

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

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**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 (*Choristoneura fumiferana* (Clemens); Lepidoptera: Tortricidae) 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 favour 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.

**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 rapport des sexes, et il peut stimuler la dispersion des mâles. Nous avons élevé trois générations de tordeuse des bourgeons (*Choristoneura fumiferana* (Clemens); Lepidoptera: Tortricidae) 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 rapport des sexes 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 rapport des sexes 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 rapport des sexes produit par un stress alimentaire chez la tordeuse des bourgeons de l'épinette. Nos résultats soulignent l'importance d'étudier le déséquilibre du rapport des sexes chez la tordeuse des bourgeons de l'épinette en conditions d'épidémie.

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.*

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2013), 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 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 (MacLean 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 >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.

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 × 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 × 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  $\chi^2$  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).

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 1) and adults (Table 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

**Table 1.** The effect of diet on sex ratios of three generations of spruce budworm pupae.

Diet	Number of pupae*	Generation (male:female)		
		1	2	3
Normal	7259	49.85:50.14	49.85:50.14	52.16:47.83
Stress	5878	50.09:49.90	<b>59.22:40.77<sup>†</sup></b>	<b>56.37:43.62<sup>†</sup></b>

**Notes:** Normal diet: likelihood-ratio  $\chi^2 = 3.12$ ,  $df = 2$ ,  $P = 0.2097$ ; stress diet: likelihood ratio  $\chi^2 = 39.05$ ,  $df = 2$ , bold values:  $P < 0.0001$ .

\*Number of individuals in the three generations.

<sup>†</sup>Departure of sex ratio from 1:1 with the  $\chi^2$  test,  $P < 0.0001$ .

**Table 2.** The effect of diet on sex ratios on three generations of adult spruce budworm.

Diet	Number of moths*	Generation (male:female)		
		1	2	3
Normal	6305	49.83:50.16	49.15:50.84	51.38:48.61
Stress	4238	51.64:48.35	<b>60.83:39.16<sup>†</sup></b>	<b>56.21:43.78<sup>†</sup></b>

**Notes:** Normal diet: likelihood-ratio  $\chi^2 = 1.85$ ,  $df = 2$ ,  $P = 0.3959$ ; stress diet: likelihood-ratio  $\chi^2 = 21.65$ ,  $df = 2$ , bold values:  $P < 0.0001$ .

\*Number of individuals in the three generations.

<sup>†</sup>Departure of sex ratio from 1:1 with the  $\chi^2$  test,  $P < 0.0001$ .

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 with 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 nonmating 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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