other *Bombus* queens and by *Psithyrus* (a subgenus of bumblebees specializing in this strategy) is common (Hobbs 1965, 1966a,b, 1967, 1968; Richards 1978), and, when successful, jeopardizes the reproductive success of the foundress queen (Richards 1994). Larger foundress queens may be more apt at defending the colony against usurpers, as seen in dominance conflicts of polygynous colonies of certain social Hymenoptera (see references above).

In the subsequent growth phase of the colony, there is an exponential increase in worker numbers, and colonies of larger queens may grow faster if fecundity is a limiting factor and if, as for many insects (see references above), fecundity is positively related to size. During this period, the queen must also maintain dominance over workers, accomplished through pheromones and aggressive behaviour (Bloch and Hefetz 1999). Large queen body size may be helpful in these physical interactions, as in small queenless colonies large workers are more successful in gaining dominance (Van Doorn 1989). Once the colony switches from worker to sexual production, prolonged dominance by the queen could lengthen colony life and extend the period of gyne production by preventing or delaying egg laying by workers. If queen body size positively affects at least some of the factors mentioned above, then one may expect larger queens to have a higher reproductive success.

The primary goal of this study was to determine if, at the intraspecific level, the body size of foundress queens affected the reproductive success of bumblebee species under field conditions. We then explored some of the possible associations between body size and reproductive success by determining if larger foudresses i) started colonies sooner, ii) produced larger colonies (and this at a faster rate of development), and iii) were less susceptible to usurpations by *Psithyrus* and other *Bombus* queens.

3.4 Methods

3.4.1 Colonies

We followed the development of 185 bumblebee field colonies in the Quebec City area, Canada, in 1999 and 2000, consisting of (number of colonies, species code): *B. bimaculatus* (5, BIM), *B. fervidus* (34, FER), *B. impatiens* (45, IMP), *B. perplexus* (29, PER), *B. rufocinctus* (10, RUF), *B. ternarius* (33, TRN), *B. terricola* (19, TRC), and *B. vagans vagans* (10, VAG). Most *B. impatiens*, *B. ternarius*, and *B. terricola* were from laboratory colonies established using queens caught in the spring, and transferred to the field when the first brood of workers began emerging (about seven workers). All other colonies were naturally established by queens in above-ground nesting boxes in 2000. We marked each queen (foundresses and usurpers) with a coloured, numbered tag (Operlith®) on the thorax. We censused colonies every 12-15 days, and counted the numbers of workers, gynes, and males. We marked sexuals on the thorax with correction fluid (Liquid Paper®) to prevent double counting, and left them in the colony. We estimated the reproductive success of a colony, RS, using $RS = M + bG$, where M = number of males, G = number of gynes (young queens), and $b = 3$, a coefficient of differential *per capita* investment between the two sexes (see Beekman & van Stratum 1998). The maximal colony size (number of workers), W_{max}, was used as an index of colony development.

For colonies established naturally, we estimated the date of nest establishment retroactively, by assessing the level of brood development upon discovery of a colony in a nest box, and by allowing 1 d for the build-up of the first wax cell, 5 d for egg hatch, and up to 10 d based on the degree of larval development.

3.4.2 Body size measurements

Queens were held steady in a clear sandwich plastic bag (without anasthesizing or chilling them to prevent deleterious effects, see Kukuk et al. 1997) and measured with an electronic caliper (precision of instrument = ± 0.01 mm, but reproducibility = ± 0.1 mm). Due to wear, absolute wing length could not always be obtained. Therefore, as an estimate of body size we measured the length from the anterior end of the tegula (the point of articulation with the thorax) to the distal end of the median cell, at the junction of the basal and costal veins on the right forewing. The advantage of these two points is that the tip of a caliper can be slightly pressed on the tegula, and that the basal vein is thick and visible. There was a strong correlation between the mid and total lengths of the forewing (Pearson's $r = 0.92$, $P < 0.0001$, $n = 216$ queens). Thirty-four of 298 queens could never be measured.

3.4.3 Statistical analyses

Only colonies that had not been subjected to major disturbances (e.g., by floods, theft, vandalism, predation, abandoned by the queen during the early stage of colony development) during the period of interest were included in analyses; for example, a colony that was flooded in late summer could be used for an analysis on early colony development but was obviously excluded from the analysis on reproductive success. We only used foundresses of naturally established colonies to test if body size influenced (i) the time of establishment (ii) the rate of growth of colonies, using only those that were not usurped, and (iii) the probability of being usurped by other *Bombus* queens, as this occurs early in colony development. We used all colonies to determine whether the foundress body size affected i) the probability of being usurped by *Psithyrus,* as this occurred later in overall colony development, and ii) the maximum size of colonies, using only those that were not usurped. To determine if the difference of size between resident and usurper *Bombus* queens affected the outcome of usurpation encounters, we only used those in which one queen survived and the outcome was not affected by other *Bombus* or *Psithyrus* usurpers. We considered two queens to differ in size only when their forewing measurements differed by > 0.2 mm.

Since colonies grow exponentially (at least in the ergonomic part of colony development), we modelled colony growth over the first 65 days with the one-parameter exponiential model $p = e^{kt}$, where p = number of pupae (pupae of gynes are given a weight of 3 compared to pupae of workers and males, see Beekman & van Stratum 1998), k = constant of growth, and t = time in days since nest establishment. We estimated k by using the regression wizard of SigmaPlot 2000 ver 6.00 (SPSS Inc. 2000). It should be noted that modeling the growth of the colony using the number of pupae provides similar results to using the mass of the brood (e.g., strong correlation between values of k, n = 7 colonies of *B. fervidus*, Pearson's r = 0.98, P < 0.0001,). We conducted all of the other statistical analyses using SAS for Windows, version 8.0 (SAS Institute Inc. 1999). For logistic models, we used the logit link function. We started with the models including all main effects and, if significant, added interactions significant at $\alpha = 0.15$. Most interactions in logistic regressions could not be tested because they caused quasicomplete separation of data (no frequency counts in some cells). In the general linear models, we transformed the values of reproductive successes and maximal colony sizes by the square-root to comply with assumptions of homoscedasticity. All analyses contained species as an obligatory factor to account for the inherent interspecific body size differences, and we required a minimum of three valid colonies to include a species in an analysis. All trends presented are at the intraspecific level, unless noted otherwise. Tests on the effect of body size were unilateral, whereas tests for all other effects were bilateral.

3.5 Results

3.5.1 Reproductive success

There was intra- and interspecific variability in queen body size (Table 1). Of 100 foundress queens whose colonies were not disturbed during the entire season, 59 produced sexuals. For any given species, the probability that a colony produced sexuals increased significantly with queen body size (Table 2 and Figure 1): a 1 mm increase of the mid-length of the fore wing of foundress queens (a large difference if we refer to the ranges of body sizes in Table 1) increased the probability of producing sexuals by a factor of 5.0 (adjusted odd ratio; 95% CI = 1.4 – 16.0) (Figure 1). Furthermore, of the queens that successfully reproduced, larger ones produced more sexuals (Table 3 and Figure 2).

3.5.2 Nest establishment

The timing of nesting establishment varied interspecifically, but not intraspecifically as a function of the queen body size (Table 4 and Figure 3). Furthermore, nesting earlier did not significantly increase the probability of producing sexuals (overall logistic regression model not significant, n = 35 queens (species: FER, PER, RUF, VAG), model likelihood ratio $\chi^2_4 = 2.98$, $P = 0.56$).

3.5.3 Colony ergonomics

The size of colonies increased with queen body size (Table 4 and Figure 4): a 1 mm increase in the mid-length of the fore wing increased W_{max} by a factor of 1.4. There was a high correlation between W_{max} and RS (Pearson’s r = 0.72, $P < 0.0001$, n = 119, all species pooled together) underlining the importance of investing in the production of a large worker force, as noted by Müller & Schmid-Hempel (1992). We were unable to determine if the rate of colony development in naturally established colonies was related to the queen body size, due to considerable inter- and intraspecific variability.

3.5.4 Usurpations

Psithyrus and *Bombus* queens usurped 49 of 101, and 11 of 34 colonies respectively, underlining the importance of usurpation on colony success. The probability of being usurped by *Psithyrus* decreased with an increase in queen body size (weakly significant at $\alpha = 0.10$, Table 5), while the probability of being usurped by *Bombus* queens in early stages of colony development was not significantly related to the queen body size (Table 5). It should be noted that these probabilities do not represent the outcome of individual attempts to usurp a queen (unlike the following analyses), but rather the incidence of different types of social parasitism over the entire life of colonies.

When examining the outcome of usurpation fights between pairs of *Bombus* queens, there was no indication that resident queens benefited from being larger than usurpers as resident queens had about the same probability of winning the combat (57 – 67%), independently of size differences between the two protagonists (Table 6).

3.6 Discussion

Our results demonstrate the intraspecific importance of the body size of foundress *Bombus* queens on their reproductive success under field conditions (Figures 1 and 2, Tables 2 and 3). The effect was clear despite the large variability caused by many other important factors, such as ecto- and endo-parasites (Müller & Schmid-Hempel 1992; Imhoof & Schmid-Hempel 1999), brood parasites, usurpations, food availability (Schmid-Hempel & Schmid-Hempel 1998; Goulson et al. 2002; Pelletier & McNeil, in press), microclimate, and predation. Thus, queen body size may explain part of the variability in reproductive success observed among laboratory colonies, reared with ample food and in the absence of enemies (e.g., Duchateau & Velthuis 1988; Müller et al. 1992; Beekman & van Stratum 1998).

The higher success of larger queens can be related to the fact they developed larger colonies (Figure 4, Table 4). A larger worker force may benefit colonies by increasing food provisioning, the quality of brood care, and thermoregulatory capabilities of the nest during the production of sexuals; and by lengthening the duration of sexual production. Furthermore, a larger number of workers provides a better protection against *Psithyrus* usurpations (Fisher 1984, 1985). *Psithyrus* usually prevent their hosts from reproducing altogether (Küpper & Schwammberger 1995 and refs therein; pers. obs.), so preventing their establishment in colonies increases the chances of colony success. It should be noted that a larger maximal colony size cannot confer a better protection against *Bombus* usurpers because these usurpations usually occur in the early stage of colony development, before the emergence of the first workers. Finally, a larger colony size may increase protection against some brood parasites, but this remains to be explored.

How can larger queens obtain larger colonies, and thus ensure higher success? First, larger queens may nest earlier to increase the length of colony ergonomic development, but this was not the case in the present study (Figure 3, Table 4). Furthermore, regardless of body size, we found no evidence that nesting earlier increased reproductive success. If nesting earlier was beneficial, then one would expect nest establishment to occur over a brief period, as in subarctic species where the summer is short (see Vogt et al. 1994). However, not only do temperate species establish nest over many weeks (Richards 1978; pers. observ.), but certain species such as *B. fervidus* do not start until June (pers. obs.). Hence, other factors, such as finding a suitable nest site close to abundant food, may be of greater importance for temperate species than nesting as early as possible.

Second, larger queens may delay the onset of sexual production to increase the length of colony ergonomic development. However, this strategy seems unlikely as a delay could (i) increase the risks of colony failure because brood parasites such as wax moths (*Vitula edmandsae* in North America and *Aphomia sociella* in Europe) can destroy the whole comb in late summer, and (ii) the late emergence of sexuals could decrease their probability of finding suitable mates. While our census schedule did not permit us to determine the precise moment when sexual production occurred, there does appear to be selection against extending ergonomic colony development in our area as most species (except *B. impatiens*) begin producing sexuals well before the end of summer (Macfarlane et al. 1994; pers. obs.).

Third, colonies of larger queens may develop faster. Fast rates of colony development are useful, and even a necessity, where summers are very short (e.g., in the Arctic), but the importance of maintaining a fast rate of development decreases with warmer temperatures and longer summers (Laverty & Plowright 1985). This does not mean that a relationship between queen body sizes and rates of colony development do not exist in temperate areas, such as in our study site, but this remains to be verified with larger sample sizes of naturally-established colonies.

Fourth, larger queens may be able to dominate workers longer; thereby preventing or retarding selfish behaviour from workers (e.g., oophagy, egg laying, matricide; see Bourke 1994; Bloch 1999; Bloch & Hefetz 1999; Bourke 2001). This issue also remains to be explored.

Queens did not benefit from being larger than *Bombus* usurpers in usurpation fights (Table 6). This is in opposition to suggestions currently in the literature (Plowright & Laverty 1984; Owen 1988; Sutcliffe & Plowright 1988), which are based on data in which the status (resident or usurper) of competitors was unknown (Richards 1978). Our finding is similar to that reported on the outcome of dominance contests during temporary polygynous phases in *B. atratus*, a Neotropical bumblebee (Cameron & Jost 1998). Other factors must, thus, determine the outcome of such fights. In the sweat bee *Lasioglossum figueresis*, it is the level of ovarian development rather than body size that appears to determine the outcome of encounters between reproductively active females (Wcislo 1997).

We have provided evidence that body size significantly affects the reproductive success of queens of many species of bumblebees under field conditions. Additional research is required to obtain a clearer understanding of the mechanisms involved, including the effect of queen size on the growth rates of colonies and dominance over workers. Another challenge will be to explain, especially with regard to life history strategies, what makes queens of species such as *B. vagans vagans* successful despite their smaller size. Finally, since larger queens have a higher reproductive potential and that their body size appears to be determined by the amount of food received during larval growth (Plowright & Jay 1977; Sutcliffe & Plowright 1988, 1990), there should be a colony trade-off, depending on prevailing ecological conditions, between producing a large number of smaller gynes and a smaller number of larger gynes with greater reproductive potential. This colony trade-off to balance the costs and benefits of gyne production also merits further attention.

3.7 Acknowledgements

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Table 3.1 Queen body size (mid-length of the fore wing in mm; mean ± variance, range) of eight *Bombus* species in the Quebec City area in 1999 and 2000, for foundresses of naturally established colonies, usurpers, and the pool of all queens (including foundresses of laboratory established colonies and queens whose role (foundress or usurper) was not established).

Species	Foundresses of naturally established colonies					Usurper queens					All queens						
	n	mean	var	min	max	n	mean	var	min	max	n	mean	var	min	max		
B. impatiens	2	10.2	0.1	10.0	10.3	11	10.0	0.2	9.3	10.6	57	10.1	0.2	8.9	10.8	A	
B. fervidus	28	9.6	0.1	8.7	10.4	37	9.7	0.1	8.9	10.5	73	9.7	0.1	8.7	10.5	B	
B. perplexus	26	9.3	0.4	7.1	10.4	11	9.6	0.4	8.6	10.2	46	9.3	0.4	7.1	10.4	C	
B. terricola	1	9.0				1	8.2				14	8.9	0.3	7.8	9.8	D	
B. bimaculatus	4	8.8	0.5	7.8	9.4						7	8.6	0.3	7.8	9.4	D	E
B. ternarius											27	8.4	0.1	7.7	9.1		E
B. rufocinctus	13	8.4	0.1	7.8	8.7	7	8.2	0.1	7.8	8.7	22	8.3	0.1	7.6	8.7		E
B. vagans vagans	11	7.8	0.7	6.0	8.6	2	7.4	1.6	6.5	8.3	14	7.8	0.7	6.0	8.6	F	

Species with the same letter are not significantly different (least-squares means, Tukey-Kramer adjustment, $\alpha = 0.05$).

Table 3.2 Logistic regression testing for the effect of foundress *Bombus* queen body size (mid-length of the fore wing) on the probability of producing sexuals (n = 100; all eight species).

Source	χ^2	df	P
Model	15.08	8	0.029
Species	12.25	7	0.093
Body size	5.90	1	0.008

Table 3.3 ANCOVA testing for the effect of *Bombus* queen body size (mid-length of fore wing) on the index of reproductive success for foundress queens that successfully produced sexuals (n = 56 ; species: FER, IMP, PER, RUF, TRN, TRC), r^2 = 0.33. The interaction was not kept in the final model because P > 0.50.

Source	F	df	P
Model	4.08	6, 49	0.0021
Species	4.47	5, 49	0.0019
Body size	5.45	1, 49	0.012

Table 3.4 ANCOVAs testing for the effect of the foundress *Bombus* queen body size on the timing of nest establishment (n = 68; species: BIM, FER, PER, RUF, VAG), $r^2 = 0.58$, and maximal colony size (n = 88; all eight species), $r^2 = 0.23$. The interactions were not kept in the final model because $P > 0.50$.

Parameter	Source	F	df	P
Nest establishment	Model	17.20	5, 62	< 0.0001
	Species	19.56	4, 62	< 0.0001
	Body size	1.39	1, 62	0.12 [a]
Maximal colony size	Model	2.98	8, 79	0.0057
	Species	2.97	7, 79	0.0081
	Body size	4.26	1, 79	0.021

[a] Power = 0.318, more than 200 queens would be required to obtain a power of 0.60

Table 3.5 Logistic regressions testing for the effect of *Bombus* queen body size (mid-length of the fore wing) on the incidence of usurpations by other *Bombus* queens (n = 50 contests; host species: FER, PER, RUF, VAG), or *Psithyrus* females (n = 49 contests; host species: IMP, TRN, TRC).

Usurper subgenus	Source	χ^2	df	P
Bombus	Model	4.86	4	0.30
	Host species	1.85	5	0.60
	Body size	0.48	1	0.25
Psithyrus	Model	5.85	3	0.12
	Host species	4.87	2	0.09
	Body size	2.27	1	0.07

Table 3.6 Observed probabilities of winning a usurpation contest for resident *Bombus* queens when fighting against another *Bombus* queen, by differences of size between the resident and usurper queens. A queen is considered larger when the mid-length of the fore wing is longer by more than 0.2 mm.

	All contests	Intraspecific contests only
Resident queen larger	10/15	7/11
Same size	2/3	1/2
Resident queen smaller	8/14	6/10
Total	20/32	14/23

3.9 Legends of figures

Figure 3.1 Positive relationship between the predicted probability of producing sexuals and the body size (mid-length of fore-wing in mm) of foundress queens for eight *Bombus* species (n=100) in the Quebec City area, Canada, in 1999 and 2000. The logistic regression model is presented in Table 2.

Figure 3.2 Predicted reproductive success of foundress queens as a function of body size (mid-length of fore wing in mm) in six *Bombus* species that reproduced successfully (n = 59) in the Quebec City area, Canada, in 1999 and 2000. Based on the ANCOVA presented in Table 3.

Figure 3.3 Julian date of nest establishments as a function of the body size (mid-length of fore wing in mm) of foundress queens in four *Bombus* species nesting in the Quebec City area, Canada, in 2000.

Figure 3.4 Predicted maximum colony size (in number of workers) as a function of the foundress queen body size (mid-length of fore wing in mm) in eight *Bombus* species in the Quebec City area, Canada, in 1999 and 2000. Based on the ANCOVA presented in Table 4.

Figure 1.

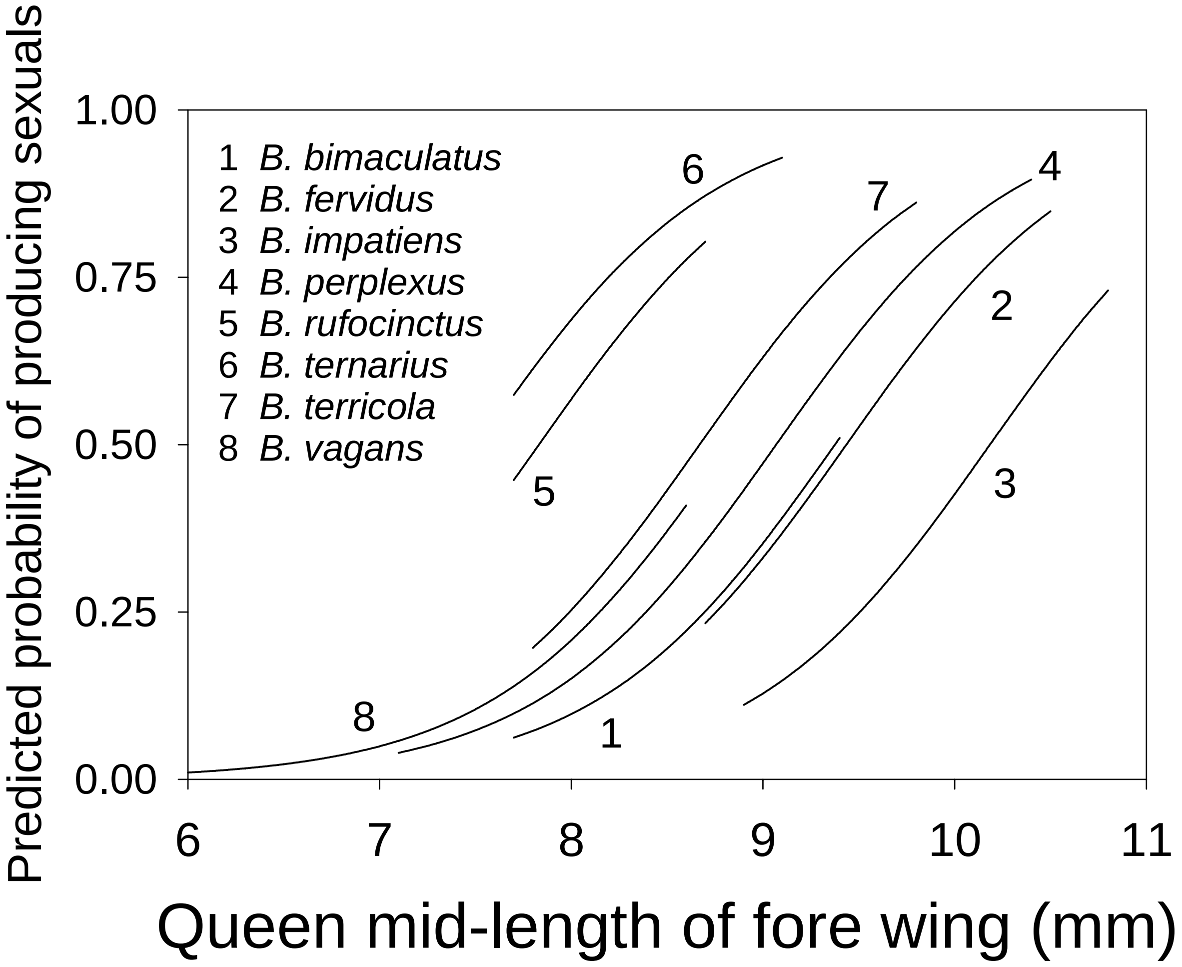

Predicted probability of producing sexuals
1.00
0.75
0.50
0.25
0.00
6
7
8
9
10
11
Queen mid-length of fore wing (mm)
1 B. bimaculatus
2 B. fervidus
3 B. impatiens
4 B. perplexus
5 B. rufocinctus
6 B. ternarius
7 B. terricola
8 B. vagans
6
7
4
2
5
3
8
1

Figure 2.

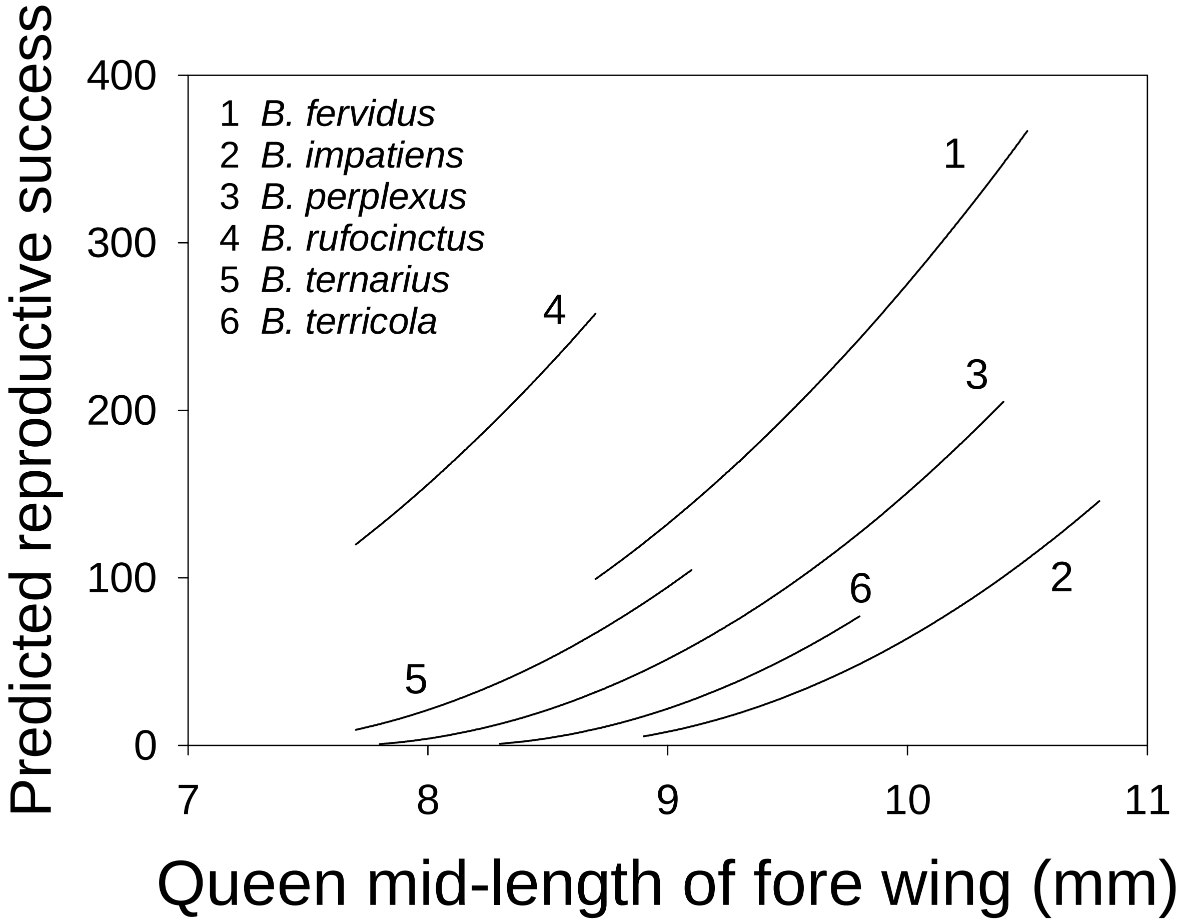

Predicted reproductive success
400
300
200
100
0
7
8
9
10
11
Queen mid-length of fore wing (mm)
1 B. fervidus
2 B. impatiens
3 B. perplexus
4 B. rufocinctus
5 B. ternarius
6 B. terricola
1
2
3
4
5
6

Figure 3.

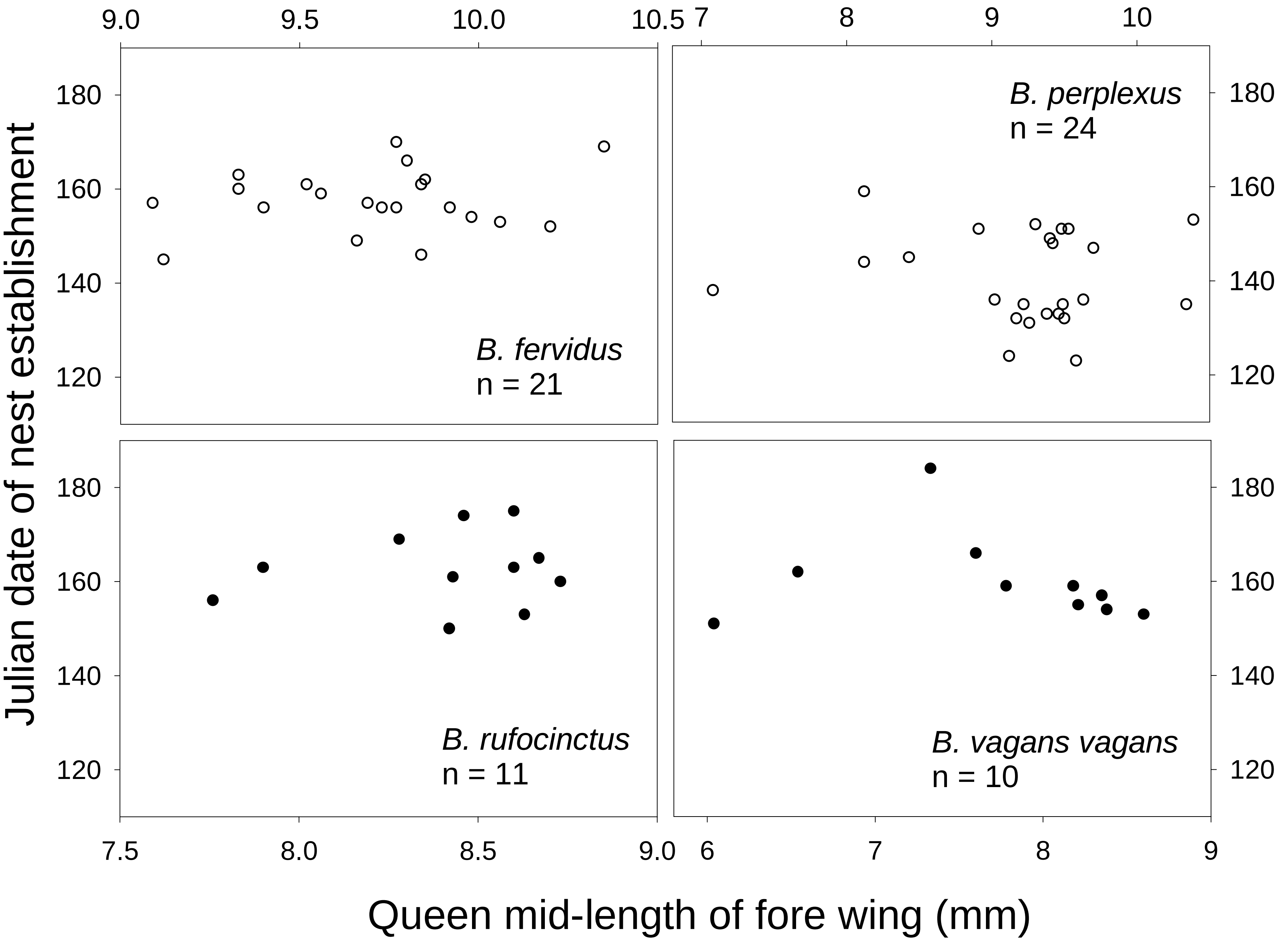
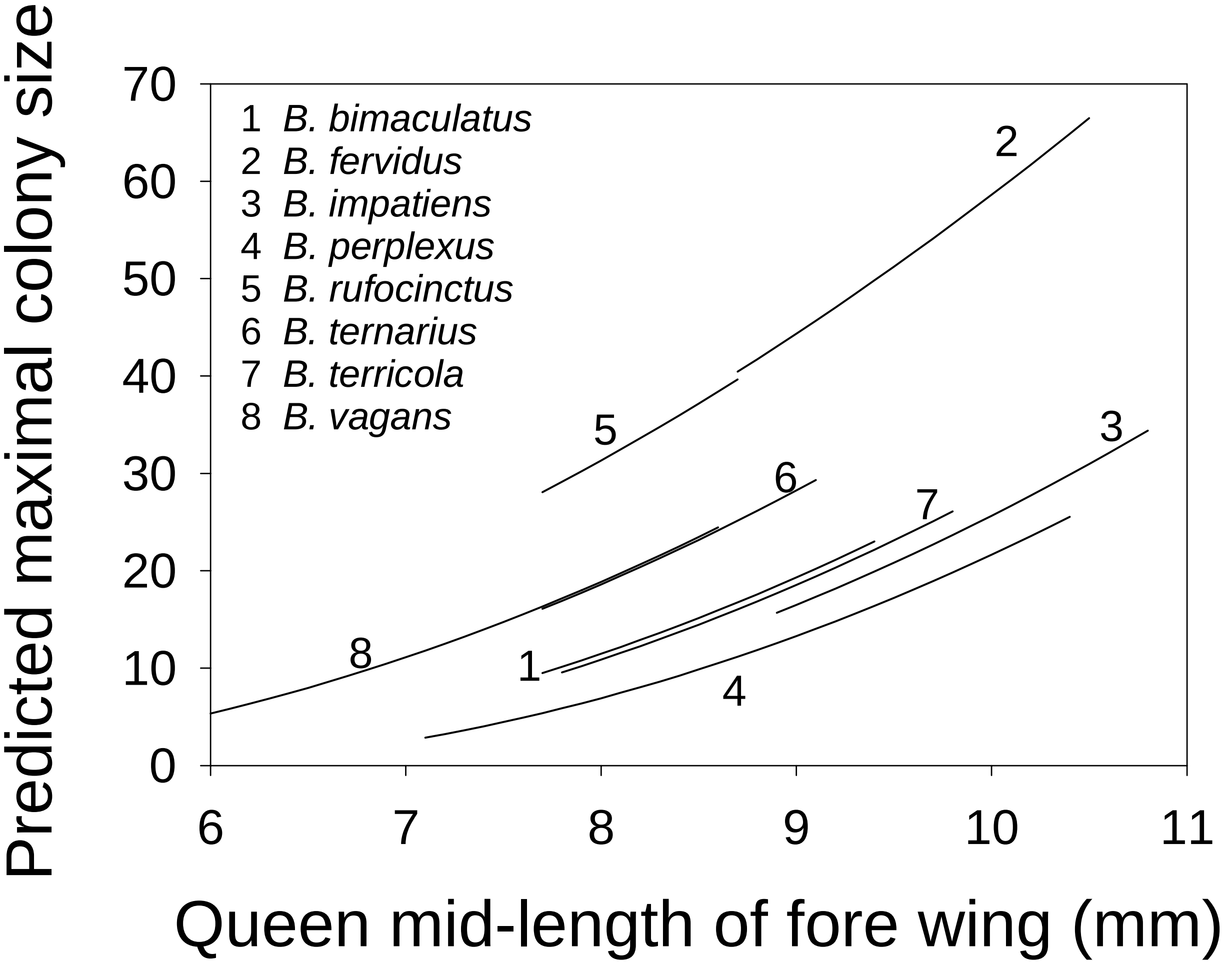

9.0
9.5
10.0
10.5
7
8
9
10
180
160
140
120
B. fervidus
n = 21
B. perplexus
n = 24
B. rufocinctus
n = 11
B. vagans vagans
n = 10
7.5
8.0
8.5
9.0
6
7
8
9
Julian date of nest establishment
Queen mid-length of fore wing (mm)

Figure 4.


Predicted maximal colony size
70
60
50
40
30
20
10
0
1 *B. bimaculatus*
2 *B. fervidus*
3 *B. impatiens*
4 *B. perplexus*
5 *B. rufocinctus*
6 *B. ternarius*
7 *B. terricola*
8 *B. vagans*
2
5
3
6
7
8
1
4
6
7
8
9
10
11
Queen mid-length of fore wing (mm)

Chapitre 4

The effect of food supplementation on reproductive success in bumblebee field colonies.

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Pelletier, L. et J. N. McNeil. The effect of food supplementation on reproductive success in bumblebee field colonies. Oikos. *Sous presse.*)

4.1 Résumé

La qualité d’un habitat est en grande partie déterminée par la disponibilité en nourriture. Pour les colonies de bourdons en milieu naturel, on ne sait pas dans quelle mesure leur succès reproducteur est limité par la disponibilité en nourriture relativement à d’autres facteurs tels que le parasitisme. Pour évaluer l’importance de la disponibilité en nourriture, nous avons fait une expérience avec 45 colonies de *Bombus impatiens* et *B. ternarius* sur le terrain, dans la région de Québec, en 1999 et 2000. Les colonies dont nous avons augmenté les réserves de nectar et pollen tout au cours de la saison sont devenues plus grosses (en nombre d’ouvrières) et ont eu un meilleur succès reproducteur que les colonies témoins, par 51% et 86% respectivement. Plus spécifiquement, l’ajout de nourriture a permis d’augmenter le nombre de mâles produits et la probabilité de produire des gynes (jeunes reines). Le ratio des sexes était fortement en faveur des mâles en général, et la proportion de gynes augmentait suite à l’ajout de nourriture chez *B. ternarius*, mais pas chez *B. impatiens*. Ces résultats suggèrent que les colonies assurent leur reproduction en produisant au moins des mâles et que lorsque l’occasion se présente (disponibilité en nourriture suffisante), elles produisent des gynes. Des raisons possibles pour expliquer le plus grand succès des colonies ayant eu un apport en nourriture sont explorées. Malgré que l’ajout de nourriture a permis de produire des colonies plus grosses, cela n’a pas permis aux colonies de mieux se défendre contre les macroparasites (*Psithyrus*, *Fannia canicularis*, *Brachicoma devia* et *Vitula edmandsae*) car les colonies avec ajout de nourriture et les colonies témoins ont eu des niveaux de parasitisme semblables.

4.2 Abstract

Food availability is a major component of habitat quality. For bumblebee field colonies, it is unknown to what extent reproductive success is limited by food availability relative to other factors such as parasites. To assess the importance of food availability, we carried out a field experiment in the Quebec City area, Canada, in 1999 and 2000, using 45 colonies of *Bombus impatiens* and *B. ternarius*. Colonies whose nectar and pollen supplies were increased regularly throughout the season reached larger sizes (in number of workers) and had a higher reproductive success than controls, by 51% and 86% respectively. In particular, food supplementation increased the number of males produced and the probability of producing gynes (young queens). The sex ratio was highly skewed in favour of males overall, and the relative proportion of gynes increased with food supplementation in *B. ternarius*, but not in *B. impatiens*. These results suggest that colonies ensure reproduction by producing some males and, given the opportunity (sufficient food availability), will produce gynes. Possible reasons for the increased success of food supplemented colonies are explored. However, despite some clear advantages of having larger food supplies such as the build-up of larger worker populations, food supplementation did not appear to help colonies defend themselves against macroparasites because experimental and control colonies experienced similar levels of parasitism by *Psithyrus*, *Fannia canicularis*, *Brachicoma devia*, and *Vitula edmandsae*.

4.3 Introduction

Food availability is a major component of habitat quality and in the event of shortfalls the impact is generally evident. For bumblebees, a group of primitively eusocial bees that feed on pollen and nectar, short-term food shortfalls may have a detrimental effect on numerous parameters leading to lower colony success. These include a drop in brood temperature and torpor in workers (Heinrich 1979; Cartar & Dill 1991), an increase in immature developmental time (Plowright & Pendrel 1977; Sutcliffe & Plowright 1990; Cartar & Dill 1991), production of fewer and/or smaller individuals (Sutcliffe & Plowright 1988, Schmid-Hempel & Schmid-Hempel 1998), as well as the production of fewer sexuals (Schmid-Hempel & Schmid-Hempel 1998). Under extreme conditions of food shortage, larvae are ejected from the nest

(Plowright & Plowright 1999). These negative effects may also result in an increased vulnerability of colonies to predators and parasites (Cartar & Dill 1991).

The importance of food availability may also be studied when organisms experience enhanced resources. This may be achieved by comparing the performance of individuals translocated to a richer habitat with those in the original one (e.g., Komdeur 1996) or by directly supplementing food (e.g., Moo-Valle et al. 2001). This approach permits one to determine if any aspects of life history are limited by ‘normal’ levels of food availability, which may include: spacing behaviour (Fortier & Tamarin 1998), timing of reproduction (Komdeur 1996; Kelly & Van Horne 1997), number of reproductive attempts (Komdeur 1996), offspring number, mass and survival (Boland, Heinsohn & Cockburn 1997; Abell 1999).

The question of whether bumblebees are limited in any way by ‘normal’ levels of food availability has been raised repeatedly, especially in a context of resource competition (e.g., Bowers 1985 and references therein). The importance of food availability on bumblebees reproduction in the field has been inferred from the earlier appearance of sexuals at flower rich sites (Bowers 1985, 1986; Schmid-Hempel & Durrer 1991), but has never been tested experimentally under field conditions. Bumblebees are known as effective and efficient foragers, and there are a multitude of factors including predators and parasites (Alford 1975; Macfarlane, Lipa & Liu 1995) and intracolonial conflicts (Bourke & Franks 1995; Crozier & Pamilo 1996; Bloch 1999; Bourke & Ratnieks 2001) that could limit reproductive success. In other words, if other factors predominate then colony success would remain unchanged if the only parameter manipulated was the addition of food.

However, if food availability is limiting then one would predict that bumblebee colonies supplemented with food should initially produce more workers to ensure food provisioning and brood care, subsequently resulting in the production of more sexuals. Gynes (young queens) require a greater investment of time and resources to produce than males as they take longer to develop, weigh more at emergence, and stay longer in the nest after emergence (see Beekman & van Stratum 1998). Thus, colonies constrained by food should invest relatively more in the production of males to ensure that at least some sexuals are produced (see Aron et al. 2001; Gaume & McKey 2002 in ants). However, colonies with large food supplies could afford, and would benefit from, a relatively higher investment in gynes. Gynes are more valuable than males because they should have a greater probability of mating (the sex ratio is strongly biased to males (Bourke 1997), and gynes generally only mate once (Schmid-Hempel & Schmid-Hempel 2000)) and found the monogynous colonies.

Bumblebees have evolved various effective defence mechanisms to reduce invasion of the nest by parasites, such as the ability to sting and the presence of guards at the colony entrance. These have a cost, at least with respect to the time allocated to defence rather than other activities such as foraging and brood care. Therefore, we could expect colonies with sub-optimum food supplies to be more susceptible to nest parasites than those with ample resources.

The objectives of this study were to evaluate experimentally the effects of richer long-term food availability on the development and reproductive success of bumblebee field colonies, and to determine whether colonies with increased resources were able to reduce the incidence and severity of nest parasitism.

4.4 Methods

4.4.1 General

We collected *Bombus impatiens* Cresson and *B. ternarius* Say queens in the spring of 1999 and 2000 to establish colonies in the laboratory. Queens were marked with a Operlith bee tag on the thorax and their body size estimated by measuring the mid-length of the right forewing from the anterior end of the tegula to the distal end of the median cell, at the junction of the basal and costal veins. Once the first brood workers emerged (5-10 workers), colonies were moved to a 400 ha meadow in St-Jean-Chrysostome, Qc, Canada, in above-ground wooden nest boxes. The habitat was rich in wild flowers with a succession throughout the summer; the most common flowers were *Epilobium angustifolium* L., *Aster* spp., *Solidago* spp., *Vicia cracca* L., *Eupatorium* spp., *Asclepias syriaca* L., and *Arctium* spp. To our knowledge, no pesticides were applied and no other major unnatural disturbances occurred in this field. A small apiary was located within 1.5 km of the nest boxes.

4.4.2 Experimental design

In 1999, prior to the onset of the experiment, we decided to include only colonies with more than 30 workers and a live queen. However, this resulted in the exclusion of too many colonies. So, in 2000 we retained all colonies with a live queen and whose worker number increased from the time placed in the meadow to the first field census. Overall, for the two years we had a total of 45 colonies (details in Table 1). Each year, and for both species, we assigned the treatment (food addition or control) at random for the first colony and alternated for subsequent ones. We censused colonies every 12 – 15 days, looked for the presence of the queen and usurpers, and counted the number of workers, sexuals, and pupal cells of different sizes.

Those colonies receiving additional resources were provided a sucrose solution (sucrose:water 1:1 V/V) *ad libitum* from a 130-ml gravity feeder that was refilled at least every week, and a 5 g fresh pollen pellet was placed beside clumps of young larvae and eggs at the time of each census. Pollen was collected fresh from honeybee traps, and stored frozen until use in bumblebee colonies. The bees used the resources provided, as honey pots were filled with the sucrose solution (evident as the viscosity and colour differs from nectar) and the pollen pellets were partially or completely eaten. We were unable to obtain an accurate measure of the volume of sucrose solution taken from the feeder, as initially planned, because other insects (mainly ants and wasps) often exploited the food source despite our efforts to exclude them. We also attempted to measure and compare food reserves in control and treatment colonies, but dropped this as it is very difficult to obtain unbiased estimates in mature colonies without destructive sampling.

4.4.3 Reproductive success

We estimated the reproductive success of a colony using $RS = M + 3G$, where M = number of males, G = number of gynes. We set the coefficient of differential investment at 3 to compensate for the *per capita* differential investment between males and gynes (see Beekman & van Stratum 1998). We used the total number of sexual pupal cells and adults to estimate reproductive success; using adults alone would result in a marked underestimation as some sexuals could emerge and leave between census dates. Once adult males were observed within a colony, we assumed that all subsequent small cocoons would give rise to males, an assumption confirmed by examining the genitalia of random subsamples of pupae. Some individuals would be counted twice (as pupae and adults) so our measure of reproductive success is only an index. Values of RS obtained were very strongly correlated to equivalent estimates of reproductive success based on either the number of adults or pupae only (Pearson's $r > 0.95$, $P < 0.001$, $n = 45$ colonies) and all results were consistent. The sex ratio of each colony was expressed as $G/(G + M)$. The maximal colony size (number of workers), W_{max}, was used as an index of colony development. We could not examine the effects of food supplementation on the timing (onset, ending, duration) of sexual production as differences between fed and control colonies were < 14 days, a time frame similar to our censusing schedule.

4.4.4 Parasitism

We observed four major groups of nest parasites during this study: *Bombus (Psithyrus)* spp. (Hymenoptera: Apidae), the lesser house fly *Fannia canicularis* L. (Diptera: Muscidae), *Brachicoma devia* Fallen (Diptera: Sarcophagidae), and the bumble bee wax moth *Vitula edmandsae* Packard (Lepidoptera: Pyralidae).

Bumblebees of the subgenus *Psithyrus* are obligate social parasites usurping a colony of a *Bombus* species. When a *Psithyrus* female invades a colony she may kill numerous workers and the founding queen. If successful, she then destroys all *Bombus* eggs and larvae before laying her own eggs (Fisher 1985, 1988). We observed two species of *Psithyrus* in our study, *P. citrinus* Smith and *P. insularis* Smith. As both species affected *Bombus* colonies in similar ways, we did not differentiate between them in subsequent analyses.

F. canicularis larvae are generalist scavengers feeding on debris, faeces, and dead bodies in the bottom of nest boxes, and only become a brood pest at high densities (> 100 larvae, pers. obs.). In contrast, *B. devia* maggots feed directly on host larvae and pupae (Alford 1975) and thus impact on the colony even at low densities. Wax moth larvae generally feed on the old comb, but in later instars, especially at high densities (> 50 larvae), they will readily attack the brood (pers. obs.).

In 1999, nearly all colonies were heavily parasitised (especially by *Psithyrus*), so in order not to lose them, we removed the parasites at the time of each census. In contrast, in the following year we left all parasites in colonies and estimated their number by taking the whole comb out of the nest box with the help of a removable plastic bottom.

4.4.5 Statistical analyses

For the general linear models, we transformed *RS* by the square root and W_{max} with the natural logarithm. We used a logistic regression to test the effect of food supplementation on gyne production because of the prevalence of null data (more than half of the colonies did not produce gynes). We included queen body size as a covariable. We did not have measurements on queen body size for two of the 45 colonies and these were excluded from analyses including this parameter. The other factors included in the model were (i) year, as there were slight differences in our protocol and in natural conditions (e.g., climate, incidence of parasitism), ii) species, because of possible different life strategies, and iii) whether or not a *Psithyrus* queen attempted to invade the colony. Tests for the effect of additional resources, queen body size, and *Psithyrus* are one-tailed, whereas those for the effect of species and year are two-tailed. We conducted all statistical analyses using SAS ver 8.0 (SAS Institute 1999).

4.5 Results

4.5.1 Reproductive success

The addition of food significantly increased both W_{max} and *RS* (Tables 1 and 2), by 51% and 86% respectively (based on least square means). These two parameters were strongly associated (Pearson's $r = 0.81$, $P < 0.001$, $n = 45$ colonies), showing the importance of investing in a larger workforce prior to sexual production. Alone the addition of food explained 7.9% and 6.5% of the variance in W_{max} and *RS* respectively**.**

Food supplementation increased male production in a nearly identical way to *RS* (*RS* and *M* are strongly correlated, Pearson's $r = 0.94$, $P < 0.001$, $n = 45$ colonies), so this analysis is not presented further. Adding food increased the probability of producing gynes by a factor of 3.97 (adjusted odds ratio, 95%CL = 1.11 – 16.67; logistic regression controlling for the same factors listed in Table 2, global likelihood ratio $\chi^2_5 = 16.16$, $P = 0.0064$; effect of food addition: Wald $\chi^2_1 = 2.89$, $P = 0.045$). The addition of food increased gyne relative to male production in *B. ternarius* (female to male sex ratio was significantly larger, Wilcoxon's one-sided $P = 0.035$, both years combined), but did not in *B. impatiens* (Table 1)*.* All colonies but one, unless usurped by a *Psithyrus*, produced males while only some of these also produced gynes (Table 1).

4.5.2 Parasitism

Of the 55 observed invasion attempts by *Psithyrus* spp., we could identify 47 to species. Forty-four were *P. citrinus*: 39 in *B. impatiens* colonies and five in *B.*

ternarius. The three attempts by *P. insularis* were all in *B. impatiens* colonies and, as two usurpations were successful, this represents a new host record for *P. insularis*. In 1999, the incidence of *Psithyrus* was very high with almost all colonies being attacked, independent of the treatment and host species (Table 1). The intensity of parasitism per colony was also high, with up to 3 and 11 attempts on individual *B. ternarius* and *B. impatiens* colonies, respectively. In 2000, the incidence and intensity of *Psithyrus* attacks were much lower with attempts occurring in less than half of the colonies and only one attempt per colony, except for one fed *B. impatiens* colony which had three. Consequently, we only used 2000 data to examine the effect of food availability on *Psithyrus* invasions. The probability of being successfully invaded by a *Psithyrus* was independent of the level of food availability for *B. impatiens* and *B. ternarius* colonies (probabilities were the same, Table 1). However, reduced values of W_{max} and *RS*, and reduced probabilities of producing gynes were significantly associated with higher probabilities of usurpation attempts by *Psithyrus* **(**Table 2 and previous section of results**)**, which shows that *Psithyrus* may have had negative impacts on colony development even when usurpations were not successful and/or that they avoided larger colonies.

There was no indication that higher food availability helped to reduce the incidence (both years) or severity (2000 only) of brood parasitism by wax moths, *F. canicularis*, and *B. devia* (Table 3). In 1999, the incidence of brood parasitism was very high with *F. canicularis* and wax moths being present in all colonies and *B. devia* being present in all colonies but one.

4.6 Discussion

It was evident, from regular seasonal observations of more than 230 bumblebee field colonies from 1999 to 2001, that they frequently have no nectar and/or pollen reserves. The most abundant food supplies we found, about 25 ml of nectar and 5g of pollen, would not last more than a few days for a mature, non-foraging colony (see Heinrich 1979). Hence, bumblebee colony reserves appear to serve only as short-time buffers for the usual periods of inactivity (e.g., nights and rainy days) rather than to provide secure backups for the longer periods of food scarcity. Therefore, given low reserves and the variability in food availability in the field, bumblebee colonies must often be on the verge of food shortfalls (with the potential negative effects presented in the introduction). Under such conditions, one would expect bumblebees to have evolved flexible strategies, as seen with larval growth (Plowright & Pendrel 1977), that allow optimal use of available resources. Flexibility would not only be useful in the face of shortfalls, but also when states of abundance occur.

This study shows that bumblebee colonies benefit from increased food supplies in terms of colony size and reproductive success. This may result from increased i) rate of egg laying by the queen (and maybe by workers too), ii) survival of immatures, e.g., by reducing larval ejection, iii) developmental rate of immatures, e.g., by increasing brood temperature at night (night temperatures dropped as low as 7^{o}C during our experiments which resulted in torpor of workers from small colonies), and iv) life expectancy of workers due to reduced foraging activity. These factors should result in a larger worker population, which is a logical prerequisite to a subsequent increased production of sexuals (see Oster & Wilson 1978, but also empirical support in Owen, Rodd & Plowright 1980; Müller & Schmid-Hempel 1992a). In particular, a stronger workforce would provide more help in accomplishing the duties of eusocial life, including food provisioning, brood care, and defence of the colony. It may also extend the period of sexual production.

The increase in reproductive success does not appear to result from an increased ability to prevent parasitism, as the occurrence and severity of these events were independent of food availability (Tables 1 and 3). Thus, an increase in colony size (and thus colony defence) does not influence the proliferation of brood parasites. In the case of *B. devia* and wax moths, workers may be unable to prevent their intrusion and/or to get rid of them, as the parasite larvae are well hidden under and within the comb. In addition, wax moth larvae quickly retreat to a safe location when disturbed (e.g., within a cocoon or silken tunnel). The case of *F. canicularis* is different as bumblebees may not even consider it as a nest enemy. When present in low numbers, this scavenger may actually be beneficial by reducing potential mould infections of the comb through the elimination of faeces, debris, and cadavers. It seems that *F. canicularis* only negatively affect the brood at high densities (pers. obs.), and high densities of this parasite are not frequent (Table 3).

In contrast to the above parasitic species, *Psithyrus* are specialist and obligatory parasites of bumblebees, and face a strong opposition from colony occupants upon invasion (Fisher 1985; pers. obs.). Thus, one might expect the larger colonies resulting from the addition of resources to be less frequently attacked as *Psithyrus* queens have greater difficulty usurping large colonies (Fisher 1985). However, this was not the case, as there were no observed differences in susceptibility to attack (Table 1). It is possible that *Psithyrus* may locate and attack faster growing colonies sooner, an aspect we could not examine during the present study as the time differences between attacks on treated and control colonies was less that the intervals between each census.

Despite correcting for a number of quantifiable sources of variability, there is considerable residual variation in our final model testing for the effect of food availability (as shown by the low r^2 value in Table 2). This reflects the complexity of the social system under study and its interaction with the environment. For instance, factors not included in the model which could impact on reproductive success are: i) the microclimate due to the specific location of nest boxes, e.g., solar radiation on nest boxes and heat loss at night, ii) brood microparasites and diseases, iii) predation and endo-parasitism of individuals (Schmid-Hempel & Durrer 1991), iv) the proximal foraging environment, and v) intrinsic and endogenous factors of the colony, e.g., the queen’s fecundity and intracolonial conflicts (see Bloch 1999; Cnaani et al. 2000). Intrinsic and endogenous factors are also of major importance, as high levels of variation in reproductive success have been reported in laboratory studies where environmental factors are controlled, food availability provided *ad libitum*, and no parasites are present (e.g., Owen, Rodd & Plowright 1980; Müller & Schmid-Hempel 1992b).

The highly skewed male-biased sex ratios we obtained (Table 1) are consistent with previous field studies on *Bombus* (e.g., review in Bourke 1997; Imhoof & Schmid-Hempel 1999). These results partially support the hypothesis that colonies invest relatively more in the cheaper production of males when resource are limited, but that they increase their investment in gynes when the resources are available.

This study raises a number of questions. First, we used colonies started under laboratory conditions and, thus, could not address the importance of food during the initial stage of colony establishment. Food availability during this stage must be critical as the queen must both forage and care for the brood at a time when climatic conditions are less favourable, and when usurpations by other *Bombus* queens are frequent (Richards 1978; pers. obs.). Hence, increased food availability would not only favour initial colony growth, it would allow the founding queen to remain with the brood for longer periods of time and thus decrease the risks of being usurped by other *Bombus* queens. Obviously, these possibilities need to be tested using naturally established colonies.

Second, adult body size is determined by food provisioning during larval development (Sutcliffe & Plowright 1988) and larger sexuals (at least gynes) have higher survival (Owen 1988) and reproductive success (unpubl. data), so food supplemented colonies could also increase their fitness by producing sexuals of higher quality rather than just producing more of them. Therefore, experiments designed to address the question of whether increased food supplies also result in the production of larger sexuals (of better quality) would be worthwhile.

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Table 4.1 Effect of the long-term addition of food on the worker population and reproductive success (mean ± SD) of field colonies of *B. impatiens* and *B. ternarius*, in St-Jean-Chrysostome, Qc, Canada, in 1999 and 2000. All colonies that produced gynes produced males.

Species	Year	Treatment	n	$n_{\psi att}$	$n_{\psi suc}$	n_G	n_M	G_p	M_p	G_a	M_a	RS	W_{max}	Sex ratio
B. impatiens	1999	fed	2	2	1	0	1	0	106.5 ± 150.6	0	45.0 ± 63.6	151.5 ± 214.3	53.5 ± 37.5	0
		control	3	3	3	0	2	0	17.0 ± 25.2	0	5.0 ± 6.2	22.0 ± 31.4	34.3 ± 4.9	0
	2000	fed	12	5	4	7	9	14.3 ± 20.4	104.7 ± 105.6	5.3 ± 8.0	42.2 ± 46.3	205.6 ± 212.8	55.4 ± 48.8	0.124 ± 0.111
		control	12	5	4	6	8	9.0 ± 13.3	70.4 ± 69.3	4.7 ± 8.3	18.7 ± 21.0	130.1 ± 125.9	41.3 ± 31.3	0.164 ± 0.237
B. ternarius	1999	fed	2	1	0	1	2	2.5 ± 3.5	102.5 ± 53.0	4.5 ± 6.4	35.5 ± 7.8	159.0 ± 31.1	65.0 ± 19.8	0.060 ± 0.085
		control	4	4	3	1	4	0.8 ± 1.5	29.8 ± 6.7	0	6.5 ± 6.5	38.5 ± 2.6	49.0 ± 17.7	0.020 ± 0.040
	2000	fed	5	1	0	4	5	10.0 ± 11.6	67.6 ± 94.1	4.6 ± 6.3	22.2 ± 28.6	133.6 ± 170.2	49.0 ± 42.2	0.124 ± 0.098
		control	5	0	0	2	5	5.0 ± 7.5	90.0 ± 41.2	2.6 ± 4.8	26.8 ± 19.7	139.6 ± 80.5	30.2 ± 6.3	0.042 ± 0.069

n = number of colonies

$n_{\psi att}$ = number of colonies with at least one invasion attempt by a *Psithyrus*

$n_{\psi suc}$ = number of colonies successfully invaded by a *Psithyrus*

n_G, n_M = number of colonies that produced gynes (adults or pupae) and males (adults only) respectively

G_p, G_a = total number of gynes pupae and adults respectively

M_p, M_a = total number of males pupae and adults respectively

RS = reproductive success = $M + 3G$, where $M = M_p + M_a$ and $G = G_p + G_a$

W_{max} = maximal number of workers

Sex ratio = $G/(M + G)$ (n.b. based only on colonies that produced sexuals)

Table 4.2 General linear models testing for the effect of food addition on the square root of the reproductive success, *RS* ($r^2 = 0.37$), and on the natural logarithm of the maximal colony size, W_{max} ($r^2 = 0.33$), in *B. impatiens* and *B. ternarius* field colonies, in 1999 and 2000, in St-Jean-Chrysostome, Qc, Canada (*n* = 43 colonies). No interactions were significant below $\alpha = 0.15$. Predictive models are of the form: $\hat{y}' = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + ... + \beta_5 x_5$, where $\hat{y}'$ is the estimate of $\sqrt{RS}$ or Ln(W_{max}+1), and x_5 = mid-length of the queen's fore-wing in mm (see Methods).

		RS		W_{max}	
Source	df	F	P	F	P
Model	4, 38	4.38	0.003	3.68	0.008
Year	1, 38	1.51	0.226	9.08	0.005
Species	1, 38	5.13	0.030	5.01	0.031
Food addition	1, 38	3.83	0.039	4.51	0.020
Psithyrus	1, 38	10.84	0.002	8.11	0.004
Queen body size	1, 38	6.75	0.007	7.17	0.011

Parameter	*i*		x_i	β_i (*RS*)	β_i (W_{max})
Intercept	0			-59.52	-3.467
Year	1	1999	1	3.44	0.828
		2000	0		
Species	2	B. impatiens	1	-11.63	-1.131
		B. ternarius	0		
Treatment	3	Food addition	1	3.81	0.407
		Control	0		
Psithyrus	4	No invasion	1	7.34	0.625
		Invasion	0		
Queen body size	5			7.30	0.741

Table 4.3 Frequencies of bumblebee colonies that reached a given level of infestation (maximum number of larvae) by the bumblebee wax moth *Vitula edmandsae*, the lesser house fly *Fannia canicularis*, and *Brachicoma devia*, per treatment and host species in St-Jean-Chrysostome, Qc, Canada, in 2000. Also presented are the right-sided probabilities of 2 x 2 Fisher's exact tests for the effect of food supplementation on the incidence (*I*) and severity (*S*) of parasitism (see notes under the table).

			V. edmandsae					*F. canicularis*					*B. devia* *		
			Max no. larvae					Max no. larvae							
Host species	Treatment	*n*	0	1–49	≥ 50	*I*	*S*	0	1–99	≥ 100	*I*	*S*	None	Present	*I*
B. impatiens	fed	12	4	3	5	0.50	0.80	3	7	2	0.81	1	4	8	0.80
	controls	12	3	5	4			4	8	0			5	7	
B. ternarius	fed	5	3	1	1	0.08	1	3	2	0	0.26	-	1	4	0.78
	controls	5	0	5	0			1	4	0			1	4	

n = number of colonies

I - H_a: supplementing colonies with food reduced the probability that a parasite became present in a colony; categories > 0 were pooled to make 2 x 2 tables

S - H_a: supplementing colonies with food reduced the probability that a parasite reached a high level of infestation in a colony; categories < 50 (*V. edmandsae*) or < 100 (*F. canicularis*) were respectively pooled to make 2 x 2 tables

* Analysis restricted to presence vs. absence because we could not estimate the number of *B. devia* immatures in the brood and this parasite impacts the colony even at low densities.

Chapitre 5

Do bumblebees always forage as much as they could?

(Article scientifique soumis à la revue Insectes Sociaux, actuellement en évaluation. La présentation orale de cet article a gagné le Prix du Président au congrès annuel 2001 de la Entomological Society of America.
Pelletier, L. et J. N. McNeil. Do bumblebees always forage as much as they could?)

5.1 Résumé

Les bourdons doivent butiner pour approvisionner la colonie en nourriture. Toutefois, butiner est coûteux car la longévité des ouvrières est inversement reliée à l’effort dépensé à butiner. Étant donné ce compromis, les ouvrières des colonies dont les réserves en nourriture sont abondantes pourraient soit continuer à butiner pour augmenter ces réserves pour usage futur, soit butiner moins pour réduire les coûts associés à cette activité. Nous avons testé ces hypothèses avec 13 paires de colonies de *Bombus impatiens* sur le terrain. Nous avons fourni à la moitié des colonies une solution sucrée à volonté et du pollen à intervalle régulier, tout au cours de leur développement, alors que l’autre moitié des colonies servait de témoins. Nous avons mesuré le taux d’activité de butinage des colonies avec des détecteurs de mouvement à infra-rouge situés dans l’entrée des nichoirs. Toutefois, pour des raisons hors de notre contrôle (perte de la reine, usurpation par *Psithyrus*, débris dans le tunnel d’entrée, etc.), nous n’avons pu nous servir que des données sur deux paires de colonies pour l’analyse. Les colonies avec ajout de nourriture ont eu un taux d’activité de butinage par ouvrière 25% plus bas que les colonies témoins, ce qui appuie l’hypothèse que les ouvrières préfèrent minimiser les risques de butinage.

5.2 Abstract

Bumblebees must forage to provide food to the colony. However, foraging is costly as worker longevity is inversely related to foraging effort. Given this trade-off, workers from colonies with abundant food supplies could either maintain foraging to increase reserves for future use or forage less to avoid the associated costs. We tested these hypotheses over one summer, using 13 pairs of field colonies of *Bombus impatiens*. Half of the colonies were provided with a sucrose solution *ad libitum* and pollen at regular intervals throughout their entire development, while the other half served as controls. We measured the foraging activity rates in colonies with infra-red motion detectors fit in nest box entrances. However, due to

reasons beyond our control (loss of the queen, usurpation by *Psithyrus*, debris in the entrance tunnel, etc.), we could use data from only two pairs of colonies for the analysis. Food supplemented colonies had a foraging activity rate per worker 25% lower than controls which supports the hypothesis that workers prefer to minimise risks.

5.3 Introduction

Pollen and nectar are the two food resources necessary for bumblebees. Pollen is their primary protein source and is necessary for larval growth and egg production by reproductive females (Plowright and Pendrel 1977; Sutcliffe and Plowright 1988, 1990), whereas nectar is their major source of energy for activity and thermoregulation of the hive (Heinrich 1979). Both resources are initially acquired by the queen during colony development, and later by foraging workers. However, despite the apparent abundance of flowers in the field they are also exploited by numerous other insect flower visitors (e.g., honeybees, wasps, flies, and other bumblebees). Therefore, competition for limited resources (Morse 1982; Bowers 1985; Thomson 1989 and references therein) could lead to food shortages in bumblebee colonies.

Short-term food shortfalls affect colony development in many ways. They may increase the vulnerability of colonies to predators and parasites (Cartar and Dill 1991), provoke a drop in brood temperature and torpor in workers (Heinrich 1979), lengthen the development time of immatures (Plowright and Pendrel 1977; Sutcliffe and Plowright 1988, 1990; Cartar and Dill 1991), and result in the production of smaller adults (Sutcliffe and Plowright 1990). Colony nutritional status also affects forager activity as depriving the colony of a particular resource increases the collection of this commodity at the expense of the other resource (Schmid-Hempel et al. 1993; Plowright et al. 1993; Plowright et al. 1999; Landry et al. 2000; Plowright and Silverman 2000; Dogterom and Winston 1999 in honey bees). Furthermore, workers in nectar-depleted bumblebee colonies may switch from in-colony to foraging tasks (Cartar 1992a), take more risks foraging in the face of predators (Cartar 1991a), and be less aggressive towards predators and social parasites (Cartar and Dill 1991). Moreover, given equal means they prefer flowers with variable rewards over those with constant rewards (Cartar 1991b).

The effect of surpluses of one or both resources, especially in the long-term, are less well understood. How would foragers react to such surpluses? One hypothesis is that foraging could remain high to favour a higher production of offspring and/or to accumulate more reserves as protection against possible future adverse foraging conditions (e.g., a week of bad weather). An alternative hypothesis is that foraging would decline in order to reduce the costs associated with this activity (Schmid-Hempel et al. 1993). There are many risks and costs associated to foraging, notably a reduced longevity as foragers only live about two weeks (Garófalo 1978; Rodd et al. 1980; Goldblatt and Fell 1987) whereas workers in the hive may survive for a few months (Brian 1952). This reduced longevity may result from higher risks of predation and parasitism when outside the nest (Pouvreau 1974; Schmid-Hempel et al. 1990), faster wing wear caused by increased flight activity (Cartar 1992b), a depression of the immune system caused by increased energetic efforts (König and Schmid-Hempel 1995), and/or to the total expenditure of energy (Neukirch 1982 in honey bees). Workers, by prolonging longevity at the expense of not maximising instantaneous food delivery, could thus increase their lifetime contribution to the colony. Increased worker longevity could result in benefits at the colony level if it extends the period for sexual production after the colony stops producing workers. At the individual worker level, increased time spent in the hive would allow foragers more opportunities to lay their own eggs. The objective of this study is to test these hypotheses with respect to foraging behaviour through a long-term manipulation of food levels of field colonies.

5.4 Methods

5.4.1 General

Queens of *Bombus impatiens* Cresson were collected from the field in the spring of 2000 and used to establish colonies in the laboratory. Once the first brood of workers had emerged, 26 colonies were transferred to a 400 ha meadow in St-Jean-Chrysostome, Qc, Canada. We set colonies in above-ground wooden nest boxes, and boxes were on stakes brushed with Tanglefoot® to prevent invasions by ants. There was a succession and abundance of wild flowers throughout the summer in this meadow, the commonest being *Epilobium angustifolium* L., *Aster* spp., *Solidago* spp., *Eupatorium* spp., *Asclepias syriaca* L.. We censused colonies every 12 - 15 days to minimise disturbance.

5.4.2 Food addition

Colonies were paired: each pair being transferred to the field the same day and set about 20 – 30 m apart to reduce the effect of habitat variability. Within a pair, we assigned treatments at random. One was fed *ad libitum* a sucrose solution (sucrose:water 1:1 V/V) from a 130 ml gravity feeder, in a chamber at the back of the nest box. We also provided it a 5 g fresh pollen pellet at each census, the pellet placed directly on the brood, beside clumps of young larvae and eggs. The other colony served as a control. The bees clearly used the sucrose solution and pollen provided: honey pots were full of sucrose solution and pollen lumps had signs of feeding or had completely disappeared. However, we could not use the amount of sucrose solution taken as a measure of energetic consumption by the colony for despite all attempts to exclude them, other insects (mainly ants and wasps) often found the feeders. We allowed one month from the onset of resource manipulation before we started monitoring foraging activity, thereby ensuring that any long term effects of adding food would be apparent.

5.4.3 Forager activity

We had four activity meters, each consisting of an infra-red motion detector fitted to the entrance tunnel of the nest box and linked to a Hobo® event datalogger. Hence, we could measure activity on two pairs of colonies at a time. As data loggers were one channelled, we could not differentiate between entries and exits. There was a minimum delay of 5 s between each event recorded, reducing the risks of double-counting the same individuals as they went along the pipe, and extended the measurement period as the datalogger had a limit of 8000 recordable events. The foraging index was calculated as the hourly forager activity rate per number of workers in the colony. Colony size is known for any census date, and for intervening days we estimated it from a simple linear interpolation based on colony size at the previous and next census.

We tested the effect of food addition on the hourly activity of foragers with a mixed analysis of variance model, using PROC MIXED on SAS ver 8.00 (SAS Institute 1999), to take into account the temporal autocorrelation of data. Treatment (the addition of food), day, hour, and their interactions were considered fixed effects; whereas the colony and interactions of fixed effects with colony were random effects. We transformed the hourly forager activity by the square root. We calculated the coefficients of spectral contrasts according to Little and Hills (1978) and Littell and al. (1996). For each treatment, foraging activity is predicted using the Fourier equation (to take into account the daily cycle in the data):

$$\hat{y} = a_0 + a_1 \cdot \cos(C \cdot X) + b_1 \cdot \sin(C \cdot X) + a_2 \cdot \cos(2 \cdot C \cdot X) + b_2 \cdot \sin(2 \cdot C \cdot X) + \ldots$$

where $\hat{y}$ = predicted hourly foraging activity rate per worker, $C = 2\pi/16$, X = hour (0600 = 0), a_i and b_i = coefficients of spectral contrast for the ith degree cosine and sine functions, and a_0 = least-square mean for the given treatment (intercept).

During the period between setting the colonies out in the field and recording their activity, we lost at least one nest in 11 of the 13 pairs. The losses resulted from events beyond our control, including predation by skunk (one colony), usurpations by *Psithyrus* that eliminated the queen and/or a substantial part of the worker population (11 colonies), and loss of the queen for unknown reasons (5 colonies). In addition, we occasionally lost data from the intact colonies when the infra-red motion detector was obstructed by debris or nesting material workers had moved into the entrance tunnel during a given recording period. Consequently, the analysis was limited to data from two pairs of undisturbed colonies, differing only in the effect of the treatment, collected on the 16 – 18 and 21 – 24 August 2000, a period coincident with the emergence of the first sexuals.

5.5 Results and Discussion

There was a strong temporal periodicity in forager activity in all nests, with a peak between 1200 and 1700 (Figure 1; significant effect of Hour in Table 1). There was 25% less foraging activity in fed colonies compared with controls (ratio of least-square means = 0.75, Figure 1; significant effect of Food addition in Table 1) which was apparent throughout the day (Figure 1; interaction Food addition x Hour not significant in Table 1). Given the small final sample size (data on two pairs of colonies out of 13 pairs), these results must be interpreted with caution, although the number of colonies we used for analysis in our long-term field experiment is similar to most studies examining the short-term effects of providing additional food to bumblebee colonies (Cartar & Dill 1990, 1991; Cartar 1991a, 1992a; Plowright et al. 1993; Plowright et al. 1999; Landry et al. 2000; Plowright & Silverman 2000).

The results support the hypothesis that workers decrease foraging risks and costs by reducing their outdoor activity when there is no risk of colony food shortage. This finding corroborates earlier anecdotal reports that the addition of food to colonies made workers “lazy” (Sladen 1912) which results in them staying inside the nest (Brian 1952). Cartar (1992a) reported a 23% short-term decrease in the frequency of foraging trips after feeding colonies, which is very close to the 25% difference we observed between fed and control colonies.

The reduced foraging activity in fed colonies also supports the idea that workers are able to assess the colony nutritional state, and that they adjust their behaviour consequently (e.g., Cartar 1992a; Cartar and Dill 1990; Landry et al. 2000; Plowright et al. 1993; Plowright et al. 1999). However, the mechanisms involved remain unknown (Schmid-Hempel et al. 1993; Plowright and Plowright 1999). Since bumblebee workers are highly flexible in their task allocation (O'Donnell et al. 2000), one possibility is that less efficient foragers (e.g., the smaller workers) always remained in the nest, as seen in short-term feeding studies with sugar solutions (Cartar 1992a). Alternatively, all workers may simply have foraged less than under normal food availability conditions (see Fewell and Winston 1992 for honey bees). The answer probably lies somewhere between these two extremes, but will only be resolved by observing directly the contribution of each worker to the overall foraging activity in treated vs. control colonies.

Unfortunately, our monitoring system did not permit us to examine how the behaviour of individual workers contributed to the overall lower activity in fed colonies. It is difficult to conduct such observations in a natural setting as the presence of an observer within a few meters of the nest box disturbs foragers' behaviour and the effect appears to increase the closer one is to the nest (pers. obs.). Blinds set permanently beside nest box entrances may be a solution, but research would have to be carried out on private rather than on public land (we conducted our research on public land). The presence of blinds would draw attention to the colonies and, thus, increase the probability of vandalism.

Whether other social bees minimise foraging risks and costs when food supplies are sufficient remains to be examined. Honeybees may do so if, as proposed, they regulate food storage levels around a homeostatic, intermediate set point (Fewell and Winston 1992; Dogterom and Winston 1999). Other benefits from reduced foraging when food reserves are sufficient must also be explored in future studies.

For example, colonies with fewer food reserves would offer a lesser reward to predators, such as skunks.

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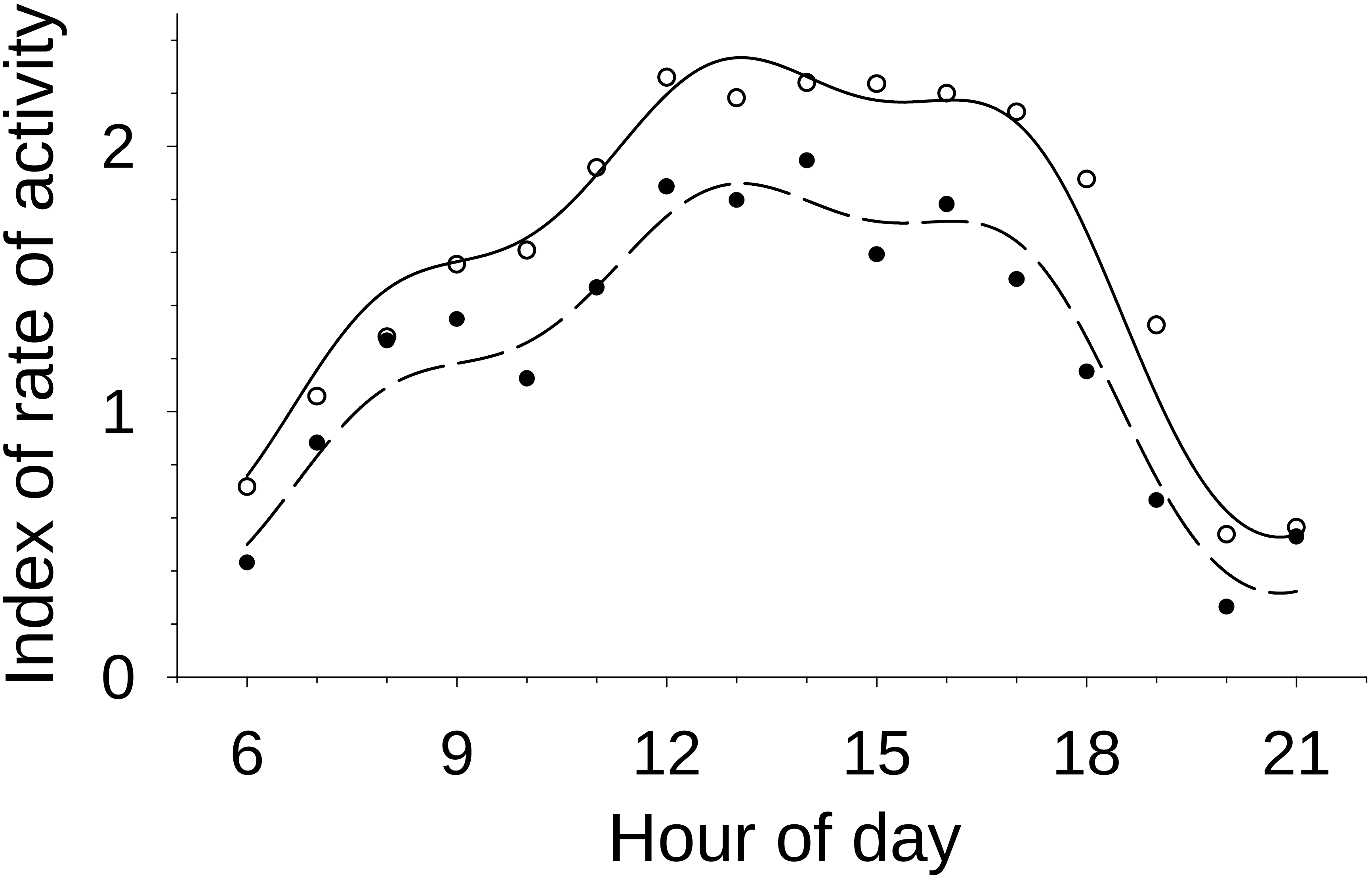
Table 5.1 Tests of fixed effects of the mixed analysis of variance looking at the effect of the long-term supplementation of food to *B. impatiens* field colonies on the index of hourly forager activity between the 16 – 18 and 21 – 24 August 2000, in St-Jean-Chrysostome, Qc, Canada.

Effect	df	F	P
Food addition (F)	1, 24.3	10.52	0.0034
Day (D)	5, 24.3	0.94	0.4709
F x D	5, 24.3	1.43	0.2478
Hour (H)	15, 121	17.17	< 0.0001
F x H	15, 121	1.02	0.4401
D x H	75, 121	1.82	0.0017
F x D x H	75, 121	0.98	0.5369

5.8 Figure legend

Figure 5.1 Observed (symbols – least-square means) and predicted (curves) hourly forager activity of control (n = 2, o, continuous line) and food supplemented *B. impatiens* field colonies (n = 2, •, dashed line) between the 16 – 18 and 21 – 24 August 2000, in St-Jean-Chrysostome, Qc, Canada. The index of foraging activity is the total number of entries and exits per hour corrected for colony size.



Index of rate of activity
0
1
2
6
9
12
15
18
21
Hour of day

Chapitre 6

Conclusion générale

Malgré la littérature abondante sur les bourdons, un nombre relativement restreint d'études se penche sur l'écologie des colonies, et très peu fournissent une information quantitative fiable à ce sujet. Cela est en partie dû à la difficulté de trouver des colonies et de suivre leur développement tout au cours de la saison. Grâce à beaucoup de persévérance et à l'élaboration de protocoles originaux, cette thèse s'attaque à cette lacune et génère des connaissances de base originales sur plusieurs aspects de l'écologie des colonies. Voici plus particulièrement les points saillants qui se dégagent de chacun des projets présentés et certaines perspectives de recherches qui en découlent.

6.1 Techniques de manipulation de colonies

Une certaine quantité d'information existait déjà sur l'élevage de bourdons en laboratoire et le marquage des bourdons, mais cette information était disparate ou incomplète. La manipulation de colonies, elle, n'était jamais abordée dans la littérature, autre que brièvement pour le transfert de colonies naturelles vers des nichoirs (Heinrich 1979; Kearns & Thomson 2000). La présentation des techniques au chapitre 2 permet enfin de faire le point sur l'ensemble de ces techniques et, surtout, de fournir une méthode complète pour manipuler les colonies de bourdons sur le terrain qui minimise le dérangement dû aux observateurs. La publication de cette information fournira un point de départ pour les néophytes désirant entâmer des projets de recherches sur les colonies de bourdons, tout en établissant une base de discussion pour les chercheurs ayant déjà une certaine expérience du sujet.

6.2 Taille corporelle de la reine

On ne savait pas si un attribut physique de la reine, tel que sa taille corporelle, pouvait affecter son succès à se reproduire. Le chapitre 3 démontre clairement que les reines plus grosses réussissent mieux, en général, à se reproduire que les reines plus petites de la même espèce. Si le plus grand succès des colonies des plus grosses reines semble provenir de leur capacité à produire de plus grosses colonies et d'une vulnérabilité moindre de leur colonies aux invasions de *Psithyrus*, la date d'établissement de la colonie et les invasions de reines *Bombus* ne semblent pas jouer comme facteurs. Fait notable, ces connaissances ont été générées grâce au suivi routinier d'un large échantillon de 185 colonies de 8 espèces, dont 81 colonies établies naturellement par la reine dans un nichoir (ce qui a entre autres permis d'établir les connaissances relatives au début de la colonie: date d'établissement, invasion par d'autres reines *Bombus* et taux de développement des colonies). Ce travail à grande échelle, mais avec des observations espacées de 12 – 15 jours sur chaque colonie, a permis de détecter l'effet de la taille de la reine et d'éliminer certains mécanismes potentiels par lesquels les reines pourraient bénéficier d'une plus grande taille. Ainsi, les plus grosses reines ne réussissent pas à mieux repousser les invasions d'autres reines *Bombus*, ce qui leur aurait permis de garder plus souvent le contrôle de leur colonie, et elles ne nichent pas plus tôt au printemps, ce qui aurait permis une plus longue durée du développement pour leur colonie. Il sera maintenant possible de passer à une échelle plus fine, en faisant des observations plus fréquentes sur un nombre moindre de colonies, pour tenter de tester d'autres mécanismes d'action possibles. En particulier, il faudrait explorer si les plus grosses reines sont plus fécondes et si elles dominent mieux leurs ouvrières.

6.3 Disponibilité en nourriture

Avant le projet du chapitre 4, on ne savait pas si la disponibilité en nourriture dans les milieux naturels où vivent les bourdons pouvait limiter le développement de leurs colonies et leur succès à se reproduire ou si, au contraire, d'autres facteurs tels que les parasites avaient une importance prépondérante et confondante. Cette expérience à long terme, en milieu naturel, a démontré qu'en effet l'acquisition de nourriture était en soi un facteur très important car deux espèces de bourdons, *B. impatiens* et *B. ternarius*, ont réussit à mieux se reproduire lorsque la nourriture leur était fournie directement dans la colonie. De plus, cette expérience a démontré que, pour les colonies, avoir plus de nourriture à leur disposition ne leur permettait pas de mieux se défendre contre plusieurs parasites de couvain, incluant la pyrale de la cire, *Fannia canicularis*, *Brachicoma devia*, et même les *Psithyrus* semble-t-il.

S'il est maintenant établi que les bourdons se reproduisent mieux lorsqu'il y a plus de nourriture, il faudrait dans un deuxième temps tenter de quantifier les ressources nutritives nécessaires pour que les colonies atteignent certains niveaux déterminés de croissance et reproduction. Cela pourrait s'avérer fort difficile à réaliser sur le terrain dû à la difficulté de restreindre le mouvement des butineuses. Une approche serait peut-être de faire les expériences en serres, en restreignant chaque colonie à une salle contenant un lot de ressources donné. Il faudrait également retenter l'expérience faite, mais cette fois sur des colonies établies naturellement, car la première phase de développement des colonies peut être critique au niveau de l'importance de la disponibilité de la nourriture. Une telle expérience pourrait donner des résultats encore plus marqués que ceux obtenus dans le cadre de mon expérience avec des colonies transférées du laboratoire sur le terrain.

6.4 Activité des butineuses

Jusqu'à présent, on ne savait pas si les ouvrières vivant constamment dans un monde plus riche en ressources auraient un comportement différent de celui d'ouvrières vivant dans un monde où les ressources sont limitées. L'expérience du chapitre 5 démontre que les ouvrières sont conscientes de la quantité de nourriture emmagasinée dans la colonie, et qu'elles modifient leur comportement pour éviter de gaspiller leurs efforts lorsque le butinage n'est pas requis. Si cela a déjà été en quelques sortes démontré à court terme (1 jour) (Cartar & Dill 1990; Cartar 1992a; Plowright et al. 1993; Plowright et al. 1999; Landry et al. 2000), on ne savait pas ce qu'il adviendrait lorsque les bourdons vivent continuellement dans ces conditions. En effet le contexte est différent car toute la vie de l'ouvrière se déroule alors dans l'abondance, éliminant ainsi tout repère de ce que constitue une mauvaise journée au plan alimentaire. Fait original, ces résultats sont basés sur les patrons d'activité de butinage au niveau de la colonie, et non seulement de quelques ouvrières. En outre, les patrons de butinage obtenus sont en continu sur plusieurs jours à la fois, sans dérangement par l'observateur, grâce aux compteurs électroniques développés pour l'expérience.

Toutefois, les bénéfices pour les ouvrières de réduire leur activité en situation d'abondance restent à démontrer. De même, il reste à élucider les mécanismes sous-jacents par lesquels la baisse d'activité au niveau de la colonie survient. C'est-à-dire, la baisse d'activité de butinage est-elle plutôt due i) à un évitement complet du butinage par seulement quelques individus qui en conditions normales (ressources limitées) auraient butiné, ii) par une fréquence de butinage moindre pour toutes les ouvrières que ce qu'elles auraient fait en conditions normales, ou encore iii) par un mélange des deux options ci-haut? Finalement, les résultats de cette expérience permettent de suggérer une hypothèse supplémentaire pour expliquer les bénéfices d'une disponibilité plus élevée en nourriture pour la colonie: comme il semble que les ouvrières des colonies ayant des réserves abondantes de nourriture réduisent leur activité de butinage, cela pourrait permettre à ces ouvrières de vivre plus longtemps et donc de se rendre utile plus longtemps pour la colonie (et peut être même d'étendre la durée de la colonie).

6.5 Parasitisme social

Les parasites de colonies sont connus depuis longtemps (Alford 1975) et plusieurs ont déjà fait l'objet d'expériences (ex. Fisher 1987a,b, 1988; Imhoof & Schmid-Hempel 1999). Toutefois, ce qui est resté méconnu est l'incidence de ces parasites sociaux et pratiquement tout ce qui touche leur établissement dans les colonies en milieu naturel. Malgré qu'aucun chapitre n'est dévoué spécifiquement au parasitisme, j'ai abordé le phénomène lors de l'étude de l'importance de la taille de la reine et de la disponibilité en nourriture.

Mes études permettent entre autre de quantifier l'incidence des usurpations par les *Psithyrus* et les reines *Bombus* étrangères. Ces résultats démontrent que l'ampleur du phénomène est énorme et que tout projet en milieu naturel doit tenir compte de ce facteur sous peine d'être voué à l'échec. La première étape qui vise à décrire le phénomène n'est pas encore complétée et devrait être une priorité étant donné l'importance des usurpations. Il s'agira entre autres de décrire i) le moment d'invasion (en fonction du calendrier et du degré de développement des colonies en nombre d'ouvrières) et les espèces hôtes privilégiées par chacune des espèces d'usurpatrices, ii) le taux de succès d'usurpation et le succès reproducteur des usurpatrices (en fonction de l'espèce hôte et du timing d'invasion), iii) les conséquences des usurpations pour les colonies, en ce qui a trait à la mortalité de la reine et des ouvrières et l'effet sur le succès reproducteur de la colonie, et iv) le taux de succès des colonies à repousser les usurpatrices et les variables pouvant affecter ce taux de succès. Des expériences devraient également être envisagées pour aider à déterminer les mécanismes aidant la localisation et l'usurpation de colonies, particulièrement en ce qui touche le comportement envers l'hôte et l'utilisation d'odeurs par les *Psithyrus* (Fisher 1988; Fisher et al. 1993a,b; Küpper & Schwammberger 1995).

J'ai également rapporté l'incidence et la sévérité d'infestations de pyrales de la cire, *Brachicoma devia* et *Fannia canicularis*, trois importants parasites de couvain; et déterminé que même si la colonie a accès à plus de nourriture, elle ne réussit pas à réduire la présence de ces parasites. Ce type d'information n'avait jamais été rapporté jusqu'à présent, mais ce n'est qu'un premier pas car il faudrait répertorier l'abondance des parasites de couvain dans d'autres habitats et régions étant donné qu'il est presque certain que leur abondance varie selon la localité. Tout comme pour les usurpatrices, il reste aussi comme première étape à décrire l'information de base sur l'établissement des parasites de couvain dans les colonies: i) le moment d'apparition dans les colonies (en fonction du calendrier et du développement des colonies en nombre d'ouvrières), ii) les espèces hôtes, iii) le succès des parasites à se reproduire (cela en fonction de différentes variables comme l'espèce hôte), iv) le succès des colonies à se reproduire malgré la présence de parasites. Comme expérience possible, on pourrait tenter de manipuler artificiellement le nombre de parasites (parmi ceux énumérés ci-haut) dans les colonies, par exemple en en retirant de certaines colonies pour les ajouter dans d'autres.

6.6 Intégration des connaissances acquises

On expliquait très mal jusqu'à présent ce qui pouvait causer l'énorme variabilité dans le succès reproducteur des colonies de bourdons en milieu naturel, ou du moins les explications plausibles n'étaient pas étayées quantitativement. Des projets expérimentaux ont bien été tentés par d'autres chercheurs, mais n'ont pas fonctionné (ex. Imhoof & Schmid-Hempel 1999). Les projets d'observations ont permis de suggérer des pistes, mais pas de déterminer quels facteurs spécifiques ont une importance et encore moins de quantifier leur effet (ex. Müller & Schmid-Hempel 1992a; Goulson et al. 2002). Mes projets, eux, ont permis d'expliquer sur une base factuelle une partie de cette variabilité du succès reproducteur en l'attribuant à l'espèce de *Bombus*, la taille corporelle de la reine, la disponibilité en nourriture (régulation « bottom-up ») et l'invasion par des *Psithyrus* (régulation « top-down »). La grande variabilité inexpliquée après avoir pris en compte ces facteurs démontre que plusieurs facteurs importants restent à identifier et explorer. Tel que mentionné dans l'introduction générale, on peut entre autre soupçonner une contribution du climat, d'autres ennemis naturels et de facteurs intrinsèques à la colonie.

Malheureusement, l'étude de ces autres facteurs peut s'avérer difficile. En effet, la plupart des facteurs pouvant jouer sur le succès de la colonie sont souvent dynamiques dans le temps (ex. taille de la colonie, disponibilité en nourriture), non-contrôlables (ex. niveaux de parasitisme, Imhoof & Schmid-Hempel 1999), difficilement observables (ex. suivi des butineuses sur le terrain), non-quantifiables (ex. certains parasites cachés dans le couvain et quantité de nourriture stockée non-quantifiables à moins de détruire le couvain) ou leur effet peut s'avérer compensatoire. La solution sera peut-être simplement de recourir à des protocoles expérimentaux ingénieux ou à des innovations technologiques, tel qu'observé récemment avec le développement du radar harmonique pour le suivi des déplacements des butineuses à moyenne échelle (Osborne et al. 1999; Riley et al. 1999). En outre, l'étude de colonies en laboratoire, une tendance de plus en plus répandue grâce au perfectionnement des méthodes d'élevage, peut s'avérer fort utile pour répondre à certaines questions (ex. pour la dominance de la reine sur les ouvrières), en autant qu'il y ait reconnaissance des artefacts produits par un tel dispositif expérimental (ex. longévité anormalement longue des ouvrières).

On note depuis quelques décennies une diminution de l'abondance et de la biodiversité des bourdons et plusieurs autres abeilles indigènes dans plusieurs régions d'Europe et d'Amérique du Nord (Kearns & Thomson 2001; Kremen et al. 2002). Une agriculture industrielle intensive (incluant l'utilisation de pesticides) et une urbanisation accroissante sont les principaux facteurs pointés du doigt, entre autre à cause de la dégradation des habitats naturels qui en résulte (Kearns & Thomson 2001; Thompson 2001).

On ne sait pas dans quelle mesure les facteurs naturels peuvent contribuer à l'érosion de l'abondance des bourdons. Ce qui est sûr est qu'on doit viser à mieux comprendre l'effet de tous ces facteurs, naturels et anthropiques, sur le développement des colonies. Ceci devrait permettre de prévoir des stratégies efficaces pour accroître l'abondance des bourdons, là où désiré, pour leur permettre de reprendre une partie de la place qui leur revient dans l'environnement et pour mieux les intégrer dans la pollinisation de certaines cultures, telles que la luzerne, le trèfle rouge, la canneberge et le bleuet (Holm 1966; Hobbs 1967; Macfarlane et al. 1994; Macfarlane & Patten 1997). En Amérique du Nord, la valorisation des populations de bourdons prend une importance accrue suite à la forte décroissance des populations d'abeilles domestiques au cours de la dernière décennie, due à leurs parasites et pathogènes (Webster & Delaplane 2001; Kremen et al. 2002).

Deux stratégies sont communément suggérées pour favoriser les populations de bourdons: l'installation de nichoirs et assurer la présence de fleurs mellifères tout au cours de la saison (Hobbs 1967a; Fussell & Corbet 1992; Macfarlane et al. 1984, 1994; Macfarlane & Patten 1997; Goulson et al. 2002). Malgré que le succès de ces stratégies reste à démontrer, elles n'en demeurent pas moins très sensées, surtout dans la mesure où on peut maintenant reconnaître l'importance de la disponibilité de la nourriture et en sachant que les nichoirs semblent bien combler un besoin pour des sites de nidification (plus de 50% de mes nichoirs en ville et en banlieue ont été occupés). En plus de ces deux stratégies, tout programme visant à accroître l'abondance des bourdons dans une région donnée devrait tenir compte de la pression exercée par les ennemis naturels et devrait, en particulier, tenter de limiter la pression exercée par les *Psithyrus* et les parasites de couvain (surtout la pyrale de la cire). Toutefois, on devra auparavant trouver comment y réussir.

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Annexe A.

Pourquoi le faire soi-même alors que d'autres peuvent le faire pour soi?

(Cet article de vulgarisation scientifique a gagné le premier prix du concours de vulgarisation scientifique du Centre de Recherches en Biologie forestière de l'Université Laval et a été publié dans le Bulletin de la Société d'entomologie du Québec :

Pelletier, L. 2002. Pourquoi le faire soi-même alors que d'autres peuvent le faire pour soi? Bulletin de la Société d'entomologie du Québec 9(3):19-20.)

Certains bourdons, fourmis et guêpes parasites ont résolument pris leur décision: faire travailler les autres pour eux. Chez les bourdons, un groupe d'abeilles sociales, cette stratégie est adoptée par des parasites du sous-genre *Psithyrus*. Ceux-ci sont de vrais bourdons (un néophyte ne saurait les distinguer les uns des autres), à la différence qu'ils doivent obligatoirement parasiter des colonies de bourdons pour se reproduire car ils en sont incapables seuls, de par leur inaptitude à récolter du pollen et à produire des ouvrières.

Leur stratégie est simple. Ils attendent qu'une reine bourdon consacre ses efforts à fonder une colonie et lorsqu'enfin plusieurs ouvrières sont présentes dans la colonie, l'opportuniste tente alors de prendre la place de la reine. L'usurpation comporte toutefois des risques car la reine n'a vraiment pas intérêt à se laisser déposséder des fruits de son travail en laissant la place au parasite. La bataille peut donc être meurtrière mais le parasite est bien équipé pour le combat. Le *Psithyrus* possède entre autres une cuirasse plus épaisse pour résister aux piqûres des ouvrières et de la reine et un dard plus long pour riposter aux attaques. Lors de l'entrée dans la colonie de bourdons, les ouvrières se ruent sur le parasite et forment une boule autour de lui. Le *Psithyrus* se défend tant bien que mal et tente de se cacher dans le couvain qui est plein de trous, un peu comme du gruyère. Une fois caché, le *Psithyrus* a beau jeu car il devient alors très difficile pour les ouvrières de le pourchasser. L'intrus passerait quelques jours à attendre caché jusqu'à ce qu'il soit imprégné de l'odeur de la colonie. Cela est très important car chez les insectes sociaux, ceux qui n'ont pas l'odeur de la colonie sont rejetés. Après quelques temps, les ouvrières ne voient plus le *Psithyrus* comme un ennemi de la colonie et ce dernier peut sortir de sa cachette. La reine vit maintenant côte à côte avec le *Psithyrus*, sauf que ce n'est plus elle qui contrôlera la destinée de la colonie. Une fois sa domination sur la colonie établie, le *Psithyrus* continue son plan machiavélique en détruisant systématiquement tous les œufs et larves de la reine et se met à pondre. La colonie n'y voit que du feu car elle s'occupera de la progéniture de l'intrus comme de la sienne. Des rejetons *Psithyrus* émergeront de la colonie quatre à six semaines après l'invasion de la colonie et les jeunes femelles *Psithyrus* hiverneront dans le sol pour être prêtes à envahir, à leur tour, de nouvelles colonies au printemps suivant. La reine et la colonie sont quant à elles bien en peine car elles n'auront pas réussi à produire de nouvelles reines.

Les *Psithyrus* sont très fréquents dans les colonies de bourdons. Tellement que cela peut compromettre l'existence de certaines espèces, surtout lorsqu'elles font face à d'autres inconvénients tels que la perte d'habitat et l'empoisonnement par les insecticides. Les bourdons étant très prisés pour la pollinisation de plusieurs cultures telles que les petits fruits, la luzerne et le trèfle rouge, nous aurions donc avantage à exclure les *Psithyrus* des colonies de bourdons pour favoriser le succès de ces valeureux pollinisateurs. Malheureusement, aucune solution n'a encore été trouvée pour combattre ce fléau.

C'est en s'attardant au comportement des reines au printemps que l'on peut soupçonner comment un groupe de parasites tel que les *Psithyrus* a pu évoluer. En effet, au printemps une grande proportion des reines ont tendance à attendre que d'autres reines fondent une colonie pour ensuite prendre leur place. La stratégie des reines bourdons paresseuses est donc la même que celle des *Psithyrus* avec la différence que, contrairement à ces derniers, les reines paresseuses ont toujours le choix de fonder leur propre colonie. Devant ce dilemme, les reines pourraient attendre le plus longtemps possible avant de fonder leur colonie en ayant toujours espoir d'usurper une autre reine. C'est en effet ce qui semble se produire: la période au printemps où les reines fondent une colonie s'étale sur plus d'un mois (il n'y a donc pas de hâte apparente à fonder une colonie) et les combats entre reines sont nombreux. Les bourdons ne sont donc pas si dociles entre eux qu'ils semblent l'être à nos yeux lorsqu'ils butinent dans les fleurs!

Tel que mentionné initialement, ce type de parasitisme social survient également, avec quelques variantes, chez d'autres groupes d'insectes sociaux tels que les guêpes et les fourmis. Il est toutefois surprenant qu'on ne répertorie pas de tels parasites chez les abeilles domestiques et les termites qui sont également des insectes sociaux. Bien sûr, l'humain étant un animal social, il n'y a qu'un pas àfranchir pour tenter de savoir si l'équivalent d'un tel opportunisme existe dans notre propre société...

Annexe B.

Entomophily of the cloudberry (*Rubus chamaemorus*)

(Paru comme article scientifique dans la revue *Entomologia experimentalis et applicata*:
Pelletier, L., A. Brown, B. Otrysko et J. N. McNeil. 2001. Entomophily of the cloudberry (*Rubus chamaemorus*). Entomologia experimentalis et applicata 101: 219-224.)

Abstract

Cloudberry (*Rubus chamaemorus* L.), a dioecious perennial plant of boreal circumpolar distribution, is greatly prized for its berries.We crossed two treatments, pollinator exclusion and supplementary hand-pollination, to determine i) the relative importance of insects as pollinators, ii) if pollinator activity was a limiting factor for the sexual reproduction of the plant, and iii) the relative contribution of diurnal vs. nocturnal visitors to pollination. The activity of natural pollinators resulted in 97.5 and 88.5% fruit set, along with 76.7 and 62.5% seed set in 1998 and 1999, respectively. When insects were excluded, fruit-set dropped significantly to 18.4 (1998) and 12.8% (1999) and seed-set to 5.4 (1998) and 5.4% (1999) showing the importance of mid- and large-sized insects as pollinators. Natural levels of insect activity were sufficient to ensure complete pollination in both years as supplementary hand-pollination did not significantly increase either parameter in plots where pollinators had free access. Nocturnal insects may serve as pollinators (fruit-set = 41%), although they were less effective than diurnal pollinators (fruit-set = 93%).

Introduction

Cloudberry (*Rubus chamaemorus* L., Rosaceae) is a dioecious perennial plant of boreal circumpolar distribution. The plant spreads mainly by means of an extensive rhizome system (Rapp et al., 1993), with 94% of total biomass going to underground organs responsible for vegetative propagation and only 0.05% allocated to sexual reproduction (Dumas & Maillette, 1987). While the plant allocates few resources to sexual reproduction, the fruits are a prized commodity and so the factors limiting fruit production have received some attention.

Frost appears to be an important abiotic factor limiting fruit production, as late spring frosts can kill a significant proportion of the flowers and fruits (Dumas & Maillette, 1987; Rapp et al., 1993), which occurred in Baie Saint-Ludger, Qc, in 1998 (pers. observ.). The availability of nutrients may also limit yield as fertilization of bogs with superphosphate or complete fertilizers increased yields several fold (Rapp et al., 1993). Herbivory and parasites may also limit the reproductive success (see Ågren, 1987, 1989), as well as insufficient pollination, especially if the plant is principally entomophilous since female flowers are reported to offer little reward (no pollen and small amounts of nectar) to insect visitors (Ågren et al., 1986).

There is an abundant and diversified diurnal insect fauna known to frequent cloudberry flowers. In Fennoscandia, Hippa et al. (1976, 1981a, 1981b, 1981c) differentiated two groups of insects found on cloudberry flowers: flower stayers and flower visitors. Flower stayers, such as thrips and staphylinids, were very poor cloudberry pollen-carriers while the best pollen-carriers were considered to be the diurnal medium- and large-sized flower visitors such as hymenopterans (Apidae and Formicidae) and dipterans (Syrphidae, Muscidae, Empididae, Fanniidae, Coelopidae, and Heleomyzidae) (Hippa et al., 1981c). However, these observations only provide indirect evidence that insects serve as pollinators as anemogamy may also play a role, especially in windswept coastal areas (Rapp et al., 1993).

We therefore conducted a study to examine cloudberry pollination and the three objectives of this study were to experimentally determine:

1. the relative importance of insect visitors vs. wind and small insects in the pollination of cloudberry by comparing the reproductive success in quadrats with and without cages that excluded medium and large-sized insects;
2. if natural pollination, presumably by insects, was sufficient to ensure that all flowers were pollinated by comparing the reproductive success in quadrats where all flowers had supplemental hand pollination with those with natural pollinators only; and
3. if nocturnal visitors contributed to the pollination, and quantify their relative effectiveness to diurnal ones by comparing the reproductive success in

quadrats that were accessible to pollinators only during the photophase or the scotophase.

Material and methods

We conducted our experiments in a *Sphagnum fuscum* peat bog at Hâvre-Saint-Pierre (50°16’N, 63°34’W), on the north shore of the St-Lawrence River, Québec, Canada. The site is a wet, coastal windswept area, bordered to the north by boreal forest. The cloudberry is one of the first plants to flower in the bog, with the peak of female flowering occurring about a week after that of males (pers. observ.).

To address the first two objectives, we crossed the exclusion of pollinators and supplemental hand-pollination treatments. Each of the four combinations of treatments was assigned randomly to ten 1.2 m x 1.2 m (2.44 m^2) quadrats, for a total of 40 quadrats. We marked all female flowers within quadrats with a numbered vinyl tag attached around the base of the shoot. Only quadrats with more than three female flowers were kept for the analysis. We conducted this experiment twice, in 1998 and in 1999.

To address the third objective, we added ten insect exclosure quadrats in 1999. Flowers in five exclosures were available to pollinators only during the day (official photophase from about 04:00 to 20:30), and the other five only at night (official scotophase from about 20:30 to 04:00). This was accomplished by removing the exclusion cages for the designated pollination period each day. We compared the reproductive success of these two groups with that of the permanently open and permanently closed quadrats.

To hand-pollinate flowers, we collected pollen by brushing dehiscent anthers of male flowers with a small paintbrush (damp in 1998, dry in 1999), and checked for the presence of pollen with a 10x magnifying glass before brushing the stigmas of the female flower several times. For each female flower, we repeated the operation with the pollen of at least two males. Different individuals hand-pollinated flowers in the two years of the study.

Insect exclosure cages (1.2 m long x 1.2 m wide x 0.4 m high) were made of a wooden frame and vinyl insect netting (size of mesh = 1.4 mm). The surface area of openings in the insect netting (1.96 mm^2) was 2700 times larger than the planar surface of a cloudberry pollen grain (7.1×10^{-4} mm^2) based on an approximate diameter of 30 µm (Eide, 1981). Hence, openings in the netting were large enough to allow pollen to pass. Furthermore, there were male plants within the enclosure cages that could serve as a source of pollen.

To reduce any possible beneficial or deleterious effect of enclosing plants on fruit development we minimised enclosure time. We set up quadrats and exclosures on 3-5 June 1998 and 1 June 1999, with marking and hand-pollination occurring a week later (10-12 June 1998, 6-16 June 1999). We removed exclosures two weeks after set-up, after the last female flowers had shut. We harvested the fruits prior to complete maturation (5-6 July 1998, 7-8 July 1999) as locals would harvest mature fruits, despite notices that these were research plots.

Variables. We used three variables as indices of the reproductive success of cloudberry flowers per quadrat:

a) fruit-set: the proportion of female flowers which developed into a fruit.

b) mean seed-set: the average of the individual seed-sets. The individual seed-set being the ratio of the number of drupes (a fruit being composed of drupes and each drupe contains one seed) on the total number of ovules for each fruit. The total number of ovules was determined by dissecting the unripe fruits under the binocular (10x).

c) the mean fresh weight of unripe fruits. Within a given year, we collected all of the fruits at the same date and weighed them on a pan balance (precision = 1×10^{-2}g). As noted above it was not possible to wait and harvest ripe fruit, so our estimate of fresh weight should only be considered as complementary information to the other two measures of reproductive success.

Statistics. We tested the effect of hand-pollination, insect exclosures, of the year, and of all possible interactions among these factors, on fruit set with multiways analyses of variance (ANOVA). We used the general linear model (GLM) procedure in SAS (SAS Institute, 1996) to conduct the ANOVAs because of the unequal cell sizes. The unbalanced design resulted from the fact that we did not include quadrats with less than three flowers in the analyses. We used the same analyses for seed set and fresh weight but the data for the two years were analysed separately due to difference in hand pollination efficacy and the fact that fruits were collected before they were fully mature. We tested the effect of the regime of day or night exclosure on fruit-set in 1999 with a one-way ANOVA.

We transformed proportions of the fruit-set per quadrat with the arcsine transformation of Freeman and Tukey (1950, cited in Zar, 1999):

$$p' = \frac{1}{2}\left[\arcsin\sqrt{\frac{X}{n+1}} + \arcsin\sqrt{\frac{X+1}{n+1}}\right] \quad (1)$$

where p’ is the transformed proportion and X/n = p is the actual proportion. We transformed proportions of the mean seed-set per quadrat with the common arcsine transformation (Zar, 1999): $p' = \arcsin\sqrt{p}$. Standard errors (s.e.) were calculated using the transformed data and then reconverted to proportions with $p = (\sin(p'))^2$. We checked normality in the distribution of residuals with the Kolmogorov-Smirnov’s test and homoscedasticity with Levene’s test. We used three multiple comparisons tests: the Tukey’s studentized range test, the Bonferonni t-tests, and the Student-Newman-Keuls (SNK) test. We set the level of significance to $P < 0.05$ for all statistical tests.

Results

Medium to large sized insects are necessary for effective cloudberry pollination (significant effect of exclosure in Table 1), as in both 1998 and 1999 fruit-set (Table 2), seed-set (Table 3), and the fresh weight of unripe fruits (Table 4) were greatly and significantly reduced in quadrats with exclusion cages compared with those in open controls. Wind and small insects (e.g., blackflies, thrips) only pollinated a very small fraction of flowers, as seen in exclosure quadrats without supplemental pollination (Table 2). Furthermore, the flowers pollinated by these vectors gave poor quality fruits as shown by the comparison between open habitats and exclosures of the seed-set and fresh weight of unripe fruits of flowers which succeeded to develop into a fruit (Table 5).

Insect activity was sufficient to fully pollinate female flowers in both years of the study as supplementary hand-pollination in open habitats did not significantly increase any of the parameters of reproductive success (Tables 2, 3, 4). This was not due to an ineffective technique as hand-pollination significantly increased fruit production in exclosures (Tables 2, 3, 4).

There is a highly significant difference in the efficacy of noctural and diurnal pollinators, as measured by fruit set (one-way ANOVA: $F = 45.90$, $df_{model} = 3$, $df_{error} = 26$, $P = 0.0001$) (Fig. 1). While nocturnal pollinators significantly increased fruit set over the controls where insects were always excluded, they were not as effective as diurnal pollinators (Fig. 1). The activity of diurnal pollinators, such as bumblebees and syrphids, was sufficient to fully pollinate cloudberry flowers, for fruit-set was as high in quadrats open only during the day as in those always open (Fig. 1).

Discussion

Fruit set was high (>94%) in our study when compared with previous reports in northern Quebec (57%) (Dumas & Maillette, 1987) and in spruce mires of northern

Sweden and Finland (72.5 – 75.6%) (Ågren, 1989), although values >90% have been reported (Ågren, 1987). The results of the present study clearly demonstrate the importance of insects relative to anemogamy for the pollination of the cloudberry. Thus some of the site and year differences reported in fruit-set may well be due to factors influencing the density and/or activity of potential pollinators. This is particularly true when one considers that cloudberry grows in circumboreal regions where the climatic conditions in spring are highly variable, and that individual flowers only last for 2-3 days (Ågren, 1987). The high fruit-set obtained in the present study may, at least in part, be attributed to the mild spring conditions encountered in both years. Higher temperatures would not only have decreased losses due to spring frost but would also have favoured the activity of potential insect pollinators.

It is clear from the comparison of fruit-set in quadrats open only at night and those always closed (Fig. 1) that nocturnal pollinators do exist in the habitat. The fact that we found unidentified lepidopteran larvae (Tortricidae) feeding on leaves and fruits in both years make different Lepidoptera potential candidates. However, their relative contribution to cloudberry reproductive success was minor compared to diurnal pollinators (Fig. 1). In other words, in 1999 diurnal visitors were sufficient for pollination, and nocturnal visitors could be seen as complementary pollinators. Nonetheless, if the short duration of exclosure opening at night (7.5 h) compared to the duration of opening at day (16.5 h) were taken into account, nocturnal insect visitors could prove to be as efficient pollinators as diurnal visitors. In years where overall insect numbers are low in the habitat of the cloudberry, the activity of noctural pollinators could significantly contribute to the sexual reproduction of the plant. Furthermore, noctural pollinators are believed to have a lower frequency of flower visitations than diurnal ones during foraging and to transfer pollen over greater distances (Herrera, 1987). If true, then this could be important as it might increase the genetic heterozygosity of a plant with a high degree of clonal propagation.

The next step will be to determine experimentally the effectiveness and efficiences of the most important groups of diurnal and nocturnal insects visiting cloudberry flowers.

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Table 1. Multiway ANOVAs of the effect of the year, insect exclosures, hand-pollination, and all possible interactions among these factors, on three different measures of reproductive success: a) the fruit-set (combined analysis for 1998 and 1999), and b) the seed-set and the fresh weight of unripe fruits (distinct analyses for 1998 and 1999, see Methods for details) of cloudberry in Hâvre-Saint-Pierre. Power of the test provided when not significant.

		df	F	P	Power
Fruit-set	Model	7	36.44	0.0001 ***	
	Year	1	0.86	0.3575 n.s.	0.534
	Exclosure	1	125.92	0.0001 ***	
	Hand pollination	1	47.63	0.0001 ***	
	Year x Exclosure	1	3.30	0.0746 n.s.	0.503
	Year x Hand pollination	1	8.89	0.0042 **	
	Exclosure x Hand pollination	1	42.46	0.0001 ***	
	Year x Exclosure x Hand pollination	1	1.92	0.1707 n.s.	0.505
	Error	59			
	Total	66			

		1998			1999		
		df	F	P	df	F	P
Seed-set	Model	3	34.88	0.0001 ***	3	40.42	0.0001 ***
	Exclosure	1	82.64	0.0001 ***	1	40.88	0.0001 ***
	Hand pollination	1	4.47	0.0447 *	1	46.42	0.0001 ***
	Exclosure x Hand pollination	1	8.76	0.0067 **	1	30.07	0.0001 ***
	Error	25			34		
	Total	28			37		
Fresh weight	Model	3	21.19	0.0001 ***	3	20.01	0.0001 ***
	Exclosure	1	48.51	0.0001 ***	1	21.85	0.0001 ***
	Hand pollination	1	4.64	0.0411 *	1	24.57	0.0001 ***
	Exclosure x Hand pollination	1	4.91	0.0361 *	1	11.82	0.0016 **
	Error	25			34		
	Total	28			37		

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ n.s. non-significant

Table 2. Effect of insect exclosures and supplementary hand-pollination on the fruit-set (%) of cloudberry in Hâvre-Saint-Pierre in 1998 and 1999. Mean per combination of treatments ($\overline{X}$) ± standard error (s.e.) and number of quadrats (n). Means with the same letters are not significantly different ($P \geq 0.05$). Results of the SNK, Tukey, and Bonferonni multiple comparisons tests are identical

		Exclosure			Open habitat		
		n	$\overline{X}$ ± s.e.		n	$\overline{X}$ ± s.e.	
Hand-pollination							
	1998	7	61.1% ± 12.0%	B	6	95.4% ± 2.5%	A
	1999	9	89.4% ± 4.7%	A	9	94.3% ± 2.2%	A
Control							
	1998	9	18.4% ± 7.6%	C	7	97.5% ± 1.6%	A
	1999	10	12.8% ± 5.0%	C	10	88.5% ± 3.2%	A

Table 3. Effect of insect exclosures and supplementary hand-pollination on the seed-set (%) of cloudberry in Hâvre-Saint-Pierre. Separate analyses for 1998 and 1999 (see methods for explanations). Mean per combination of treatments ($\overline{X}$) ± standard error (s.e.) and number of quadrats (n). Within years, means with the same letters are not significantly different. Results of the SNK, Tukey, and Bonferonni multiple comparisons tests are identical

		Exclosure			Open habitat		
		n	$\overline{X}$ ± s.e.		*n*	$\overline{X}$ ± s.e.	
1998	Hand-pollination	7	31.3% ± 7.6%	B	6	70.1% ± 5.6%	A
	Control	9	5.4% ± 2.9%	C	7	76.7% ± 3.3%	A
1999	Hand-pollination	9	67.4% ± 5.2%	A	9	73.2% ± 3.2%	A
	Control	10	5.0% ± 2.7%	B	10	62.5% ± 7.1%	A

Table 4. Effect of insect exclosures and supplementary hand-pollination on the fresh weight (g) of unripe cloudberry fruits in Hâvre-Saint-Pierre. Separate analyses for 1998 and 1999 (see methods for explanations). Mean per combination of treatments ($\overline{X}$) ± standard error (s.e.) and number of quadrats (n). Within years, means with the same letters are not significantly different. Results of the SNK, Tukey, and Bonferonni multiple comparisons tests are identical

		Exclosure			Open habitat		
		n	$\overline{X}$ ± s.e.		*n*	$\overline{X}$ ± s.e.	
1998	Hand-pollination	7	0.15 ± 0.04 g	B	6	0.28 ± 0.02 g	A
	Control	9	0.03 ± 0.02 g	C	7	0.26 ± 0.03 g	A
1999	Hand-pollination	9	0.44 ± 0.06 g	A	9	0.50 ± 0.04 g	A
	Control	10	0.04 ± 0.02 g	B	10	0.43 ± 0.06 g	A

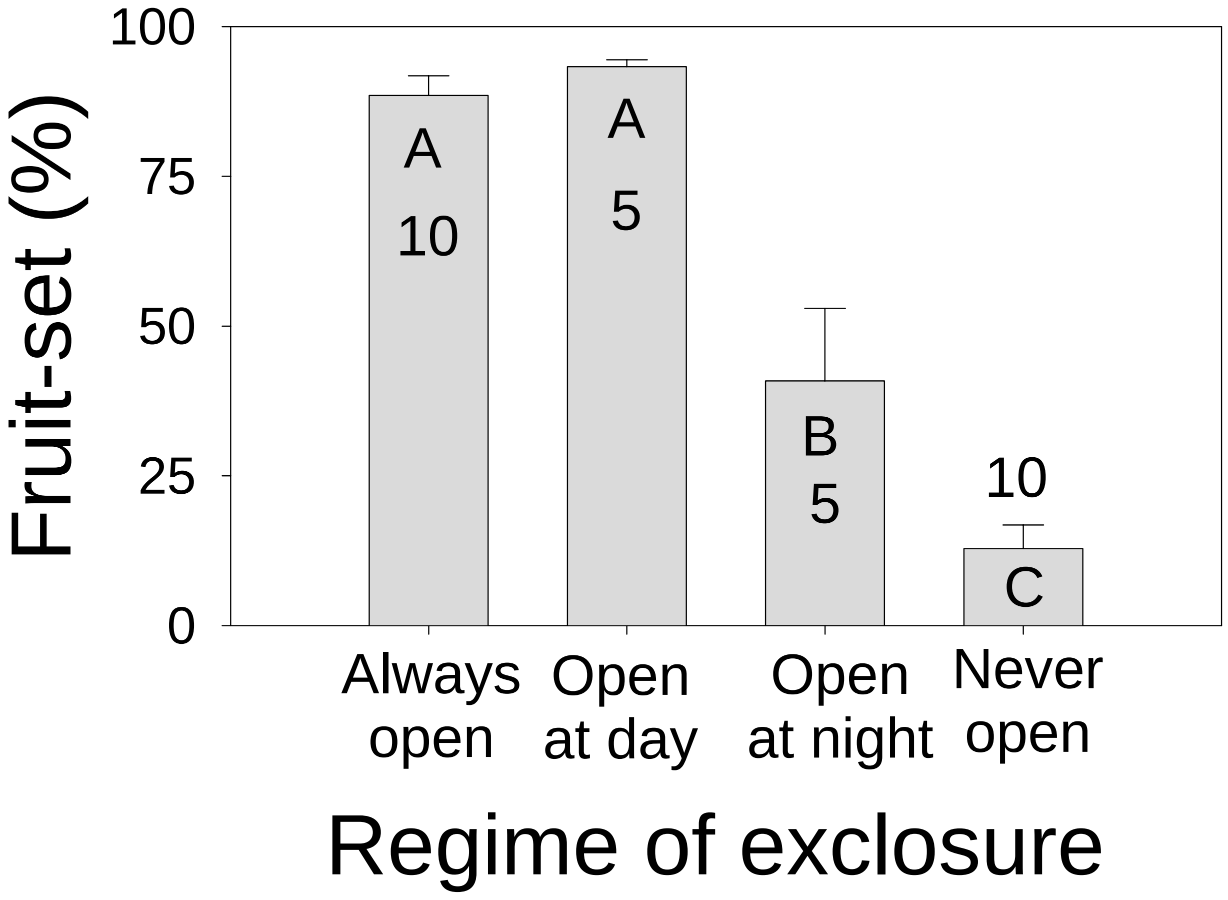
Table 5. T-tests of the effect of insect exclosures on the seed-set (%) and fresh weight (g) of unripe fruits of the cloudberry flowers that gave rise to a fruit at Hâvre-Saint-Pierre in 1998 and 1999. Mean per treatment ($\overline{X}$) ± standard error (s.e.) and number of quadrats (n)

		Exclosure		Open habitat			
		n	$\overline{X}$ ± s.e.	*n*	$\overline{X}$ ± s.e.	t	P
1998	Seed-set	6	32.4 ± 11.4 %	7	78.6 ± 2.5 %	3.86	0.0096 **
	Fresh weight	6	0.20 ± 0.06 g	7	0.29 ± 0.03 g	1.46	0.17 $^{n.s.}$
1999	Seed-set	10	31.6 ± 5.6 %	8	84.1 ± 7.8 %	5.21	<0.0001 ***
	Fresh weight	10	0.26 ± 0.06 g	8	0.55 ± 0.05 g	3.99	0.0011 **

* P < 0.05 ** P < 0.01 *** P < 0.001 n.s. non-significant

Figure legend

Figure 1. Effect of four different regimes of exclusion of insect visitors on the fruit-set of cloudberry in Hâvre-Saint-Pierre in 1999. Mean fruit-set per combination of treatments ± standard error (error bars) and number of quadrats. Means with the same letters are not significantly different. Results of the SNK, Tukey, and Bonferonni multiple comparisons tests are identical.


Fruit-set (%)
100
75
50
25
0
A
10
A
5
B
5
10
C
Always open
Open at day
Open at night
Never open
Regime of exclosure