



Étude sur la pression de sélection alimentaire chez la tordeuse des bourgeons de l'épinette

Thèse

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Résumé

La détérioration de la qualité de la nourriture affecte la performance biologique des insectes. Ce facteur pourrait être un moteur évolutif qui amène les insectes à entrer dans des processus adaptatifs à court terme. Les épidémies de la tordeuse des bourgeons de l'épinette (TBE) altèrent considérablement la qualité nutritionnelle des hôtes. Lors d'une infestation qui s'étend sur plusieurs années, le manque de nourriture et sa mauvaise qualité exercent une pression de sélection importante sur les populations. Sous ces conditions, on peut penser que certaines stratégies adaptatives sont sélectionnées qui permettent à la TBE de persister dans l'environnement. Ces stratégies sont intimement liées à des traits de vie, tels que la fécondité, la survie et le poids des chrysalides ainsi qu'à la durée de vie.

La présente étude a pour objectif d'étudier le potentiel d'adaptation de la TBE soumise à un stress nutritionnel en analysant particulièrement les traits associés à l'histoire de vie.

Une colonie de tordeuses des bourgeons de l'épinette a été élevée sous deux types de régimes alimentaires artificiels (une diète témoin et une autre induisant un stress en sucres et en azote) pendant trois générations successives. Les variables mesurées étaient : la mortalité, le temps de développement, la fécondité, le poids des chrysalides, le taux de croissance, et la fertilité. Les résultats montrent qu'il y a effectivement une pression de sélection. La forte mortalité causée par le stress alimentaire fait en sorte que les insectes les mieux adaptés ont le potentiel de se reproduire. En moyenne les femelles résiduelles sont plus lourdes et donc la fécondité moyenne est plus élevée dans la dernière génération. Les résultats montrent une forte variabilité additive en ce qui concerne la fécondité, la fertilité des œufs et le temps de développement, et une plus faible au niveau du poids des chrysalides. Il semble que les stratégies évolutives qui sont sélectionnées via le stress alimentaire chez la tordeuse des bourgeons de l'épinette soient particulièrement liées au temps de développement, à la fécondité, au taux de croissance et au poids des chrysalides. Les différences de performances biologiques entre les sexes indiquent une distorsion en faveur des mâles. Ce facteur pourrait stimuler la dispersion des mâles vers des zones où les ressources alimentaires seraient plus abondantes. L'ensemble des résultats obtenus démontre de façon évidente que la TBE possède une importante capacité d'adaptation et d'évolution en fonction de la qualité du régime alimentaire de l'insecte.

Abstract

Deterioration in food quality can affect insect performance. This factor could be an evolutionary engine that forces insects to go through short-term adaptive processes. Outbreaks of spruce budworm (*Choristoneura fumiferana* (Clem.)) significantly affect the quantity and quality of food that is provided by host trees. This phenomenon exerts a strong selection pressure on the population, especially when there is a shortage of food or when food is of poor quality. Thus, various adaptive strategies can be selected for so the species can persist in the environment. These strategies are closely linked to life-history traits such as fecundity, survival, pupal mass and developmental time.

The present study investigated the potential for adaptation in the spruce budworm, which had been subjected to nutritional stress, by analyzing particular features that are associated with life-history traits.

A colony of spruce budworm larvae was reared on two kinds of artificial diet over three generations (control versus a stress diet with low sugar and high nitrogen contents). The variables that were considered included developmental time, fecundity, pupal mass, growth rate and fertility. Our results showed that a selection pressure was indeed being exerted; the high mortality produced by the stress diet resulted in females having higher pupal mass and fecundity in the last generation.

The results revealed high additive variability for fertility, fecundity and developmental time, and a lower additive variability for pupal mass. It appeared that the evolutionary strategies of spruce budworm selected for under diet stress are related to developmental time, fecundity, growth rate, pupal mass. Differences in biological performance between the sexes indicate a bias in favour of males. This factor could stimulate dispersal of males to other locations where resources are more abundant. The overall results clearly showed that this insect has high adaptability and evolutionary potential when food quality affects its biological performance. This phenomenon should be further considered in studies of population dynamics.

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Avant-propos

Malgré les nombreuses recherches sur la tordeuse des bourgeons de l'épinette, les processus adaptatifs de cet insecte défoliateur restent méconnus. Pourtant, la connaissance des stratégies adaptatives de ce ravageur forestier est primordiale en considérant l'ampleur des infestations au Canada, la grande variabilité de la composition forestière et les changements climatiques. Sachant que la performance biologique de la TBE dépend de la disponibilité de la nourriture et de la qualité nutritionnelle, l'idée d'étudier les processus d'adaptation de cet insecte par rapport à sa ressource alimentaire a surgi.

La présente thèse est composée de quatre chapitres. L'auteur principal des quatre chapitres est le candidat au doctorat. Pour chacun des chapitres, l'auteur principal a planifié, réalisé, analysé et interprété les résultats. Il a également écrit les manuscrits. Le Dr. Éric Bauce a établi les hypothèses de travail, planifié l'étude et contribué à l'interprétation des résultats dans tous les chapitres. Le M.Sc. Lukas Seehausen a contribué dans l'analyse et l'interprétation des résultats dans le deuxième chapitre, dans le troisième chapitre Dr. Fuentealba a contribué à l'analyse et l'interprétation des résultats et finalement la Dre Pureswaran a contribué à l'interprétation des résultats dans le dernier.

La présente thèse est composée de quatre chapitres. Le premier chapitre traite de l'héritabilité impliquée dans les caractères d'histoire de vie. Autrement dit, il y sera question du potentiel d'adaptation de la TBE. Le deuxième chapitre porte sur la vitesse d'adaptation de la TBE à un stress nutritionnel continu. Le troisième sur les stratégies adaptatives que l'insecte emploie dans l'utilisation de la nourriture. Finalement, le quatrième chapitre examine le déséquilibre du rapport des sexes chez la tordeuse après un stress nutritionnel. Ce déséquilibre nous laisse entrevoir une mortalité différentielle entre les mâles et les femelles. Chaque chapitre est composé d'un article en anglais et de son résumé en français. Le premier, le deuxième et le quatrième sont déjà publiés, le troisième a été soumis à une revue. L'introduction, les résumés de chaque chapitre et la conclusion sont écrits en français.

1 Introduction Générale

La tordeuse de bourgeons de l'épinette (TBE) [*Choristoneura fumiferana* Clemens (Lepidoptera : Tortricidae)] est l'un des insectes les plus problématiques pour l'industrie forestière en Amérique du Nord (Blais 1983, Montgomery *et al.* 1983, Sanders 1991). Cet insecte apparaît périodiquement sur de grandes superficies forestières causant des impacts économiques considérables. Pendant les années 1970, la TBE a causé de sévères défoliations sur plus de 50 millions d'hectares au Québec (Sanders 1991). Les infestations de ce lépidoptère représentent un phénomène naturel résultant d'une coévolution entre l'insecte et leurs plantes hôtes (Blais 1985b), qui date de plusieurs siècles (Krause et Morin 1997, Burleigh *et al.* 2002). À cause de sa grande capacité destructrice, la TBE a été l'objet de nombreuses recherches, afin de découvrir la cause des infestations. La réponse n'est pas encore clarifiée. Toutefois, plusieurs variables pourraient y jouer un rôle important. Par exemple les variations périodiques de la composition forestière, l'âge des peuplements, les conditions climatiques, les feux de forêt, la sécheresse et la disponibilité des nutriments. Depuis le début du vingtième siècle, la fréquence et l'étendue des infestations augmentent dans l'est de l'Amérique du Nord. Les dommages causés par la TBE sont très préoccupants en raison des pertes potentielles de ressources ligneuses et non ligneuses qu'ils peuvent entraîner dans les forêts boréales (Ressources Naturelles Canada 2010). Cependant la TBE joue un rôle important dans l'écologie des forêts du Canada, en favorisant la régénération des peuplements (Blais 1983). Des études montrent que des défoliations successives d'une année à l'autre s'accompagnent de changements dans les concentrations foliaires en nutriments (Bauce et Hardy 1988). Ainsi, un tel changement de la qualité de la nourriture pourrait exercer une pression de sélection. Les individus seraient donc soumis à des processus évolutifs à court terme (Carroll *et al.* 2007). Lors d'une infestation, le grand nombre d'individus réduit la quantité et la qualité du feuillage, ce qui affecte considérablement la performance biologique des défoliateurs (Tuomi *et al.* 1984, Haukioja *et al.* 1991). Après quelques années de défoliation constante, la qualité de la nourriture diminue. En conséquence, la performance des insectes est de plus en plus négativement affectée (Haukioja 1991). Tauber *et al.* (1986) mentionnent que les insectes qui sont éloignés de l'équateur sont plus sensibles à la qualité et la disponibilité des ressources, car ils complètent leur cycle de vie en fonction du climat et de la biologie de leurs hôtes. Les insectes vivants dans les plus

grandes latitudes seraient donc plus exposés à des contraintes lorsque la qualité et la disponibilité des ressources sont affectées.

D'un point de vue écologique, une infestation est considérée comme une augmentation du nombre des individus dans une population. Les populations d'insectes sont tellement vastes qu'elles nuisent négativement aux hôtes sur lesquels ils se nourrissent (Berryman 1987). Depuis le dernier siècle, de nombreuses études ont permis d'acquies de l'information substantielle sur l'écologie des populations et les conséquences des infestations (Yang 2012). Ainsi, des hypothèses sur les causes des épidémies ont été avancées. Celles-ci ont conduit Berryman (1987) à proposer une classification des types d'épidémie (cyclique, graduelle, éruptive, etc.). Ces recherches sur la théorie des infestations ont mené à des méthodes de lutte contre les épidémies (Berryman et Stark 1985).

1. 1 Biologie de la tordeuse des bourgeons de l'épinette

La TBE est un défoliateur indigène, lequel se trouve dans toutes les provinces canadiennes, de la Colombie-Britannique jusqu'à Terre-Neuve. Elle se nourrit principalement de sapin baumier (*Abies balsamea* Mill.), d'épinette blanche (*Picea glauca* Voss.) et parfois d'épinette noire (*Picea mariana* Mill.) et d'épinette rouge (*Picea rubens* Sarg.) (Harvey 1985). Le cycle de vie de la TBE est complété en douze mois. Il est caractérisé par un stade œuf, six stades larvaires (L₁ au L₆), un stade chrysalide et un stade adulte (Miller 1975).

En juillet, les femelles s'accouplent et déposent leurs œufs sur les aiguilles. Une fois les œufs pondus, la larve de premier stade émerge et trouve un abri dans les lichens corticaux ou dans les cicatrices florales (Miller 1975). La TBE passe ensuite l'hiver dans un petit cocon de soie appelé *hibernaculum* avant de muer en deuxième stade larvaire (L₂). Cet insecte passe les trois quarts de sa vie en diapause. Pendant cette période, la TBE ne se nourrit pas (Han et Bauce 2000), ainsi la survie hivernale des larves dépend directement des réserves qui leur ont été fournies par leurs mères.

À la fin du mois d'avril ou au début du mois de mai, les jeunes chenilles sortent de leur état d'hibernation (NRCAN 2015). Attirées par la lumière, elles se dirigent vers les extrémités des branches où elles se nourrissent des fleurs en attendant l'ouverture des bourgeons (Burleigh *et al.* 2002). C'est au cinquième et sixième stade larvaire que les larves

mangent le plus, causant 85 % de la défoliation. C'est donc durant ces stades que leurs dégâts sont les plus apparents. À la fin du mois de juin, elles se transforment en chrysalides sur l'une des branches inférieures d'un arbre-hôte. Dix à quatorze jours plus tard, les papillons émergent. Une femelle pond environ 200 œufs qu'elle dépose en masses de 10 à 50 œufs. Elle pond principalement sur les aiguilles de ses hôtes. L'incubation des œufs dure de dix à quinze jours (NRCAN 2015).

Les populations de TBE augmentent graduellement pour atteindre un niveau épidémique tous les 30 ans environ (, Boulanger et Arseneault 2004, Rauchfuss et Ziegler 2011). Ces changements périodiques dans la densité de population de tordeuses peuvent se produire sur de vastes territoires. Lors d'une épidémie, les arbres les plus faibles meurent généralement après trois ou quatre années de défoliations graves; la majorité des arbres meurent de six à dix ans après la première attaque (Blais 1983).

Le déclin d'une épidémie est associé au manque de nourriture, en situation endémique, les larves se nourrissent des bourgeons de l'année courant ou des bourgeons encore fermés. Cependant en conditions épidémiques, le grand nombre d'individus affecte négativement les hôtes (Nealis et Régnière 2004). Il y a donc une réduction significative du nombre des bourgeons et de la qualité nutritionnelle. Ces deux facteurs ont comme conséquence la réduction de la performance biologique des individus (Nealis et Régnière 2004, Régnière et Nealis 2007). De plus, de nombreux ennemis naturels attaquent la TBE comme : les oiseaux prédateurs et les parasitoïdes (Thireau et Régnière 1995). Les espèces de parasitoïdes contribuent à faire baisser les populations de tordeuses à la fin des infestations (Royama 1984, Serikaya et Avci 2005, Régnière et Nealis 2007).

Les effets biologiques sur les populations de TBE après une épidémie sont encore méconnus, notamment les processus adaptatifs. Malgré que la tordeuse des bourgeons de l'épinette est un sujet d'étude depuis plus de 60 ans, Les effets biologiques sur les populations de TBE après une épidémie sont encore méconnus, notamment les processus adaptatifs. La qualité de la nourriture est invariablement un des facteurs les plus importants, car la performance biologique dépend directement des nutriments acquis pendant le stade larvaire. L'identification des mécanismes d'adaptation par rapport au stress nutritionnel, pourrait donner des pistes sur la trajectoire adaptative favorisée par cette pression de sélection lors du déclin d'une épidémie.

1.2 Les infestations de la TBE et la qualité nutritionnelle de la plante hôte

Pour les insectes phytophages, la plante-hôte constitue la seule ressource alimentaire. Cette relation est plus profonde pour ces insectes qui ne se nourrissent qu'au stade larvaire et que très peu au stade adulte (Boggs 1992). Donc, la variabilité alimentaire en qualité peut avoir un impact significatif sur les performances biologiques (Carisey et Bauce 2002, Frago et Bauce, 2014). Une mauvaise qualité de la nourriture peut engendrer des chrysalides plus petites, un nombre restreint d'œufs pondus par les femelles, moins de réserves en nutriments pour les larves et un taux de mortalité plus élevé durant les périodes de stress (Price *et al.* 1980, Ritchie 2000).

Pour la tordeuse de bourgeons de l'épinette, un prérequis très important pour engendrer une infestation est la présence de sapins baumiers et/ou d'épinettes blanches matures sur de grandes superficies (Blais 1985a). Plusieurs études ont démontré qu'après une infestation, les arbres souffrent d'un stress nutritionnel considérable (Schultz 1988, Mattson *et al.* 1997). La qualité de la nourriture influence la valeur sélective (le fitness) des insectes en affectant leur performance biologique et leur fécondité (Mattson *et al.* 1982, Mattson et Scriber 1987, Clancy *et al.* 1995). Des études ont montré que la qualité de l'alimentation possède un impact négatif sur la tordeuse occidentale de l'épinette (*Choristoneura occidentalis* Freeman) (Kemp et Moody 1984, Clancy 1992a-b,) ainsi que sur la tordeuse des bourgeons de l'épinette (Carisey et Bauce 2002, Fuentealba et Bauce, 2012, Frago et Bauce, 2014). L'effet de la qualité nutritionnelle sur les populations a un impact important sur l'adaptation des individus aux conditions des hôtes (Raubenheimer *et al.* 2012). Le comportement du coléoptère *Megacerus eulophus* (Coleoptera : Bruchidae), représente un exemple d'adaptation locale face à un stress nutritionnel. Cet insecte se nourrit de deux hôtes : *Convolvulus chilensis* et *C. bonariensis*. Dans certaines régions, ces deux hôtes sont séparés géographiquement, cette situation prédispose à une adaptation locale. Les individus ont toujours une meilleure performance biologique sur l'hôte qui prédomine une région donnée. À l'inverse, ils connaissent une performance biologique moindre sur l'hôte co-dominant présent dans cette même région (Stotz *et al.* 2013). Cette adaptation est causée par des différences nutritionnelles entre les hôtes, notamment l'azote et les sucres. L'azote et le

sucres sont les nutriments les plus importants pour la bonne performance des insectes herbivores.

1.2.1 Le rôle de l'azote

Le contenu en azote des plantes est fondamental pour le développement des herbivores (Mattson 1980, Scriber et Slansky 1981, Lovett *et al.* 2002). L'azote est présent dans plusieurs composés organiques impliqués dans différents processus métaboliques et physiologiques chez les végétaux. Par exemple le développement de la structure cellulaire, l'ADN et les processus de transcription et la croissance et la reproduction (Mattson 1980). Une mauvaise concentration en acides aminés chez les plantes peut engendrer de graves conséquences sur la croissance, la survie et la fécondité des insectes (Scriber et Slansky 1981, Awmack et Leather 2002). Les recherches ont déjà démontré que les insectes ont la capacité de réguler l'ingestion de protéines dans leur diète, pour obtenir l'optimum dans leur alimentation au cours de leur vie larvaire (Simpson et Raubenheimer 2000, Raubenheimer et Simpson 2004). Cependant, un mauvais équilibre nutritionnel peut réduire les taux de croissance des insectes. Par exemple un excès d'azote impose des coûts métaboliques, ce qui représente une augmentation du catabolisme et du taux d'excrétion des nutriments (Slansky et Scriber 1985, Karowe et Martin 1989, Purnell 2007). La question réside à savoir si ce comportement face à un déséquilibre nutritionnel peut influencer les autres générations quant à l'utilisation de la nourriture.

Chez la TBE, un déficit en azote affecte négativement la survie, le poids des chrysalides, la taille des adultes et le temps de développement (Shaw et Little 1977, Frago et Bauge, 2014). L'azote contenu dans le feuillage est un facteur qui affecte la performance de la tordeuse (Montgomery 1985). Albert et Bauge (1994) ont démontré que le contenu en azote et en sucres du feuillage a une importance primordiale pour la performance de la larve de la TBE. Les diètes avec des concentrations adéquates en azote permettent de bonnes performances de la TBE (Albert et Bauge 1994).

1.2.2 Le rôle des sucres

Le sucre constitue la principale source d'énergie de la TBE. Une nourriture riche en sucre contribue à diminuer le temps de développement, à augmenter le poids des chrysalides

et des adultes (Bidon 1993, Harvey 1983). La préférence alimentaire des TBE aux derniers stades larvaires est principalement affectée par le contenu en sucrose dans le feuillage (Albert et Bauce 1994). Pour survivre à l'hiver, la TBE accumule du glycérol dans ses cellules afin de résister au gel (Han et Bauce 1995, Han *et al.* 2000).

1.3 La pression de sélection

La défoliation constante provoque un stress sur les hôtes, ce qui en retour représente une pression de sélection importante pour les insectes via une modification de la qualité nutritionnelle de la diète. Dans ces conditions, une pression s'exerce sur les insectes qui résulte en une sélection de traits ayant un caractère adaptatif (Rundle et Nosil 2005, Carroll *et al.* 2007). Dans le cas de la TBE, la qualité de la nourriture affecte la fécondité, le poids et le temps de développement (Carisey et Bauce 1997a, 2002, Fuentealba et Bauce 2012). Ces caractères sont associés au vécu de l'insecte. L'équilibre nutritionnel chez les bourgeons est important pour la performance des insectes. Les basses concentrations en nutriments incitent à une meilleure utilisation de la nourriture chez les insectes phytophages (Crawley 1983). Chew (1975) a observé que les variations nutritionnelles chez les hôtes de *Pieris rapae* (Lepidoptera :Pieridae) ont influencé le développement, le taux de survie et la croissance des insectes. Les résultats de cette étude suggèrent que cette espèce de lépidoptère possède un potentiel d'adaptation associé au statut nutritionnel des hôtes étudiés.

Schmidt et Lauer (1977) ont observé que chez la tordeuse occidentale de l'épinette, les femelles montrent un grand degré de polymorphisme dans leur développement. Cet effet est possiblement associé aux espèces d'arbres qu'elles colonisent. Cette réponse pourrait être le résultat d'une pression de sélection due à l'hôte. Dans le cas de la TBE, Tremblay (2007) a effectué une étude sur la pression de sélection de type alimentaire avec l'azote. Ses résultats ont démontré qu'un stress alimentaire peut constituer une pression de sélection. Cette étude suggère une adaptation et un effet parental sur le poids des chrysalides chez les femelles dus à un stress alimentaire.

1.4 Les stratégies adaptatives

Selon la théorie de la sélection naturelle, toute population constituée d'individus ayant des propriétés de reproduction, d'hérédité et de variation à le potentiel d'évoluer en fonction des conditions biotiques et abiotiques retrouvées dans l'environnement de cette population (Fischer 1930). Ainsi l'évolution est le résultat d'une sélection qui s'exerce sur les stratégies présentes au sein d'une population. Ces stratégies constituent un amalgame des caractères comportementaux, morphologiques, physiologiques et ceux associés au vécu des insectes,

lesquels permettent de favoriser la survie et la reproduction des individus (Southwood 1988, Nylin et Gotthard 1998). Certains caractères influencent davantage l'adaptation des insectes dans des situations précises, telles que le climat, la disponibilité de la ressource alimentaire et la présence des prédateurs. Ainsi, les stratégies dépendent d'une situation et d'une population données. (Stearns 1976). Par exemple, le changement climatique affecte alors significativement la phénologie du chêne pédonculé (*Quercus robur* L.). Cette essence présente une désynchronisation phénologique (une période de décalage) qui affecte considérablement la disponibilité de la ressource alimentaire pour le lépidoptère *Operophtera brumata* L. (Geometrydae). Ainsi, l'insecte adopte une stratégie qui consiste à décaler son temps de développement pour ajuster sa phénologie à celle de l'hôte (Visser et Holleman 2001).

Pour comprendre les voies évolutives des insectes, il est important d'identifier les situations où les insectes répondent à une condition environnementale et sa relation avec son hôte (Jermy 1984). La nutrition est profondément impliquée dans les processus adaptatifs des insectes (Slansky et Feeny 1977). En effet, tous les processus de développement, de reproduction et de comportement dépendent directement des ressources nutritives (Reznick *et al.* 2000) et le compromis qu'il y a entre la croissance et la reproduction (Roff 2000). Dans un environnement donné, la sélection naturelle favorise les meilleures stratégies ou phénotypes par rapport aux autres alternatives. Ainsi, face aux contraintes d'ordre physiologique et écologique, les stratégies sélectionnées seront celles qui présentent la valeur d'adaptation la plus élevée (Southwood 1988), c'est-à-dire, celles qui permettront aux individus d'atteindre le stade adulte et se reproduire. Ensuite, ces individus transmettront leur bagage génétique aux prochaines générations.

1.5 L'héritabilité

Tous les organismes sont portés à transmettre leurs caractéristiques à leur descendance, ce qui fait que la progéniture va ressembler à ses parents, ce qui est lié à l'hérédité. L'héritabilité (h^2) ne concerne cependant pas un individu, mais plutôt un ensemble d'individus. L'héritabilité est représentée par la proportion de la variation dans la population qui est attribuable à des différences génétiques entre les individus (Falconer et Mackay 1996, Kempthorne et Tandon 1953). L'héritabilité est exprimée par la formule ($h^2 = V_A / V_P$), où V_A

représente la variance additive et V_P , la variance phénotypique (Zobel et Tarbert 1984). La variance additive est la partie de la variance phénotypique qui est due aux effets additifs de tous les allèles qui codifient le phénotype. Cette variance est une grande composante d'hérédité. De plus, la variance additive est la partie de la variance totale génotypique qui répond à la sélection, en conséquence elle est très impliquée dans le processus d'adaptation (Jones 1987). L'héritabilité d'un caractère est spécifique à une population donnée, dans un environnement particulier et à un moment précis. Sa valeur est comprise entre 0 et 1, plus la valeur se rapproche de 1, plus l'héritabilité est déterminante dans le caractère transmis à la progéniture. Une hérédité faible correspond à une valeur plus petite que 0,2, une h^2 moyenne entre 0,4 et 0,6 et dans de très rares cas elle est plus de 0,7 (Carles 2005).

Il existe deux méthodes couramment utilisées pour estimer l'héritabilité (h^2) : la première consiste à estimer les covariances entre les apparentés (frères et sœurs) et ensuite une analyse d'ANOVA et la deuxième consiste à une régression entre les parents et les enfants (Falconer et Mackay 1996).

Les analyses démontrent que les apparentés semblent être plus affectés par la variable environnementale (Willham 1963). Ainsi, la régression parent-enfant, semble être la plus convenable. Cependant, il existe une différence constante entre le nombre de rejetons dans chaque famille, dans ce cas-là, il est convenable d'appliquer une des méthodes proposées par Ollivier (1974) :

- 1) Répéter la valeur du parent autant de fois qu'il a des descendants;
- 2) Utiliser la moyenne des descendants;
- 3) Pondérer la moyenne des descendants.

1.6 Indices gravimétriques

Le processus d'alimentation chez les insectes est divisé en cinq phases distinctes : l'ingestion, la digestion, l'assimilation, la conversion de la nourriture en biomasse et l'excrétion (Waldbauer 1968). White (2003) suggère que la qualité nutritive est un facteur clé qui déclenche une épidémie, tandis que d'autres études suggèrent que la qualité nutritionnelle est un facteur impliqué dans le déclin d'une épidémie (Miller 1977). Lors d'un déséquilibre nutritionnel, on observe chez les insectes différentes stratégies afin d'utiliser efficacement la nourriture (Behmer 2009). Ces stratégies comprennent la réduction du temps de développement lorsque la nourriture n'est pas disponible durant une longue période, une augmentation du taux de consommation et une plus grande efficacité à digérer la nourriture et la convertir en biomasse. Un exemple de cette adaptation est l'insecte *Omocestus viridulus* (Acrididae), il augmente sa capacité digestive (Behmer *et al.* 2005). Ces indices nutritionnels mesurent l'efficacité de l'insecte à convertir la nourriture en biomasse, ce qui a des répercussions sur les taux reproductifs et les réserves (Simpson et Raubenheimer 1993). Il a été observé que la tordeuse occidentale de l'épinette (Brewer *et al.* 1985, Clancy 1992,) et la TBE (Bauce *et al.* 1994, Tremblay 2007) ingèrent plus de nourriture pour compenser les faibles quantités d'azote contenues dans la nourriture. Pour les besoins de la recherche présente, les indices gravimétriques ont été pris lors du sixième stade larvaire, stade auquel la larve ingère environ 85% de toute son alimentation (Bauce 1996). Les indices gravimétriques sont tirés de Waldbauer (1968).

L'observation des variations liées à l'utilisation de la nourriture peut donner des pistes sur les stratégies adaptatives quant à l'utilisation de la nourriture par la TBE.

- ❖ La digestibilité approximative (DA) : Proportion de la nourriture ingérée qui a été assimilée et absorbée par l'organisme.

$$DA = \frac{NI}{P*T} * 100$$

- ❖ Le taux relatif d'ingestion (TRI) : Quantité de nourriture ingérée en fonction du temps

$$TRI = \frac{NI}{P*T}$$

- ❖ Le taux relatif de croissance (TRC) : Croissance de l'insecte en fonction du temps.

$$TRC = \frac{GP}{P*T}$$

- ❖ L'efficacité de conversion de la nourriture digérée (ECD) : Mesure de la capacité de l'insecte à convertir la nourriture digérée en biomasse.

$$ECD = \frac{GP}{NI - FP} * 100$$

- ❖ L'efficacité de conversion de la nourriture ingérée (ECI) : Mesure de la capacité de l'insecte à convertir la nourriture ingérée en biomasse.

$$ECI = \frac{GP}{NI} * 100$$

GP : Gain en poids (poids initial – poids final)

P : Poids moyen (GP/log GP)

NI : Quantité de nourriture ingérée (Poids secs)

FP : Quantité de fèces produites (Poids secs)

Avant que les indices de chacun des insectes puissent être comparés, il faut ajuster le numérateur des traitements, des sexes et des rapports (y/x). Pour cela, les variables d'une équation de régression linéaire ou non linéaire [f(x)] qui prédisent le numérateur y en fonction du dénominateur x, ont été estimées. Pour chaque rapport (y/x), la moyenne du

dénominateur X a été calculée (Bauce *et al.* 1994). Pour chaque ratio, chaque traitement et chacun des sexes, les valeurs observées du numérateur (y_i) ont été ajustées à la valeur de X en utilisant l'équation suivante :

$$\text{Rapport ajusté} = \frac{y_i + f(x) - f(x_i)}{X}$$

1.7 Le rapport des sexes

Dans la plupart des espèces, le rapport des sexes est de 1:1 (Shaw et Mohler 1953, Kolman 1960). Fischer en 1930, a démontré que la sélection naturelle tend à équilibrer cette proportion, qui s'explique par une contribution parentale égale. De nombreuses recherches ont tenté d'observer ce phénomène dans la nature, depuis la théorie de Fischer (Jennions *et al.* 2012). Le rapport de sexes de 1 :1 n'est pas atteint dû aux variations environnementales. Ainsi, les recherches ont suggéré que les populations acquièrent des mécanismes d'adaptation qui modifient ce rapport (Shaw et Mohler 1953, Andersen 1961, Hamilton 1967, Clark 1978). Du point de vue adaptatif, la théorie de l'attribution du sexe prédit que les femelles devraient réguler le rapport lorsqu'un sexe dépasse l'autre sexe en proportion, ce qui pourrait amener l'équilibre entre les deux sexes (Trivers et Willard 1973). Mais, les contraintes environnementales peuvent modifier ce rapport, car les femelles et les mâles se différencient en termes de coûts métaboliques (House *et al.* 2011). Donc, les facteurs externes jouent un rôle important dans ce rapport. Un rapport autre que 1 :1 peut aider à maintenir l'équilibre de la population. Par exemple, chez les hyménoptères la régulation du sex-ratio est possible durant la période de la reproduction. En dehors de cette période, les mâles n'ayant qu'une faible contribution au bon fonctionnement de la colonie, sont générés en moins grand nombre (Meunier *et al.* 2008). D'autre part, la présence de mâles de la saison précédente peut assurer l'accouplement des premières femelles qui émergent de la saison en cours, lors de variations de température favorables (Werren et Charnov 1978, Wiklund *et al.* 1991). De plus, le fait que les mâles sont plus sensibles que les femelles aux maladies ou aux prédateurs, crée aussi un déséquilibre dans le rapport des sexes (Scali et Masetti 1973; Jiggins *et al.* 1998, Tabadkani *et al.* 2012). Lors des épidémies, le déséquilibre du rapport des sexes peut indiquer que l'infestation est en déclin, et peut aider à réduire la taille de la population

d'insectes (Mauffette et Jobin 1985, Cipollini 1991). Cela s'explique par les attaques répétées aux hôtes au cours de plusieurs années qui affectent la qualité nutritionnelle de la ressource. Par conséquent, cette situation peut affecter un sexe plus que l'autre (Robinson 1983), en raison des besoins métaboliques différents entre les mâles et les femelles (Mopper et Whitham 1992) et causer une mortalité différentielle (House *et al.* 2011).

2 Hypothèses

L'hypothèse principale de la présente étude propose que l'effet d'un stress alimentaire soit suffisamment important pour induire une pression de sélection. Cette hypothèse sera testée en simulant un stress alimentaire en azote et en sucre et en vérifiant si ce stress peut induire une pression de sélection sur une population de tordeuses des bourgeons de l'épinette. Selon la théorie décrite par White (1978), un stress chez les arbres-hôtes dû à une défoliation excessive, peut causer des changements nutritionnels importants. Ces variations nutritionnelles dans les bourgeons de l'hôte créent des changements qui touchent les caractères associés au vécu des insectes (Bauce et Hardy 1988, Clancy *et al.* 2004). Les hypothèses sont les suivantes :

- a) La fécondité, le temps de développement et le poids des chrysalides sont les caractères les plus susceptibles d'être héréditaires. L'héritabilité de ces caractères permet une probabilité plus grande de survivre, de se reproduire et de donner une descendance performante pour continuer le cycle biologique (Roff 1992). Mousseau et Roff (1987) mentionnent que les caractères associés au vécu de l'insecte ne représentent pas toujours une faible hérédité, cela dépend plutôt de la biologie de l'organisme et sa relation avec l'environnement.
- b) Sous l'influence d'une pression de sélection du type nutritionnel, les caractères associés au vécu des insectes s'adaptent, tels que la fécondité, le temps de développement, le taux de croissance, et le poids des chrysalides. Les adaptations rapides peuvent être observées dans des environnements où la qualité de la nourriture se détériore rapidement (Mousseau et Fox 1998).
- c) L'efficacité dans l'utilisation de la nourriture augmente sous un régime alimentaire de mauvaise qualité au cours des générations. Ce type de stratégie d'adaptation alimentaire est souvent utilisé par les insectes spécialisés (Simpson *et al.* 2002, Behemer 2009, Warbrick *et al.* 2009), tels que la TBE.
- d) Il existe un déséquilibre de la proportion de sexes en faveur des mâles et il devient plus évident au fil des générations. Les femelles ont des coûts métaboliques plus élevés (p. ex., la ponte d'oeufs, une longévité plus longue, un poids plus élevé, etc.) (Bauce *et al.* 1994, Koller et Leonard 1981 et Carisey et Bauce 2002). Ainsi, le stress nutritionnel peut avoir un impact plus grand sur les femelles. Un déséquilibre

progressif sur le rapport de sexes dû à un stress environnemental peut causer une chute de la population des insectes (Robinson 1983).

3 Objectifs

- ❖ Mesurer le niveau d'adaptation de la population de TBE à la qualité de la nourriture.
- ❖ Déterminer si un stress alimentaire subi par les parents affecte la performance de leurs descendants.
- ❖ Identifier les caractères qui ont les degrés d'héritabilité les plus élevés.
- ❖ Déterminer s'il y a une adaptation de la TBE à travers des générations lors d'une pression de sélection nutritionnelle constante.
- ❖ Évaluer la plasticité phénotypique aux changements nutritionnels.
- ❖ Observer les stratégies adaptatives liées à l'utilisation de la nourriture consommée par les insectes, au cours de plusieurs générations.
- ❖ Examiner s'il y a un déséquilibre des proportions des sexes lors d'un stress nutritionnel.

4 Matériel et méthodes

4.1 Méthode d'élevage

Pour la première partie de l'expérience, des larves diapausantes de TBE de deuxième stade (post-diapause) provenant de l'Unité de Production d'Insectes du Service Canadien des Forêts (Sault Sainte-Marie, Ontario) ont été élevées. Il s'agit d'une population issue de dix générations élevées depuis 2001. Un total de trois croisements (A, B, C) ont été effectués avec cette population. Afin d'assurer un niveau constant de familles (plus de 50), chaque croisement était constitué de 2 000 organismes soit environ 150 familles dans chaque traitement et une moyenne de 30 descendants par famille, ce qui se traduit dans un total de 6 000 individus répartis en 450 familles pour débiter l'étude. Toutes les expériences ont été menées au laboratoire d'entomologie forestière de l'Université Laval.

Deux diètes artificielles ont été choisies pour réaliser l'expérience :

- a) Une diète « témoin » telle que décrite par McMorran (1965) contenant 12 % de sucre et 5,5 % d'azote. Le pourcentage représente la concentration de ces éléments par rapport à la diète totale. Cette composition de diète permet de reproduire en laboratoire, les performances retrouvées sur le terrain, sur les hôtes de qualité (p. exp. sapins baumiers matures).
- b) Une diète « stress » simulant une pression de sélection dont les concentrations en sucres et en azote sont de 1,5 et 7 % respectivement. Ce type de stress a été établi afin de maximiser la pression de sélection, cette diète provoque un taux de mortalité similaire aux conditions trouvées à la fin d'une épidémie (Raske 1985).

Tous les insectes ont été élevés sous conditions contrôlées dans des chambres de croissance à une température de 23°C, à 65 % d'humidité relative et avec une photopériode de 16 heures de lumière et 8 heures de noirceur. On a utilisé la technique d'élevage proposée par Robertson (1985). Toutes les larves ont été déposées dans des contenants en plastique de 30 ml. Chaque contenant regroupait quatre larves afin de minimiser le risque de cannibalisme et de compétition pour la nourriture. À la fin du sixième stade larvaire, le temps de développement des larves a été observé tous les jours. La mortalité a été notée pour chaque stade larvaire. Les chrysalides ont été déposées dans des contenants individuels avant d'être

identifiées, numérotées, pesées et sexées. Les adultes étaient sexés et enregistrés au moment de leur émergence. Pour garantir le succès des accouplements, deux mâles ont été placés avec une femelle. Les accouplements étaient intra-traitements. Les adultes non accouplés ont été gardés jusqu'à leur mort naturelle.

Les accouplements ont été faits dans des contenants en plastique de 7,5 cm par 11 cm de diamètre. Pour assurer la disponibilité de nourriture aux adultes, deux fioles en plastique de 2,0 ml étaient placées à l'intérieur de la cage. Ces tubes ont été remplis avec de l'eau sucrée à 5 % et fermés avec du coton. Les œufs ont été récoltés tous les trois jours.

Après l'éclosion des œufs, les larves de premier stade ont été exposées durant une semaine à 23°C avant d'être placées dans une chambre de croissance à 18°C pour deux semaines, sous les mêmes conditions de photopériode et d'humidité, décrites précédemment. Après cette période, les insectes ont été placés à 2°C dans l'obscurité pendant 25 semaines afin de compléter la diapause (Stehr 1954, Harvey 1985, Schmidt et Reese 1986).

Une fois que les techniques d'élevage ont été standardisées, une deuxième génération de larves a été formée par trois croisements (Fig. 1.1). Les larves ont été élevées sur les deux types de diètes. L'élevage de départ a constitué les familles. Le poids des chrysalides, le temps de développement, la survie durant la diapause et celle post diapause ainsi que la proportion de sexes ont été mesurés.

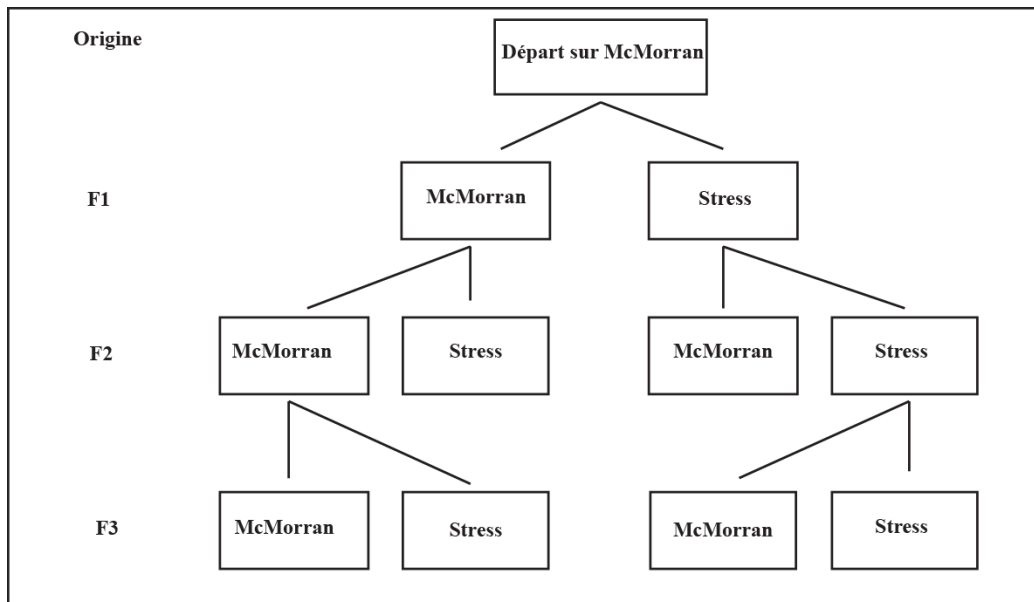


Figure 1.1 Diagramme d'étude pour les trois générations de TBE. Tous les élevages sont conçus de la même manière.

Pour les analyses gravimétriques, un sous échantillon de 80 individus par génération, a été pris selon le type de diète et selon le croisement. Dans l'ensemble, cela représente 480 individus par génération.

Les variables prises en considération sont les suivantes :

- **Poids des chrysalides** : poids 24 heures après la pupaison;
- **Temps de développement** : durée des stades de développement et de transformation (larve-pupe-adulte), jusqu'à la mort de l'adulte;
- **Fécondité** : nombre d'œufs pondus par les femelles au cours de leur vie. Le taux de fécondité est une mesure permettant d'évaluer le potentiel d'une population à augmenter ou à diminuer sans facteur externe. Cet indice est généralement calculé en faisant le rapport entre le nombre total d'œufs à la naissance et le nombre moyen de femelles fécondes (Krebs 1978). Dans le deuxième chapitre, cette variable correspond à *realised fecundity* ;
- **Fertilité** : nombre d'œufs fertiles pondus par la femelle. Dans le deuxième chapitre, cette variable correspond à *fecundity* ;
- **Fertilité (2^{ème} chapitre) : présence ou absence d'accouplement de la femelle ;**
- **Survie hivernale** : nombre d'organismes vivants avant, durant et après la diapause ainsi qu'au stade de larve (L3-L6), chrysalide et adulte;
- **Indices gravimétriques** : indices décrits auparavant dans la section sur la gravimétrie. Ces indices sont effectués au cours du 6^{ème} stade larvaire.
- **Mortalité** : échantillon des larves du 6^{ème} stade larvaire, mis sur les indices gravimétriques.
- **Proportion de mâles et femelles** : rapport du nombre de mâles et de femelles qui ont atteint les stades de chrysalide et d'adulte.

4.2 Analyses statistiques

L'héritabilité a été calculée afin de déterminer s'il existe la probabilité d'une adaptation. Cette analyse permet d'observer la relation entre les apparentés père-enfants et mère-enfants sous forme d'une régression linéaire. Étant donné que le nombre d'enfants est inégal, la méthode d'Ollivier (1974) a été utilisée pour standardiser les données. Elle consiste à répéter la valeur du parent autant de fois qu'il y a d'enfants. Une fois la régression linéaire calculée, l'héritabilité a été estimée à deux fois la valeur de la pente, et l'erreur standard de h^2 a été estimée à deux fois l'erreur standard de la pente (Klein *et al.* 1973, Falconer et Mackay 1996). Ce calcul a été effectué à l'aide de la procédure proc REG (SAS-Institute 1988).

Pour observer l'existence d'une adaptation, une analyse d'ANOVA à trois facteurs a été réalisée. Les variables étudiées étaient le temps de développement, le poids des chrysalides et le taux de croissance. Étant donné qu'il existe une différence au niveau de la performance biologique entre les mâles et les femelles (Koller et Leonard 1981, Carisey et Bauce, 2002), l'analyse a été réalisée pour chaque sexe. Ainsi, les facteurs d'analyse étaient la génération (F2 et F3), la diète (témoin et stress) et la diète parentale (témoin et stress). Les analyses ont été réalisées pour les générations F2 et F3 car elles sont issues d'insectes élevés respectivement sur la diète témoin et la diète stress. La procédure GLM (SAS Institute 2003) a été utilisée pour ces analyses. La fécondité et la fertilité des œufs ont été analysées par une régression de Poisson avec la procédure GENMOD (SAS Institute 2003). La survie hivernale et la mortalité ont été analysées sous forme d'une régression logistique, à l'aide de la procédure GLIMMIX (SAS Institute 2003). Les différences entre les moyennes ont été comparées avec le test de Tukey.

Pour déterminer s'il existe une adaptation à l'utilisation de la nourriture, les indices gravimétriques ont été analysés avec une ANOVA factorielle à trois facteurs. Ces facteurs sont les mêmes que pour l'analyse précédente. Lorsque les données n'ont pas satisfait les postulats de l'analyse de variance (Conover et Iman, 1981), elles ont été transformées en rang avec la procédure RANK (SAS Institute 2003). Puis, une régression linéaire ou non linéaire a été effectuée pour chaque indice gravimétrique, afin d'ajuster le ratio et pouvoir faire les comparaisons correspondantes (Bauce *et al.* 1994). Une régression logistique a été réalisée afin d'analyser la mortalité à l'aide de la procédure GENMOD (SAS Institute 2003).

Enfin, l'hypothèse que la variation de la qualité de la ressource alimentaire induit une mortalité différentielle a été vérifiée à l'aide d'une régression logistique afin de tester si le rapport de sexes se maintient 1:1. La procédure proc LOGISTIC (SAS Institute 2003) a été utilisée dans ce cas-ci. En somme, toutes les analyses ont été réalisées avec le logiciel statistique SAS 9.3 (SAS Institute 2003).

Chapitre I

Heritability of life-history traits in the spruce budworm

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Résumé

L'héritabilité dans les caractères d'histoire de vie revêt une grande importance pour les insectes qui dépendent directement de la condition de leurs hôtes. Les défoliations sévères causées par la tordeuse des bourgeons de l'épinette ont un impact négatif sur la ressource alimentaire, ce qui représente une contrainte environnementale pour l'insecte. L'héritabilité dans les caractères d'histoire de vie peut aider à élucider les différents processus évolutifs des espèces. Elle identifie également quels sont les caractères qui possèdent une variance additive, ce qui peut aider à comprendre les effets de la sélection naturelle. Des individus ont été élevés sous conditions contrôlées de laboratoire pendant trois générations. La fertilité et la fécondité ont présenté une héritabilité significative, suivies du temps de développement et du poids des chrysalides. Les résultats suggèrent un pourcentage important de variance additive dans les caractères d'histoire de vie. Cette étude contribue à mieux comprendre la relation de ce ravageur forestier avec les conditions environnementales. Finalement, il révèle une architecture génétique importante dans les caractères d'histoire de vie pour la tordeuse des bourgeons de l'épinette.

Abstract

The heritability of life-history traits is of particular importance for insects that are very dependent on host conditions. Severe defoliation caused by the spruce budworm negatively impacts its food source, which in turn imposes environmental constraints on the insect. The heritability of those traits can help elucidate this species' evolutionary process. Heritability also helps identify which traits exhibit significant additive variance and can be key to understanding natural selection effects. Individuals were reared under laboratory conditions over three generations on an artificial diet. Heritability was estimated by parent-offspring regression. Fertility and fecundity demonstrated significant heritability followed by larval development, while pupal mass showed minimal heritable variation. These results suggest an important percent of additive variance in life-history traits. This study contributes to our understanding of the relationship of this forest pest to its environmental conditions. This study also reveals an important genetic architectural structure of life-history traits in the spruce budworm.

INTRODUCTION

Some of the most important questions in evolutionary biology are concerned with explaining the phenotypic variation observed in a population. Adaptation processes have minimum requirements for bringing about evolutionary change, that is, the presence of additive variance in the trait in question (Mousseau & Roff 1987; Hoffmann & Merilä 1999). Falconer and MacKay (1996) defined additive variance as the resemblance between relatives and, therefore, the chief determinant of observable genetic properties of the population and of the response of the population to selection. A percentage of this information will be transmitted to the next generation. Heritability thus can provide answers to many questions regarding evolutionary mechanisms, such as the potential adaptiveness of traits (Jones 1987). The fundamental theorem of natural selection, as proposed by Fisher (1930), suggests that low heritability of life-history traits is due to the action of natural selection. However, recent studies have shown a significant amount of heritability for traits (Price & Schluter 1991; Roff 2000) such as developmental time (Hammerschmidt *et al.* 2012), fecundity (Long *et al.* 2009), fertility (Kamrul & Rahman 2008) and pupal mass (Tanaka 1991). One of the most important features of heritability is its predictive nature. This value is not constant and changes in response to environmental conditions, gene frequencies and generations. Thus, it determines the intrinsic rate of change for traits that are studied within a population and indicates the evolutionary direction that the population takes; it can also show whether or not a trait is subject to selection processes (Roff 1992; Falconer & MacKay 1996).

Evolutionary processes can be observed on an ecological time scale. Insect outbreaks are a clear example of that type of evolution, since a continuous pest attack causes significant stress to host trees (Barbosa & Baltensweiler 1987). Thus, evolutionary changes can occur in insect populations in a matter of a few generations (Hendry & Kinnison 1999; Carroll *et al.* 2007). For insect pests in the boreal forest, the challenge of surviving is greater because resources and weather play a selective and restrictive role in fitness (Bonan & Shugart 1989). The boreal forest is characterized as having a long, cold winter and a short summer. Consequently, resources are available for a limited time and space (Chen *et al.* 2003). Insects are forced to synchronize their periods of growth, development and reproduction within this time frame (van Asch & Visser 2007; Wilczek *et al.* 2010). Phenotypic variation therefore affects biological performance by slowing development, and decreasing fertility and survival,

among other responses. The way in which a forest pest responds to environmental change determines which ecological strategies its population will follow (Ehrlich & Raven 1964; Jermy 1984). In this study, we use spruce budworm *Choristoneura fumiferana* (Clemens) as a model of a widespread North American forest pest. This species is very sensitive to variation in the environment (Régnière 1987; Dupont *et al.* 1991), the host (in terms of age, availability, nutritional quality, and the concentration of secondary compounds in the vegetative buds) (Blais 1984; Bauce *et al.* 1994; Nealis 2003) and phenological synchronization (Lawrence *et al.* 1997; Nealis & Régnière 2004), thereby making it an ideal candidate for this type of study.

Biology of spruce budworm

The spruce budworm is an insect defoliator that is native to the boreal forest of North America and is one of its most significant outbreak pests (Blais 1965; Royama 1984). Severe and widespread budworm infestations have been observed over many centuries (Boulanger & Arseneault 2004; Rauchfuss & Ziegler 2011). Its principal hosts are balsam fir *Abies balsamea* (L.) Mill. and white spruce *Picea glauca* (Moench) Voss (Sanders 1991). Infestations on trees can continue for six to seven years or until the host trees die. A previous study of heritability in the spruce budworm demonstrated high heritability for egg mass weight (Harvey 1983) and low heritability for diapause emergence (Volney 1999). These studies suggest that life history traits are under strong genetic control, possibly due to winter climatic conditions incurred during diapause. The evolutionary processes that are associated with life history traits in spruce budworm have yet to be studied. Our study thus considers the heritability of traits that are associated with the insect's life history. We test the hypothesis that there is an important additive variance in fecundity and developmental time because they are directly influenced by food source availability. This can lead to consequences for wintertime survival and the synchronization of tree and insect, two major selective forces. This study aims to determine the importance of heritability as a mechanism of adaptation for this defoliator.

MATERIAL AND METHODS

Study organism

A colony of the spruce budworm *Choristoneura fumiferana* was obtained from the Forest Pest Management Laboratory, Canadian Forest Service, Sault Ste. Marie, Ontario. Insects were reared on the artificial diet described by McMorran (1965) under controlled conditions at 23°C, 55–60% relative humidity, under conditions of 16 h light : 8 h dark (LD 16:8) (Robertson 1985). The diet was changed every 10 days to maintain its quality. Pupae were individually weighed and separated by sex in 4 cm x 4 cm cups. When adults had emerged from their pupae, fertilization was assured by grouping two males with each female; for purposes of analysis we used the mean of the two males. Each mating group was housed in an 11 cm x 7.5 cm cage. A flask with a 5% sugar solution was placed in the cage to allow insects to feed. Eggs were collected every two days until the female died. Once larvae had emerged and completed their *hibernaculum* construction, they were placed into diapause for a period of 25 weeks at 2°C.

Experimental design

Four hundred and fifty full-sib families were randomly formed, with couples being formed from individuals with unrelated parents. The laboratory culture was maintained for three generations and stable laboratory conditions were maintained to minimize environmental variability. Mating was done randomly with unrelated parents because this does not introduce bias into heritability estimates; moreover, this method decreases standard errors (Klein *et al.* 1973). Because all individuals were reared under the same environmental conditions, genetic correlations were not considered important (Falconer & MacKay 1996; Sgrò & Hoffmann 2004). Variables that were taken into account in subsequent analyses included fecundity (total eggs per female) and egg fertility (total fertile eggs; we considered only females that were mated and had at least one offspring), pupae fresh mass (mg), and developmental time (days from 3rd to 6th larval instar, pupal time).

Statistical analysis

We considered only families with more than four offspring. As the number of offspring varied among families, we applied the method of Ollivier (1974), which consisted of repeating the value of the parent as many times as there were offspring. Then, narrow sense heritability (h^2) was calculated by regression analysis between parent and offspring for each trait, since this method uses only one parent. A trait's narrow-sense heritability (h^2) was defined as the proportion of phenotypic variance (VP) that was accounted for by additive genetic effects (VA), i.e. $h^2 = VA/VP$ (Falconer & MacKay 1996). The narrow sense heritability determines the degree of resemblance between relatives and it was estimated at twice the regression line slope (Falconer & MacKay 1996). The standard error (SE) of the regression coefficient sampling variance was also estimated to be twice the standard error of the estimate (SEE) of the slope. Data analysis was performed in SAS v6.12 (SAS Institute 1988). Homogeneity of variance was not equal so data were logarithmically transformed and then normality assumptions were verified prior to the implementation of linear regression (Proc GLM and proc REG).

RESULTS

In the current study, significant heritable variance was exhibited by all traits that were analyzed (Table 1-1). For the relationship between mothers and daughters; the highest values of heritability were observed in fertility ($h^2 = 0.61 \pm 0.11$; $F_{1,77} = 33.92$, $P < 0.0001$; Fig. 1-1 A), in fecundity ($h^2 = 0.50 \pm 0.26$; $F_{1,77} = 25.91$, $P < 0.0001$; Fig. 1-1 B), and in developmental time during the larval period ($h^2 = 0.27 \pm 0.06$; $F_{1,780} = 34.17$, $P < 0.0001$; Fig. 1-1 C). Low heritability was exhibited by pupal mass ($h^2 = 0.01 \pm 0.04$; $F_{1,1565} = 14.69$, $P < 0.0001$; Fig. 1-1 E) and pupal developmental time was not significant ($h^2 = 0.001 \pm 1.7$; $F_{1,1245} = 1.22$, $P = 0.2705$; Fig. 1-1 D). We observed low heritability between fathers and sons during the larval period ($h^2 = 0.01 \pm 0.001$; $F_{1,753} = 39.96$, $P < 0.0001$; Fig. 1-1 C) and pupal mass ($h^2 = 0.24 \pm 0.02$; $F_{1,1575} = 19.45$, $P < 0.0001$; Fig. 1-1 E) and pupal development times ($h^2 = 0.006 \pm 0.02$; $F_{1,1305} = 4.52$, $P = 0.0337$; Fig. 1-1 D).

DISCUSSION

Our results demonstrate underlying additive variation related to life-history traits, especially with regards to fertility, fecundity and developmental time. The narrow-sense heritability indicates that an important percentage of resemblance is due to genetic variation in loci with additive effects. Mousseau and Roff (1987) noted that estimates of heritability may be inflated since laboratory conditions reduce environmental variation compared to that of field conditions. However, heritability for life-history traits in the spruce budworm is poorly understood, and our study points to the usefulness of investigating the genetic architecture of this forest pest. Possible maternal effects provide another source of variability, since these effects are a combination of genotype and environment covariance; therefore, the correlation between mothers and offspring may contain significant non-genetic elements (Mousseau & Roff 1987; Falconer & MacKay 1996). However, under stressful conditions, maternal effects may increase the covariance between relatives, thereby inflating the genetic variance (Mousseau & Roff 1987; Potti & Merino 1994). Our results suggest that maternal effects did not play an important role because heritability scores (h^2) were not high ($h^2 < 0.6$) and all insects were reared under non-stress conditions. Several irruptive insects in the boreal forest share similar life history tactics. The harsh climatic conditions oblige the insects to enter into a diapause state (e.g. the hemlock looper *Lambdina fiscellaria fiscellaria* (Guenée), gypsy moth *Lymantria dispar* (L.) and the forest tent caterpillar moth *Malacosoma disstria* (Hübner) (Fitzgerald 1995; Gray *et al.* 2001; Delisle *et al.* 2009). Thus, winter conditions, nutritional resources and predators represent the main force of natural selection acting on these boreal insect species (Royama 1984, Régnière & Duval 1998). High heritability in fecundity and egg fertility may be associated with those factors to compensate for losses incurred through mortality. Volney (1999) investigated the genetic structure of *Choristoneura* and discovered that the developmental process in early spring (April–May) exhibits significant genetic variance. Over several weeks (3 to 4 weeks of difference in bud availability), this variability is probably related to differences in host phenology (Nealis 2003). Thus, genetic variance likely retains an ample array of optimal phenotypes that respond to different times of bud availability (Kause *et al.* 2001). Harvey (1977) demonstrated that initial mean egg mass has an important additive variance. Moreover, Harvey (1983) noted spatial variation in the means of two spruce budworm populations. The

mean egg mass from the first egg laid was higher in the northwest than the southeast population, and it has been further hypothesized that the decrease in egg mass may be an adaptation to winter conditions (Harvey 1985).

Developmental time appeared to be more important for the larval stage than for the pupal stage. The spruce budworm feeds on a limited number of hosts and, consequently, nutritional resources are restricted to a short period of the year. Thus, its performance depends on food of high nutritional quality and availability, unlike other more generalist species that can look to other hosts (e.g. hemlock looper, gypsy moth). These results in developmental times also correspond with other studies in silkworm *Bombyx mori* L. heritability. This species has also exhibited significant heritability with respect to moth emergence, and the duration of larval and pupal stages (Narasimharaju *et al.* 1990; Singh *et al.* 2011). The presence of additive variance in larval duration also can be related to the presence of secondary metabolites and the manner in which nutrients are allocated during the host's growing season (Feeny 1970; Kause *et al.* 2001). These factors directly affect insect performance (Carisey & Bauce 2002; Kumbasli *et al.* 2011) and can have severe consequences on offspring fitness. Thus, heritability may lead insects to achieve optimal development.

Spruce budworm and several other boreal forest insects use a “fast-growth” adaptive strategy, where host-plants are exploited when they have lower levels of defense compounds at the beginning than the end of the season and correspondingly high initial allocation of nutrients with which to grow (Herms & Mattson 1992). The low level of secondary metabolites and low rates of lignification only permit insects to have a short window of time in which to grow and develop. Phenological variation in buds can directly affect the biological performance of the spruce budworm (Fleming & Voney 1995; Rauchfuss & Ziegler 2011). Several studies have revealed that moth species from high latitudes have important heritable variation, which permits them to synchronize their development with their hosts (van Asch *et al.* 2007; Delisle *et al.* 2009). This variability is closely implicated in the adaptation of many species (van Asch *et al.* 2010). Unlike generalist moths, the spruce budworm feeds on a limited number of host species, and, therefore, its biological performance depends on bud availability and quality (Carisey & Bauce 2002). Once a bud is open, it begins to release toxic compounds, thus making it difficult for the insect to digest

and assimilate food (Feeny 1970; Bauce *et al.* 1994); the heritability involved in larval development probably determines the degree of synchronism with the environment. The minimal heritable variation encountered in pupal development indicates that this trait could be also important in natural selection because only a single optimal genotype remains after generations of selection. The relationship between males and their male offspring could explain the synchronization between males and females. In a spruce budworm population, males emerge before females. The low heritability for pupal mass indicates that the optimal genotype is closely related to the fitness of the population. The pupal development stage is a metabolically active period during which the insect prepares for adulthood (Koller & Leonard 1981); variation in traits does not yield a wide range of possibilities. The same pattern is seen in terms of pupal developmental time. Similar results have been found in the mosquito *Wyomyia smithii* (Coq.). Females of this species have greater heritability than males because they exhibit more genetic variation in growth rate than do males (Bradshaw *et al.* 1997). These species are considered semelparous: their life cycle is characterized by a single reproductive period. Thus, females have to allocate all the energy to reproduction, while males also disperse.

In general, heritability for females was greater and more frequent, in terms of the reproductive process. Females usually expend more energy because they search for a place to oviposit, they live longer, and they provide nutritional resources to progeny (Ehrlich & Ehrlich 1978). The larval developmental period is crucial specially for females because the spruce budworm does not feed much as an adult; fitness is thus based primarily on resources acquired during the larval period (Mattson *et al.* 1991). In conclusion, this study shows there is a significant amount of genetic variation in spruce budworm life-history traits. The presence of this additive variance, which operates on phenotypes, indicates which traits are most important to conserve for reasons that are related to environmental constraints (e.g. climate, hosts, phenology) affecting the insect population. Knowledge of the architectural genetic structure has strong predictive value and can greatly assist in understanding the evolution process in spruce budworm. This information facilitates the monitoring of adaptation of insect populations, and climatic and host conditions, which in turn assists with more appropriate measure of pest control.

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Table 1-1 Life history traits of spruce budworm

Trait	Means ± SE							
	Mothers	<i>n</i>	Fathers	<i>n</i>	Daughters	<i>n</i>	Sons	<i>n</i>
Pupal developmental time (days)	6.1 ± 0.8	1305	6.5 ± 0.7	1305	6.1 ± 1.2	1247	6.4 ± 1.6	1307
Larval developmental time (days)	25.0 ± 0.7	1305	24.5 ± 1.3	1305	23.3 ± 3.3	1348	22.1 ± 3.3	1422
Pupal mass (g)	0.1 ± 0.02	1300	0.07 ± 0.01	1566	0.1 ± 0.2	1568	0.07 ± 0.03	1580
Fecundity	191 ± 81	79	–	–	173 ± 111	79	–	–
Fertility	118 ± 73	79	–	–	75 ± 47	79	–	–

–, not applicable.

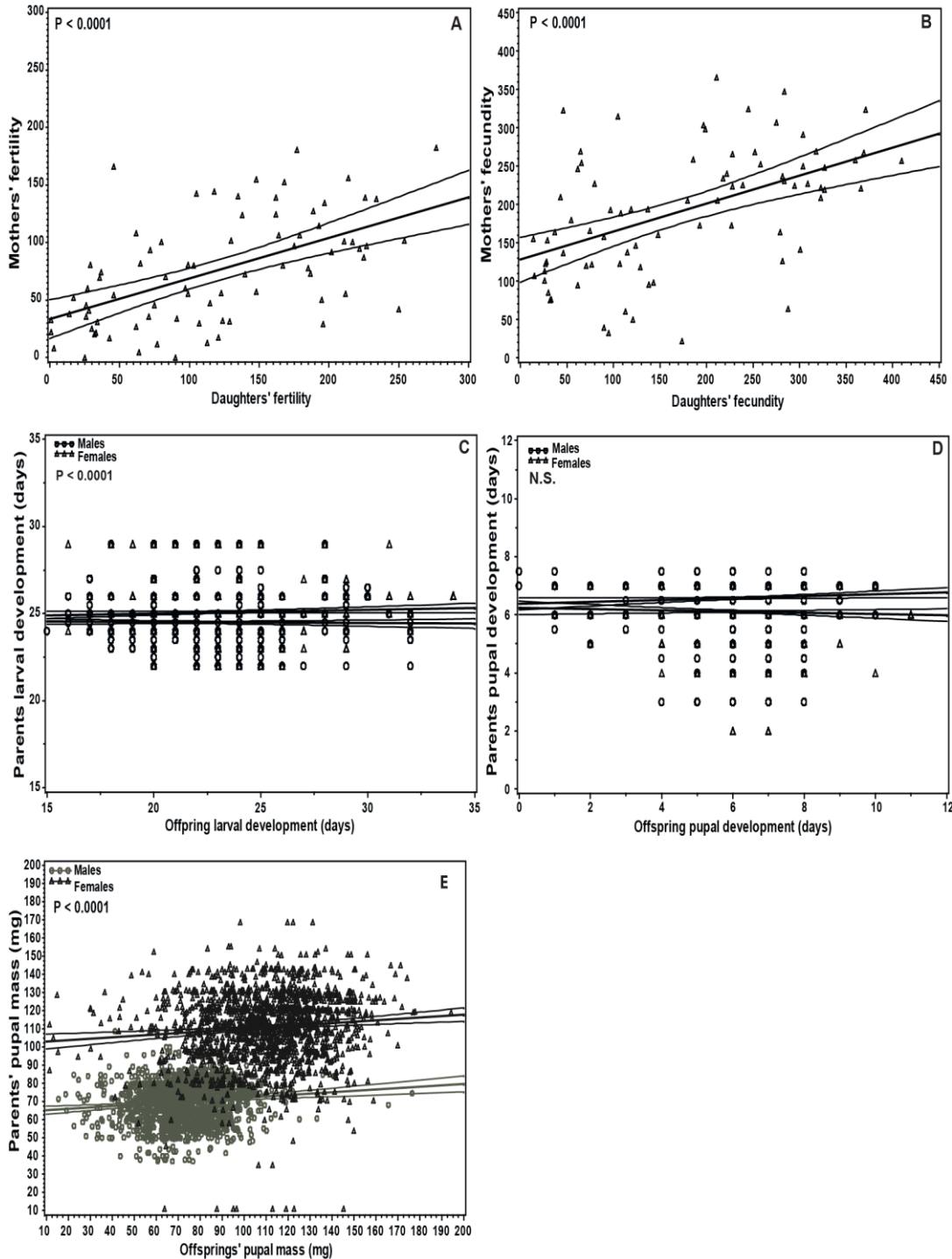


Figure 1-1 Relationships between parent and offspring and the SE. (A) Mean fertility of daughters and mothers, (B) mean fecundity of daughters and mothers, (C) mean of larval developmental time of offspring and parents, (D) mean of pupal developmental time of offspring, (E) mean pupal mass of offspring and parents. Triangles represent females, circles represent males.

Chapitre II

Adaptation of an outbreaking insect defoliator to chronic nutritional stress

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Résumé

Lors d'une épidémie d'insectes, le grand nombre d'individus qui se nourrissent sur l'arbre hôte provoquent une diminution de la ressource alimentaire. La nourriture est non seulement réduite en terme de quantité, mais également en terme de qualité ce qui a une grande influence sur les caractères d'histoire de vie des insectes. Des stratégies adaptatives sont donc sélectionnées afin que leur population survive dans l'environnement. Des expériences ont été menées en laboratoire pour tester l'effet d'un stress nutritionnel au cours de trois générations chez la tordeuse des bourgeons de l'épinette. Les résultats montrent que la mauvaise qualité de la nourriture affecte négativement tous les caractères d'histoire de vie testés (mortalité, temps de développement, poids des chrysalides, taux de croissance et la fécondité) excepté la fertilité. Les femelles de la troisième génération élevées avec un stress nutritionnel constant présentent une adaptation comparée à celles élevées une première fois sur la diète stress. Le temps de développement larvaire a été réduit significativement, tandis que le poids des chrysalides, le taux de croissance et la fécondité ont augmenté significativement. Cette étude montre que la tordeuse des bourgeons de l'épinette possède la capacité de s'adapter dans des conditions de stress nutritionnel. Cette information contribue à comprendre le processus adaptatif de cette espèce spécialement au pic et à la fin d'une épidémie.

Abstract

During insect outbreaks, the high number of individuals feeding on its host plant causes a depletion of the food source. Reduced availability and decreased quality of nutrients negatively influence life-history traits of insects, suggesting that adaptive strategies are selected in order to persist in the environment. In a laboratory experiment with three repetitions, we tested the effect of chronic nutritional stress on spruce budworm performance during three generations to determine the adaptive strategies employed by the insect to deal with a selection pressure produced by low-quality diet. Our results show that all tested life-history traits (mortality, developmental time, pupal mass, growth rate and female fecundity) but female fertility were negatively influenced by the low-quality diet simulating food depletion during outbreak conditions. However, especially females in the third generation under chronic nutritional stress show an adaptive response in life-history traits when compared to those reared only one generation on low-quality diet. Larval developmental time significantly decreased and pupal mass, growth rate and fecundity significantly increased. The study demonstrates the capacity of spruce budworm to react to chronic nutritional stress with adaptations that may be caused by epigenetic parental effects. This information can help to understand the course of an outbreak especially at peak densities and during the collapse.

Introduction

Several studies have documented rates of evolution happening sufficiently fast to represent adaptation (Rundle & Nosil, 2005; Carroll et al., 2007). In general, adaptation of organisms can be observed when populations are exposed to constant environmental constraints (Hairston et al., 2005; Warbrick-Smith et al., 2009; Loxdale, 2010). One example is the continuous attack of host plants by outbreaking insects (Yang, 2012). Some forest insects quickly transition from endemic to epidemic population levels. The high quantity of individuals feeding on trees causes a depletion of its own food source, such as a reducing quantity of foliage and a decreasing quality of nutrients (Tuomi et al., 1984; Haukioja et al., 1991). Nutritional quality is related to the fitness of populations (Warbrick-Smith et al., 2009; Raubenheimer et al., 2012; Reddiex et al., 2013). After several years of severe defoliation, the insect's performance may be negatively affected by the poor nutritional supply (Awmack & Leather, 2002). Several studies revealed that the nutritional composition and availability of food in host trees can be a major factor contributing to the end of an insect outbreak (Morris, 1963; Nealis, 2003; Nealis & Régnière, 2003). However, the altered allocation of nutrition in host plants as a result of insect attacks can constitute a selection pressure leading to new insect phenotypes adapted to the altered resource (Mopper et al., 1995; Hoffmann & Merilä, 1999). Adaptive changes can happen through generations, resulting in adaptive differentiation within and between populations (Schulter, 2009; Warbrick-Smith et al., 2009). Many studies on forest pests focus on ecological aspects such as tree age (Bauce et al., 1994), host plant phenology (Lawrence et al., 1997; Carisey et al., 2004) and natural enemies (Royama, 1984; Venier & Holmes, 2010), even if it has been observed that quality of food affects performance of forest pests (Carisey & Bauce, 2002; Carisey et al., 2004). The adaptive processes related to selection pressure caused by the nutritional quality of the host have been widely neglected. In this study, we focus on spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae), because it is one of the most significant outbreaking insects in North American conifer forests (Morris, 1963; Blais, 1984; Rauchfuss & Ziegler, 2011) and because it is easy to rear in the laboratory (Robertson, 1985). This makes it a good model insect to study selection pressure by nutrients. Spruce budworm is a univoltine insect feeding on current-year foliage of several boreal host trees (Morris, 1963). Its main host tree is balsam fir, *Abies balsamea* (L.) Mill., followed by white spruce, *Picea*

glauca (Moench) Voss, black spruce, *P. mariana* (Mill.) BSP, and red spruce, *P. rubens* Sarg. Quantity and quality of available food sources is strongly related to the synchronization of spruce budworm phenology with its host plants, which affects its development and performance (Blais, 1952; Bauce et al., 1994). During spruce budworm outbreaks, host trees are weakened through the insect's attack and cannot produce adequate foliage, qualitatively and quantitatively. Therefore, spruce budworm larvae experience nutritional stress. As a result, insect performance is severely affected and can initiate the decline of spruce budworm populations (Blais, 1958; Mattson et al., 1991; Carisey & Bauce, 2002), which eventually results in the collapse of an outbreak through the additional action of natural enemies (Régnière & Nealis, 2007). Numerous studies have addressed the effects of various nutrients on spruce budworm performance, principally the effects of nitrogen (Durzan & Lopushanski, 1968; Mattson et al., 1991) and sugars (Harvey, 1974; Albert & Jerrett, 1981; Albert et al., 1982; Albert & Bauce, 1994). These studies suggest that sugars and nitrogen are the most important nutrients acting on spruce budworm performance, influencing weight, fecundity and mortality, creating selection pressure on life-history traits. The existence of phenotypic variation in spruce budworm (Volney, 1999), and a strong heritability in fecundity, pupal mass and developmental time has been demonstrated (Quezada-Garcia & Bauce, 2014). However, despite the extensive literature on this species, no study has reported differential adaptation of spruce budworm under a constant selection pressure, for example caused by nutritional stress. We conducted experiments under laboratory conditions using two artificial diets differing in nutritional quality. The objective was to study the mechanism of adaptation caused by a nutritional selection pressure and to estimate the direction and magnitude of the adaptation for spruce budworm. We focused on life-history traits such as mortality, developmental time, pupal mass, growth rate, female fecundity and fertility over three successive generations. The hypothesis was tested that under continuous selection pressure through nutritional stress, an adaptation of life-history traits to the low-quality diet will take place.

Materials and methods

Insect rearing

Spruce budworm larvae were obtained from the Forest Pest Management Institute, Canadian Forest Service (Sault Ste. Marie, ON, Canada). Post-diapause second instar larvae were reared according to the technique described by Robertson (1985; 23 °C, 55–60% relative humidity, 16 h D:8 h N photoperiod). The first generation of larvae was reared on the standard rearing diet for spruce budworm (McMorran, 1965) to standardize all population variables (Fig. 2.1, Origin). Subsequent generations were reared on one of two artificial diets, with at least 2000 insects per diet. The first diet was the standard rearing diet for spruce budworm with a 12% sugar and 5% nitrogen content (McMorran, 1965). This diet provides spruce budworm larvae with all necessary nutrients for optimal performance (McMorran, 1965; Bidon, 1999) and is hereafter referred to as high-quality diet. The second diet had a lower sugar (1.5%) and a higher nitrogen content (7%). This diet induces detrimental impact on insect survival and development representative of conditions found at the end of an outbreak (Bidon, 1993; Frago & Bauce, 2014) and will hereafter be referred to as low-quality diet. Lower nitrogen contents have been shown to induce very high mortality (close to 100%; Bidon, 1999) and were therefore not suitable for this study. All other ingredients such as minerals, vitamins and water content remained the same in both diets. Experimental design A reciprocal transference experimental design (Fig. 2.1) with three repetitions was used to measure the influence of the two diets on spruce budworm life-history traits. First, individuals were reared on high-quality diet until adulthood to standardize all variables (Fig. 2.1, Origin). Thereafter, the offspring was divided into two parts and one thousand individuals were reared on high- and low quality diet, respectively (Fig. 2.1, F1). The resulting offspring from each diet were then again divided into two parts and randomly assigned to a high- or low-quality diet (Fig. 2.1, F2). The offspring of the F2 generation consecutively reared on high-quality and low-quality diet were then again divided into two parts and randomly assigned to one of the two diets (Fig. 2.1, F3). This procedure was used to compare the performance of individuals in the first generation on low-quality diet with those in the second or third consecutive generation under nutritional stress. This process was repeated three times with larvae of three different generations originating from the same population.

Biological variables

Seven variables were taken for each F2 and F3 generation of the experiment: mortality, larval developmental time, pupal mass, growth rate, realized fecundity, fecundity and female fertility. A subsample of 1760 larvae (960 from the F2 generation and 800 from the F3 generation) was taken to estimate insect mortality from sixth instar larva to imago for each treatment. Larval developmental time was counted in days from larval emergence after hibernation until the pupal stage. Pupal mass was taken as fresh weight 24 h after pupation with a precision of 0.1 mg. Growth rate was calculated as described in detail by Frago & Bauce (2014) to measure the efficiency of individuals to use developmental time to reach adult mass. Realized fecundity was measured by counting the total number of eggs produced by a female, and egg fertility by counting all viable eggs. Female fertility represents the percentage of fertile females per diet and generation. Female fertility was estimated by considering each coupled female as fertile when it laid at least one viable egg.

Statistical analysis

Three-way analyses of variance (ANOVAs) were performed separately for each sex to analyse developmental time, pupal mass and growth rate because diet affects performance of spruce budworm males and females differently (Koller & Leonard, 1981; Carisey & Bauce, 2002). Factors included in the analysis were generation (F2 and F3), diet (high and low quality), parental diet (high and low quality) and all possible interactions (PROC GLM, SAS Institute, 2003). Realized fecundity and fecundity were analysed with Poisson regressions (PROC GENMOD, SAS Institute, 2003), and fertility and mortality with logistic regressions using binomial distributions (PROC GLIMMIX, SAS Institute, 2003). Means were compared using Tukey's range test for significant factors.

Results

Mortality was significantly influenced only by diet ($X^2_{1,12} = 10.38$; $P = 0.0073$) and generation ($X^2_{1,12} = 21.32$; $P = 0.0006$). Low-quality diet caused significantly higher mortality compared with high-quality diet (Fig. 2.2a), and mortality was significantly higher in the F2 generation when compared to the F3 generation (Fig. 2.2b). Larval developmental time was significantly longer on low-quality diet compared with high-quality diet for both sexes. It was also longer for males when larvae of the previous generation were fed low-quality diet. However, this carry-over effect was not observed for females. For both females

and males, all interactions significantly influenced larval developmental time, including the interaction between all three main factors (Table 2.1; Fig. 2.3a,d). Pupal mass was significantly lower when larvae of both sexes were fed low-quality diet. However, the interaction between diet and precedent diet was not significant for both sexes (Table 1). Pupal mass was significantly lower in the second generation (F2) when compared to the third (F3), and diet as well as parental diet had a significant interaction with generation. Only in the F3 generation low-quality parental diet significantly increased pupal mass for both females (Fig. 2.3b) and males (Fig. 2.3e). The interaction between all three main factors was not significant. Growth rate of males and females was significantly reduced by the low-quality diet. Diet of the parental generation alone did not have a significant influence on growth rate. However, a significant interaction between diet and parental diet and between diet and generation was found for both males and females (Table 2.1). Compared with males consecutively reared on high-quality diet, the growth rate of males on high quality diet but low-quality parental diet was significantly reduced (Fig. 2.3f). A significant influence of the interaction of all three main factors on growth rate was found for females only (Table 2.1; Fig. 2.3c). The total number of eggs laid (realized fecundity; Table 2.2) and the number of viable eggs laid (egg fertility; Table 2.2) by females were significantly reduced when larvae were reared on low-quality diet. When larvae of the parental generation were fed low-quality diet, realized fecundity and fecundity in the present generation were also significantly reduced. The interaction of diet and parental diet as well as the interaction between generation and parental diet significantly influenced fecundity and realized fecundity. Only in the F3 generation realized fecundity and fecundity were significantly increased by low-quality parental diet (Fig. 2.4). Female fertility was significantly higher on low-quality diet compared with high-quality diet (Fig. 2.5). All other main factors and interactions did not significantly influence female fertility; however, generation and the interaction between diet and generation were nearly significant (Table 2).

Discussion

Several studies have shown that nutritional stress can have negative effects on spruce budworm performance over several generations (Carisey & Bauge, 2002; Fuentealba & Bauge, 2012; Frago & Bauge, 2014). In our study, mortality and larval developmental time

were increased and pupal mass and growth rate were significantly reduced by nutritional stress. When released from nutritional stress after two generations, a carry-over effect became evident for both males and females, expressed by a decreased growth rate as a result of an increased developmental time. Carisey & Bauce (2002) showed that low food quality can negatively affect early larval development of spruce budworm progeny, suggesting a carry-over effect of nutritional stress to the next generation. Fuentealba & Bauce (2012) also suggest that effects of nutritional quality on spruce budworm performance can carry over to the next generation. This can be explained because life-history traits such as fecundity, fertility, larval developmental time and pupal mass exhibit high heritability (Quezada-Garcia & Bauce, 2014). We show in this study that chronic nutritional stress can also result in adaptations of spruce budworm life-history traits. An increased pupal mass but shorter developmental time resulted in an increased growth rate for spruce budworm females three generations under chronic nutritional stress, relative to females of the same generation but the first time under nutritional stress (low-quality diet and high-quality parental diet). In contrast, only pupal mass of males increased after three generations and no adaptations occurred in developmental time and growth rate. However, low-quality diet did not result in an accumulative negative effect for males, contradicting the findings of Frago & Bauce (2014). The above-described adaptations are likely to be caused by epigenetic changes triggered by poor food quality in the parental generation, even if rapidly evolving genetic adaptations cannot be excluded given the present experimental design. Non-Mendelian parental effects, often described for simplicity as maternal effects, are common in insects (Mousseau & Dingle, 1991) and make fast adaptations to environmental changes such as decreasing food quality possible (Mousseau & Fox, 1998). Because food quality may improve as spruce budworm population levels decrease, the adaptations to nutritional stress have to be reversible to ensure an optimized exploitation of the food source. Maternal effects can be reversible as they are not necessarily changes in the DNA sequence which can only be changed through mutation, but they can also be changes in gene expression (epigenetic changes) and therefore be revocable. Therefore, they provide a logical explanation for the cause of adaptations reported in this study. Maternal effects leading to adaptations of insects to their environment are a frequently studied phenomenon (Mousseau & Dingle, 1991), but the role of maternal effects in Lepidopteran pests has received relatively little attention

(Hunter, 2002). Maternal effects influencing the time of egg hatch in winter moths *Operophtera brumata* L. (Lepidoptera: Geometridae) have been found to lead to an adaptation of the insect to host plant phenology (van Asch et al., 2010). A classic empiric example of maternal effects caused by food quality is the variation of egg size and egg provisioning, which transmits the mother's experienced food quality to the next generation. A classic example is described by Rossiter (1991b) for gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Erebidae). But maternal effects caused by food quality go reportedly beyond the egg and first larval instars. For example, consumption and pupal mass of the outbreaking autumnal moth *Epirrita autumnata* Borkhausen (Lepidoptera: Geometridae) can be influenced through maternal effects by food quality the mother experienced (Alonso et al., 2001). And also pupal weight and developmental time of gypsy moth progeny can be influenced by the mother's diet (Rossiter, 1991a). The above-described maternal effects have an immediate effect on the next generation, whereas in our study the improved performance on low-quality diet becomes only visible in the third generation. However, examples can be found in the literature describing maternal effects influencing progeny over three generations (Hercus & Hoffmann, 2000). Because we cannot conclusively demonstrate that maternal effects over three spruce budworm generations are responsible for the adaptive processes associated with nutritional stress found in this study, this hypothesis should be tested experimentally in a subsequent study. Differences in the reaction to nutritional stress between males and females may be produced by the fact that females have different nutritional requirements than males (Koller & Leonard, 1981; Stockhoff, 1992). Females have higher metabolic costs than males incurred in connection with their longer lifespan and reproductive processes, for example allocation of nutritional resources to offspring, location of oviposition sites, oviposition, etc. (Thornhill, 1976). This may make females more susceptible to unbalanced diets. In addition, there are higher metabolic costs related to the consumption of poor-quality food (Behmer, 2009). This may force larvae to increase the efficacy to utilize the low-quality diet. Quezada-Garcia et al. (submitted) found that, when subjected to low-quality food, spruce budworm females but not males increased their consumption rate in the second generation to meet their nutritional requirements. However, in the third generation, females managed to reduce their consumption rate and, at the same time, increase their pupal mass and decrease their developmental time. These results suggest that an increase in the

efficacy to utilize low-quality diet plays an important role in the adaptation of spruce budworm females to nutritional stress. There is a trade-off between developmental time and final insect size. The longer the developmental time of an insect, the more it is exposed to mortality factors such as predation or parasitism (Sibly, 1986).

To optimize chances of survival, the developmental time of insects to the adult stage should be as short as possible (Nylin & Gotthard, 1998). In general, less time is needed for smaller individuals to develop, but smaller individuals may have disadvantages compared with bigger ones, such as fewer chances to mate (Reznick et al., 2000) or a lower fecundity (Honěk, 1993). Therefore, the insect has to find a balance between developmental time and its optimum size. As shown in this study and several others (Carisey & Bauce, 2002; Fuentealba & Bauce, 2012; Frago & Bauce, 2014), spruce budworm has generally a longer developmental time and a lower body mass under nutritional stress and therefore biological and ecological disadvantages. Our data suggest that after three generations on low-quality diet, spruce budworm is able to respond to nutritional stress by improving the management of the trade-off between developmental time and pupal mass.

Insects subjected to environmental constraints can resort to two strategies to increase offspring survival. They can either produce many small eggs or fewer large eggs. For example, Harvey (1983) found that spruce budworm females laid fewer but bigger eggs in northern latitudes and more but smaller eggs in southern latitudes. Large spruce budworm eggs provide more metabolic reserves to overwintering second-instar larvae, which can then exhibit higher survival under harsh winter conditions than larvae emerging from small eggs (Harvey, 1985). Carisey & Bauce (2002) found that egg mass weight of females on low-quality diet is significantly lower. In the present study, we show that spruce budworm females on low-quality diet decrease the total number of eggs and the number of viable eggs compared with females on high-quality diet. However, after three generations of nutritional stress, realized fecundity and fecundity increased compared with females which parents were fed on high-quality diet. This result suggests that the adaptive strategy of spruce budworm in response to nutritional stress is to produce more eggs, which may increase the probability of offspring survival. Under nutritional stress conditions, spruce budworm females may not have met all the nutritional requirements to complete their reproductive process. During mating, spruce budworm males do not only transfer a spermatophore containing sperm but

also accessory gland secretions which represent an additional nutritional resource for females (Deslisle & Hardy, 1997). These male donations may play an important role for females fed on low-quality diet to complete their nutritional requirements, making them more prone to mate to assure fertility of their eggs, even if also the nutritional value of the male's secretions may be influenced by the low-quality diet. Studies on *Musca domestica* L. (Diptera: Muscidae) revealed that under detrimental nutritional stress, mated females lived significantly longer, suggesting that they could get nutritional resources from males that increase the fitness of the population (Hicks et al., 2004). Also females of several lepidopteran species were found to increase their longevity due to male donations (Kaitala & Wiklund, 1994; Wedell, 1996). As spruce budworm populations rise and reach epidemic levels, negative density-dependent feedbacks caused by heavy defoliation lead to population declines (Régnière & Nealis, 2007). However, spruce budworm populations remain at epidemic levels for several years (Morris, 1963; Royama, 1984; Rauchfuss & Ziegler, 2011) and therefore, to persist in the environment under these detrimental conditions, the insect has to find adaptive strategies to compensate its low performance. Our study demonstrates that spruce budworm can react to such detrimental conditions with adaptations of life-history traits to chronic nutritional stress. Spruce budworm population series compiled and discussed by Régnière & Nealis (2007) show that after an initial decline of population levels following the peak of an outbreak, often a recovery occurs after only a few years. This repeatedly observed phenomenon (Royama, 1984; Nealis & Régnière, 2004) has been associated with fluctuations of spruce budworm populations near the carrying capacity of the forest (Régnière & Nealis, 2008). Our study can shed some light on the adaptive strategies that might be involved in allowing spruce budworm populations to maintain population levels so near the carrying capacity of the forest despite excessive depletion of their food source through severe defoliation.

The present study demonstrates that spruce budworm can react to chronic nutritional stress with adaptations of life-history traits that allow this species to improve overall performance on low-quality diet. These findings may help to not only better understand the role of nutritional stress in spruce budworm dynamics but also its importance in the dynamics of other outbreaking insects.

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Table 2.1 Factors introduced to ANOVA as fixed effects and their levels of significance for larval developmental time, pupal mass and growth rate of spruce budworm males and females.

Source of variation	Larval developmental time						Pupal mass						Growth rate					
	males			females			males			females			males			females		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Model	359.04	7,716 7	<.000 1	350.9 1	7,816 1	<.000 1	180.43	7,716 0	<.000 1	105.5 2	7,815 2	<.000 1	574.86	7,878 1	<.000 1	539.66	7,997 5	<.000 1
Diet	1553.7 7	1,716 7	<.000 1	774.0 3	1,816 1	<.000 1	1150.2 6	1,716 0	<.000 1	637.1 4	1,815 2	<.000 1	2589.2 7	1,878 1	<.000 1	1954.7 6	1,997 5	<.000 1
Parental diet	1.72	1,716 7	0.190 0	7.15	1,816 1	0.007 5	10.45	1,716 0	0.001 2	9.94	1,815 2	0.001 6	0.18	1,878 1	0.673 5	1.57	1,997 5	0.210 1
Diet* Parental diet	77.10	1,716 7	<.000 1	15.49	1,816 1	<.000 1	3.24	1,716 0	0.071 7	0.57	1,815 2	0.449 2	36.61	1,878 1	<.000 1	23.71	1,997 5	<.000 1
Generation	571.50	1,716 7	<.000 1	774.0 3	1,816 1	<.000 1	42.70	1,716 0	<.000 1	13.70	1,815 2	0.000 2	580.20	1,878 1	<.000 1	860.37	1,997 5	<.000 1
Generation*Diet	37.27	1,716 7	<.000 1	112.4 8	1,816 1	<.000 1	62.79	1,716 0	<.000 1	31.27	1,815 2	<.000 1	200.18	1,878 1	<.000 1	288.99	1,997 5	<.000 1
Generation*Parental diet	39.76	1,716 7	<.000 1	31.92	1,816 1	<.000 1	33.03	1,716 0	<.000 1	33.58	1,815 2	<.000 1	0.04	1,878 1	0.833 3	0.07	1,997 5	0.793 4
Diet*Parental diet* Generation	57.86	1,716 7	<.000 1	26.61	1,816 1	<.000 1	0.09	1,716 0	0.765 2	1.51	1,815 2	0.218 8	6.49	1,878 1	0.010 8	0.44	1,997 5	0.505 9

Table 2.2. Factors introduced to the logistic regression as fixed effects and their level of significance for realised fecundity, fecundity and fertility of spruce budworm females.

Source of variation	Realised fecundity			Fecundity			Fertility		
	χ^2	df	<i>P</i>	χ^2	df	<i>P</i>	χ^2	df	<i>P</i>
Diet	182.45	1,2468	<.0001	66.63	1,2468	<.0001	26.16	1,16	<.0001
Parental diet	7.21	1,2468	0.0073	5.78	1,2468	0.0163	0.14	1,16	0.7159
Generation	54.08	1,2468	<.0001	16.38	1,2468	<.0001	4.02	1,16	0.0621
Diet*Generation	5.46	1,2468	0.0195	1.14	1,2468	0.2850	4.14	1,16	0.0587
Diet* Parental diet	9.28	1,2468	0.0023	9.48	1,2468	0.0021	1.07	1,16	0.3161
Parental*diet Generation	8.52	1,2468	0.0035	8.73	1,2468	0.0032	0.03	1,16	0.8597
Diet*Parental diet* Generation	0.63	1,2468	0.4266	0.19	1,2468	0.6642	1.35	1,16	0.2620

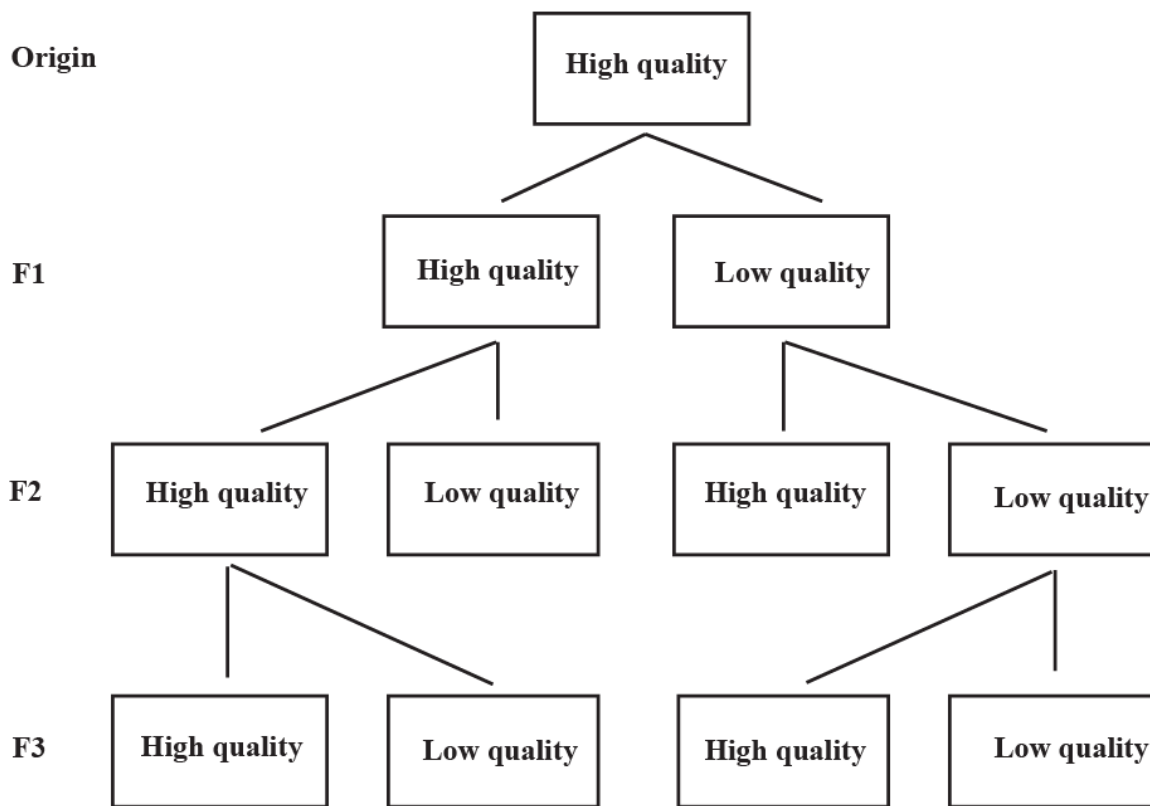


Figure 2.1 Schematic diagram of the experiment to estimate performance and adaptive responses of spruce budworm to chronic nutritional stress.

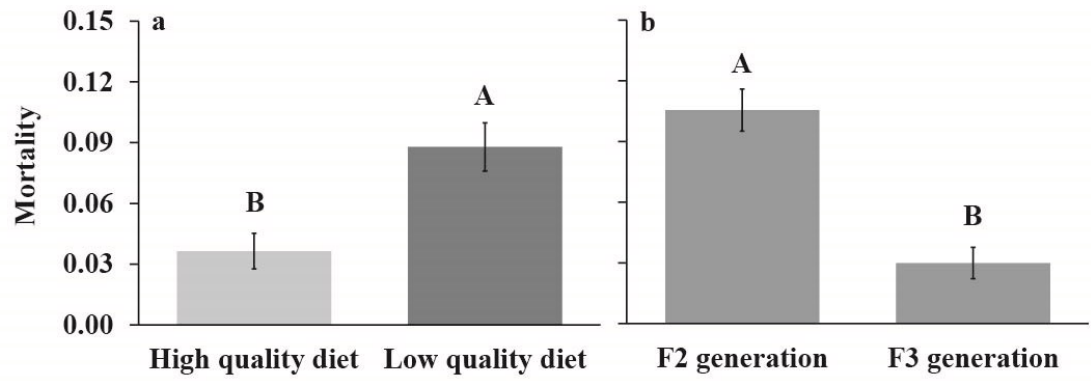


Figure 2.2. Mean (\pm SEM) ratios of mortality from sixth instar spruce budworm larva to imago influenced by high and low quality diet (a) and generation (b). Bars followed by the same letter do not differ significantly at $P < 0.05$ according to Tukey's range test.

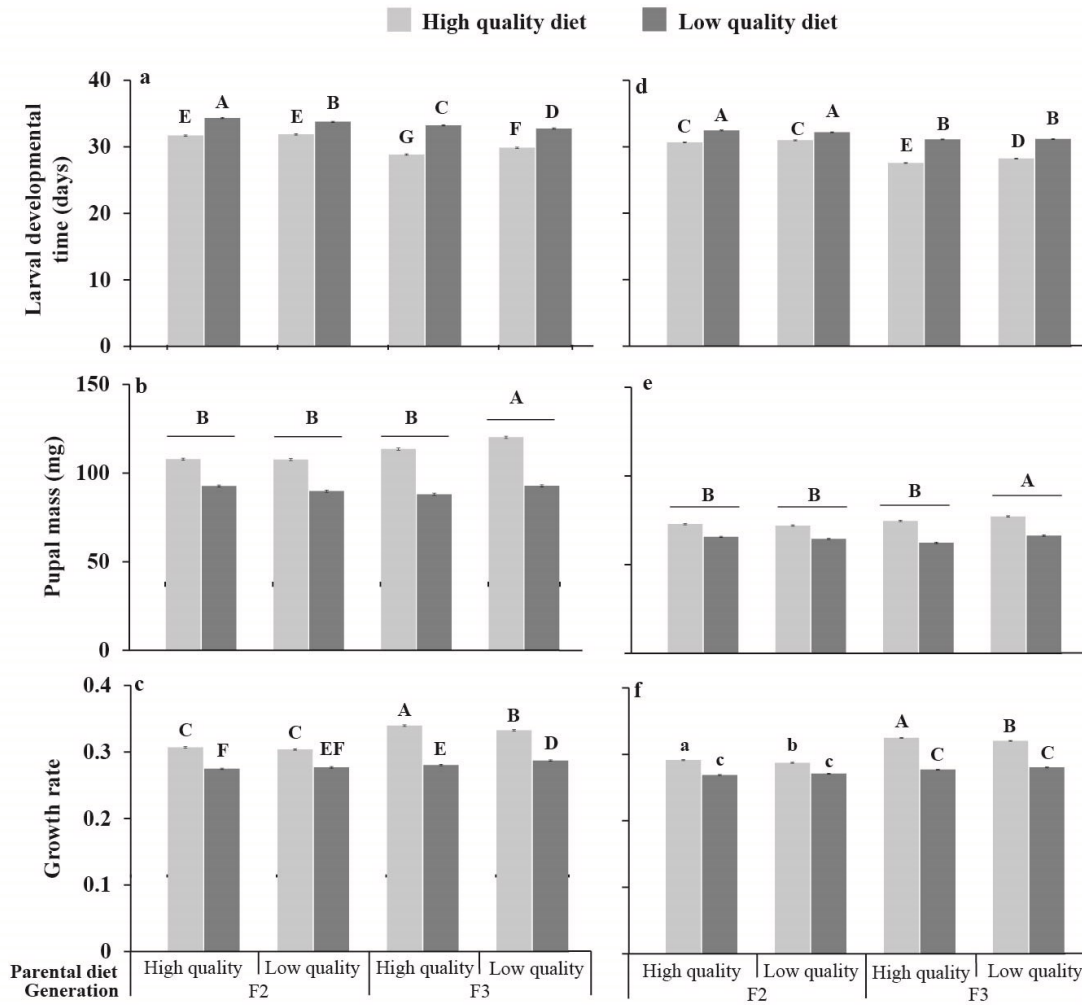


Figure 2.3. Mean (\pm SE of the interaction) developmental time, pupal mass and growth rate (mg /day) of spruce budworm influenced by diet, parental diet and generation for females (a to c) and males (d to f). Bars followed by the same letter do not differ significantly at $P < 0.05$ according to Tukey's range test.

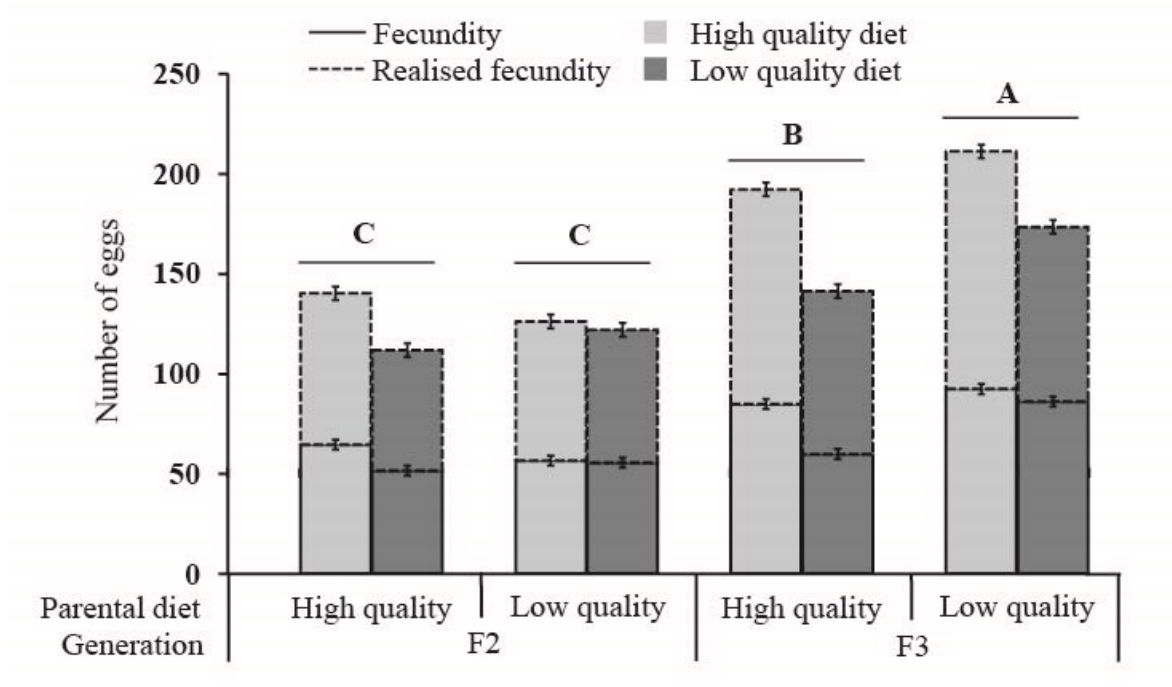


Figure 2.4. Mean (\pm SE of the interaction) fecundity and realised fecundity of spruce budworm females influenced by diet, parental diet and generation. Bars followed by the same letter do not differ significantly at $P < 0.05$ according to Tukey's range test.

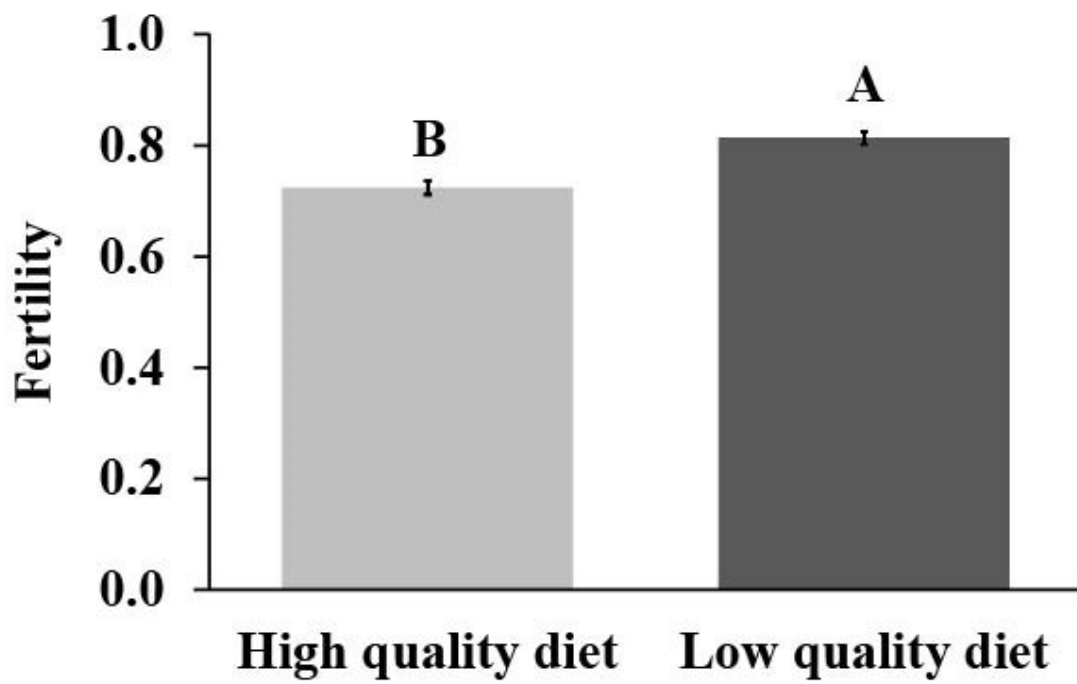


Figure 2.5. Mean (\pm SEM) ratios of fertile spruce budworm females affected by high and low quality diet. Bars followed by the same letter do not differ significantly at $P < 0.05$ according to Tukey's range test.

Chapitre III

Increasing consumption rate or digestion efficiency? Responses of an outbreaking herbivore insect to nutritional selection pressure over multiple generations.

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Résumé

À la fin d'une épidémie, les insectes herbivores sont soumis à une pression de sélection nutritionnelle élevée en raison du grand nombre d'individus présents par rapport à la quantité et à la qualité de la ressource alimentaire disponible. Dans la présente étude, nous avons testé l'effet d'un stress nutritionnel constant sur la capacité de la TBE à utiliser de la nourriture. L'effet d'un stress nutritionnel constant a été testé sur la TBE pendant trois générations afin de déterminer les stratégies qui sont sélectionnées pour contrer la pression de sélection engendrée par la mauvaise qualité de la nourriture qui peut causer un impact négatif sur la survie et le développement à la fin d'une épidémie. Les résultats ont montré que la TBE de deuxième génération augmente son taux de consommation (RCR) lorsqu'elle s'alimente à partir d'une diète de mauvaise qualité. Cependant, cette stratégie ne compensait pas suffisamment les carences pour que les larves nourries de la diète stress atteignent un poids similaire à ceux des larves nourries avec une diète de bonne qualité. Une réduction du temps de développement, une réduction de la RCR et une augmentation du taux de croissance (RGR) ont été observées dans la troisième génération, ce qui suggère que les insectes commencent à ajuster leur capacité à assimiler la nourriture de mauvaise qualité. Ce phénomène peut s'expliquer en partie par la légère augmentation de l'efficacité de conversion de la nourriture digérée (ECD) et par la conversion de la nourriture ingérée (ECI) observées dans la troisième génération. Ces observations pourraient s'expliquer par la présence de plasticité phénotypique chez la TBE. Ces résultats suggèrent que la tordeuse possède la capacité d'adapter l'utilisation de la nourriture, ce qui permet à l'insecte de persister dans l'environnement après l'effondrement d'une épidémie. Cette information peut représenter une clé importante pour comprendre l'adaptation de ce ravageur forestier pendant les épidémies.

Abstract

1. Insect herbivores are subjected to nutritional selection pressure at the end of outbreak episodes because of the great number of individuals that deplete the quantity and quality of food available.
2. The effect of constant nutritional stress was tested on spruce budworm for three generations to determine the strategies that were employed by the insect to deal with a selection pressure produced by low quality food that induces detrimental impact on insect survival and development representative of conditions found at the end of an outbreak.
3. Results showed that spruce budworm increased its consumption rate (RCR) when fed on low quality diet in the second generation. However, this compensatory strategy was not enough for larvae fed on low quality diet to reach the mass observed in larvae fed on high quality diet.
4. We observed a reduction in developmental time, together with a reduction in RCR and an increase in growth rate (RGR) in the third generation, suggesting that insects start to adjust their utilization of food on low quality diet. This phenomenon may be explained in part by the slight increase in the efficiency of conversion of digested food (ECD) and ingested food (ECI) observed in the third generation, suggesting the presence of phenotypic plasticity in spruce budworm.
5. This study suggests that food utilization in the spruce budworm can be subject to adaptations, which allows the insect to persist in the environment after the collapse of an outbreak. This information may represent a key to understanding adaptation of this forest pest during outbreaks.

Introduction

Food nutritional value has an important influence on the rate and extent of insect herbivore development (Haukioja *et al.*, 1991; White, 1993). Food quality affects ecological, physiological, and behavioural processes that play an important role in determining the future of the population. This could lead to the start (*e.g.*, White, 1993) or decline (*e.g.*, Miller, 1977; Régnière & Nealis, 2008) of insect outbreaks. However, the quality of the food encountered by insects is not always suitable for their development and reproduction, forcing some species to adjust their feeding strategies to improve the efficiency of food utilisation or increase consumption rates to meet their nutritional requirements. One example of this sort of adaptation is *Omocestus viridulus* (Acrididae), which can employ compensatory feeding to overcome nitrogen deficiencies in wheat (Berner *et al.*, 2005).

Many studies of forest insect pests focus upon ecological aspects such as tree age (Bauce *et al.*, 1994), host plant phenology (Lawrence *et al.*, 1997), and mortality factors (Royama, 1984), even when it has been observed that food quality affects the performance of these insects (Carisey & Bauce, 2002). Adaptive processes related to selection pressures that are imposed by host nutritional quality have been widely neglected.

Spruce budworm is the most significant irruptive insect pest in the boreal and maritime coniferous forests of eastern North America (Morris, 1963; Blais, 1984; Rauchfuss & Ziegler, 2011). Larvae normally feed on current-year foliage; therefore, their development directly depends upon the quantity and quality of the foliage (Blais, 1952; Bauce *et al.*, 1994). During insect outbreaks, the high number of individuals consuming plant tissues reduces the quantity and quality of food, which can induce the collapse of the outbreak episode, in tandem with the effects of natural enemies (Miller, 1977; Régnière & Nealis, 2008). Therefore, spruce budworm larvae are often forced to feed on mature foliage (*i.e.*, from the previous year), with negative consequences for performance (Blais, 1958; Mattson *et al.*, 1991; Carisey & Bauce, 1997; 2002).

Inherently low nutritional quality of host foliage that has been subjected to repeated defoliation over the years is a selection pressure on spruce budworm, which has been implicated in the decline of outbreak episodes of this insect (Miller, 1977; Régnière & Nealis, 2008). Low quality food is conducive to changes through selection of the insect's

feeding behaviour and digestive efficiency to meet nutritional requirements (Carisey & Bauce, 1997). This adjustment must be done over few generations to permit the insect to persist in the environment. Otherwise, the selection pressure may result in insect population extinction at the local level (Jermy, 1984). Insects that exhibit high phenotypic plasticity are more prone to adjust quickly to changes in the environment (Barbosa & Baltensweiler, 1987). Another way to adjust to volatile environments is through maternal effects. The importance of maternal effects has been widely observed in Lepidoptera (Mousseau & Fox, 1998). Yet knowledge regarding the adaptive capacity of spruce budworm is scarce. It was recently reported that spruce budworm has the capacity to adapt to low quality food after a few generations (Quezada-Garcia *et al.* 2015). The adaptive strategy that was used by the insect to cope with low quality food, however, remains to be elucidated. A good understanding of the strategy that is employed by an insect facing this nutritional selection pressure may help us understand its adaptive capacity and the evolutionary direction that the population may take in the future under various climate change scenarios.

The main objective of this study was to determine the strategies employed by spruce budworm to deal with a selection pressure associated with low quality food. Spruce budworm larvae were reared on two types of diets (a high quality diet vs a low quality diet. The latter diet produces a mortality rate as high as 90 % (Frago & Bauce, 2014), which is similar to the mortality rate found at the end of outbreak episodes (> 80 %; Raske, 1985), over three consecutive generations and three repetitions of the experiment. We hypothesised that the insect will increase its efficiency for using the low quality food. This kind of strategy is used frequently by specialist insects (Simpson *et al.*, 2002; Behmer, 2009), but it has not been documented for spruce budworm.

Materials and Methods

Insect rearing

Larvae of spruce budworm (*Choristoneura fumiferana* (Clemens)) were reared under laboratory conditions. Insects were obtained from the Forest Pest Management Institute, Canadian Forest Service (Sault Ste. Marie, Ontario). Larvae were reared over three consecutive generations under controlled conditions, according to the technique

described by Robertson (1985) (23 °C, 60 ± 5 % relative humidity, 16 h D:8 h N photoperiod). Two artificial diets were formulated, differing only in nitrogen and sugar content. All other ingredients such as minerals, vitamins and water content remained the same in both diets. The first diet contained 12 % sugar and 5 % nitrogen (high quality diet), whereas the second contained 1.5 % sugar and 7 % nitrogen (low quality diet). The high quality diet corresponds to McMoran's diet (McMoran, 1965), while the low quality diet was obtained by modifying McMoran's diet, as described by Bidon (1999). The low quality diet was chosen because it has a negative effect on larval survival, development and growth. This diet produces a mortality rate as high as 90% (Frago and Bauce, 2014), which is similar to the mortality rate observed at the end of outbreak episodes (> 80 %) (Raske, 1985). This low quality diet, therefore, exerts a considerable selection pressure on spruce budworm, but it allows the insect to reproduce and have viable offsprings. Other low quality diets were tested (7.8 % sugar and 1.7 % nitrogen; 6 % sugar and 2.7 % nitrogen), but the high mortality rates that were incurred (almost 100 %) prevented us from using them. High quality diet provides spruce budworm larvae with all of the necessary nutrients for good performance (McMorran, 1965; Bidon, 1999). Spruce budworm larvae that were obtained initially from the Canadian Forest Service were reared on high quality diet in order to standardise all population variables. Subsequent generations were reared on each of the two artificial diets.

Insects were reared in Petri dishes (100 x 15 mm), with 10 individuals per dish. Pupae and adults were separated according to sex (Robertson, 1985). Mortality was recorded every day. Couples were installed in circular plastic cages of 11 cm x 7.5 cm. Adults were fed a 5 % sugar water solution and allowed to mate. Eggs were collected two or three days after the females died. Substrates that were provided included wax paper for ovipositing and cheese-cloth for overwintering. Larvae that hatched were held at 18 °C for two weeks and were then transferred to 2 °C for 25 weeks to overwinter in dark growth chambers (Robertson, 1985).

Experimental design

A reciprocal transfer experimental design with three replicates was used to measure the influence of the two artificial diets on spruce budworm food utilisation. Individuals

were randomly selected and reared until adulthood on high quality food to standardise all variables (Fig. 3.1, Origin). Thereafter, the offspring were divided into two groups: one was assigned to the high quality diet and the second to the low quality diet. They formed the first generation (F1) (hereafter referred to as parental diet) that was used in this study (1000 individuals per diet). Progeny from individuals that were reared on high and low quality diet were then again divided into two groups: one was assigned to the high quality diet and the second to the low quality diet, forming the second generation (F2) that was used in this study (Fig. 3.1, F2). The offspring of individuals from the F2 generation that were reared on either the high or low quality diet for two consecutive generations was then again divided into two groups and randomly assigned to one of the two diets (Fig. 3.1, F3). This procedure was used to compare the performance of individuals from the first, second and third consecutive generations under nutritional stress. This process was repeated three times with larvae from three different generations that originated from the same population.

Biological variables

For each generation and treatment combination, 80 sixth-instar larvae were randomly chosen to determine relative growth rates and relative consumption rates, which were estimated on a dry-mass basis (gravimetric experiment). Newly-moulted sixth-instar larvae were weighed and placed in Petri dishes (100 x 15 mm). Larval developmental time was monitored twice daily. Ingested food and excreted dry faeces that had been produced during larval development were quantified, as described by Bauce et al. (1994). Relative growth rate (RGR), relative consumption rate (RCR), approximate digestibility (AD), the efficiency of conversion of digested food (ECD), and the efficiency of conversion of ingested food (ECI), were determined from the following formulae:

$$\text{RGR} = G/MW * \text{hours (developmental time)},$$

$$\text{RCR} = I/MW * \text{hours (developmental time)},$$

$$\text{AD} = 100 * (I - F) / I$$

$$\text{ECI} = G * 100 / I$$

$$\text{ECD} = G * 100 / (I - F)$$

where:

$$G = \text{gained mass} = (\text{final mass} - \text{initial mass})$$

MW = mean larval mass = $G/\log(\text{final mass}/\text{initial mass})$

I = ingested food

F = faeces

Nutritional indices were expressed on a dry-mass basis according to Waldbauer (1968).

Statistical analysis

Three-way analysis of variance (ANOVA) was performed for each of the aforementioned variables throughout with generations (F2 vs F3), diets (high quality vs low quality) and parental diet (high quality and low quality) as factors with three replicates (PROC GLM). When data did not satisfy ANOVA requirements, they underwent rank transformation (Conover & Iman, 1981) (PROC RANKS). Means were compared using the Tukey adjustment test (Zar, 2010) (PROC GLM). Linear or non-linear regression was computed for each ratio of nutritional index to determine an adjusted ratio and allow meaningful comparisons of indices (Bauce *et al.*, 1994). A logistic regression was performed to assess treatment effects on mortality (PROC GENMOD). Data were analysed using SAS 9.3 (SAS Institute, 2012).

Results

Mortality

Female mortality was significantly affected by the interaction between generation and diet on female mortality ($\chi^2_{1,839} = 5.43, P = 0.0200$). Indeed, the highest mortality was observed in those individual that were fed on low quality diet whereas the lowest mortality was observed in those individual that were fed on high quality diet in the second generation. However, a great reduction in insect mortality was observed in those females fed on low quality diet in the third generation, being this mortality rate the lowest observed in the aforementioned generation (Fig. 3.2h). In contrast, male mortality was not affected by the treatments tested ($\chi^2_{1,765} = <0.00, P = 0.99$; Fig. 3.3h).

Pupal mass

Results showed a significant effect of diet, generation, and the interaction between these two factors on female pupal mass (Table 3.1). Females reared on low quality diet exhibited lower pupal mass than those reared on high quality diet. Furthermore, females showed an increase in pupal mass in the third generation as compared with the second generation in both type of diets but this increase was higher on high quality diet (Fig. 3.2a). As for male pupal mass, this variable was significantly affected by generation, diet, parental diet and the interaction between generation and diet (Table 3.2). The impact of generation x diet interaction on male pupal mass was equivalent to that observed on female pupal mass (Fig. 3.3a). Parental diet had an interesting impact on male pupal mass. Individuals whose parents were reared on low quality diet showed greater pupal mass than those individuals whose parents were reared on high quality diet.

Developmental time

Developmental time in females was significantly affected by generation, diet, and the generation x diet and diet x parental diet interactions (Table 3.1). Females that were reared on low quality diet exhibited a longer developmental time than those reared on high quality diet. A reduction in female developmental time was observed in the third generation as compared to the second generation in both type of diets (Fig. 3.2b). Furthermore, individuals that were fed on the same diet upon which their parents were reared showed a slightly shorter developmental time than those that were given the other diet. With respect to males, developmental time was affected by generation and diet (Fig. 3.3b). Individuals that were reared on low quality diet had longer developmental times than those reared on high quality diet; individuals from the second generation took longer to complete their life cycle than those from the third generation.

Relative growth rate (RGR)

RGR in females was significantly affected by generation, diet, together and generation x diet and generation x parental diet interactions (Table 3.1). Females reared on low quality diet had a lower RGR than those reared on high quality diet. Further, it was

observed an increase in female RGR in the third generation compared with the second generation in both type of diets, but this increase was higher in those individuals that were fed on high quality diet (Fig. 3.2c). The generation x parental diet interaction in females indicated that the type of parental diet influenced RGR of the future generation, being the progeny of parents fed on high quality the one that exhibited the highest RGR. In the case of males, RGR was significantly affected by generation, diet and parental diet (Table 3.2). Males that were reared on high quality diet had a higher RGR than those reared on low quality diet. However, individuals whose parents were fed on low quality diet showed higher RGR. Finally, third generation individuals had a higher RGR than those from second generation (Fig. 3.3c).

Relative consumption rate (RCR)

There were significant effects of diet and parental diet on RCR, as well as the effects of generation x parental diet and diet x parental diet interactions (Table 3.1). Females that were fed on low quality diets showed a slight decrease in RCR in the third generation compared with the second generation (Fig. 3.2d). The generation x parental diet interaction in females indicated that the type of parental diet had an influence on RCR of the future generation, being the progeny of parents fed on high quality the one that exhibited the lowest RCR. Moreover, individuals that were fed on the same low quality diet upon which their parents were reared showed the highest RCR, while those that were fed on the same high quality diet as their parents exhibited the lowest RCR (Fig. 2d). In the case of males, RCR was significantly affected by generation, diet, parental diet, and the generation x diet, generation x parental diet and diet x parental diet interactions. Males that were reared on low quality diet had a higher RCR than those on high quality diet (Fig. 3.3d). Individuals reared on low quality diet showed a substantial reduction of RCR in the third generation relative to the second generation (Fig. 3d). A similar response was observed in the interaction between generation and parental diet (Fig. 3.3d). Moreover, individuals that were fed on the same low quality diet upon which their parents were reared showed the highest RCR whereas those individuals that were fed on the same high quality diet as their parents exhibiting the lowest RCR (Fig. 3.3d).

Approximate digestibility (AD)

Only the interaction between generation and diet, and generation and parental diet did not significantly affect AD in females (Table 3.1). We observed that females feeding on low quality diet had a lower AD than females feeding on high quality diet in both generations (Fig. 3.2e). It should be noted that individuals whose parents were fed on low quality diet had the highest AD when they were reared on high quality diet; individuals whose parents were fed on high quality diet showed the lowest AD when they were reared on low quality diet in both generations (Fig. 3.2e). Male AD was significantly affected by generation, diet, and the generation x parental diet and diet x parental diet interactions (Table 3.2). Males exhibited a lower AD when feeding on low quality diet compared to when feeding on high quality diet. Insects whose parents were fed on low quality were capable of increasing their AD in the third generation compared with the second generation, while individuals whose parents were fed on high quality maintained a similar AD between generations (Fig. 3.3e). Furthermore, individuals that were fed on the same low quality diet upon which their parents were reared showed the lowest AD, while those that were fed on the same high quality diet as their parents exhibited the highest AD (Fig. 3.3e).

Efficiency of conversion of digested food (ECD)

Female ECD was significantly affected by generation, diet, and the generation x diet and generation x parental diet interactions (Table 3.1). Females that were fed on high quality diet had a higher ECD than those individuals fed on low quality diet in both generations. Females feeding on high quality diet could increase their ECD in the third generation (Fig. 3.2f). Furthermore, individuals whose parents were fed on high quality diet exhibited a higher ECD than individuals whose parents were fed on low quality diet in both generations (Fig. 3.2f). ECD of males was significantly affected by generation, diet, and the generation x diet, generation x parental diet and generation x parental diet interactions (Table 3.2). ECD was higher in males that were fed on high quality diet compared with those that were fed on low quality diet in both generations (Fig. 3f). Males increased their ECD in the third generation as compared to the second generation (Fig. 3.3f). Furthermore, males whose parents were supplied with high quality diet exhibited

higher ECD than males whose parents were fed on low quality diet in both generations (Fig. 3.3f). Finally, individuals that were fed on the same low quality diet upon which their parents were reared showed the lowest ECD, while those fed on the same high quality diet as their parents exhibited the highest ECD (Fig. 3.3f).

Efficiency of conversion of ingested food (ECI)

Results show that generation, diet, generation x diet and generation x parental diet had a significant impact on females ECI (Table 3.1). Females exhibited a greater ECI when they were reared on high quality diet in both generations. Females feeding on high quality diet could increase their ECI in the third generation, whereas no significant increase in ECI was observed in larvae that fed on low quality diet (Fig. 3.2g). Parental diet has an influence on ECI in the third generation on high quality diet, exhibiting the highest ECI (Fig. 3.2g). In males, generation, diet, parental diet x diet and generation x parental diet had significant effect (Table 3.2). Male individuals feeding on low quality diet had lower ECI than those feeding on high quality diet (Fig. 3.3g). Parental generation had similar effects on males than it had on females in both generations (Fig. 3.3g). Moreover, individuals that were fed on the same low quality diet upon which their parents were reared showed the lowest ECI whereas those that were fed on high quality diet just as their parents exhibited the highest ECI (Fig. 3.3g).

Discussion

The nutritional heterogeneity hypothesis states that generalist insect herbivores cope with low quality food by increasing their consumption rate, whereas specialist insect herbivores tend to eat a relatively small amount of low quality food, but they use it more efficiently (Simpson *et al.*, 2002). Spruce budworm feeds mainly on balsam fir but can also consume foliage of three spruce species; therefore, we expected the insect to resort to the second strategy. The results of this study show that ingestion of low quality food exerted a selection pressure on spruce budworm, which forces the insect to employ an adaptive strategy to cope with it. This strategy consists of increasing its consumption rate when feeding on low quality diet relative to those individuals that were reared on high quality

diet to meet its nutritional requirements during the second generation. However, the budworm appeared to improve the efficacy of digestion during the third generation, probably to reduce the metabolic cost that is incurred in digesting great amounts of nutritionally unbalanced food (Behmer, 2009).

We observed that spruce budworm larvae that were fed on low quality diet increased their consumption rate compared with those fed on high quality diet in the second generation. This compensatory feeding offset to some degree the negative effects on insect performance that were incurred by ingesting the low quality diet. However, this compensatory strategy was not sufficient for individuals that had been fed the low quality diet to attain a mass equivalent to that observed in larvae fed on the high quality diet. A similar response has been reported in spruce budworm that fed on 1-year-old balsam fir foliage (Carisey & Bauce, 1997), on foliage of the less suitable host black spruce (Fuentealba & Bauce 2012), and on low quality diet (Carisey & Bauce, 2002) over several generations (Quezada-Garcia *et al.* 2015). The low pupal mass that was observed in individuals fed on the low quality food in the second generation may have negative impacts on insect fecundity because pupal mass is often related to fecundity (Miller, 1957; Bauce & Carisey, 1996). Furthermore, Carisey & Bauce (2002) found a reduction in egg mass when individuals of the parental generation fed on a low quality diet, which represented old foliage. However, offspring that fed on this type of food demonstrated greater larval survival and resistance to starvation. Quezada-Garcia *et al.* (2015) found that insects reared on low quality food exhibited an increase in fecundity after three generations under constant nutritional stress. When taken together, these results suggest that spruce budworm can adapt to variation in food quality.

Insects that were fed on low quality diet had longer developmental times than those that were fed on high quality diet. Longer developmental time implies that the insect spends more time feeding (with an increase in defoliation) and is therefore exposed for a longer period to natural enemies, which may increase mortality (Slansky, 1982). Nevertheless, we observed a reduction in developmental time, together with a reduction in RCR and an increase in RGR in the third generation, suggesting that the insect begins to adjust its digestive behaviour towards low quality food. This kind of adjustment should produce an increase in AD, ECD or ECI (Waldbauer & Friedman, 1991). Indeed, our results showed

a small increase in ECD (Fig. 2f) and ECI (Fig. 3.2g) in females and an increase in ECD in males (Fig. 3.3f). Feeding on a diet with high nitrogen content implies an increase in metabolic costs to eliminate excess nitrogen, which may result in an increased developmental time and lower pupal mass (Behmer, 2009). A continuous stress may drive insects to take adaptive strategies to improve their digestion. Our results show that larvae fed on low quality diet had lower ECD. This suggests in turn that the insect allocated relatively more assimilated food towards energy metabolism than growth, possibly because it needed the extra energy to eliminate excess nitrogen. The increase in ECD in the third generation suggests that the insect has started an adaptive process, likely attributable to phenotypic plasticity to eliminate the excess nitrogen, which is reflected in the increase in pupal mass (Fig. 3.2a and 3.3a), and decreases in developmental time (Fig. 3.2b and 3.3b) and RCR (Fig. 3.2d and 3.3d). A similar response has been observed in the gypsy moth *Lymantria dispar dispar* (L.). Larvae that were fed on high nitrogen diet had a lower ECD compared to larvae that were fed on a low nitrogen diet (Stockhoff, 1992).

The strategy used by spruce budworm to compensate for low diet quality is consistent with the predictions made by the nutritional heterogeneity hypothesis for specialist insects (Simpson *et al.*, 2002). However, it took three generations for budworm to start showing signs of increasing efficiency of food utilisation. Consuming a greater amount of food when forced to feed on low quality food appears to be a temporary response in spruce budworm until it can adjust its digestive system to nutritionally unbalanced food. This adaptive behaviour has been observed in other specialist insects. For example, the small tortoiseshell *Aglais urticae* L. (Nymphalidae) feeds on old leaves of stinging nettle (*Urtica dioica* L.). These leaves of low nutritional quality forced this specialist butterfly to increase its consumption rate to compensate for the low levels of water and nitrogen while attempting to attain its ideal mass (Pullin, 1987). Woods (1999) reported that fifth-instar larvae of the tobacco hornworm *Manduca sexta* (L.) likewise resorted to compensatory feeding to maintain growth rates when fed a low-protein diet.

The rapid response that was observed in the present study suggests phenotypic plasticity may play an important role in the processing of food by spruce budworm, as environmental conditions (diet quality) clearly had an important effect on digestive indices such as AD, ECD and ECI. Increases in these indices seem to be responsible for the better

performance that was exhibited by insects fed on low quality food in the third generation. Better performance resulted in increased pupal mass, and decreased RCR and developmental time. Spruce budworm phenotypic plasticity that is related to changes in the environment has been reported in the past. For example, earlier studies reported significant phenotypic variation in mean egg mass of spruce budworm (Harvey, 1983; 1985), which was associated with climate conditions. Female spruce budworm from higher latitudes (longer winters) laid heavier (larger) eggs than females from lower latitudes. The mechanism that was responsible for this relationship probably involves temperature because egg size tends to increase with decreasing environmental temperature (Harvey 1985). A certain degree of phenotypic plasticity was also observed in spruce budworm spring emergence, which occurs over a long period (Volney & Fleming, 2007). This guarantees that some individuals will be able to emerge synchronously with host budbreak, which is critical for spruce budworm performance and survival (Lawrence et al., 1997). However, given that our experimental design did not permit us to estimate the influence of maternal effects on the observed response, we cannot rule out that maternal effects are implicated in this adaptive response.

Previous studies in Lepidoptera have reported that males are in general more susceptible than females to unbalanced diets (*e.g.*, Roeder & Behmer, 2014), perhaps because females are more efficient at utilising proteins (Lee, 2010) and can store them, which reduces the metabolic costs of eliminating excess nitrogen (Telang *et al.*, 2001). However, females appeared to be more susceptible to nutritional stress during the second generation than did the males in this study. Females exhibited the highest mortality rate when they were fed on low quality diet during the second generation but this phenomenon was reversed in the third generation (Fig. 3.2h), whereas male mortality was unaffected by the treatments (Fig. 3.3h). This phenomenon can be explained by the fact that females have higher metabolic costs (longer lifespan, higher mass, reproduction, etc.) than males, which increase the nutritional and energetic requirements of the former (Thornhill, 1976). For example, Koller & Leonard (1981) found that spruce budworm females consumed more energy than males to increase their fat reserves, a major component of additional female pupal mass that is necessary for reproductive processes (Thornhill, 1976). Furthermore, carbohydrates are more important than nitrogen for older spruce budworm larvae because

the former provide the energy that is required to complete their development (Albert & Bauce, 1994). This is also more important for females because the consumption of these compounds allow the female to increase its lipid reserves (Roeder & Behmer, 2014), which are an important component of eggs (Chapman *et al.*, 2013). Given that the low quality diet used in this study had very low carbohydrate content (1.5 %), females may have experienced more problems than males in consuming the required amounts of these compounds in the second generation. However, the increase in spruce budworm digestive efficiency that was observed in the third generation probably renders females more resilient to nutritional stress produced by low quality diet than males because they can deal better than males with the excess in nitrogen found in this diet (Lee, 2010).

The strategy employed by spruce budworm to deal with low quality food may help us to understand what happen during the last stage of an outbreak. This information may be of great utility to understand the adaptive mechanisms that insects take to persist in the environment and the role of nutritional selection pressure on spruce budworm population dynamics. Previous studies have suggested that spruce budworm has the ability to adapt to feed on low quality food by increasing its consumption rate and its efficacy to use nitrogen (Carisey & Bauce, 1997, 2002). However, they studied spruce budworm responses to low quality food for one generation. This study suggests that spruce budworm have the capacity to adapt its digestive system after three generations feeding on a low quality food that induces detrimental impact on insect survival and development representative of conditions found at the end of an outbreak. This fact needs to be tested in the field, and it could represent an important key to understand the adaptation of this forest pest in North America.

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Table 3.1. Summary of three-way analysis of variance (ANOVA) for all variables that were measured in female spruce budworm.

Source of variation	Pupal mass			Developmental time			RGR			RCR			AD			ECD			ECI		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Generation	63.46	1,777	<.0001	73.53	1,775	<.0001	110.54	1,751	<.0001	1.28	1,725	0.2574	18.38	1,729	<.0001	31.31	1,723	<.0001	24.57	1,722	<.0001
Diet	128.62	1,777	<.0001	374.42	1,775	<.0001	504.71	1,751	<.0001	182.93	1,725	<.0001	216.49	1,729	<.0001	164.43	1,723	<.0001	244.83	1,722	<.0001
Generation*Diet	9.87	1,777	0.0017	6.78	1,775	0.0094	23.29	1,751	<.0001	0.02	1,725	0.9018	1.17	1,729	0.2791	10.55	1,723	0.0012	11.63	1,722	0.0007
Parental diet (PD)	0.45	1,777	0.5024	0.12	1,775	0.7284	2.61	1,751	0.1064	19.19	1,725	<.0001	15.63	1,729	<.0001	0.07	1,723	0.7903	0.02	1,722	0.8826
Generation*PD	3.01	1,777	0.0830	3.79	1,775	0.0520	4.25	1,751	0.0396	8.74	1,725	0.0032	3.16	1,729	0.0759	4.53	1,723	0.0336	4.72	1,722	0.0301
Diet*PD	0.22	1,777	0.6382	6.21	1,775	0.0129	2.91	1,751	0.0886	11.90	1,725	0.0006	8.21	1,729	0.0043	0.52	1,723	0.4724	0.91	1,722	0.3417
Generation*Diet*PD	1.75	1,777	0.1868	0.89	1,775	0.3455	2.95	1,751	0.0862	2.61	1,725	0.1068	5.89	1,729	0.0154	1.55	1,723	0.2135	1.06	1,722	0.3046

Table 3.2. Summary of three-way analysis of variance (ANOVA) for all variables that were measured in male budworm.

Source of variation	Pupal mass			Developmental time			RGR			RCR			AD			ECD			ECI		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Generation	24.54	1,709	<.0001	98.93	1,709	<.0001	114.08	1,691	<.0001	12.54	1,676	0.0004	5.67	1,683	0.0176	7.67	1,673	0.0058	4.69	1,672	0.0307
Diet	119.98	1,709	<.0001	99.64	1,709	<.0001	129.64	1,691	<.0001	186.90	1,676	<.0001	345.54	1,683	<.0001	200.59	1,673	<.0001	289.22	1,672	<.0001
Generation*Diet	19.95	1,709	<.0001	0.04	1,709	0.8467	1.48	1,691	0.2248	6.91	1,676	0.0088	2.94	1,683	0.0868	4.36	1,673	0.0371	2.59	1,672	0.1079
Parental diet (PD)	6.95	1,709	0.0086	2.24	1,709	0.1345	7.44	1,691	0.0066	15.12	1,676	0.0001	3.69	1,683	0.0551	0.00	1,673	0.9623	0.76	1,672	0.3846
Generation*PD	3.06	1,709	0.0809	1.71	1,709	0.1909	3.82	1,691	0.0509	10.41	1,676	0.0013	6.36	1,683	0.0119	8.13	1,673	0.0045	9.56	1,672	0.0021
Diet*PD	0.01	1,709	0.9227	0.06	1,709	0.8092	0.24	1,691	0.6235	26.16	1,676	<.0001	11.42	1,683	0.0008	8.34	1,673	0.0040	11.83	1,672	0.0006
Generation*Diet*PD	0.45	1,709	0.5002	0.38	1,709	0.5372	0.22	1,691	0.6362	2.22	1,676	0.1364	1.17	1,683	0.2793	0.11	1,673	0.7430	0.61	1,672	0.4361

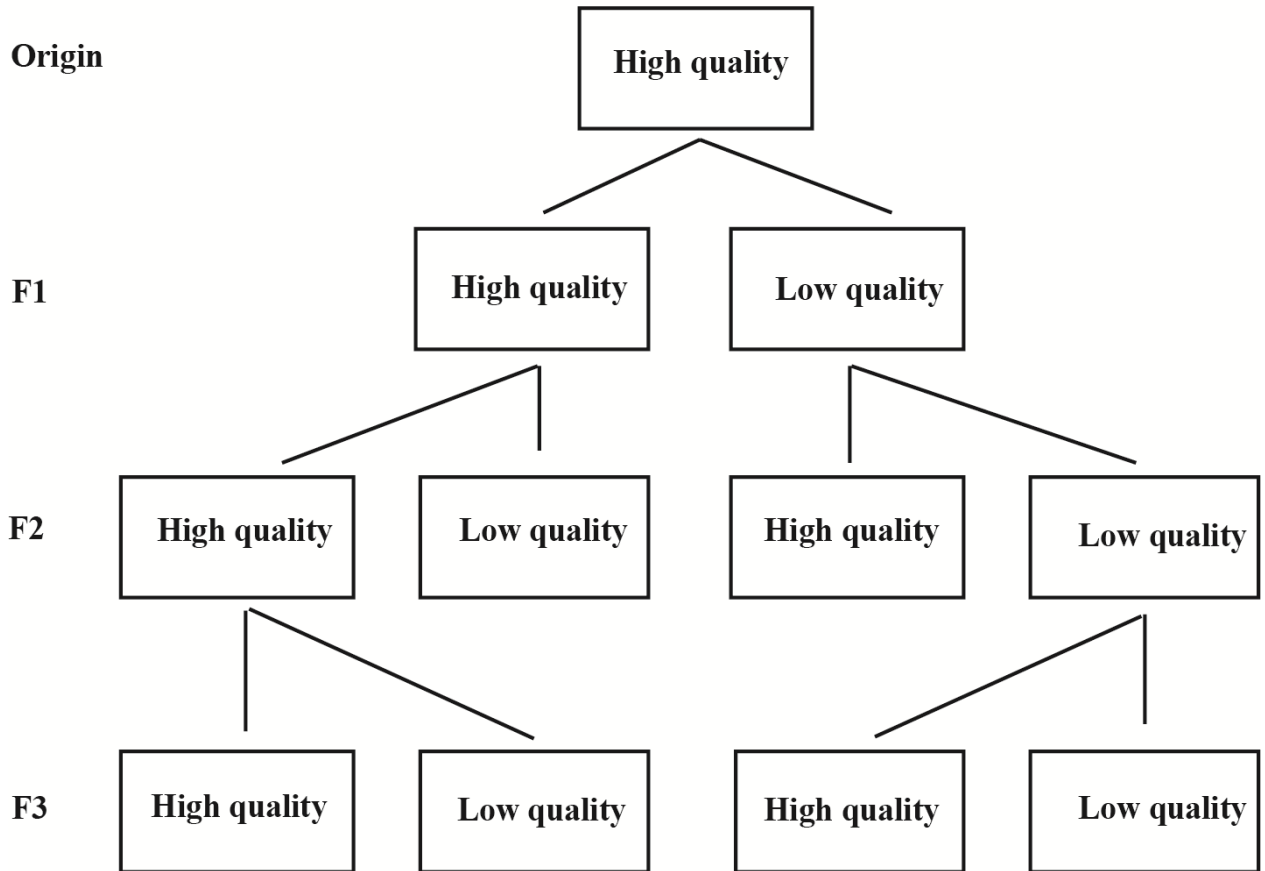


Figure 3.1. Schematic of the experiment to estimate performance and adaptive responses of spruce budworm to high quality and low quality diets

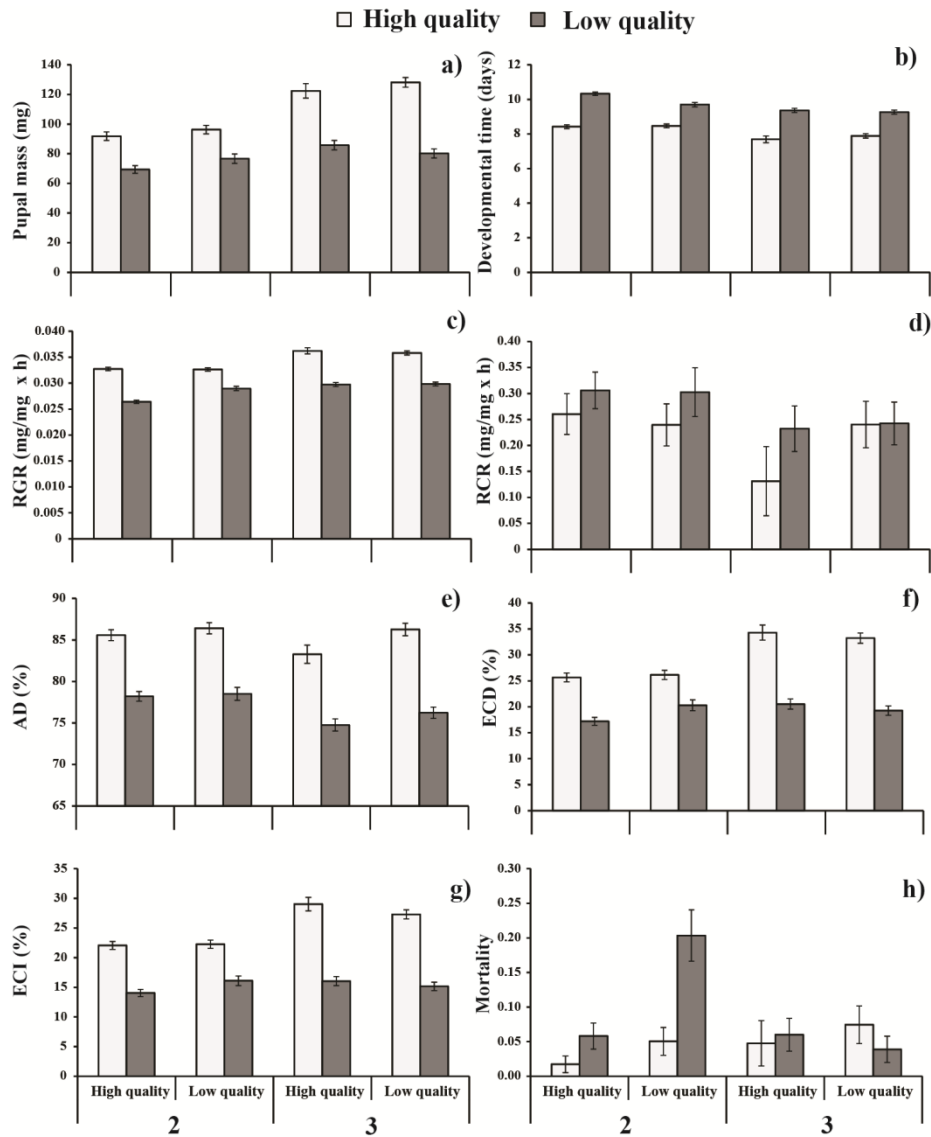


Figure 3.2. Mean (\pm SEM) Pupal mass (a), developmental time (b), relative growth rate (RGR) (c), relative consumption rate (RCR) (d), approximate digestibility (AD) (e), the efficiency of conversion of digested food (ECD) (f), the efficiency of conversion of ingested food (ECI) (g), and mortality (h), which were tabulated by diet, parental diet and generation for females

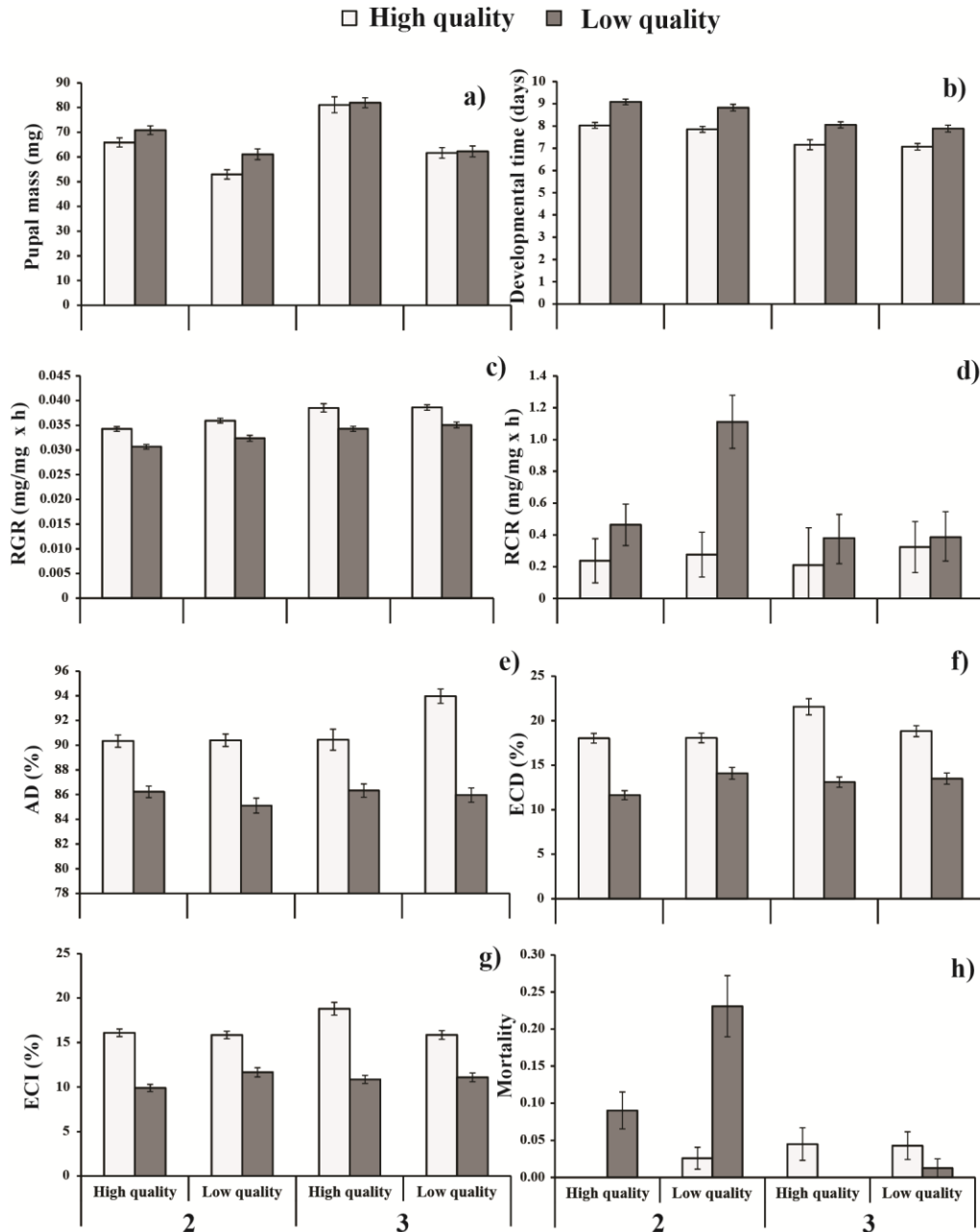


Figure 3.3. Mean (\pm SEM) Pupal mass (a), developmental time (b), relative growth rate (RGR) (c), relative consumption rate (RCR) (d), approximate digestibility (AD) (e), the efficiency of conversion of digested food (ECD) (f), the efficiency of conversion of ingested food (ECI) (g), and mortality (h), which were tabulated by diet, parental diet and generation for males.

Chapitre IV

Nutritional stress causes male-biased sex ratios in eastern spruce budworm (Lepidoptera : Tortricidae)

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Résumé

La variation de la qualité de la ressource alimentaire peut entraîner une mortalité différentielle entre les sexes chez la tordeuse des bourgeons de l'épinette. En effet, si la valeur sélective (fitness) des mâles et des femelles diffère, alors l'augmentation de la mortalité du sexe le plus sensible pourrait entraîner un biais dans le sex-ratio. Nous avons élevé trois générations de tordeuse des bourgeons en les nourrissant avec deux diètes artificielles: une diète équilibrée qui répond à tous les besoins nutritionnels pour soutenir un bon développement et une diète présentant un stress, soit un déficit de sucre et d'azote. Cette diète qui simulait la baisse de la qualité de la nourriture présente en conditions d'infestation a eu un impact négatif sur la survie des larves, leur développement et leur croissance. Nous avons testé les effets d'un stress nutritif continu sur le sex-ratio de chrysalides et d'adultes. Ce type de diète favorise les mâles par rapport aux femelles. Ainsi, une faible qualité de la nourriture se traduit par une diminution du nombre de femelles. Ce déséquilibre du sex-ratio a été observé à partir de la deuxième génération avec un pourcentage plus faible de femelles ayant atteint les stades de chrysalide et d'adulte. Ces résultats démontrent clairement que la variation de la qualité alimentaire provoque une mortalité différentielle entre les sexes, les femelles étant plus sensibles à une faible valeur nutritive des ressources alimentaires. Cette étude montre pour la première fois la présence d'un déséquilibre dans le sex-ratio produit par un stress alimentaire chez la tordeuse des bourgeons de l'épinette. Nos résultats soulignent l'importance d'étudier le déséquilibre du sex-ratio chez la tordeuse des bourgeons de l'épinette en conditions d'épidémie.

Abstract

Nutritional variability in resources may cause differential mortality between sexes resulting in biased sex ratios. If males and females differ in fitness, then mortality of the more sensitive sex can cause a bias in sex ratios, and can stimulate dispersion of males. We reared three generations of spruce budworm on two artificial diets: a “normal” diet that provided all nutritional requirements for development and a “stress” diet (deficient in sugars and slightly higher in nitrogen), that simulated deterioration of food quality during outbreak conditions and had a detrimental impact on larval survival, development and growth. We tested the effects of continued nutritional stress on the sex ratio of pupae and adults. We found biased sex ratios in favor of males related to diet. Low quality food resulted in fewer females. This distortion was observed from the second generation onward, with a lower percentage of females reaching the pupal and adult stage. These results provide evidence that nutritional variation causes differential mortality between sexes, suggesting that females are more sensitive to nutritional stress. This is the first study that demonstrates sex-ratio distortion due to nutritional selection pressure in spruce budworm. Our results indicate the importance of studying sex ratio distortion of spruce budworm in outbreak conditions.

INTRODUCTION

In species where differential mortality between sexes occurs under certain environmental constraints, biased sex ratios can be observed (House *et al.* 2011). External factors such as seasonal changes (Charnov *et al.* 1981), disease (Jiggins *et al.* 1998), predators (Tabadkani *et al.* 2012), protandry (Wiklund *et al.* 1992), and food availability (Charnov *et al.* 1981) can play a role in determining sex ratios. In outbreaking insects that significantly compromise their host, nutritional variation may directly affect insect biological performance such as body size and fecundity (Awmack and Leather 2002). Differences in fitness costs and metabolic requirements between males and females suggest that nutritional quality would differentially affect the survival of the two sexes (Mopper and Whitham 1992; Carisey and Bauce 2002). Therefore continuous nutritional stress over several generations may result in sex ratio distortion and eventually, a local reduction in population size (Robinson 1983; Mauffette and Jobin 1985; Lobinger 1996). The eastern spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) is one of the most destructive outbreak insects in North America (Rauchfuss and Ziegler 2011). This pest attacks balsam fir (*Abies balsamea* (Linnaeus) Miller; Pinaceae), white spruce (*Picea glauca* (Moench) Voss; Pinaceae), black spruce (*Picea mariana* (Miller); Pinaceae) and red spruce (*Picea rubens* Sargent) (Montgomery *et al.* 1983). Continuous severe defoliation significantly reduces growth of the host and can result in mortality (Morris 1963). Following an outbreak, foliage production decreases considerably because removal of foliage through defoliation causes growth loss of trees and decreased wood production (McLean 1984). Nutritional quality of foliage in heavily defoliated trees is diminished in terms of nitrogen, carbon, and defensive compounds (White 2004). Lack of food availability accompanied by changes in natural enemy composition and increase in incidence of diseases due to overcrowding can cause local decline of outbreaks (Morris 1963; Royama 1984). There is evidence of a slight sex ratio bias in the western spruce budworm, *Choristoneura occidentalis* (Walsingham), in sites where defoliation of current-year shoots was greater than 50% (Campbell *et al.* 1983). In this study, we evaluated the effect of nutritional stress on sex ratio in the eastern spruce budworm. We tested the hypothesis that poor nutritional quality favours survival of males because females have higher fitness and metabolic costs.

MATERIALS AND METHODS

We reared a total of 14 000 insects over three consecutive generations under controlled conditions, at 23 °C, 60 ±5% relative humidity in a 16:8 hour light:dark photoperiod cycle (Robertson 1985). Two artificial diets were formulated differing only in nitrogen and sugar content (Bidon 1999). They are hereafter referred to as “normal” and “stress” diet and contain 12% sugar, 5% nitrogen and 1.5% sugar, 7% nitrogen, respectively. This stress diet was chosen because it has a negative impact on larval survival, development, and growth that is representative of food quality deterioration in outbreak conditions (Bidon 1999). Rearing was initiated with 2000 individuals in each diet in the first generation. Insects were reared in the laboratory in Petri dishes (100 x 15 mm) with 10 individuals per dish. Pupae and adults were separated according to sex (Robertson 1985). Mortality was recorded every day. Couples were installed in plastic cages of 11 cm x 7.5 cm. Adults were fed a 5% sugar water solution and allowed to mate. Eggs were collected two or three days after females died. Substrates provided were wax paper for oviposition and cheese cloth for overwintering. Larvae that hatched were held at 18 °C for two weeks and were then transferred to 2 °C for 25 weeks to overwinter in dark growth chambers (Robertson 1985). The laboratory culture was maintained for three generations and laboratory conditions were kept stable to minimise environmental variability. We did not consider larval mortality because sex of individuals is not evident in all larval stages. Individuals used for mating were selected randomly. A χ^2 test and a logistic regression were performed to assess treatment effects using log-likelihood ratios (Zar 2010). We tested the null hypothesis that sex ratio is maintained at 1:1 across generations. Data were analysed using PROC FREQ and PROC LOGISTIC modules of SAS 6.12 (SAS Institute Inc. 2003).

RESULTS AND DISCUSSION

We observed equal sex ratios for pupae and adults reared on normal diet in all three generations. However, when larvae were reared under stress diet conditions, from the second generation onwards, strong distortions in favour of males were observed both for pupae (Table 4.1) and adults (Table 4.2). It appears that sex ratio distortion in favour of males was caused by higher mortality of females reared on the stress diet. Poor nutritional quality leads to differential mortality (Charnov *et al.* 1981) with greater impact on the larger-sized sex because larger individuals require

more energy for development (Caswell and Weeks 1986). Female spruce budworms are larger and have a longer lifespan than males. They also require additional resources to produce progeny (Miller 1975). Sex distortion on stress diet found in this study may be related to higher energy costs for female development compared to males. Females may not have been able to derive enough energy from the stress diet for their development and therefore failed to reach the pupal and adult stages. From the second generation onwards, sex ratio distortion was significant. Our results indicate that mortality of females would increase during the course of an outbreak if nutritional stress, as caused by severe defoliation over consecutive years, continues for several generations. Decline in host nutritional quality can trigger emigration from outbreak areas resulting in moth dispersal into uninfested areas (Greenbank *et al.* 1980) causing outbreaks to spread.

The sex ratio difference that we observed was interpreted as a deficit of females and not as an excess of males. Hamilton's (1967) local mate competition theory posits that when a population is subject to environmental stress, sex ratio distortion can be beneficial because when there are more males the likelihood of fertilising females increases. Variance in reproductive success is expected to be greater among females in polygynous animals because males have more than one mate available (Godfray and Werren 1996). High reproductive performance by males as opposed to females may increase overall reproductive success in local populations because males are more likely to successfully mate with several females than vice versa. A non-mating female would represent an energy loss to the population.

From an ecological point of view, defoliation during spruce budworm outbreaks causes significant variability in food resources (Morris 1963). Under these conditions, unequal sex ratios may offer an advantage for auto-regulation of the population. Linear decreases in sex ratios were observed in several insect species when population densities increased or when food quality and quantity decreased (Lobinger 1996) and this negative feedback may contribute to auto-regulation of a population (Dingle 1966). Female forest insect pests generally tend to produce an equal sex ratio under favourable conditions (Robinson 1983). In this study we observed that the lower food quality results in a higher mortality of females from the second generation onwards, so it was less likely that females reached pupal and adult stages. For outbreak insects that attack their host over the course of several years, causing foliage quality to deteriorate over time, a high proportion of males could indicate that the population is in decline (Lobinger 1996). Decline in foliage quality has been documented as a factor contributing to population crashes during spruce budworm

outbreak cycles (Nealis and Régnière 2004; Régnière and Nealis 2007), but sex ratio bias has neither been considered as an effect of deteriorating resources nor as a cause for population decline. Our study provides evidence that consumption of poor quality resources over a few generations causes male-biased sex ratios suggesting that occurrence of this phenomenon should be tested under natural conditions.

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Table 4-1 The effect of diet on sex ratios of three generations of spruce budworm pupae. Normal diet: Likelihood-ratio $X^2 = 3.12$, $df = 2$, $P = 0.2097$; Stress diet: Likelihood ratio $X^2 = 39.05$, $df = 2$, $P < 0.0001$.

* Departure of sex ratio from 1:1 with the X^2 test, $P < 0.0001$.

- Number of individuals in the three generations.

Diet	Number of pupae [•]	Generation		
		1	2	3
		male:female	male:female	male:female
Normal	7259	49.85: 50.14	49.85: 50.14	52.16: 47.83
Stress	5878	50.09: 49.90	59.22: 40.77*	56.37: 43.62*

Table 4-2 The effect of diet on sex ratios on three generations of adult spruce budworm. Normal diet: Likelihood-ratio $X^2 = 1.85$, $df = 2$, $P = 0.3959$; Stress diet: Likelihood-ratio $X^2 = 21.65$, $df = 2$, $P < 0.0001$.

* Departure of sex ratio from 1:1 with the X^2 test. $P < 0.0001$.

- Number of individuals in the three generations.

Diet	Number of moths [•]	Generation		
		1	2	3
		male:female	male:female	male:female
Normal	6305	49.83:50.16	49.15:50.84	51.38:48.61
Stress	4238	51.64:48.35	60.83:39.16*	56.21:43.78*

Conclusion Générale

La présente thèse a mis en évidence que la TBE possède un potentiel d'adaptation sous une pression de sélection nutritionnelle constante. Les caractères associés au vécu des insectes ont une héritabilité et une plasticité importantes, ce qui fait que la TBE peut s'adapter rapidement. Les processus adaptatifs chez la TBE sont aussi observés avec l'utilisation de la nourriture. Lorsque la nourriture est de mauvaise qualité, les insectes en ingèrent plus, pendant une période plus longue. Cependant, les insectes de troisième génération sont plus efficaces pour convertir la nourriture ingérée en biomasse et leur temps de développement diminue. Cette adaptation affecte principalement les femelles, car elles ont des coûts métaboliques plus grands (elles sont plus grandes que les mâles, elles vivent plus longtemps et elles ont plus de coûts reproductifs).

La réponse adaptative chez la TBE est en lien avec sa biologie. En effet, cet insecte indigène de l'Amérique du Nord est adapté aux conditions environnementales présentes dans cette région, tels un hiver long et un été court, qui limitent son développement et celui de ses hôtes. En conséquence, la TBE complète sa croissance et sa reproduction dans une période de quelques semaines, c'est-à-dire, $\frac{1}{4}$ de son cycle de vie et les $\frac{3}{4}$ restants, elle le passe au stade L₂ larvaire. La principale ressource alimentaire de la TBE consiste à manger les bourgeons de l'année en cours, il y a donc une synchronisation entre l'insecte et l'hôte, ce qui limite encore plus les périodes d'alimentation. Ainsi, le succès biologique de la TBE est déterminé par la disponibilité et la qualité de la nourriture, car elle se nourrit principalement au stade larvaire (du L₃ au L₆) et pratiquement pas au stade adulte. Durant la diapause, les larves de TBE (L₂) doivent faire l'allocation des réserves nutritives qu'elles ont obtenues de leurs mères pour passer l'hiver. De plus, cet insecte présente des cycles épidémiques, où le nombre d'individus augmente à tel point que les ressources nutritionnelles adéquates « bourgeons de l'année en cours » ne sont pas disponibles pour tous les individus, affectant négativement leur performance biologique. En fait, le manque et la mauvaise qualité de la nourriture sont des facteurs clés dans le déclin d'une épidémie. En conséquence, la vie évolutive de la TBE est régulée de manière importante par la qualité nutritionnelle.

Jusqu'à maintenant, il y a beaucoup de controverse sur l'héritabilité des caractères associés au vécu. Dans la présente thèse, les caractères associés au vécu des insectes montrent une grande héritabilité. Cependant, il faut signaler que dans un milieu où les prédateurs et

l'environnement exercent des pressions de sélection naturelle, les caractères morphologiques possèdent une héritabilité plus grande que les caractères associés au vécu. Dans ce cas-là, conserver un caractère morphologique est plus important. La TBE possède donc une grande héritabilité dans ces caractères associés au vécu en conditions de laboratoire, où il n'y a aucun effet délétère tel que le manque de nourriture, la présence de composés secondaires, de prédateurs et de parasitoïdes. Ainsi, l'héritabilité est amplifiée, car elle n'est pas érodée par une sélection naturelle. Cette recherche laisse voir que les caractères associés au vécu ont une importance dans les processus évolutifs, cependant d'autres recherches sur l'héritabilité des caractères morphologiques, physiologiques et de comportements seraient appropriées afin d'approfondir cette étude.

L'étude d'adaptation réalisée dans le deuxième chapitre illustre que le stress alimentaire constant induit une adaptation chez la TBE. Cette réponse adaptative prend un délai de deux générations et devient plus évidente dans la troisième. Cette capacité d'adaptation se traduit par des phénotypes ayant une fécondité, un poids et un taux de croissance plus élevé ainsi qu'un temps de développement plus court. Les effets délétères sont plus évidents chez les femelles que les mâles. Il y a aussi une résilience importante des insectes de la troisième génération nourris avec la diète témoin dont les deux générations précédentes avaient été alimentées avec la diète stress. Ainsi, les insectes ont le potentiel de se rétablir si les conditions stressantes diminuent. Cette stratégie serait la plus adéquate pour la TBE, quand la qualité de nourriture diminue à court terme. Ce mécanisme d'adaptation pourrait être efficace en conditions épidémiques à cause du manque de nourriture. Les résultats ont suggéré qu'il existe une plasticité phénotypique et des effets maternels impliqués dans les processus adaptatifs. Ces résultats ouvrent la porte à un éventail de possibilités de recherches sur la plasticité phénotypique et les effets maternels chez la TBE.

Les résultats du troisième chapitre sur l'utilisation de la nourriture sont complémentaires à ceux observés dans le deuxième chapitre. Les bourgeons de l'année en cours sont disponibles seulement durant une période de temps précise. En conséquence, les périodes de croissance de la TBE sont limitées en temps et en valeur nutritive. Ce dernier facteur représente de l'énergie pour les individus. De cette manière, la sélection devrait maximiser l'efficacité de la conversion de la nourriture en biomasse et en énergie. Cette étude a démontré que les processus adaptatifs associés à l'utilisation de la nourriture représentent

des coûts métaboliques importants, tels qu'un temps de développement plus long et un poids des chrysalides plus petit. L'étude a également montré que l'ajustement du métabolisme pour avoir une meilleure efficacité de conversion de la nourriture s'étend au moins sur deux générations. Ces coûts sont en lien avec les caractères associés au vécu des insectes tels que les processus reproductifs. Le manque et la mauvaise qualité de la nourriture lors d'une infestation sont des éléments ayant une pression de sélection sur les individus. Cette réponse adaptative observée dans cette étude est une invitation à produire d'autres recherches axées sur l'adaptation de la TBE en nature avec les hôtes. (p. exp. Ce qui amène à se poser la question, est-ce que les coûts métaboliques peuvent être plus élevés sur un type d'hôte par rapport à une autre espèce « le sapin baumier vs l'épinette blanche ou noire »)

Le quatrième chapitre démontre que la pression de sélection affecte plus les femelles que les mâles, car les femelles ont des besoins métaboliques plus élevés que les mâles. Pour plusieurs espèces de papillons, le rapport mâle : femelle varie en fonction de la mortalité dans les stades larvaires et le décalage dans l'émergence des adultes, la prédation et la dispersion d'un sexe. La distorsion de ce rapport amène l'équilibre dans la taille des populations. Une pression de sélection nutritionnelle altère le rapport des sexes. En conséquence, plus de mâles peuvent assurer la reproduction des femelles. Il existe l'hypothèse (Mauffette et Jobin 1985) que pour les ravageurs forestiers, un déséquilibre dans le rapport mâle : femelle, peut indiquer que la population est en déclin. Cette recherche nous a permis de constater cet effet en laboratoire. Cependant, la recherche sur le sex-ratio en nature est une tâche très difficile à réaliser, car cela représente un contrôle important des individus et beaucoup de logistique.

L'ensemble des recherches faites dans le cadre de cette thèse nous révèle que la TBE a la capacité de s'adapter rapidement, lorsqu'elle subit une forte pression de sélection. Il faut signaler que la diète stress avait la fonction de reproduire un taux de mortalité similaire aux conditions rencontrées lors du pic d'une épidémie. On observe au travers des chapitres que la TBE possède une malléabilité adaptative importante, ce sont notamment les femelles les plus sensibles à la pression de sélection. En conséquence, elles s'adaptent plus rapidement (elles ingèrent plus de nourriture, elles deviennent plus efficaces à transformer la nourriture en énergie et en biomasse et elles réduisent leur temps de développement). Cette étude permet de mieux comprendre les mécanismes évolutifs de ce ravageur forestier et elle offre aussi un large éventail de possibilités de recherches sur l'adaptation de la TBE. Par exemple, la

plasticité phénotypique dans les caractères physiologiques et comportementaux en présence des différents hôtes, l'influence des hôtes sur ces stratégies. Cette information sera d'une grande utilité afin de prédire l'adaptation de la TBE à des conditions particulières de composition forestière et climatique, une information qui pourrait être aussi utile dans l'aménagement des forêts canadiennes.

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