Life history evolution in a bivoltine butterfly

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List of papers


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Abstract

Evolution is not always straight-forward, as selection pressures may differ between different generations of the same species. This thesis focuses on the evolution of life history of the model species, the Green-veined White butterfly *Pieris napi*. In central Sweden *P. napi* has two generations per year. The directly developing summer generation is short-lived and time stressed, compared to the diapausing generation.

In **paper I** polyandry, defined as female mating rate, was shown to differ between generations but was unaffected by environmental factors. In **paper II** both males and females of the direct developing generation were shown to eclose more immature than the diapausing generation, indicating larval time constraints. Consistent with this, diapausing males mated sooner than direct developers. Directly developing females, however, mated sooner after ecllosion than diapausing females, even though they are more immature. This was shown to negatively affect fecundity, but can pay off when the season is short.

**Paper III** shows that directly developing males have less sex pheromones at ecllosion than diapausers, and the differences in sex pheromone production is consistent with developmental time constraints and the differences in mating system.

In *P. napi* and other polyandrous butterflies, males transfer a large, nutritious ejaculate at mating. Large ejaculates confer advantages under sperm competition, but as they are costly, males should adjust ejaculate size to the risk of sperm competition. In **paper IV** we found that males transfer on average 20% larger spermatophores under high male competition than at low competition. The same effect could be observed if we added male sex pheromone to the air in a mating cage without male-male competition. **Paper V** shows that males of the two generations respond differently to an increase in male-male competition, with diapausing males transferring larger spermatophores than direct developers at high male competition risk.

**Key words**  Bivoltine, Diapause, Lepidoptera, Life history, Mating system, Pheromone, Polyphenism, Population density, Sexual selection, Sperm competition
Introduction

“Nothing in Biology Makes Sense Except in the Light of Evolution”

These eloquent words by Dobzhansky (1973) provide an excellent backdrop for this thesis. Ecology provides the stage where evolution takes place and integrating the two is essential for a deeper understanding of the interactions we observe around us today.

When we think about evolution what usually comes to mind is natural selection. It is even in the title of Charles Darwin’s seminal book “On the Origin of Species by Means of Natural Selection” (1859). By this beautiful and skilful integration of several pre-existing ideas and his own observations Darwin suddenly made previously unexplained patterns come together into one unifying theory that still, 150 years later, holds true. Any trait that was heritable and conferred an advantage in the struggle for existence and survival could now be explained by natural selection.

More difficult for Darwin were highly evolved and complicated features that conveyed apparently no adaptive advantage to the organism. In his famous words from his correspondence (Darwin, 1860);

“The sight of a feather in a peacock’s tail, whenever I gaze at it, makes me sick!”

To reconcile the theory and these apparently maladaptive traits Darwin needed to add yet another mechanism for evolution. Sexual selection is the evolutionary process proposed by Darwin to explain traits whose primary function appears to be ensuring an individual’s success in courtship and mating. Or, in the words of Darwin himself in his book The Descent of Man, and Selection in Relation to Sex (1871);

“... [sexual selection] depends, not on a struggle for existence, but on a struggle between the males for possession of the females; the result is not death to the unsuccessful competitor, but few or no offspring”

Different characteristics could thus be selected for if they conveyed a reproductive advantage to the individual (Darwin, 1871). Sexual selection is therefore believed to have a strong effect on the evolution of animal mating systems (see Wiklund, 2003) and will be an integral part of this thesis.

For studies of mating systems, insects in general and butterflies in particular are often used model organisms. Insects display a diversity of reproductive strategies unparalleled among animals, which has put research in this field at the forefront of the study of animal mating systems (Brown et al. 1997). Butterflies offer great model systems for evolutionary studies as they are small, fecund animals with a reasonably short life span. They are also, at least my model species Pieris napi (the Green-veined White), easy to rear in the laboratory. This enables us to perform both artificial selection, life time fitness measurements and behavioural studies. Using a previously well studied model species like
**P. napi** also comes with the advantage of having very good background information on the species, regarding everything from host plant use (Forsberg 1987) and courtship behaviour (Forsberg & Wiklund 1989) to polyphenism (eg. Wiklund et al. 1991) and chemical signaling (Andersson et al. 2007), which is not very common (see Nieberding et al. 2008).

**Life history evolution**

Life history theory sees the scheduling of events such as growth, sexual maturation, and reproduction as the result of strategic decisions over an organism’s life (Stearns 1992). Organisms have limited time, energy and nutrients at their disposal. Investing time or energy into mating, for example, decreases time and energy available for foraging and these trade-offs in allocation form the life histories of a species. When to mature, how many eggs to lay or how much to invest in each mating are all classical examples of life history traits and the allocation pattern that produces as many successful offspring as possible will be selected for during evolution. Life histories thus balance trade-offs between current and future reproduction, representing the best solution of conflicting demands on the organism.

However, in an organism with several life cycles per year selection pressures may differ between different generations of the same species. Multivoltine insects appear in two or more discrete generations per year. In temperate regions the different generations will most likely experience varying selection regimes, depending on the season. Many multivoltine butterfly species do indeed show seasonal polyphenisms not only in appearance (e.g. wing colouration) but also in life history traits, such as adult weight and larval development time (Nylin et al. 1989), fecundity and dispersal (Karlsson & Johansson 2008) and female mating propensity (Friberg & Wiklund 2007). These seasonal polyphenisms might either be the result of adaptive plasticity triggered by reliable generation-specific environmental cues, or passively induced phenotypes caused by generation-specific environmental constraints. Therefore, the particular life history traits that are targeted by selection or affected by developmental constraints are likely to differ between species, and relate to the species-specific life cycle. An interesting example of this is that female *Leptidea reali* (sensu lato) butterflies (Lepidoptera: Pieridae) of the time constrained directly developing summer generation accept mating faster than the less time constrained spring generation females that have spent the winter in pupal diapause (Friberg & Wiklund 2007). An explanation for this behavioural difference could be that theoretical models predict a context-dependent female mate choice behaviour and relaxed selection on female choosiness when females are time constrained (Johnstone 1997). The generality of these life history effects, the mechanisms behind them and their effects are examined in **paper I and II**.
In butterflies, as a general rule, incoming resources are used in preference to stored reserves (Boggs 2003). Butterfly life history is also constrained by the shift in diet between life stages. Essential amino acids are, in most *Lepidoptera*, derived from larval feeding whereas sugars predominate in adult nectar diet (Erhardt & Rusterholz 1998). This has several important effects on life history evolution. Leimar *et al.* (1994) have shown that if the larval resource environment becomes more variable, male-derived nutrients become more important for the female to ensure reproduction and thus affects mating system. Allocation patterns (see figure 1) may also be differentially affected if larval resources are decreased; all life history traits could be affected to the same degree or allocation of larval nutrients could be unevenly distributed to support some important traits whereas others receive a disproportionately small part of the resources (Boggs 2003). If reserves, measured as initial adult body mass, are large the butterfly is able to maintain fecundity during adult food deprivation. If reserves are small, it cannot. This shows the importance of larval feeding on adult life history patterns (Boggs 2003). In \textbf{paper III} we study the effect of larval and adult derived nutrients on sex pheromone production and relate it to developmental pathway and mating system.

![Figure 1. The allocation of nutrients to different life history traits.](image)

**Polyphenism**

Biotic and abiotic factors vary over space and time. These variations affect an organism’s allocation of time and nutrients to reproduction, survival, growth, storage and foraging. Allocation patterns hereby link environmental variation with life histories and population dynamics. Allocation studies are being carried out in a wide variety of animals; butterflies, orthopterans, lizards and plants among others.
As mentioned above, for multivoltine insects in temperate regions the different generations will most likely experience varying selection regimes, depending on the season. In a seasonally changing environment active periods, such as development and reproduction, must occur during the favourable time of year and diapause during unfavourable periods. As a consequence of this the production of more than one generation per year involves a choice between alternative developmental pathways, direct development or diapause. If a genotype produces individuals who are able to complete additional generations within a given period of time, it will have a higher intrinsic rate of increase.

In most organisms a single genotype can produce many different phenotypes and which phenotype is expressed is dependent on the environment in which the organism develops and phenotypic plasticity is believed to be the primitive character state for most or all traits (Nijhout 2003). Evolution of plasticity can then move in one of two directions; stabilization of the phenotype and loss of plasticity or exploitation of the plasticity. During evolution plasticity has been advantageous as a mechanism that enables an organism to develop different phenotypes that can be adapted to and optimized for two or more environments (Nijhout 2003). In insect polyphenisms the environmental variable that induces one of the phenotypes is a stimulus that only serves as a predictor of, but is not itself, the environment to which the polyphenism is an adaptation. The environmental stimulus changes the endocrine mechanism of metamorphosis and the altered pattern of endocrine interactions results in the execution of the alternative developmental pathway (Nijhout 2003).

Polyphenisms are adaptations to predictable variations in the environment. However, the inducing environment is not the same as the environment to which the phenotype is adapted. Seasonal polyphenism, for example, may be an adaptation to cold or food shortage but is typically induced mainly by a change in photoperiod (Wiklund et al. 1992). The change in photoperiod is not in itself an unfavourable environment but it is a good predictor of seasonal change. In adult polyphenisms, the critical period typically occurs during the larval stages (Nijhout 2003).

To fully understand how these polyphenisms evolve we need to separate the effects of the environment the two generations live in and the effect of the induced phenotype in itself. For example, food availability may change between generations, giving different patterns of effect on life history and allocation and hence, polyphenism. But the induced phenotypes may also have inherently different allocation patterns. This is a common theme for all the articles in this thesis.

**Mating system**

Mating systems represent an array of adaptations selected to maximize the reproductive rate of individuals of each sex (Brown et al. 1997). Life history theory tries to explain how natural selection
has shaped traits to produce life cycles, including mating patterns, that fit the demands of the environment. Polyphenism, physiological processes and sexual conflict are three areas that all have direct or indirect effects on animal mating systems. The evolution of mating systems depends on many variables, including resource and mate distribution, the presence and extent of transferable genetic and material benefits and the degree of control exerted by each sex over events at different stages in the mating sequence (Brown et al. 1997, see figure 2).

The common view was, for a long time, that male fitness increased through copulating with multiple partners, whereas female fitness only increased with the quality of, and care for, offspring. However, several studies have now shown that females receive direct fitness benefits, *i.e.* increased offspring production, from mating polyandrously (*e.g.* Wiklund et al. 1993, Kaitala & Wiklund 1994, Karlsson 1998).
Whether an individual maintains monandry or polyandry could be dependent on a number of factors. Sexual selection affects both male and female life history traits. Sexual conflict also has an effect on the optimal mating frequency and different generations may experience different selection regimes, leading to a seasonal polyphenism in mating system. Välimäki et al. (2006) have shown that monandrous females perform better than polyandrous during unpredictable conditions and when pressed for time. Consistent with these findings, Välimäki et al. (2008) have found that females with a low mating frequency are more likely to produce an additional summer generation in seasonal environments than the females with a high degree of polyandry. This contributes to explaining the low mating frequencies in P. napi and shows that life history, polyphenism and mating system are closely intertwined. Comparing life histories of females with varying degree of polyandry Välimäki & Kaitala (2007) concluded that polyandrous females developed at a faster rate than monandrous females, in both direct and diapausing cohorts.

If females mate more than once during a reproductive period, it has profound effects on females’ role in sexual selection. It could generate sperm competition (Parker 1970) which in turn could lead to several evolutionary responses in males, females and features of the ejaculate. It could, for example, create the possibility for females to, via cryptic female choice, choose which male’s sperm will fertilize the eggs (Eberhard 1996). Polyandry, and mixed paternity in offspring, have been recorded in organisms as different as snails, honeybees, mites, spiders, fish, frogs, lizards, snakes, birds and mammals (Birkhead & Møller 1998). In polyandrous species the last male to mate with a female generally enjoys higher fertilization success than previous males (Wiklund 2003). Male size has also been shown to be associated with success in sperm competition because large males transfer more sperm (Wiklund 2003).

**Sexual selection**

Natural selection favours traits that increase survival, but an individual’s fitness is also dependent on how many offspring that are produced. Sexual selection theory therefore predicts, as mentioned earlier, that a trait that enhances reproductive success will be favoured and spread in the population.

If a female mates more than once, as in P. napi, this confers that sperm from different males will compete for fertilizations (Parker 1990a, b, 1998, Parker et al. 1997, Parker & Ball 2005). Sperm competition has been recognized as a particularly powerful force that can lead to adaptations in male behaviour, morphology and physiology that contribute to competitive fertilization success (e.g. Parker 1970, Birkhead & Møller 1998). Game theory has been used to develop predictions about how male ejaculation strategies should be influenced by variation in sperm competition risk (Parker 1990a, b, 1998, Parker et al. 1997, Parker & Ball 2005). Sperm competition game theory assumes that (1)
ejaculates are costly - meaning that expenditure on any fertilization must be traded against that of gaining future fertilizations, and (2) that fertilization success is proportional to the number of sperm transferred to females at mating (Parker 1998) - like the tickets in a lottery. The models predict that increased risk of sperm competition should favour the evolution of increased expenditure on ejaculates, and there is now evidence suggesting such an effect both between and within species (Gage & 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). Although this phenomenon is widespread in insects and other taxa, the mechanisms that allow males to assess sperm competition has only recently been investigated with the results that pheromones are important for assessing sperm competition risk in the beetle Tenebrio molitor (Carazo et al. 2007), the fruit fly Drosophila melanogaster (Friberg 2006), the cricket Teleogryllus oceanicus (Thomas & Simmons 2009) and the meadow vole Microtus pennsylvanicus (delBarco-Trillo & Ferkin 2004, 2007).

In P. napi males, as in most butterflies, cannot enforce copulations on females, and therefore have two, mutually non-exclusive, options to increase fertilization success: to manipulate the female into delaying her next mating or to increase the number of sperm transferred to the female. Both of these options are mediated by a large spermatophore. In contrast, under low risk of sperm competition males should benefit from saving resources which can be allocated to the next mating. How males from both developmental pathways allocate their resources under male-male competition for matings, and if they use the amount of sex pheromone as a cue for mating investment is examined in paper IV and V.

The male donation of nutrients must be regarded both as paternal investment, increasing the number and quality of the male’s own offspring up to the time the female remates, and as mating effort, delaying female remating (Wiklund & Kaitala 1995). This kind of mating system leads to a fundamental asymmetry between males and females. The male’s ability to invest in reproduction is dependent on the resources he can accumulate as a larva, capital investment, whereas females can obtain resources for reproductive investment both as larva and from nuptial gifts, a combination of capital and income investment.

Sexual conflict

Conflict arises whenever the outcome of an interaction yields differing optima for different individuals or groups/classes of individuals (Brown et al. 1997). Each individual will then be selected to manipulate the interaction in ways that bring the outcome closer to its optimum. Conflict of interest between the sexes is expected at each stage between pair formation until the end of parental care, and in all mating systems (Brown et al. 1997) and sexual conflict is a potent force in male-female coevolution (Chapman et al. 2003).
Sexual selection can be divided into two classes; in indirect models the female preference evolves as it becomes genetically associated with genes that confer either sexy sons and/or high viability offspring. In direct models the female preference itself is under natural selection. Females could for example gain resources or greater parental care. The models of sexually antagonistic coevolution also fall into this class. There are two main mechanisms for sexual conflict (Chapman et al. 2003). The intralocus conflict where the fitness optimum for a trait expressed in both sexes is different for males and females. The interlocus conflict occurs when there is conflict over the outcome of male-female interactions – the optimal outcome is different for the two sexes. Examples of this are conflicts over mating frequency, parental effort and so on. Both sexes are then expected to evolve suites of sexually antagonistic adaptations that bias the outcome towards their own respective interests. The result is sexually antagonistic coevolution between interacting traits in males and females. Because of sexual conflict, the selection pressures on males and females are different and represent an important factor in shaping the evolution of mating systems and sexual dimorphism (Rutowski 1997).

Even though females receive direct benefits by mating multiply, only two out of twenty-three investigated butterfly species, showed an average lifetime number of matings exceeding three in the wild (Wiklund & Forsberg 1991). So far we have seen that polyandrous females obtain direct benefits from mating multiply. So why then aren’t female butterflies in all species polyandrous?

The reason why females do not mate more could be that there is limited space for spermatophores in the female bursa and that they take time to break down (Oberhauser 1992). This fact indicates a conflict between the sexes in that males would profit from a slow rate of degradation which delays female remating, whereas females would benefit from fast degradation to make possible the reception of a new nutritious spermatophore (Boggs 1981).

In *P. napi*, females obtain direct fitness benefits from mating multiply. Multiply mated females of naturally polyandrous *P. napi* have been shown to lay larger eggs, live longer and have larger life time fecundity then singly mated females (Wiklund et al. 1993) since males transfer a large nutritious gift to the females that the females use to increase both their fecundity and lifespan (Arnqvist & Nilsson 2000). Despite this positive relationship between fitness and number of matings, there is a large variation in female mating frequency and 12% of old females are monandrous (Bergström et al. 2002). Monandry in females is not coupled to lack of mates (Bergström et al. 2002, Bergström & Wiklund 2005, Välimäki & Kaitala 2006). It could instead be discussed in terms of sexually antagonistic coevolution and whether environmental conditions influence optimal mating frequency (Bergström et al. 2002). The reason for the low mating frequency could either be that males have evolved the ability to manipulate females to mate at a suboptimal rate as a measure of protection against sperm competition, or as suggested by Bergström & Wiklund (2005), that female mating is suppressed by some costs.
Studies have shown that there is genetic variation in female mating rate (Wedell et al. 2002, Välimäki et al. 2006) and that female mating rate and fecundity are under genetic control and show high heritability. Even though polyandrous female *P. napi* have higher lifetime fecundity than monandrous females, some females simply abstain from remating irrespective of the number of times they have been courted. Explaining this genetic variation in mating rate requires that monandrous females perform better than polyandrous ones under some conditions.

Using high and low intrinsic mating rate females Välimäki et al. (2006) showed that during the first days of reproduction, females with a low mating rate produced more eggs than females with a high mating rate. This could lead to an evolutionary advantage for the monandrous females if the time at hand for reproduction is limited. The study also shows that unpredictable weather favours monandry, as hypothesized in Bergström et al. (2002). Since mating takes several hours (Bergström et al. 2002), there is an apparent short-term conflict between mating and egg-laying. Under extended poor weather conditions when there are few opportunities to lay eggs, optimal mating frequency may be lower than in good weather. So the explanation for the apparent suboptimal mating rate in females could be that there are costs associated with mating in the wild that have been underestimated and that optimal mating frequency covaries with environmental factors, in which case females do not in fact mate below the optimal frequency. A combination of life-history cost and unpredictability of fitness may explain the maintenance of monandry in the wild and the increasing frequency of monandry with latitude.

**Study species**

The butterflies used in these studies were the offspring of wild-caught *Pieris napi* (Green-veined white; Lepidoptera: Pieridae) from the Stockholm area. In central Sweden, *P. napi* has two generations per year, both of which are generally widespread and common. Larvae feed on a variety of crucifers, including several cultivated plants e.g. cabbages and rapeseed. The diapausing generation ecloses in spring after having spent winter in the pupal stage. The offspring of this generation undergoes direct development, eclosing during summer, and their offspring in turn pupate and diapause during winter. There are potentially very different selection regimes acting on the two generations. Individuals from the diapausing generation have plenty of time to develop physiologically in the pupal stage during winter and are not time constrained in the adult stage as they eclose in spring. In central Sweden the flight period of the direct developing generation, on the other hand, is approximately two months later than that of the diapausing generation, yielding a correspondingly higher time stress in the adult stage, as the offspring must reach pupation before the onset of winter (Abrams et al. 1996, Gotthard et al. 1999) and the direct development also imposes shorter time in the pupal stage.
At mating male *P. napi* transfer an ejaculate that, on average, corresponds to 15% of male body mass (Svärd & Wiklund 1989). The ejaculate contains both fertilizing and non-fertilizing sperm as well as significant amounts of nutrients and female fitness increases with number of matings (Wiklund *et al.* 1993, Karlsson 1998). In the wild females mate between 1 and 5 times during their lifetime, with an average of 2.7 times (Bergström *et al.* 2002). *P. napi* females usually mate on an interval of 3-5 days (Wiklund *et al.*, 1993), the duration of the refractory period being positively correlated with ejaculate mass. As in most butterflies, females control mating (Bergström & Wiklund 2005) *i.e.* males cannot mate without a female accepting him. Male *P. napi* emit a sex- and species specific pheromone, citral, which makes females accept mating with a courting male and both males and females have receptors that are sensitive to the male sex pheromone (Andersson *et al.* 2007). Citral is a citrus smelling 1:1 combination of the volatile isomers geranial and neral, with the molecular formula C_{10}H_{16}O. The adult scent composition consists to around 95% of citral.

**Methods**

By manipulating temperature and light conditions we can obtain both generations in the laboratory during the same period and could thereby exclude effects of phenotypic plasticity induced during the adult stage. Experiments were carried out at the Department of Zoology, Stockholm University, using mating cages measuring approximately 0.8x0.8x0.5m. Cages were housed in a room where the daylight regime could be controlled. All pheromone measurements where performed at KTH - Royal Institute of Technology, Stockholm, in collaboration with Prof. Borg-Karlson and her ecological chemistry group. With the *P. napi* butterfly system we can collect the sex pheromone either by Solid Phase Micro Extraction (SPME) of live emissions of volatiles or by extracting the volatiles from the wings of euthanized males and then identify the components by gas chromatography. For more detailed studies on biosynthesis, stable carbon isotopes were used. By this procedure, we can describe citral production by studying time allowed for feeding on labeled glucose together with the appearance of labeled pheromone components. This then can be compared with known geranial and neral biosynthetic pathways in plants (Iijima *et al.* 2006, Schilmiller *et al.* 2009) to investigate whether *P. napi* uses similar or different biosynthetic pathways.

**Objectives**

*Paper I* Whether an individual maintains monandry or polyandry could be dependent on a number of factors. Sexual selection affects both male and female life history traits. Sexual conflict also has an effect on the optimal mating frequency and different generations may experience different selection regimes, leading to a seasonal polyphenism in mating system. Polyandrous females have higher
lifetime fecundity compared to monandrous females (Wiklund et al. 1993). Nevertheless, 12% remain monandrous throughout their life (Bergström et al. 2002).

We investigated which factors affect polyandry in a bivoltine central Swedish population of *P. napi*. Our objective was to examine: (1) the effect of environmental factors on the level of polyandry; and (2) the effect of developmental pathway on the level of polyandry. We did this by staging experiments in which larval host plant, adult activity level, nectar and mate availability, and daylength were manipulated, and by assessing lifetime number of matings of diapause and directly developing generation females, controlling for possible variation between families in the level of polyandry.

**Paper II** The aim of this study was to test whether mating propensity differs between generations, *i.e.* do males and females of the two generations differ in time from eclosion to first mating? Along with this we also tested several physiological parameters to establish whether males and females of the time stressed direct developing generation eclose as adults less mature than the diapausing generation and if this correlates to mating propensity.

To determine if there are any differences in maturity at eclosion between the generations we first looked at female daily and lifetime fecundity. Females were allowed to mate either within the first 12 hours after eclosion, or after 13-84 hours after eclosion. Eggs were then counted daily for the duration of their life. For males we assessed male sexual maturity by their emission of sex pheromone.

**Paper III** The time constrained directly developing generation males take, on average, more than 6 hr longer to mate for the first time after eclosion than do diapausing males (23 vs. 29.5 hours) (Paper II). To study the differences in physiology between generations that experience different levels of time stress and explore the dynamics in male sex pheromone production we first set out to assess pheromone biosynthesis in *P. napi*, and to compare sexual maturity at the time of adult eclosion, between the two generations. We hypothesized that the directly-developing males would take longer to reach adult scent composition than diapausing males. Second, we also more specifically studied whether the sex pheromone components are biosynthesized from larval and/or adult-derived material by using stable carbon isotopes.

**Paper IV** In this study we investigate the reproductive behavior of male *P. napi* under different population densities to test if males 1) tailor their ejaculate expenditure to the degree of competition and 2) use the male sex pheromone citral as a cue when assessing the risk of sperm competition, predicting that males mating in high male competition treatments or citral treatment will transfer larger ejaculates.

Virgin butterflies (from the direct developing generation), 1-3 days old, were put in mating cages to test the impact of population density on male ejaculate expenditure, with the number of males (M) to
females (F) in each cage as follows, 1M:2F, 2M:2F, 10M:20F, 20M:20F, 1M:30F or 30M:1F. For the pheromone experiments large plastic jars were used as mating cages. In each of these a pair of a virgin male and a virgin female was allowed to mate. Citral solution was added to a filter paper at the bottom of the jar. After mating the ejaculate was dissected out and weighed.

**Paper V** In this study we investigate the relative mating investment in response to sperm competition risk, by male *P. napi* from the two different generations. To test whether developmental time constraints affect male ejaculate expenditure, we staged mating experiments in the two generations, with equal sex ratio and in which the number of partaking males varied from 1 to 25. The ejaculates were then dissected out and weighed as in paper IV.

**Conclusions**

**Paper I** Results showed that (1) females did not compensate for bad environmental conditions (such as larval host plant or nectar availability) by mating more often to procure nutrients; and (2) the level of polyandry was higher in the directly developing generation than in the diapausing generation. The results also indicate that there is a correlation between the time to first mating and subsequent lifetime number of matings. This could be an indication that the degree of polyandry is contingent on mating propensity and that whether an individual is monandrous or polyandrous might not be a true dichotomy but rather depend on a continuum of mating propensities.

**Paper II** From our results we conclude that neither males nor females seem to be physiologically mature when they eclosed from direct development, probably due to larval time constraints. Directly developing males take longer to synthesise the sex pheromone and longer to mate for the first time.

When comparing females mated before 12h of age, the direct developing generation has significantly lower fecundity than diapausing females. There was however no difference in fecundity between the generations when comparing all age classes. This is explained by the older females of the direct generation laying significantly more eggs than the young during the first day of egg laying. So, the females of the direct developing generation do not have lower fecundity *per se* but cannot realise their full fecundity if they are mated when they are less than 12 hours old.

Interestingly though, directly developing females mate sooner after eclosion than diapausing females, even though this reduces their fecundity. The results may seem contradictory, as these females were shown to be less mature than the diapausing females and loose in fecundity by mating early. However, laying fewer eggs but laying them early will pay off if the season is short and adult time stress substantial, and we contend that this can explain the result. The results are also in agreement with results by Friberg & Wiklund (2007) and consistent with the theory that females should reduce choosiness when time stressed (Johnstone 1997). Females can get mating out of the way as quickly as
possible, even if they are not fully reproductively mature. For males, on the other hand, mating is tightly linked to physiological maturation. Females seem to only accept mating if the male emits the species-specific sex pheromone citral (Andersson et al. 2007) and the males are therefore constrained by their physiological development.

**Paper III** The two generations are shown to have significantly different scent composition early in life. The directly developing males, who have shorter time for pupal development, need the first 24 hours after eclosion to synthesise the sex pheromone whereas the diapausing generation males eclose with adult scent composition. We could also show that the sex pheromone components can be synthesised from both larval and adult derived nutrients. In summary, paper III shows that time-stress changes the timing of biosynthesis of the sex pheromone components between generations and underpins the importance of understanding resource allocation and physiology and their effect on life histories.

**Paper IV** The results show that male *P. napi* tailored their reproductive investment in response to the risk of sperm competition; ejaculates transferred by males in the high male density treatments were on average 23% larger than ejaculates transferred at low male densities. The results also show that citral is the cue used by males to determine how much they will invest in the ejaculate; ejaculates transferred by males in presence of added citral were 19% larger than ejaculates transferred in absence of added citral.

This shows that male *P. napi* have not only evolved increasingly larger spermatophores in response to sexual selection, but also an ability to accurately determine the level of intra-sexual competition and adjust reproductive expenditure accordingly. The results also show that the sex pheromone has assumed dual functions – (1) facilitating female acceptance when dispensed by courting males and (2) allowing males to assess the degree of male competition for matings.

**Paper V** The results show that male *P. napi*, as in paper IV and in accordance with sperm competition theory, tailor their reproductive investment to the risk of sperm competition. Ejaculates transferred by males under high sperm competition risk were larger than those transferred by males without competition. Moreover, there was a significant difference between generations in response to male-male competition; in the absence of competition males invest equally, but under high sperm competition risk directly developing males increase spermatophore size by 42% and diapausing males by 67%. In conclusion, males from the two developmental pathways differ in mating investment in response to sperm competition risk and so may influence life history evolution and potentially also geographic differences in mating patterns.
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