Young Love? Mating of Parnassius smintheus Doubleday (Papilionidae)

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Butterflies exhibit a range of mating behaviors (Scott 1972, 1974) and reproductive strategies (Rutowski 1984) that can have consequences for the growth, genetic structure, and persistence of populations (Calabrese & Fagan 2004; Rhainds 2010). In this study we investigate mating in the alpine butterfly, *Parnassius smintheus* (Papillionidae). There have been several previous accounts for this species, detailing its protandry, mating with newly eclosed females, and the production of sphragides—structures affixed to females by males to prevent subsequent mating (e.g., Scott 1972, 1974; Guppy & Shepard 2001). Calabrese et al. (2008) showed that ≈10% of female *P. smintheus* never mate. Mechanistic models fit to mark-recapture data showed that the proportion of unmated females in the population increased with increasing male density and/or male age. Here, we bring together disparate data to determine whether mating success for female *P. smintheus* varies with their age.

In 2007, we conducted an experiment originally intended to examine the effects of male age on female mating success. We paired 29 field-collected virgin females with males. Pairs were housed at ambient conditions in mesh enclosures (0.5 × 0.5 × 0.5 m) at the Biogeosciences Institute which is approximately 13 km from and 700 m lower than the alpine meadow habitat (2100 m) from where the butterflies were removed (Jumpingpound Ridge, 50.957°N, 114.890°W). Enclosures contained nectar flowers (Matter et al. 2009), but did not contain host plants. If a male died before the female, the male was replaced. Trials lasted for the lifespan of the female. Surprisingly, only one mating occurred (3.5%).

Based on these results, and the fact that we rarely see mating unless females are immobile (e.g., in nets or glassine envelopes), we began to question how frequently non-pupal mating occurs. We first examined the frequency of mating of virgin females in our mark-recapture data (see Matter & Roland 2010 for details of mark-recapture methodology and the study site). Mark-recapture data from 1995–1997 and 2001–2009 comprising 3119 captures of 2303 females revealed only 51 instances where a female who was initially marked as a virgin was later recaptured. The majority of these recaptures were within a single marking period (< 2

hrs). Twenty-two recaptures were over an interval of at least 24 hrs and were thus deemed of sufficient time to potentially reveal mating. Less than one-third of these recaptures showed that mating had occurred (7 of 22). The longest time between recaptures that a female remained unmated was 11 days; three of the seven females mated with at most one day between recaptures. The mean number of days between observations did not differ between females that mated (4.7) and those that did not (3.9 days; t = 4.0, separate variance df = 8.8, P = 0.70).

To further test the frequency of adult mating, in 2010, we released 13 virgin females who were captured as larvae, reared in captivity, and released as adults into populations M and J on Jumpingpound Ridge. Releases took place between the 7th and 18th of August. Five of these females were subsequently recaptured. In contrast to the low rate of mating seen in our previous experiment and mark-recapture data, all of these butterflies mated. The mean time between release and recapture when mating was first observed was 5.0 days. In concordance with the analyses of Calabrese et al. (2008), showing an inverse relationship between female mating success and male density, total population size in these meadows was low in 2010. The maximum estimated population size in meadow M in 2010 was 84.6 versus a mean of 233.3 ± 187.1 (SD) from 1995–2009. Population size in meadow K in 2010 was 30.1 versus a mean of 143.3 ± 106.4. Despite this agreement, the results also indicate that other mechanisms may affect female mating success.

Differences in observed female mating success seen for released virgins and from mark-recapture data could relate to accurately determining mating status by the presence of a sphragis. We are certain that the butterflies we reared and released were virgins because larvae were housed individually. In the field we rely on the presence of a sphragis to indicate mating. Females with worn sphragides are difficult to distinguish from virgins and occasionally males fail to properly attach a sphragis (Guppy & Sheppard 2011). Vlasanek and Konvicka (2009) estimated that ≈ 3% of *P. mnemosyne* lose a sphragis. Thus, some females presumed to be virgin in the mark recapture data may have mated. If males avoid mated females (Gilbert 1976), and if some

females assumed to be virgins were mated, it could account for the low mating success seen in the mark-recapture data. However, there is no indication that male *P. smintheus* avoid mated females. Additionally, Vlasanek and Konvicka (2009) show evidence for remating in *P. mnenosyne* following the loss of a sphragis, and two sphragides on one female can occur (Guppy & Sheppard 2001, p.54). Thus, it is unlikely that misidentification of mated females accounts for the lack of female mating seen in the mark-recapture data.

A possible explanation for the incongruous results is that males only mate with young females. *P. smintheus* have several traits making this hypothesis plausible. First, males often mate with females who have not expanded their wings (Scott 1972). Second, *P. smintheus* are protandrous; males emerge prior to females and patrol for mates. Finally, there is evidence that females emit a pupal pheromone whose effect diminishes with time (Scott 1974).

From an evolutionary perspective, male selection for females is thought to be rare (Rutowski 1984). Because male sexual function is inexpensive compared to females, males should maximize fitness by increasing their number of copulations. Exceptions occur where there is a cost of reproduction for males. For male butterflies, costs are usually in the form of courtship time or nuptial gifts (Rutowski 1984). Male Parnassians incur a reproductive cost by the production of a sphragis. These costs involve both missed mating opportunities during the time it takes to produce a sphragis (> 2 hrs) and the physiological cost of its production (Eltringham 1925). If males are limited in the number of females with whom they can mate, they should mate with females who will optimize their fitness. Because females only mate once, lay eggs singly from emergence whether they have mated or not, and continue to mature eggs throughout their life, the youngest females have the greatest potential fitness, and should be selected by males (Rutowski 1982).

Although not explicitly designed to test it, data are consistent with this hypothesis. Virgin females released in 2010 had high mating success and were very young. They were kept cool (2 °C) to minimize physiological demand and were all released within 1–3 days of emergence. The ages of the females with low mating success in the enclosure study were known less precisely, but were older. For this experiment, females were field-collected (Matter & Roland 2010). Using a mean collection interval of 2.9 days (range 1–5) in 2007 and a capture probability of p = 0.30 seen for females in the populations from which they were removed, the mean age of these females was 4.7 days, assuming no mortality or migration. Thus, females used in the mating experiment were on average 1.8 days older than virgins released in 2010. Females encountered during mark-recapture are also of an imprecise age. From 2001, age has been assessed based on wing wear, using the categories "new," "old," and "tattered." All but one of the 20 virgins recaptured during this time were initially scored as having new wings. These data are consistent with the age hypothesis in that all those that mated were initially new and the butterfly initially captured as an old virgin did not mate. Because wing condition is a function of age and flight, sedentary females often are scored as new, even those up to 5–6 days old.

Our analyses of mating indicate that females do mate after expansion of their wings, but the window of opportunity for a female to mate appears to be short, about 3 days. Most considerations of insect mating assume that females are equally mateable (but see Rhainds 2010). Our results indicate that the mating success of female *P. smintheus* decreases as females age, possibly due to the loss of a pheromone produced during the pupal stage (Scott 1972). Thus, effects previously attributed to male age may also be due to its correlation with female age (Calabrese et al. 2008). We are currently testing this hypothesis by releasing old and young virgins reared in the lab.

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