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Field assessment of female mating success based on the presence–absence of spermatophore: a case study with spruce budworm, *Choristoneura fumiferana*

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The mating success of female spruce budworms was evaluated using the presence–absence of spermatophore in genitalia. Sampling was conducted in the 1970s in Atlantic Canada at 21 locations/years characterized by high budworm densities. More than 99.8% of in-flight females (captured at light or malaise traps) and migrant females (collected while ascending above tree canopies or in the airspace) were mated, which may reflect physiological constraints restricting flight in gravid females. About 85% of resident females (collected using sweep nets or by fogging trees with insecticide) were mated; mating success was low among young females collected early in the season and increased over time to reach a level similar (98%) to that of females that died from natural causes. These findings highlight the importance of unbiased estimates of mating success to evaluate the efficacy of mating disruption and the role of mate-encounter Allee effects on population dynamics.

Introduction

Dissection of female genitalia to assess the presence or absence of spermatophore is the most prevalent tool to determine the mating status of field-collected females in insects (Callahan 1958, Burns 1968, Pease 1968, Pliske 1973). This technique has been used to unravel the ecological consequences of female mating failure on population dynamics through the mate-encounter Allee effect (Fauvergue 2013), to develop management programs that interfere with the normal mating sequence of insect pests (e.g., pheromone-based mating disruption: Baker 2008), and to further our understanding of mating systems e.g., taxonomic patterns related to the lower rate of virginity in butterflies than in moths (Rhainds 2010). Despite the practicality and usefulness of spermatophore dissections to assess female mating status, caution is needed in the interpretation of *lifetime* mating success (defined as the probability that a female copulates at least once in her lifetime, as opposed to the probability that a female does not get inseminated and dies as a virgin).

Females have to be killed to determine the presence of spermatophore, hence the approach provides a snapshot of mating status at the time of sampling. The probability that captured females may have mated during the subsequent (post-sampling) portion of their life is difficult to assess because it depends on a combination of unknown factors, including the age of the individual at the time of sampling as well as age-dependent probabilistic functions of survival and mating. Lifetime mating success can be approximated by estimating the age of females at the time of sampling using wing wear (Ehrlich & Ehrlich 1978, Braby 1996) or the level of degradation of spermatophore (Elliott & Dirks 1979), or by sampling populations repeatedly over time to correct for the low mating success of young females early in the season (Howell 1979, Gerber & Walkof 1992). Ultimately, absolute measures of lifetime mating success can be obtained only by dissecting females at the end of their life, after they have died from natural causes. Postmortem dissection of female cadavers is a practical approach that has been used in some insects to assess lifetime reproductive success (Thomas *et al*. 1980, Rosenheim *et al*. 2008, Rhainds 2010).

Assessment of mating status must rely on an unbiased sample of the population of females, which is difficult to achieve because the proportion of mated females varies as a function of the sampling technique that is used (Elliot 1977), the habitat that is sampled (Yathom 1981, Cameron *et al*. 2005), or the nature of olfactory cues that females respond to (aggregation pheromone or host volatile; Allou *et al*. 2008, Nehme *et al*. 2010). These constraints are magnified in studies that rely on sentinel (caged or tethered) females because the mating success of feral females is usually higher than that of sentinel females (Showers *et al*. 1976, Weissling & Knight 1995, López *et al*. 2003, Rhainds *et al*. 2012).

The spruce budworm (SBW), *Choristoneura fumiferana* (Lepidoptera: Tortricidae), is a univoltine insect whose larvae feed on needles of balsam fir, *Abies balsamea*, and spruce (*Picea* spp.) (Morris 1963). The reproductive ecology of SBW has been reviewed (Greenbank *et al*. 1980, Silk & Kuenen 1984, Rhainds *et al*. 2012), as summarized below. Sixth instars pupate near the foliage that they consumed penultimately and emerge as adults about 10 days later in July. Females release pheromones to attract mates on their first night after emergence and start to lay eggs in clusters shortly after mating. Because of their heavy abdomen, young females are incapable of flying before having laid some eggs (Rhainds & Kettela 2013). Mated females migrate between oviposition bouts by ascending above the plant canopy to the atmospheric boundary layer where they can get carried several hundreds of kilometers away.

During the course of the last SBW outbreak in Atlantic Canada in the 1970s, extensive studies were carried out on the reproductive ecology and migration behavior of adults. A subset of the data included dissections of thousands of females to determine mating status, using an array of techniques to collect females. The mating data had been archived but not published in the peer-reviewed literature. The anticipated outbreak of spruce budworm in eastern Canada has revived interest in the reproductive ecology of SBW, first because interference mating using synthetic pheromones (mating disruption) represents one of few options for controlling low density populations (Rhainds *et al*. 2012), and second because low mate encounters in endemic populations (Allee effect) may affect population dynamics (Régnière *et al*. 2013). In this paper, data on SBW mating probability (defined as a binary variable with females classified as mated or unmated based on the presence–absence of spermatophore) are consolidated and compared for different sampling treatments, with the objective of providing a biologically sound framework to interpret mating probabilistic functions in SBW.

Material and methods

The data were generated in the 1970s at eight locations in two provinces in eastern Canada, New Brunswick and Nova Scotia, for a total of 21 locations/years (Fig. 1 and Table 1). All the experimental sites consisted of forest stands with hosts of SBW as dominant or co-dominant tree species. Densities of SBW were high at all locations/years as indicated by time-series of egg abundance (Royama *et al*. 2005). Insecticides were not applied to kill larvae at any of the experimental sites. The data on mating probability were a subset of experiments aimed at inves-

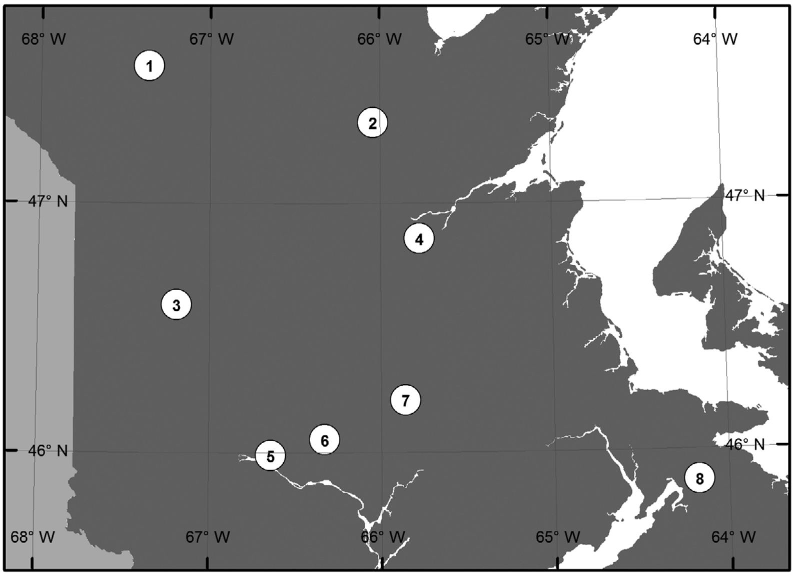

Fig. 1. Location of sites where mating probabilities of female spruce budworm were estimated in New Brunswick (NB) and Nova Scotia (NS), Atlantic Canada, during a large-scale outbreak in the 1970s. 1: Saint-Quentin, NB; 2 Heath Steele, NB; 3: Juniper, NB; 4: Renuous, NB; 5: Fredericton, NB; 6: Acadia Experimental Forest Station, NB; 7: Chipman, NB; 8: Amherst, NS.

tigating the migration behavior of adults (sites 3, 4, 6, 7 in Fig. 1; Greenbank *et al*. 1980), realized fecundity of females (sites 2, 5 in Fig. 1; Thomas *et al*. 1980), and mating disruption (sites 1, 8 in Fig. 1; Miller 1979); in the latter case, control plots isolated from treated plots were included in the experimental design.

Females were sampled using an array of techniques that are grouped in four categories (Table 1). (1) Resident females on the foliage of host trees were collected with sweep nets (manual collection) or by fogging trees with insecticides (usually a combination of pyrethrins and piperonyl butoxyde). (2) In-flight females were captured with malaise traps and light traps (Hurley & Titus 1987: fig. 1). (3) Females were classified as migrants if they were sampled while ascending above the plant canopy, on the runway of airports (tarmac) early in the morning (observations conducted the evening before revealed an absence of females on the tarmac, hence females encountered there in early morning had migrated overnight), in pyramid traps above the plant canopy, or in the atmospheric boundary layer with the use of aircrafts (Greenbank *et al*. 1980, Eveleigh *et al*. 2007). (4) Dead females were collected using drop trays placed below the canopy of host trees. Sampling was conducted during the entire flight season (> 10 days) for all sites and sampling procedures. Techniques used to collect the females are described in the following references: Miller 1979, Greenbank *et al*. 1980, Thomas *et al*. 1980, Hurley & Titus 1987.

All collected females were dissected to determine the presence or absence of spermatophore by macerating their genitalia in heated potassium hydroxide (Robinson 1976). The proportion of mated females at different locations/years was calculated for each sampling treatment. Because the experimental protocol was unbalanced, mating proportions at different locations/years were treated as independent observations. The data were not normally distributed (79.6%, or 43 of 54 observations had a mating probability of 1; Table 1), hence non-parametric one-way ANOVA followed by Student-Newman-Keuls' test were used to compare mating probabilities for different sampling protocols.

Daily records of the abundance and mating status of females were available for most locations/years and sampling methods. Because mating probabilities were high for a vast majority of observations, however, documenting temporal variation in mating success at different locations/years was either not possible (when all females were mated) or inconsequent (when overall mating probabilities exceeded 95%). Variation in mating success over time was documented for resident females at four locations/years (*see* below).

Results

A total of 12 155 females were dissected for the presence of spermatophore, only 575 (4.7%) of these were virgin at the time of sampling. The average proportion of mated females per location/year significantly varied between sampling treatments ($F_{8,47} = 16.38$, $p < 0.001$) (Fig. 2).

Table 1. Proportion of mated female eastern spruce budworm and sample size (*n*) for different locations presented in Fig. 1. Resident females were collected with sweep nets (SWP) or by fogging trees with insecticide (FOG). In-flight females were collected with malaise traps (MAL) and light traps (LIG). Migrants were collected in pyramid traps above the plant canopy (CAN), in the atmosphere using aircrafts (ATM), on platforms while moths ascended vertically above the tree canopy (ASC), or on tarmacs early in the morning (TAR). Dead females were collected using drop trays.

Location/ year	Resident		In-flight		Migrants				Dead
	FOG	SWP	MAL	LIG	ASC	ATM	TAR	CAN	DROP
1/1978			1.000 (226)	1.000 (250)					0.983 (355)
2/1975	0.909 (320)		1.000 (100)	1.000 (171)					0.990 (105)
2/1977	0.904 (625)		1.000 (58)	1.000 (172)					0.915 (94)
3/1975				1.000 (233)	1.000 (87)	1.000 (51)	1.000 (38)		1.000 (91)
				1.000	1.000	1.000			
4/1971						(73)			
4/1972						(86)			
4/1974				(39)		(267)			
4/1975					(84)	(8)			
4/1976						(45)			
4/1977						(11)			
4/1978						(24)			
5/1980								1.000 (161)	0.990 (105)
			1.000		1.000	1.000			
6/1970					(42)				
6/1971					(45)	(30)			
6/1972					(51)	(55)			
6/1976			(117)						
6/1977	0.820 (848)		1.000 (270)	0.985 (198)					
7/1973		0.798 (525)		1.000 (839)	1.000 (158)	1.000 (9)			
7/1974		0.758 (297)	0.995 (198)	1.000 (138)	1.000 (404)	1.000 (815)	1.000 (361)	1.000 (29)	
7/1975		0.794 (34)			1.000 (156)	1.000 (153)	1.000 (147)		
8/1978	0.978 (407)		1.000 (400)	1.000 (340)	1.000 (148)				1.000 (605)

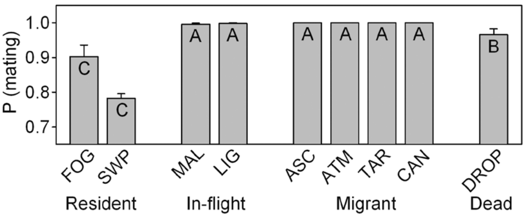

Fig. 2. Average mating probability per location/year (mean ± SE) (Fig. 1 and Table 2) for female spruce budworms collected with different sampling techniques; bars with different letter superscripts are significantly different, non-parametric ANOVA followed by Student-Newman-Keuls' test. Resident females were collected with sweep nets (SWP) or by fogging trees with insecticide (FOG). In-flight females were collected with malaise traps (MAL) and light traps (LIG). Migrants were collected in pyramid traps above the plant canopy (CAN), in the atmosphere using aircrafts (ATM), on platforms while moths ascended vertically above the tree canopy (ASC), or on tarmacs early in the morning (TAR). Dead females were collected using drop trays.

Among the 3498 females classified as migrants, none was virgin. A very low proportion (9 of 3746, or 0.2%) of in-flight females were virgin. The proportion of virgin females was intermediate among dead individuals (24 of 1350, or 1.8%) and relatively high among resident females captured in the canopy of host trees (542 of 3591, or 15.1%) (Table 1 and Fig. 2). The trend of lower mating probability for resident or dead females than for in-flight or migrant females was consistent at 9 of 10 locations/years for which the comparison could be made (Table 1). Temporal variation in the mating success of resident females was evaluated at four sites for which repeated measures over time were available during the entire flight period, and the same trend was consistently observed: the proportion of mated females was low early in the season, steadily increased over time, and peaked at a level near 100% late in the season (Fig. 3).

Discussion

Data collected using an array of techniques at 21 locations/years in Atlantic Canada in the 1970s illustrate patterns in mating success of female SBW that are consistent with its known reproductive biology. For example, the very low (< 0.2%) rate of virginity among active females (in-flight or migrant) reflects physiological constraints for flight in gravid females (*see* below). As with SBW, in-flight females captured at light traps or food bait traps in other Tortricidae are nearly all mated (> 99%) (*Platonotortryx excessana* and *Ctenopseustis obliquana*, Tomkins *et al*. 1987; *P. octo* and *Epiphyas postvittana*, Wearing *et al*. 2012), although it remains unclear whether this is due to an inherently high mating success in these insects or to physiological constraints for flight in virgin females. This situation contrasts with other tortricids e.g, *Cydia pomonella* and *Ecdytolopha insiticiana*, where up to 40% of females captured in passive or light traps are virgin (Thoeny & Nordin 1988, Weisling & Knight 1995). A guild of coniferophagous *Choristoneura* defoliators native to North America (Silk & Kuenen 1984) provides an opportunity to investigate taxonomic patterns at the genus level in terms of linkage among mating, oviposition, and dispersal behavior, but field data are available only for SBW.

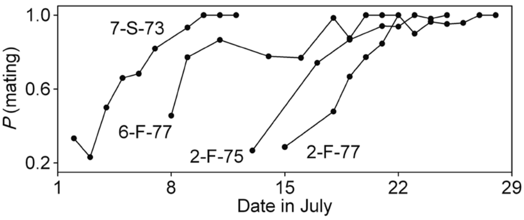

Fig. 3. Temporal variation in mating probability of resident female spruce budworms sampled on their host trees. The codes below the curves indicate the location (2: Heath Steele, 6: Acadia; 7: Chipman; *see* Table 1 and Fig. 1), the sampling collection method (S: sweep net, F: trees fogged with insecticide), and the year (1973 to 1975).

Female SBW are incapable of flying before having laid half their egg complement, because of their heavy abdomen (Wellington & Henson 1947, Rhainds & Kettela 2013). Most females in epidemic populations mate at least once (Sanders & Lucuik 1972, Kipp *et al*. 1995) and under these conditions are unlikely to fly before mating (Fig. 2). Flight is *not* physiologically impossible for virgin females, however, as indicated by the high proportion of virgin females captured at light traps (8.9%, or 31 of 347) in populations with female-biased sex ratios induced by aerial applications of insecticide (table 5 in Thomas *et al*. 1979). Egg dumping by virgin females may represent an adaptation to reduce abdominal weight and facilitate flight to locate mates when males are a limiting resource (Rhainds *et al*. 2012); this hypothesis explains otherwise counterintuitive observations such as the longer time allocated to laying eggs by virgin females than mated females (Wallace *et al*. 2004). Assuming that virgin females can fly in ecological contexts where mating opportunities are limited, the assessment of mating status of females captured at light traps may help to evaluate the efficacy of mating disruption (Rhainds *et al*. 2012) or declines in mating success as a function of population density (Régnière *et al*. 2013). This approach has not been thoroughly tested because it is generally assumed that virgin females are physiologically incapable of flying.

The high rate of virginity in resident females early in the season (Fig. 3) is likely due to the preponderance of young females that had not mated yet. Even though the proportion of mated females eventually approached 100% late in the season, the low mating success of early emergent females suggests that mating is constrained to some extent even in high density populations. Potential adaptations of females to enhance mating success include egg dumping (*see* above) or selection of micro-habitats with a higher abundance of mate-seeking males and enhanced mating probability, such as the upper strata of trees (Miller & McDougall 1973: fig. 1, Kipp *et al*. 1990). The latter behavioral adaptation may be ubiquitous in moths as indicated by the many examples of enhanced mating probability with an increasing height of females on the host plant (Showers *et al*. 1976, Weissling & Knight 1995, Lawson *et al*. 1996, López *et al*. 2003, Evenden & McLaughlin 2004, Cameron *et al*. 2005, Rhainds *et al*. 2009). Sentinel females may experience a low mating success because tethering impedes with the foraging behavior of mate-seeking females (Baker 2008, Rhainds 2010). For example, the upper threshold of mating probability of sentinel female SBW at high population densities (corresponding to > 500 males captured per pheromone trap per day) is consistently below 80% (Régnière *et al*. 2013), which contrasts with estimates of mating success averaging 98% among females dissected postmortem (Table 1 and Fig. 2).

Obtaining accurate, unbiased estimates of mating probability in female SBW is important for assessing the efficacy of pheromone-based disruption (Rhainds *et al*. 2012) and also for quantifying mate-encounter Allee effect at low density (Régnière *et al*. 2013). The relationship between population density and mating probability in female SBW is characterized by a positive sigmoid curve, and the most critical value is the inflexion point of the sigmoid function, i.e., the density that corresponds to a sharp increase in mating probability and is associated with a rise from endemic to epidemic populations. Mating probabilities derived strictly from sentinel females may systematically overestimate the rate of virginity compared with feral females (Rhainds *et al*. 2012), and if so, will not be suitable for assessing the density above which populations are released from the Allee effect. Unfortunately, demonstration of an Allee effect in SBW is strictly based on sentinel females, and mating probabilities of feral females have only been documented in epidemic populations. Future studies need to compare mating probabilities of both sentinel and feral females across a broad range of density to calibrate the mating success of sentinel females relative to that of feral females.

The strength of this study lies in the diversity of procedures that were used to sample females and assess biases in mating probabilities, e.g., in-flight or migrant females versus dead females, resident females collected early or late during the flight season, and possibly also sentinel females versus feral females. The literature on assessment of female mating status is abundant in Lepidoptera, for example in Noctuidae where hundreds of reference sources are available but have not been reviewed, or in mating disruption studies for which comparative data collected in control (untreated) plots may provide an insight into the reproductive ecology of many moth species. Ample opportunities exist to consolidate the literature and yield a general framework to interpret mating probabilistic functions in Lepidoptera, and this study represents a first step in that direction.

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