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ORIGINAL PAPER

Different mating expenditure in response to sperm competition risk between generations in the bivoltine butterfly *Pieris napi*

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Abstract Examining how the response to sperm competition risk varies in a population is essential in order to understand variation in reproductive success and mating system. In polyandrous butterflies, males transfer a large spermatophore at mating that delays female remating and confers an advantage in sperm competition. However, as large ejaculates are costly to produce—male expenditure on ejaculate size should be selected to vary with risk of sperm competition, as previously shown in the butterfly *Pieris napi*. In *P. napi*, adults can either emerge after winter diapause, or they can emerge as a directly developing generation later in the summer. Post-diapause adults have fewer developmental constraints because direct developers have to grow, develop, emerge, mate, and reproduce during a more limited seasonal timeframe, and as a result are more time-stressed. The two generations show polyphenisms in a variety of traits including polyandry, pheromone production, mating propensity, and sexual maturity at eclosion. Using these within-species, between-generation differences in ecology, we generated three important findings: (1) that both generations respond to an immediate risk of elevated sperm competition and significantly raise ejaculate investment, (2) that the diapausing generation raises this investment by a far greater 65 % increase compared with the direct generation males' 28 %, and (3) that males show a graded response relative to sperm competition risk and increase their ejaculate investment in relation to the actual level of mate competition. The difference in male mating allocation between generations may help explain life history evolution and geographic differences in mating patterns.

Keywords Ejaculate · Mating investment · Nuptial gift · Paternal investment · Resource allocation · Spermatophore

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Introduction

Sperm competition has been recognized as a particularly powerful force that can affect male behavior, morphology, and physiology to increase competitive fertilization success (Parker 1970; Birkhead and Møller 1998; Simmons 2001). Sperm competition risk arises when there is a probability that females will mate with more than one male—so that sperm will compete for fertilizations. To develop predictions on how male ejaculation strategies should be influenced by variation in sperm competition risk, game theory has been used (Parker 1990a, b, 1998; Parker et al. 1997; Parker and Ball 2005). Assuming that ejaculates are costly—so that what is spent on any one fertilization must be traded against future fertilizations, and that fertilization success is proportional to the number of sperm transferred to females at mating (Parker 1998), the models predict that an increased risk of sperm competition favors the evolution of increased expenditure on ejaculates. There is now evidence suggesting such an effect both between and within species in many taxa (Gage and Baker 1991; Gage 1991, 1994; Harcourt et al. 1995; Hosken 1997; Stockley et al. 1997; Byrne et al. 2002; Pitcher et al. 2005; Simmons et al. 2007).

Male nutrient provisioning or nuptial gifts are widespread among insects (Vahed 1998). Large ejaculates delay female remating for a longer time compared to small ejaculates (Oberhauser 1989, 1992; Wiklund et al. 1993; Kaitala and

Wiklund 1994; Wiklund and Kaitala 1995; Bissoondath and Wiklund 1997). Larger ejaculates also confer a competitive advantage in post-copulatory sperm competition as larger ejaculates can contain more sperm (Svärd and Wiklund 1989; Cook and Wedell 1996). These results all support the hypothesis that sperm competition plays a major role in the evolution of ejaculate size (Leimar et al. 1994; Bissoondath and Wiklund 1997). To better understand ejaculate tailoring and animal mating pattern evolution, a fruitful avenue is to examine responses to polyandry within cohorts of the same species, whereby the process can be experimentally investigated free of phylogenetic confounding effects. The butterfly *Pieris napi* model system offers an excellent opportunity to do this with its two different developmental pathways, direct versus diapause development, with the two phenotypes being associated with a number of different traits, especially with respect to reproductive traits (Larsdotter Mellström et al. 2010).

In butterflies, males produce a complex ejaculate during copulation that can contain both eupyrene (fertilizing) and apyrene (non-fertilizing) sperm (e.g., Silberglied et al. 1984), volatile anti-aphrodisiacs (Andersson et al. 2000), and accessory gland substances that function as paternal investment or male mating effort (Oberhauser 1989). Studies have shown that the male-transferred materials increase both female longevity (Karlsson 1998) and fecundity (Wiklund and Kaitala 1995) in our model species, the butterfly *P. napi*. The *P. napi* model is especially appropriate because it is well recognized as a species where the ejaculate is a considerable and limiting trait for males (e.g., Svärd and Wiklund 1986; Wedell and Karlsson 2003); for example, a male mating twice on the same day transfers in his second mating an ejaculate corresponding to about one third of the first and it takes at least 2 days of recuperation before the male can deliver a second ejaculate as large as the first (Bissoondath and Wiklund 1996). Hence, in view of the fundamentally different reproductive phenotypes as represented by directly developing versus diapause generation males in this species, it is relevant to test to what extent the two differ in their response to variation in mate competition.

In *P. napi*, females control mating initiation (Bergström and Wiklund 2005), as shown by the fact that females do not increase mating frequency with male courtship intensity. Hence, as males cannot enforce copulations on females, males can increase chances of paternity under competition by manipulating the female into delaying her next mating, or by increasing the number of sperm transferred. Both of these options are mediated by a large ejaculate. In contrast, under low risk of sperm competition, males should benefit from saving resources (Svärd and Wiklund 1986) which can be allocated to the next mating; hence, tailoring mating effort to the risk of sperm competition will be selected for. This pattern has been shown in *P. napi* (Larsdotter Mellström and Wiklund 2009), with males in high male competition treatments transferring larger spermatophores than males in low or no male competition treatments. The same study (Larsdotter Mellström and Wiklund 2009) also shows that male *P. napi* use the concentration of male sex pheromone (citral) in the air to tailor their ejaculate to sperm competition risk, a pattern that has been shown in several other species (Friberg 2006; Carazo et al. 2007; Thomas and Simmons 2009).

However, in many insect species, the different generations or morphs can show distinct life history polyphenisms in, e.g., behavior, sexual maturity, and/or polyandry (see Nylin and Gotthard 1998; Simmons et al. 2007; Larsdotter Mellström and Wiklund 2010; Larsdotter Mellström et al. 2010). Bivoltine butterflies like *P. napi* can exhibit polyphenisms, as the different generations experience different selection pressures in, for example, temperate environments. Several important differences in life history traits between the generations have been found in *P. napi*. Larsdotter Mellström and Wiklund (2010) show that the species is more polyandrous in the directly developing than diapausing generation. Välimäki and Kaitala (2006) found that northern populations of *P. napi* are less polyandrous than southern populations. This could be explained by differences in mating strategy between the developmental pathways of this species, as northern populations are univoltine and southern populations have two or more generations per year—selection on female mating rate or male mating expenditure in the directly developing generation could affect respective traits in the diapausing generation as well. The underlying reason is that winter can only be spent in the pupal stage in *P. napi*, and so the offspring from the directly developing generation have to reach the pupal stage before the onset of cold autumn conditions; this creates a situation in which the directly developing generation is severely stressed for time and are selected to complete development as fast as possible. Accordingly individual growth rates are higher in the directly developing generation compared to the diapausing generation which is less time-stressed (Wiklund and Forsberg 1991; Friberg et al. 2012). Indeed, Larsdotter Mellström et al. (2010, 2012) have shown that both males and females of the directly developing generation are less mature at eclosion than diapausing individuals and the directly developing generation (e.g., males lack adult sex pheromones at eclosion and females have a limited egg laying capability early in life) likely as a result of their being constrained by their short development time.

In this context, we investigate how males allocate mating expenditure in response to sperm competition risk between generations in a bivoltine insect. Two opposing hypotheses can be made; if the time-stressed directly developing generation is constrained by their short development time, males will not be able to respond to sperm competition risk by increasing spermatophore size as much as males of the diapausing generation. On the other hand, if the two generations respond

equally to existing selection pressures, the directly developing males are expected to invest relatively more in each mating, as the polyandry level is higher in this generation.

In this study, we investigate mating allocation (spermatophore size) in response to sperm competition risk (male population density), by male *P. napi* from the two different generations.

Materials and methods

Study species

The green-veined white butterfly, *P. napi*, is a common polyandrous temperate butterfly species that over-winters in the pupal stage (Tolman and Lewington 1997). The diapausing individuals fly during spring and directly developing individuals during summer. The larvae feed on a variety of species in the Brassicaceae family. In the wild, females mate between 1 and 5 times during their lifetime, with an average of 2.67 times (Bergström et al. 2002). The male transfers, on average, around 10–15 % of his body mass in the ejaculate (Svärd and Wiklund 1989). It has also been shown in several studies that larger males enjoy increased paternity share (Bissoondath and Wiklund 1997; Wedell and Cook 1998). As females gain from mating multiply in *P. napi* (Wiklund and Kaitala 1995; Karlsson 1998), many studies (e.g., Välimäki and Kaitala 2006, 2007; Välimäki et al. 2006, 2010) have investigated why the species still has around 12 % monandrous females (Bergström et al. 2002). Female mating frequency has been shown to be genetically determined (Wedell et al. 2002), and genetically polyandrous females have reduced longevity when denied remating, and this could help explain the variation in polyandry level (Wedell et al. 2002). Monandrous females have also been shown to have a higher initial reproductive output (Välimäki et al. 2006), which could be selected for under a short flight period and this could help explain why monandry is still prevalent in this species. Male *P. napi* emit a sex pheromone, citral, which is necessary for a female to accept to mate with a courting male (Andersson et al. 2007). Electroantennogram experiments have shown that not only females but also males have receptors that are sensitive to the male sex pheromone (Andersson et al. 2007).

For comparison of the two generations, we conducted two experiments with synchronized adult eclosion from both pathways according to a standard protocol (e.g., Larsdotter Mellström et al. 2010). The founders of the laboratory population are wild-caught in southern Sweden, where *P. napi* generally has two generations per year. Forty-five individuals were collected in the wild to establish the lab population. During the fall of 2010 and 2011, the light conditions in the climate cabinets (Termaks KB 8000 L) were set to induce diapause (12:12 h light:dark at 23 °C), eggs were laid and larvae reared on the natural host plant *Armoracia rusticana* (Brassicaceae) in water culture, and pupae were then overwintered in a refrigerator at −1 °C. After 5 months, half of the pupae were transferred to a climate cabinet at 23 °C, which led to adult eclosion approximately 1 week later. These adults were mated and allowed to lay eggs on *A. rusticana*, and eggs randomly chosen from >15 females founded the next generation. These larvae were reared under conditions that induce direct development (20:4 h light:dark photoperiod, 23 °C) and were allowed to feed ad libitum on *A. rusticana* throughout larval development until pupation. The remaining diapausing pupae (f1) were transferred to a climate cabinet (20:4 h light:dark, 23 °C) approximately 1 month later (having spent approximately 6.5 months in diapause) and eclosed simultaneously as the directly developing individuals (f2).

On the day of eclosion, after releasing the meconium, butterflies were weighed to the nearest mg on a Sauter AR 1014 electrobalance, individually marked and transferred to a cold room (10 °C) for a maximum of 3 days, until sufficient numbers of adult butterflies had emerged for an experiment to start. Butterflies were then randomly assigned to the treatments, and the treatments randomly allocated to the flight cages.

Experimental setup

The butterflies were kept in indoor flight cages at the Department of Zoology, Stockholm University, Sweden. The study was carried out on two occasions to increase sample size: 4–6 May 2011 and 7–16 Feb 2012. The flight cages (0.8×0.8×0.5 m) were located in a room with large windows and 400 W metal halide lamps over the cages to simulate daylight. The lamps were lit 8 h/day between 9 a.m. and 5 p.m. The cages have a transparent plastic top, three solid plastic walls with the fourth wall consisting of a black mesh net. The bottom of each cage was covered with paper soaked with water to maintain high humidity. In each cage, a Kalanchoe sp. plant, with drops of 20 % sucrose solution added on the flowers, was present for feeding.

All treatments were started 9 a.m., as we know that time of day for the mating does not correlate with spermatophore size (Larsdotter Mellström and Wiklund 2009) and did not have to be included as a factor in the analysis. All matings occurring under male densities ranging from 10 to 25 were considered as high sperm competition risk, as 10 males or more have been shown to elicit a high competition response (Larsdotter Mellström and Wiklund 2009). In the same study, it was also shown that males react instantaneously to the present population density, and that the time a male has spent in a given population density does not affect spermatophore size. Therefore, to optimize the number of experimental animals and still be able to study the effect of male–male competition also within the high competition treatment (not only between

high and low male competition treatments), we used the declining population density as mating couples are removed from the mating cage as a factor in the analysis.

Eclosion weight of males and females, spermatophore weight, time from the experiment started to mating were recorded as well as the population density at each mating.

For the high competition treatment, the number of males (M) to females (F) in each mating cage was 25 M:25 F at the start of the experiment. The cages were inspected continuously every 15 min for mating pairs and on discovery pairs were isolated in a plastic jar covered by a net, in the cage, until separation. The mated butterflies were then removed from the cage, frozen within 15 min after separating, and the females dissected to extract and weigh the spermatophore in their bursa. As the cages were not refilled during the day, population density decreased. The lowest male population density allowed for a mating was ten males (see Larsdotter Mellström and Wiklund 2009). On the second day of the experiment, new individuals were introduced into the cages to restore the population density (25 M:25 F). By this procedure, we can discern the time an individual has spent in the treatment from the time of the day and population density at mating. If spermatophore size declines continuously with time a male has spent in the experiment, over the two experiment days, it would indicate that the best males mate first and also transfer the largest spermatophores. If spermatophore size on the other hand is highest at the highest population density also on day 2, regardless of whether the male has spent 1 or 2 days in the treatment, it indicates a response to sperm competition risk per se. Any individuals that had not mated by the end of day 2 were discarded.

For the low competition treatment, males were allowed to mate under no sperm competition risk (1 M:1 F in each cage). The maximum time allowed for this treatment was 3 days. Cages were inspected and mating couples handled as above.

The spermatophores were dissected out of the females, wiped gently on a filter paper, and weighed to the nearest μg on a Cahn 28 Automatic electrobalance.

A total of 114 matings were recorded in the two experiments combined (Table 1). All statistical tests were performed in R (R Development Core Team 2009). Alpha was set at 0.05. Spermatophore weight and male eclosion weight did not meet linear test assumptions of homogenous variances. After a Box-Cox test ($\lambda \sim 0$), spermatophore weight and male weight were log transformed.

Results

Both generations increased mating allocation with increased sperm competition risk, but to a different extent. Males of the diapausing generation increased relative ejaculate size (spermatophore weight/male weight) more under higher risk of sperm competition ($\bar{x}$±95 % CI; single male; 7.5±0.6 %, high male density; 12.4±0.7 %) than directly developing males (single male; 8.5±0.9 %, high male density; 10.9±0.6 %) (see Fig. 1 and Table 1). For statistical analysis of data (R, Version 2.10.1, 2009) on spermatophore investment, we used a linear mixed-effect model (spermatophore weight ~ male weight × pathway × treatment, with experimental round as random factor, treatment levels: high/single) on the log-transformed values, including all interactions. Experimental round was non-significant ($F_{1,107}=1.39$, $P=0.25$) and subsequently removed from the model—leaving a linear model (spermatophore weight ~ male weight × pathway × treatment) analyzed with the car package in R (Fox and Weisberg 2011) using type III sums of squares. For statistics, see Table 2.

To assess factors affecting spermatophore weight within the high competition treatment and discern the time an individual has spent in the treatment from population density (10–25) at mating, we used a linear model (spermatophore weight ~ experimental day × pathway × male weight × male density) on the log-transformed values, including all interactions. Non-significant interactions and factors were then removed from the model with the step function in R, as above. Experimental day was non-significant ($F_{1,52}=0.02$, $P=0.90$) and subsequently removed from the model. Hence, although males mating on the second experiment day may have eclosed as less mature, they transferred spermatophores of a similar size to males mating on the first day. The final model, that best described the data, was (spermatophore weight ~ pathway + male weight + male density). For statistics, see Table 3. Hence,

Table 1 Number of matings and males not mated in each treatment

Treatment	No. matings	No. males not mated	Male eclosion weight (mg)	Spermatophore weight (mg)	Male mating investment (%)
Direct single	25	8	69.6±5.4	5.8±0.5	8.5±0.9
Diapause single	22	8	69.9±4.7	5.2±0.5	7.5±0.6
Direct high	32	9	61.7±4.1	6.6±0.4	10.9±0.6
Diapause high	35	7	71.3±2.8	8.8±0.5	12.4±0.7

Male eclosion weight, spermatophore weight, and male investment (spermatophore weight/eclosion weight) in each treatment given as $\bar{x}$±95 % CI

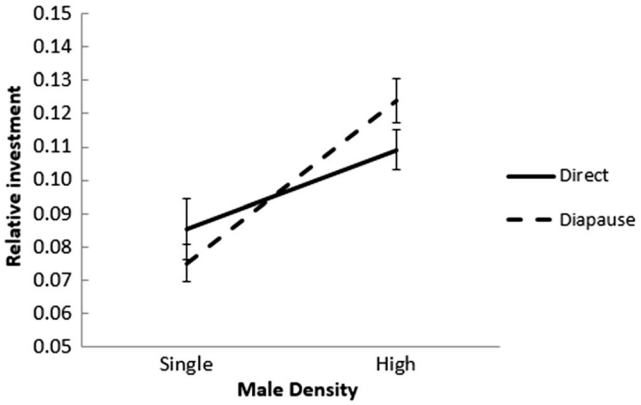

Fig. 1 Mating investment (spermatophore weight/male weight) by male population density (single male/high male density=10–25 males), given as $\bar{x}$±95 % CI. *Solid line* directly developing males, *dotted line* diapausing males

directly developing males transfer smaller spermatophores than diapausing males and male density had a significant effect on mating investment, also within the high male competition treatment; as population density decreased so did the size of ejaculate delivered (Fig. 2).

Discussion

In this study, we show that males of the two generations of *P. napi* differed in mating investment in response to sperm competition risk. Both generations reacted to sperm competition risk by increasing spermatophore investment conforming to sperm competition theory (e.g., Parker 1998), but at different levels. Using the within-species, between-generation differences in mating and ecology in *P. napi*, we generate three important findings: (1) that both generations responded to an immediate risk of elevated sperm competition and significantly raise ejaculate investment, (2) that the diapausing generation raised this investment by a far greater 65 % increase compared with the direct generation males' 28 %, and (3) that across an increasing high sperm competition risk, males showed an associated increase in ejaculate investment, with the diapausing males investing more than direct males at each given density. Contrary to prediction by sperm competition theory, directly developing males did not invest more, even though the directly developing generation has a higher polyandry level. The explanation for this might lie in the contents of the spermatophore, but this requires further study. The results of this study do however demonstrate the extent to which males are specifically adapted to sensing risks of sperm competition and thereby making possible appropriate ejaculate tailoring.

Contrary to our prediction based on Larsdotter Mellström and Wiklund (2009), we also found an effect of sperm competition risk within the high male competition treatment. This could be due to several non-exclusive options. In Larsdotter Mellström and Wiklund (2009), the population density ranged from 30 to 40, whereas in this study, the range was larger (20–50), making it easier to find a small effect. It could also be that males do not only react to male competition risk but possibly take in information of, e.g., total population density. This warrants further studies.

Table 2 Analysis of variance

	Degrees of freedom	Sums of squares	*F*	*P*
Treatment	1	0.62	105.25	**<0.001**
Male weight	1	0.30	50.73	**<0.001**
Pathway	1	0.02	3.81	**0.05**
Treatment: male weight	1	0.02	4.12	**0.04**
Treatment: pathway	1	0.10	17.18	**<0.001**
Male weight: pathway	1	0.03	5.83	**0.02**
Treatment: male weight: pathway	1	0.01	1.65	0.20
Residuals	106	0.63		

Response variable spermatophore weight. Significant effects in bold

Table 3 Analysis of variance

	Degrees of freedom	Sums of squares	*F*	*P*
Male weight	1	0.29	68.68	**<0.001**
Pathway	1	0.08	15.04	**<0.001**
Male density	1	0.06	2.95	**<0.001**
Residuals	63	0.26		

Response variable spermatophore weight. Significant effects in bold

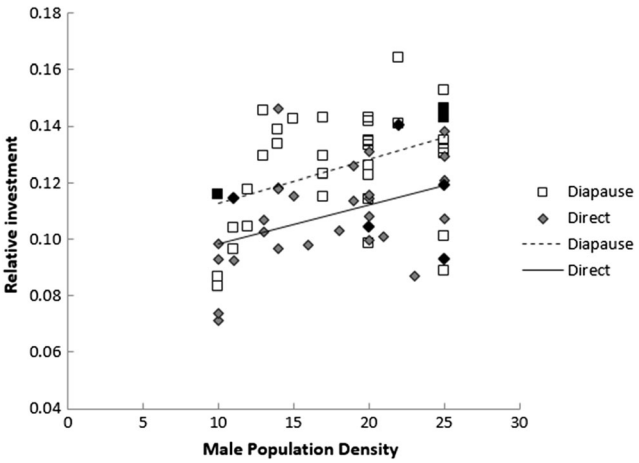

Fig. 2 Mating investment (spermatophore weight/male weight) by male population density (10–25 males). Diapausing males (*open squares and dotted line*), directly developing males (*filled diamonds and solid line*). *Black squares/diamonds* denote males mating on day 2 of the experiment

Bivoltine butterflies from seasonal environments, such as southern Sweden, are time-constrained. The present results correlate well with Larsdotter Mellström and Wiklund (2010) and Larsdotter Mellström et al. (2010) who suggest time stress as a cause of higher polyandry in the direct generation. Virgin directly developing females are shown to have a higher mating propensity (mate sooner after eclosion) than diapausing females, even though they are less physiologically mature (Larsdotter Mellström et al. 2010). All else being equal, this means that females of the directly developing generation are expected to be more polyandrous. This current study suggests that there might also be a physiological mechanism, acting on males of the directly developing generation, for the difference in polyandry in the two generations. As shown in Larsdotter Mellström et al. (2010, 2012), males are physiologically constrained by the short pupal development time in the directly developing generation. We suggest that this could constrain the directly developing males' possibilities to respond to a high risk of sperm competition impairing their ability to transfer an ejaculate as large as that of males of the diapausing generation. If males of the directly developing generation, on average, transfer smaller spermatophores to the female at mating, the females will remate sooner and achieve a higher polyandry level. Hence, time stress may be the causal factor underlying both the lower maturity of males and the higher mating propensity of females, under direct development. Alternatively—males of the two generations might experience different baseline risk (polyandry) in the wild, making the experimentally imposed level of risk proportionately higher for diapausing males relative to direct developers. Direct males may already anticipate heightened sperm competition risk but cannot match this with a bigger average ejaculate and instead opt for a smaller spermatophore which could contain more sperm (see Cook and Wedell 1996).

If we accept that directly developing males are less able than diapause generation males to increase relative ejaculate size under high mating competition, it might be asked why there is no difference between generations under relaxed competition. One possible explanation is that, since a large spermatophore is costly (Bissoondath and Wiklund 1996), diapausing males would not benefit from transferring more resources than necessary when there is no sperm competition risk. Instead, both generations seem to transfer a "minimum acceptable ejaculate," saving resources for the next mating (see Parker 1998).

Virgin males of both generations were able to increase their ejaculate expenditure under increased risk of sperm competition, which presumably must be traded against ejaculate expenditure in future matings. This is a prerequisite for game theory correctly predicting how male ejaculation strategies should be influenced by variation in sperm competition risk (Parker 1990a, b, 1998; Parker et al. 1997; Parker and Ball 2005). The results of this study, that males under high risk of sperm competition transferred larger ejaculates, whereas males under low intra-sexual competition saved their resources for future fertilizations, conform well to this theory, and also add the novel insight how differences in life histories between generations can shift the balance of what is the optimal strategy for a given set of life history traits.

We show that males were able to judge male population density, and thereby sperm competition risk, fast and accurate even though population density changed in the cages during the experiment. From Larsdotter Mellström and Wiklund (2009), we know that males adjust spermatophore size based on the amount of the male sex pheromone citral in the air. As males dispense citral in the process of flying (Andersson et al. 2007), the amount of citral in the air would be a good and honest signal of how many males are nearby and provide a mechanism for males of reacting to fast changes in male competition. To be able to judge competition accurately not only in fixed population densities but also in fluctuating environments would of course be a prerequisite for a correct sperm competition risk response, and we contend that the results indicate that there is selection for fast and accurate judgment of male competition, e.g., by pheromone assessment.

Mating rate in *P. napi* also varies geographically. Välimäki and Kaitala (2006) showed that northern (univoltine) populations are less polyandrous than southern (bivoltine) populations, the ultimate reason likely being the shorter flight period available at northern latitudes which may preclude both female opportunity to remate, and benefit from doing so—polyandry increases female fecundity only when the flight season is protracted (cf. Välimäki et al. 2006). This correlates well with the result from Larsdotter Mellström and Wiklund (2010) that diapausing females have a lower polyandry level than direct developers. Interestingly, the results of this study suggest a proximate reason for this difference; given that males of the directly developing generation are less able to increase ejaculate size under mate competition, this means that the duration of the female refractory period will be shorter, which, in turn leads to a higher level of polyandry. According to this scenario, with directly developing males being less able to increase ejaculate size with increasing male competition, it may be expected that the variance in the degree of polyandry will vary more in southern directly developing generations in accordance with variance in population density. When comparing northern and southern populations of *P. napi* in Finland, Välimäki and Kaitala (2010) found no spatial variation in relative ejaculate size between and suggested that geographical variation in polyandry probably is maintained by selection acting on female, rather than male, life history traits. However, our results show that competition-dependent variation in male ejaculate weight between generations can contribute to how a difference in number of generations in a population may influence the evolution of mating rates.

Variation in the level of sperm competition and females' chances for post-copulatory mate choice (see Parker 1970; Eberhard and Cordero 1995) and differences in mating frequency generates variation in the strength of sexual selection not only by populations as stated by Välimäki and Kaitala (2006) but also between generations. As shown by Kaitala and Wiklund (1994), mating frequency increases if the transferred ejaculate is small. When the directly developing generation is constrained by the short pupal development time, this will affect both polyandry level and associated traits and also have profound effects on life history traits in the species.

It has earlier been shown by Wedell et al. (2002) that females of *P. napi* have different, heritable reproductive tactics ranging from strict monandry to polyandry. To this, we can now add significant differences among males between generations in both mating behavior (Larsdotter Mellström and Wiklund 2010), maturity (Larsdotter Mellström et al. 2010) and response to sperm competition risk. In view of our results that male *P. napi* adjusted ejaculate size differently in the two generations, it would be interesting to also examine if they also strategically change the composition of the ejaculate in response to sperm competition risk. In the moth, *Plodia interpunctella* males increase eupyrene but not apyrene sperm numbers in sperm competition situations (Cook and Gage 1995), whereas butterfly *Pieris rapae* increases both eupyrene and apyrene sperm numbers with increased sperm competition (Wedell and Cook 1999).

The ultimate causes of the differences between generations could be better understood if we study sperm allocation within the spermatophores. This warrants further studies. Nonetheless, this study does conclusively demonstrate that time stress and seasonal polyphenisms can change male mating allocation in response to sperm competition risk.

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