Nuptial feeding in the scorpionfly *Panorpa vulgaris*: ultimate and proximate causes

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General Introduction

Natural and sexual selection

When Darwin's "Origin of species" was published in 1859 it gave naturalists a first explanation of how evolution works. While the concept of natural selection could convincingly explain the evolution of traits contributing to an individual's viability, Darwin recognised numerous characters that could not have evolved by means of natural selection, since they seemed to reduce viability (Darwin 1859). These were mostly conspicuous male ornaments which Darwin recognised to be subject to female choice in the context of reproduction. A central problem for Darwin was to explain the origin of sexually dimorphic traits that probably constrict survival and viability. His solution was the process of sexual selection. In his book "The Descent of Man and Selection in Relation to Sex" Darwin (1871, pp 256-257) defines sexual selection by contrasting it with natural selection: "We are, however, here concerned only with that kind of selection, which I have called sexual selection. This depends on the advantage which certain individuals have over other individuals of the same sex and species, in exclusive relation to reproduction.". For long time the evolutionary pathway to extraordinary male characters remained unsolved, until it received growing interest by numerous researchers during the last decades. Nowadays, the most established and accepted theories to explain sexually selected characters are the Fisherian self-reinforcing theory (Fisher 1930, Maynard Smith 1991) and the indicator mechanism theory (Zahavi 1975, Andersson 1982, 1986, Maynard Smith 1991), although the selective mechanisms behind it are still not fully understood (Andersson 1994). Meanwhile the concept of sexual selection is undoubtedly accepted to explain the evolution of sexual ornaments and as a result heavily affecting mating systems of sexual reproducing organisms. Nevertheless, it may be underestimated concerning its importance in the context of ecological diversification and radiation of taxa where the ancestral species features internal fertilisation (Andersson 1994, Sauer 1996).

Although generally accepted differences of opinion exist on the relation of natural and sexual selection (e.g. see Endler 1986). While some authors consider sexual selection being a subset of natural selection (e.g. Andersson 1994, Endler 1986), others regard natural and sexual selection as independent processes (e.g. Sauer 2002). Natural selection is non-random differential survival and/or winning of resources. In contrast, sexual selection can be defined as non-random differential access to mating partners. Therefore, it seems that these are two

dynamic processes which are independent and distinct from each other concerning their outcomes as well as the processes themselves (see Endler 1986, Sauer 2001). Moreover, they become effective during different phases of an individual's life history. Hence, it seems useless and even misleading to describe sexual selection as a subset of natural selection. In fact, the term selection should be used for the general process that comprises a natural and a sexual component (for discussion see Endler 1986, Sauer 2001).

Nuptial feeding – love for sale in the animal world

Nuptial feeding is a very common mating strategy and widespread among different animal species. It encompasses any transfer of consumable substances from the male to the female during or after courtship and/or mating. Nuptial gifts can be food items captured by the male, glandular products, substances in the ejaculate, and even parts of the male's body (Vahed 1998). But for what reasons do males "pay" for sex and why do females choose these males? Anyway, nuptial feeding obviously is a sexually selected trait. But in fact, a distinction has to be made between the evolutionary pathway that led to nuptial feeding and the reasons for its maintenance. Generally, nuptial gifts can represent mating effort (Alexander & Borgia 1979, Trivers 1972) or parental/paternal investment (Fisher 1930, Trivers 1972). While the meaning of mating effort is quite clear and easy to understand, there has been comprehensive debate about what can be termed paternal investment in literature (e.g. Alexander & Borgia 1979, Fisher 1930, Low 1978, Simmons & Parker 1989, Trivers 1972, Wickler 1985). The question of the ultimate function of nuptial feeding has been studied intensively in many different mating systems (mostly insects) resulting in a big number of publications (e.g. Gwynne 1984, 1986, 1988, Hayashi 1998, Sakaluk 2000, Sauer et al. 1998, Simmons 1988, Vahed & Gilbert 1997, Wedell & Arak 1989, see also Vahed 1998 for review). Noting the great differences of nuptial feeding between species in combination with differences in important life history characteristics (e.g. sperm precedence patterns, female remating behaviour), it becomes self-evident that no generalisations can be made about the function of nuptial gifts. It rather has to be carefully investigated for each mating system separately. Benefits for males gained via nuptial feeding are obvious. Males get access to females (= mating effort) and/or contribute to the number and/or fitness of their progeny (= paternal investment). Choosy females will gain direct benefits if the gift contains nutrients, but in some cases may also derive indirect benefits. In these mating systems gift size correlates with the ecological adaptedness of the male and therefore, is an indicator for male genetic quality (Andersson 1982, 1986, 1994,

Maynard Smith 1991, Zahavi 1975). In these cases nuptial gift production can be regarded as a sexual ornament enabling females to discriminate against "low-quality" males and through this increasing their own fitness by choosing well-adapted males (= good genes).

Studying proximate factors affecting secondary sex traits in insects

There are many studies investigating the effects of various environmental factors on traits related to male mating success in insects. Especially temperature and dietary composition as well as the amount of food available are known to influence an individual's development and viability (Wigglesworth 1972). However, studies on how environmental conditions affect the development and/or expression of sexually selected traits tend to focus on adult organisms rather than including the pre-mature phase of life history. Since before maturation the natural component of selection is effective and sexual selection becomes effective only during the reproductive phase, it may seem comprehensible to concentrate on environmental effects during adulthood when investigating sexually selected traits. Nevertheless, the outcome of an individual's performance in a natural selection context may still be of importance when becoming sexually mature and affect mating performance and/or the development of secondary sex traits. Such effects of environmental conditions during larval development on characters directly linked to mating success have been found for different insect species (e.g. Delisle & Bouchard 1995, Emlen 1997, Hunt & Simmons 1997, Moczek & Emlen 1999, Telang & Wells 2004). Therefore, larval development should be taken into account when investigating environmental effects on secondary sex traits and mating performance in insects.

The mating system of *Panorpa vulgaris*

Panorpa vulgaris (Mecoptera: Panorpidae) is one of five central European scorpionfly species which differ substantially in their mating behaviour. *P. vulgaris* represents a highly promiscuous mating system resulting in a high level of sperm competition (Sauer et al. 1990, 1997, 1998, 1999, Sindern 1996). The sperm of different males are mixed within the female's receptacle and are utilised following the fair raffle principle (Parker et al. 1990, Sauer et al. 1990, 1997, 1999). Sperm transfer rate is continuous during copulations so that long copulations lead to a high amount of transferred sperm (Sauer et al. 1997). Despite all differences between the central European *Panorpa*-species their mating systems are all characterised by resource dependent male mating tactics. Males of *P. vulgaris* can obtain

copulations by three alternative tactics. They can (i) copulate without any courtship gift, (ii) monopolise a dead arthropod and feed it to females or (iii) produce saliva secretions during copulation. The latter strategy leads to the longest copulation durations in *P. vulgaris* (Bockwinkel & Sauer 1994, Sauer et al. 1998, Sindern 1996). Saliva secretions seem to be an indicator for male genetic quality and females adjust copulation duration to the number of salivary masses they receive (Sauer 1996, Sauer et al. 1998, Sindern 1996). Since copulation duration determines the number of fertilised eggs for a given male, salivary mass production can be viewed as a sexually selected trait that is the main proximate determinant for male fitness.

Outline of the thesis

In my thesis I deal with certain aspects of male nuptial feeding in the scorpionfly *P. vulgaris*. The studies presented here aim at verifying the function of nuptial gifts in this mating system as well as investigating effects of the environment during life history on several traits linked to male mating success. Therefore, I am able to present data on questions that combine the proximate and ultimate level of evolution.

Chapter I deals with the question whether nuptial gifts in the mating system of *P. vulgaris* represent paternal investment. While their function as mating effort has been demonstrated several times and is unquestionable (Fleck 1997, Sauer et al. 1990, 1998, Sindern 1996), this is the first approach to measure an effect of salivary mass consumption on female reproductive output. If salivary mass consumption induces females to lay more eggs and therefore increases the number of a given male's progeny, saliva secretion can be regarded as a form of paternal investment (Simmons & Parker 1989).

Salivary mass production is food dependent and therefore not unlimited. Chapter II shows that male *P. vulgaris* must replenish their saliva storage in between matings or the number of gifts they are able to produce during the next mating will decrease significantly. Moreover, I present data which clearly show that the marginal costs arising from salivary mass production differ for males of different quality.

Based on the results of chapter II I investigated the effect of different food availability during life history on the development of the salivary glands and on nuptial gift production. Although

the production and storage of saliva starts only after adults have hatched, animals were exposed to different food availability during larval development as well as during adulthood. Therefore, I was able to distinguish between effects of nutrition during these stages of development. The results are shown and discussed in chapter III.

The separate chapters of this thesis should be comprehensible as they are, without need for reference to other sections. Consequently, recurrent descriptions and explanations are occasionally inevitable.

Chapter I: Love for sale and its fitness benefits: Nuptial gifts in the scorpionfly *Panorpa vulgaris* represent paternal investment

Abstract

Nuptial feeding is a very common strategy shown by males of various insect taxa in order to obtain copulations. In the majority of cases these gifts presented during or after courtship and/or copulation can be considered as mating effort. In this study I present data which indicate that nuptial feeding in *Panorpa vulgaris* (Mecoptera: Panorpidae) represents paternal investment. During copulations males produce salivary secretions which are then consumed by the females. The more salivary masses a male produces the longer copulation will last. Moreover, receiving a high number of salivary masses causes females to lay significantly more eggs compared to females receiving few or no salivary secretions. Thus, in *P. vulgaris* the nuptial gift increases the reproductive output of females and hence must not only be considered as mating effort but also as paternal investment. The mechanism by which salivary masses increase female fecundity is yet unknown. I hypothesise that the secretions may not only transfer nutrients and possibly operate as carriers for an allohormone that manipulates the females' physiology in terms of increasing egg production.

Introduction

Courtship feeding is a well known strategy shown by males of various insect taxa and has been the subject of many scientific studies (e.g. Gwynne 1984, 1986, 1988, Hayashi 1998, Sakaluk 2000, Sauer et al. 1998, Simmons 1988, Vahed & Gilbert 1997, Wedell and Arak 1989). Nuptial gifts can be food items captured by the male, glandular products, substances in the ejaculate, and even parts or the whole of the male's body (Vahed 1998). When studying mating systems in which courtship feeding occurs and considering the obvious costs of such behaviour to males naturally raises the issue of its ultimate function. Trivers (1972) was the first to introduce the concept of a subdivision of reproductive effort into mating effort and parental investment (see also Alexander & Borgia 1979). Mating effort is defined as that proportion of reproductive effort expended to find a mate or to overcome members of the own sex in order to mate with an individual of the opposite sex. Such behaviour and related male traits was already recognised by Darwin (1859, 1871) and inspired him to formulate his theory of sexual selection. The term parental investment was first used by Fisher (1930) and is, according to Trivers (1972), "any investment by the parent in an individual offspring that increases the offspring's chance of surviving (…) at the cost of the parent's ability to invest in other offspring.". In contrast to mating effort the term parental investment has been revised and specified many times by different authors. Low (1978), for example, used the phrase parental effort instead of parental investment, meaning that proportion of reproductive effort devoted to the production of progeny as a whole. Whenever I use the term parental / paternal investment, I follow the definition of Simmons and Parker (1989): Paternal investment is any increase in a male's total surviving progeny by increasing the reproductive output of a female. This accords with Low's (1978) parental effort.

Whereas many forms of nuptial gifts surely represent mating effort in the sense of Trivers (1972) and Low (1978, see Vahed 1998), there is a number of studies that show a positive effect on the female's reproductive output (e.g. Gwynne 1984, 1988, Simmons 1988, reviewed in Vahed 1998), and thus indicate that these gifts can be considered as paternal investment (Simmons & Parker 1989) or parental effort (Low 1978). However, since most of the studies did not control for substances in the ejaculate that possibly can affect the rate of oviposition following mating (see Vahed 1998 for examples), the true function of the courtship gifts in these mating systems still remains uncertain.

The scorpionfly *Panorpa vulgaris* (Imhoff & Labram 1836) represents a highly promiscuous mating system resulting in a high level of sperm competition (Sauer et al. 1990, 1997, 1998,

1999, Sindern 1996). The sperm of different males are mixed within the female's receptacle and are utilised randomly in proportion of their numerical representation in the spermatheca (Sauer et al. 1990, 1997, 1999). Sperm transfer rate is continuous during copulations so that long copulations lead to a high amount of transferred sperm (Sauer et al. 1997). Thus, copulation duration is the main proximate determinant for male fitness and males are expected to try to gain as many and as long copulations as possible to maximise their fitness (Sauer et al. 1990, 1997, 1998, 1999).

Males of *P. vulgaris* can obtain copulations by three alternative tactics. They can (i) copulate without any courtship gift, (ii) present a dead arthropod and feed it to females or (iii) produce saliva secretions during copulation. The latter leads to the longest copulation durations in *P. vulgaris* (Bockwinkel & Sauer 1994, Sauer et al. 1998, Sindern 1996). Saliva secretions are an indicator for male genetic quality and females adjust copulation duration to the number of salivary masses they receive (Sauer 1996, Sauer et al. 1998, Sindern 1996). Thus, high numbers of courtship gifts lead to long copulation durations. Since they increase copulation duration and thereby the proportion of fertilisations for a given male, their function can surely be considered as mating effort (Fleck 1997, Sauer et al. 1990, 1998, Sindern 1996). In this study I investigate whether the relevance of the salivary masses in *P. vulgaris* goes beyond this and possibly affects the reproductive output of females and thus satisfies Simmons & Parker's (1989) criteria for paternal investment.

Methods

Breeding and keeping

Animals used for this study were all offspring of the first annual generation of *P. vulgaris*, collected at a site near Freiburg, Germany in early 2002. Collected adults were held pairwise in plastic boxes (10 cm x 10 cm x 7 cm) containing moist tissue paper, ad libitum food and a small Petri dish filled with moist peaty soil for oviposition. Larvae were reared on an 18 h : 6 h light : dark cycle at 18° C on moist tissue paper with ad libitum food at a maximum density of 20 larvae per Petri dish (12 cm diameter). Third instar larvae were transferred to soil-filled, open-bottomed plastic cylinders (40 cm diameter) placed in a field ground, where they finished their development. The breeding of scorpionflies is described in detail by Sauer (1970, 1977).

After emergence 167 females were marked individually and were kept under semi-natural conditions in two perspex enclosures (150 cm x 70 cm x 70 cm) in an outside area in Bonn,

Germany. These cages contained blackberry twigs (*Rubus* spec.) and moist tissue paper as water supply. Throughout the study females had to compete for food. They were provided with ¼ segment of *Tenebrio molitor* larvae (one segment = 5.6 ± 1.3 mg) per day and animal. Males were held singly in plastic tubes (8 cm x 3.5 cm) and obtained ? segment of *T. molitor* larvae per day. The females' body weight was measured daily with a balance of Sartorius.

Experimental treatment

After ten days all females having a body weight between 48.0 and 59.9 mg were selected to be used for the experiment. Four out of 93 individuals died within the first days so that 89 individuals were included in the statistical analyses. Males and females were selected randomly for copulations and put in small plastic boxes (10 cm x 10 cm x 7 cm) containing moist tissue paper but no food. During copulations I measured copulation duration as well as the number of salivary masses consumed by the female. To measure the impact of nuptial gifts on female fecundity I manipulated the number of received salivary masses among females by separating three different groups that were allowed to mate once (N = 35), twice (N = 33) and three times (N = 21), respectively. Thus, the created variance in the number of received salivary masses ranged from zero up to a maximum of 31. After the first copulation females were still kept in population cages competing for food over day but were isolated in small plastic containers (10 cm x 10 cm x 7 cm) overnight. These boxes contained moist tissue paper and a small Petri dish filled with moist peaty soil to permit oviposition. Subsequent to copulations I measured the number of eggs laid for each female over a period of 20 days.

Statistics

For statistical analyses I separated four different time periods: 0-5 days, 0-10 days, 0-15 days and 0-20 days after last copula. Mean weights at oviposition were derived from weights on days when clutches were produced. Mean values are given as mean ± standard error (SE). All statistical tests were performed using SPSS 11.0. All tests were two-tailed and the level of significance was determined $\alpha = 0.05$ for all cases.

Results

The aim of this study was to find out whether there is more to the function of nuptial feeding in *P. vulgaris* than recently known. While its meaning as mating effort has been documented

several times, this time I was interested in its effects on female fecundity. Therefore, I first of all had to create a variance in the number of consumed saliva secretions among females. This was done by separating three female groups characterised by different numbers of matings. The achieved distribution of consumed salivary masses among females ranged from zero up to 31. Fig. 1 shows the overall number of eggs laid separated for different time periods. 42.6 % of the total number of eggs were laid within the first five days after copulation. Tab. 1 gives more detailed information on egg production during this period considering only females that consumed few (0-5) and many (15-31) saliva secretions. Although a comparison between the number of individuals that laid eggs and those that did not lay eggs delivers no significant differences between the two female groups (G-test: df = 1, G = 2.147, p = 0.143), it shows, however, that saliva consumption promotes early oviposition and increased egg numbers.

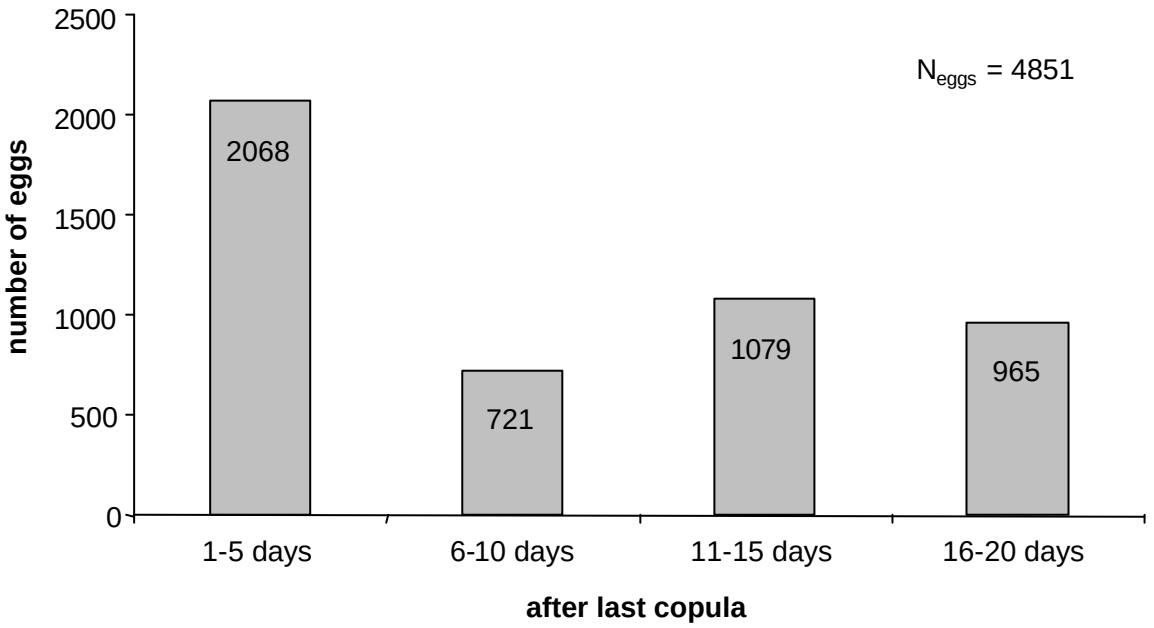

Figure 1: Overall number of eggs laid separated for different time periods.

Table 1: Egg production during the first five days after copulation considering only females that consumed few (0-5) and many (15-31) saliva secretions.

	absolute no. of eggs laid	no. of animals laying eggs	no. of animals not laying eggs	% of animals laying eggs	% of animals not laying eggs
0-5 salivary masses	595	25	11	69.44	30.56
15-31 salivary masses	976	27	5	84.38	15.63

As Tab. 2 indicates I found positive correlations between female fecundity and the following variables: number of consumed salivary masses, copulation duration and mean female body weight at oviposition. These relations are statistically significant for any of the tested time periods with exception of the 10 and 20 day period for copulation duration.

Table 2: Spearman rank correlation for different time periods after last copulation measuring the relation between the number of consumed salivary masses, copulation duration and mean weight at oviposition and the number of eggs laid.

	eggs after 5 days	eggs after 10 days	eggs after 15 days	eggs after 20 days
salivary masses	$r_s = 0.303$	$r_s = 0.255$	$r_s = 0.284$	$r_s = 0.224$
	$p = 0.004$	$p = 0.016$	$p = 0.007$	$p = 0.035$
copulation duration	$r_s = 0.264$	$r_s = 0.199$	$r_s = 0.218$	$r_s = 0.134$
	$p = 0.012$	$p = 0.061$	$p = 0.04$	$p = 0.21$
weight at oviposition	$r_s = 0.58$	$r_s = 0.547$	$r_s = 0.654$	$r_s = 0.734$
	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

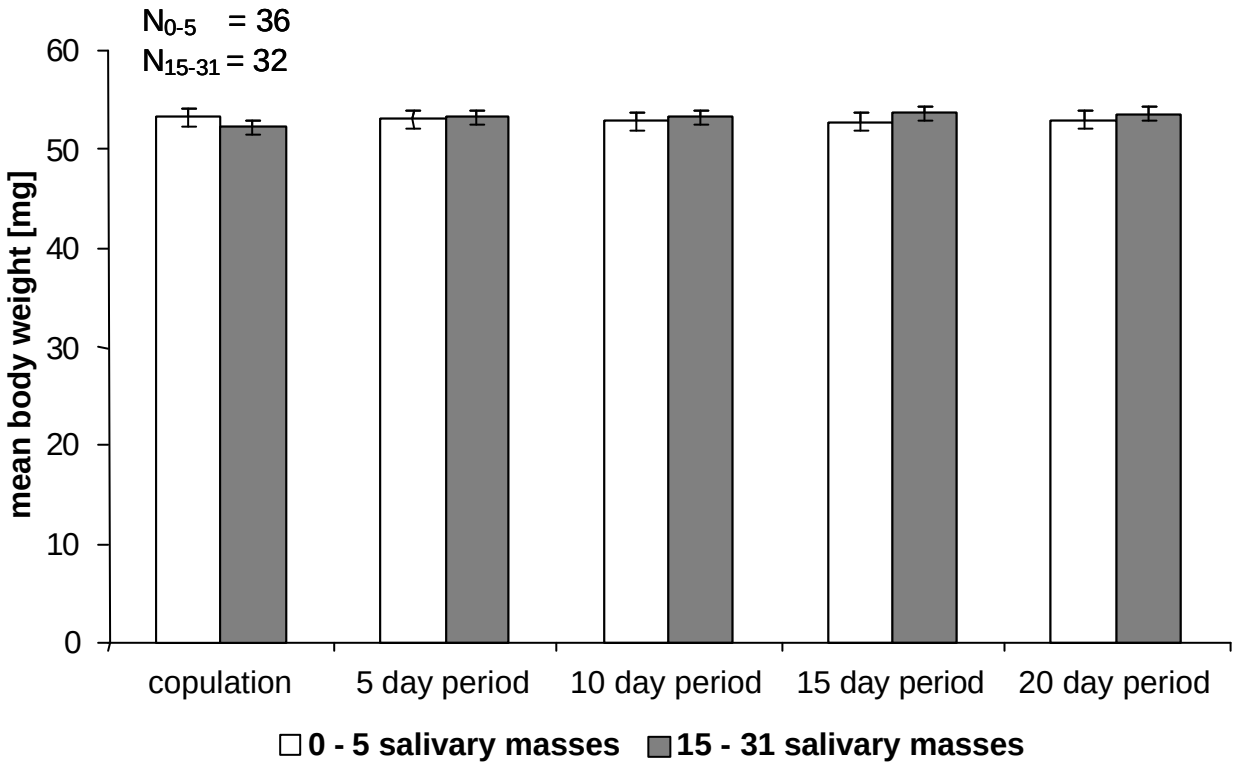

Figure 2: Comparison of mean body weight between females that received few (0-5) and many (15-31) salivary masses.

A comparison between females that received few (0-5) and many (15-31) salivary secretions shows that there were no differences in body weight (Fig. 2; Mann-Whitney U-Test: $p_{all\ cases} > 0.05$). This was true for the point of time when copulations took place and when comparing

mean body weight at oviposition for the different time periods. In contrast to this, females that received many nuptial gifts laid significantly more eggs than those that received few (Fig. 3; Mann-Whitney U-Test: 5 days: U = 362.5, p = 0.008, 10 days: U = 409.5, p = 0.04, 15 days: U = 395.5, p = 0.026, 20 days: U = 441, p = 0.097).

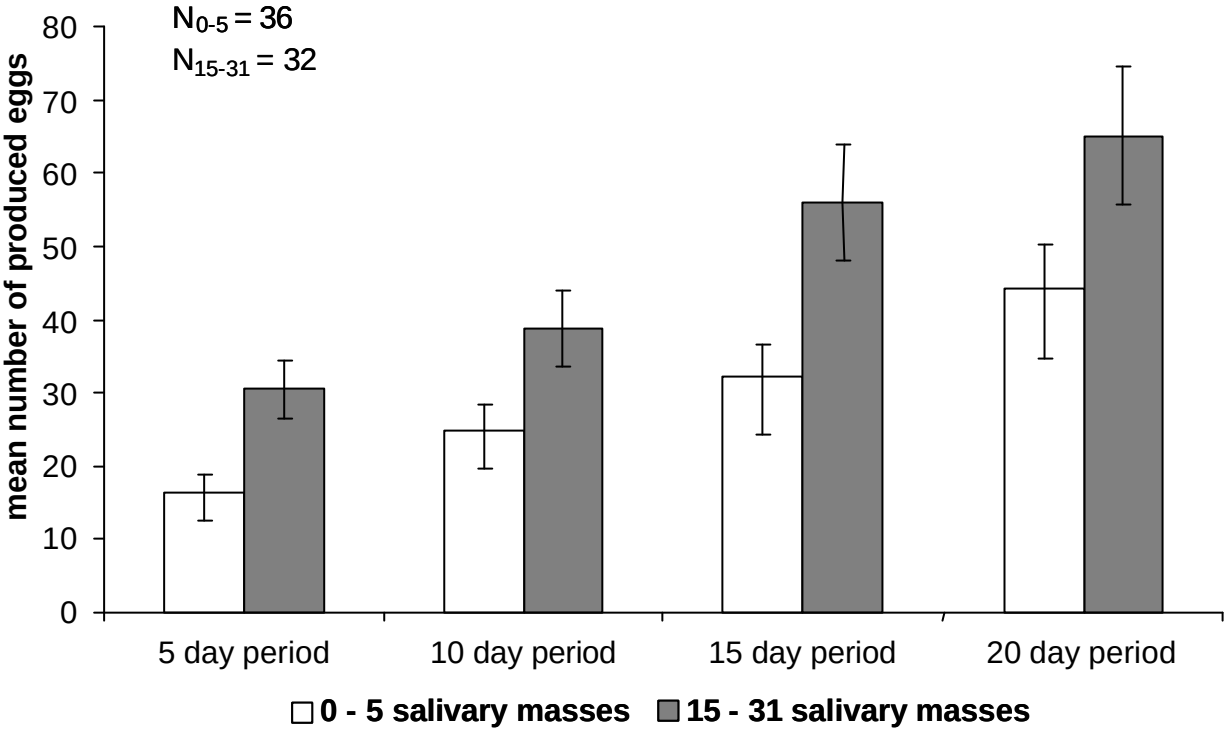

Figure 3: Comparison of the mean number of eggs laid between females that received few (0-5) and many (15-31) salivary masses for different time periods after last copulation.

Fig. 4 shows the relation between mean body weight at oviposition and female fecundity for females that received few and many salivary masses exemplarily for the 15 day period. The slopes of the trend lines for both groups differ significantly from each other (few salivary masses: b = 3.29 ± 0.665, many salivary masses: b = 6.448 ± 1.591, $F_{1,64}$ = 3.994, p = 0.05) showing that the fecundity increasing effect of the nuptial gifts increases with female body weight. This difference, however, is not found for the 5 day period (few salivary masses: b = 1.518 ± 0.38, many salivary masses: b = 2.755 ± 0.815, $F_{1,64}$ = 2.193, p = 0.144) and remains slightly above the level of significance for the 10 day period (few salivary masses: b = 2.212 ± 0.559, many salivary masses: b = 4.416 ± 1.103, $F_{1,64}$ = 3.586, p = 0.063) indicating that the interaction between saliva consumption and body weight arises and increases with age.

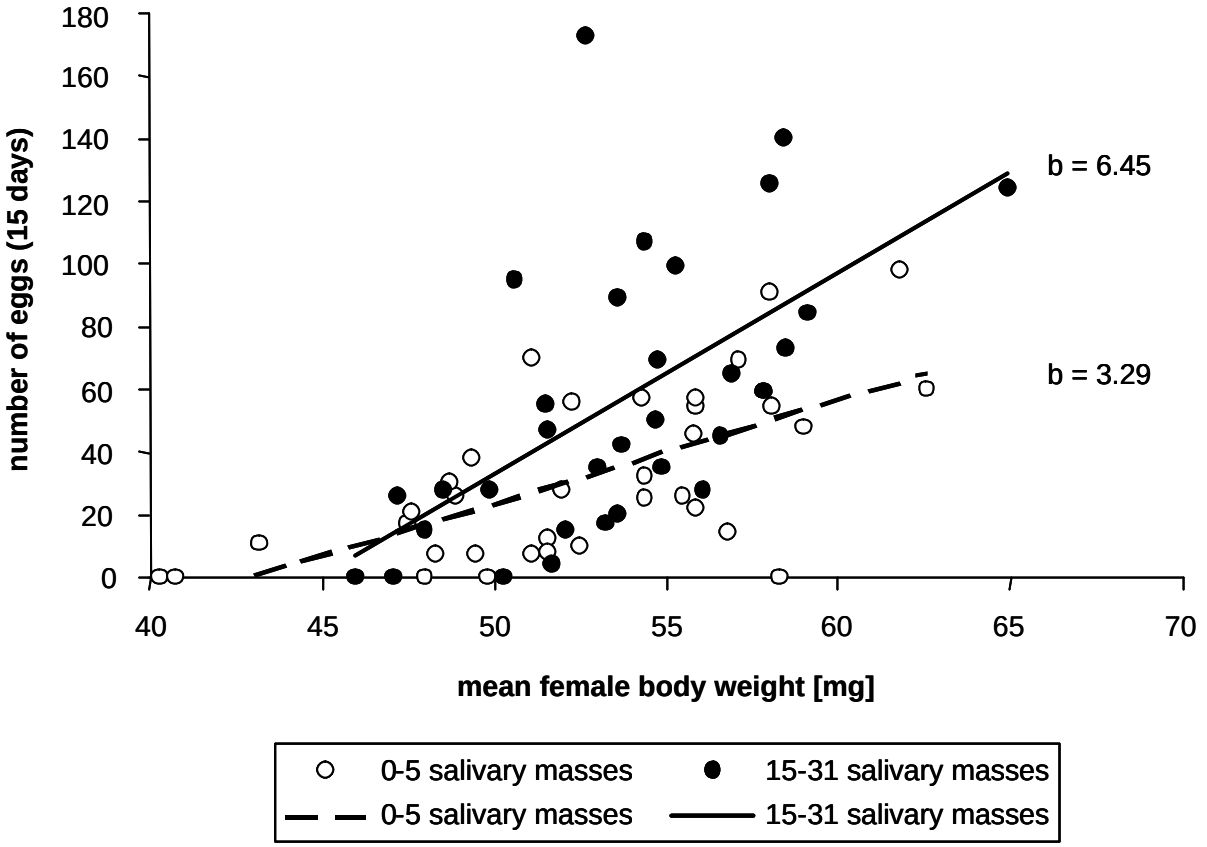

Figure 4: Relation between mean body weight at oviposition and number of eggs laid for females that received few (0-5) and many (15-31) salivary masses.

There is no correlation between nuptial gift consumption and hatching success (Spearman rank correlation: N = 81, r_s = -0.018, p = 0.876). Furthermore, a comparison of hatching rates achieved by females that consumed many (mean = 51.91 ± 3.91 %) or few (mean = 51.82 ± 3.86 %) nuptial gifts shows no significant differences (t-Test: t_{59} = -0.016, p = 0.987).

To discover if salivary masses serve females as nourishment, I tested whether there is a correlation between the number of consumed salivary masses and the change in weight before and after copulation. This analysis was done for first copulations only. The results reveal a significant relation between the variables (Spearman rank correlation: N = 89, r_s = 0.26, p = 0.014, mean ? -weight = 0.89 ± 0.29 mg).

Discussion

In this study I was able to show that the nuptial gifts of male *P. vulgaris* have a significant effect on female fecundity. There is a positive correlation between the number of salivary masses a female consumed and the number of eggs it produced. The number of eggs is also influenced by body weight at oviposition. This is not a surprise and is actually expected, since

food availability, mirrored by body weight, is known to affect female fecundity in insects (Chapman 1971, Wheeler 1996). There is no correlation between the number of consumed nuptial gifts and hatching success.

Additionally to correlative analyses I compared groups of females that received few and many salivary secretions. The results show that those females that consumed many nuptial gifts laid significantly more eggs than those that received few. I found this effect even though there were no differences in body weight between the groups and thus, all females were in the same condition concerning the degree of fecundity. From this follows that the higher number of salivary masses induced females to increase their reproductive output. This confirms my results from the first part of the study. Hatching success did not differ between the groups.

The nuptial gifts of *P. vulgaris* do not only increase female fecundity, but do so dependent on female body weight. While an effect of the salivary masses is hardly recognisable when females are in poor condition, it accelerates as body weight increases. This effect is absent directly after mating, where saliva secretions affect oviposition independently of body weight, but increases as time passes on. It follows that although males should always produce many salivary masses in accordance to their condition and possible future matings, the pay off increases with female body weight. I suppose a physiological response of females to saliva consumption to be responsible for this phenomenon. I believe that by consuming nuptial gifts females are manipulated to invest a greater proportion of their resources into egg production than they normally would. The absolute amount of additional resources that these gifts can make available naturally increases with female condition (the mechanism by that salivary masses increase egg production is further discussed below). Since female body weight itself affects fecundity and the effect of nuptial gifts on fecundity is stronger for heavy females rather than for smaller and lighter ones, males should select for mates that are in good condition. This kind of male choice has already been demonstrated for *P. vulgaris* by Sauer (1996) and Sindern et al. (1994, 1995, 1996).

Still there could be doubt about the actual cause of increased female fecundity, because the number of produced salivary masses during copulation is highly correlated with copulation duration (Sauer et al. 1998). As sperm transfer rate in *P. vulgaris* is continuous, the amount of transferred sperm to the female's receptacle increases with copulation duration (Sauer et al. 1997). Thus, a male that produces a high number of nuptial gifts, also transfers a large amount of ejaculate. Based on this fact the fecundity increasing substances could as well be located in the ejaculate. In the present study copulation duration and the umber of produced eggs are positively correlated indicating an effect of mating duration or ejaculate transfer on egg

production. But when having a closer look at the coefficients of correlation one will recognise that the coefficients for correlations between the number of consumed saliva secretions and produced eggs are higher for any of the time periods measured than in correlations between copulation duration and egg number (Tab. 2). From this I can conclude that duration of copulation is not meaningless, but that also an effect of nuptial gift consumption is definitely present and at least of equal importance for female fecundity. Moreover, even when assuming fecundity increasing substances in the ejaculate, saliva secretions would still be the proximate factor determining how much of these substances can be transferred to a female.

The results clearly show that the number of salivary masses a female consumes during copulations, has a considerable influence on egg production. The more secretions are consumed the higher the increase in a female's reproductive output gets. Therefore, I must consider the nuptial gifts in *P. vulgaris* not only as mating effort, but also as paternal investment in the sense of Simmons & Parker (1989). Since females mate with numerous males and the level of sperm competition is very high, it seems questionable whether a male benefits from increasing a female's fecundity, because the eggs could possibly be fertilised by other males. This is what Wickler (1985) refers to as pseudo-parental investment. This argument is easily disabled for *P. vulgaris* when taking into account the mechanism of sperm competition. The sperm of different males are mixed within the female's receptacle and are utilised randomly in proportion to their numerical representation in the spermatheca (Sauer et al. 1998, 1999). From this follows that the principle proximate determinant of paternity is duration of copulation which is prolonged by nuptial gifts (Sauer et al. 1998, 1999). Thus, males that invest many nuptial gifts and thereby enhance both, the female's reproductive output and their own proportion of sperm in the spermatheca, will fertilise an accordingly greater proportion of eggs than do males that invest less salivary masses. Therefore, producing many nuptial gifts can be considered as a rewarding strategy and the gifts themselves as true paternal investment. I therefore conclude that, in *P. vulgaris*, one trait performs two separate functions and consequently its maintenance is favoured twofold by different modes of selection: The production of high numbers of salivary masses, when looking upon as mating effort, is surely maintained in the context of the sexual component of selection (Sauer 1996) and additionally is favoured by the natural component of selection by raising the number of offspring of the male and its mate.

With my experimental design I cannot clearly discriminate whether nuptial gifts really increase egg production (= synthesis) or only the rate of oviposition. The average lifespan of a summer generation female is, dependent on food availability, about 15.6 to 19.7 days (Sindern

1996). Since the duration of this study exceeded 30 days and 55 out of 89 (= 61,8 %) females died before the end of the study, it is assumed that my data on egg deposition fairly represent the females' lifetime reproductive success or at least very most of their reproductive lifespan. If only oviposition rate was affected, the total number of deposited eggs throughout a female's life should not differ between individuals that consumed different numbers of salivary masses. In *P. vulgaris,* as I already emphasised, consuming nuptial gifts leads to an increase in the number of eggs laid. Assuming that females did not hold back fully developed eggs until they died, I can conclude that consumption of salivary masses does in fact benefit egg production and not only oviposition rate. Further research on this matter is currently in progress.

To date I cannot make a decision on the mechanism by which nuptial gifts in *P. vulgaris* increase egg production. Paternal investment by nuptial feeding usually means the transfer of nutrients from a male to a female which are then incorporated into the eggs (see Vahed 1998). This seems also probable for *P. vulgaris* as the change in female body weight during copulation is positively correlated with the number of consumed salivary masses during that copulation, indicating that salivary secretions can serve as nourishment. But since average ?-weight is only 0.89 mg, the transfer of nutrients is possibly not the sole reason for an increased fecundity. Furthermore nuptial gift consumption seems to trigger and accelerate oviposition resulting in the fact that 42.6 % of all eggs that were produced during the time of the experiment were laid within only five days after copulation. Moreover, 84.38 % of females that consumed at least 15 or more saliva secretions started egg laying during the first five days after mating, while only 69.44% of females that received a maximum of five secretions did so. As a consequence, the first group laid an absolute total number of 976 eggs in this time period, while the latter laid only 595. I believe that this is not a result of transferred nutrients alone and that nuptial gifts possibly transfer other substances additionally. Koene & ter Maat (2001) introduced the term allohormone for "substances that are transferred from one individual to another free-living member of the same species and that induce a direct physiological response (…)". However, there are numerous ways on which allohormones can be transferred, but in none of the cases Koene & ter Maat (2001) mention, they are transferred by nuptial feeding. They emphatically exclude nuptial gifts from their definition, because "these give either direct energetic or defensive benefits to the mating partner or the offspring (…)" and therefore do not transfer allohormones. In my view this is not mutually excluding. I propose the nuptial gifts in *P. vulgaris,* additionally to the transfer of nutrients, to operate as carriers for a substance that, based on the definition by Koene & ter Maat (2001), can be referred to as allohormone. This allohormone possibly diffuses into the

haemolymph from the digestive system and then manipulates the female's physiology in terms of increasing egg production. If this was true, we would look upon *P. vulgaris* as an example for a by now undiscovered mechanism by that nuptial feeding unfolds its function as paternal investment.

Chapter II: Resource dependent male mating effort in the scorpionfly *Panorpa vulgaris*: saliva secretion as an honest signal for male quality

Abstract

In mating systems that are characterised by resource dependent male behaviour like nuptial feeding, food limitation obviously plays a major role in male performance. In *Panorpa vulgaris* (Mecoptera: Panorpidae) the ability to produce nuptial gifts, however, implies major fitness consequences, as the number of gifts decides about copulation duration which again determines the number of fertilised eggs for a given male. As expected the results of this study show that males of *P. vulgaris* are limited in their production of salivary secretions. The number of saliva secretions males are able to produce declines in successive matings. Moreover, males of nutritionally high status produce more gifts than those of nutritionally low status. The proximate factor determining male saliva secretion is the development of the salivary gland which in turn depends on the males' capability of finding food. The degree of male mating effort corresponds to the size of the salivary gland, yet while absolute investment increases with gland size, the relative investment decreases. As a consequence mating costs are differential for males of various nutritional status. This result provides further evidence of the nuptial gift's function as a Zahavian quality indicator. Furthermore, only males of low nutritional status seem to allocate their mating effort strategically according to the female's fecundity. I conclude that cryptic male choice may exist in *P. vulgaris*, but only below a certain quality threshold of males.

Introduction

The basic assumption in modern behavioural ecology is that animals should behave in a way maximising their overall fitness. However, mating interests are often significantly different between the sexes (e.g. Andersson 1994, Arnqvist & Rowe 2002, Arnqvist et al. 2000, Chapman et al. 2003, Gavrilets et al. 2001, Parker 1979, Parker & Partridge 1998, Stutt & Siva-Jothy 2001). Generally, in polyandrous species the challenge for males is to transfer more sperm to more females than other males, thereby siring more offspring than their competitors. The way to succeed, however, can be very different depending on the species and, moreover, on intraspecific strategies. In some species intrasexual competition between males over mates may play a major role, e.g. fighting, mate guarding or some other way of monopolising access to several females (e.g. Andersson 1994, Krebs & Davies 1996). A more cryptic struggle for fertilisations that arises from polyandry is sperm competition (Parker 1970), which is very common and widespread among various animal species (see Simmons 2001). But females are far away from playing only a passive role in reproduction. There are numerous examples where females are the ones in control by choosing those males that seem to fit their claims best (e.g. Andersson 1994, Sakaluk & Eggert 1996, Sauer et al. 1998). A very conspicuous form of female choice can be observed when females choose males on the basis of secondary sexual ornaments. In general such ornaments can be viewed as a form of male advertising in order to get access to females. A special case of sexual advertising is nuptial feeding. In mating systems characterised by nuptial feeding males have to offer gifts in order to obtain copulations, which can for instance be prey items, body parts or glandular secretions (see Vahed 1998). These nuptial gifts can be associated with considerable costs for males. If this is the case, males should not tend to waste but rather allocate their resources strategically by adjusting the degree of mating effort to female quality (Engqvist & Sauer 2001, 2002, Sauer 1996, 2002).

In the present study I was concerned with the mating system of the scorpionfly *Panorpa vulgaris* (Mecoptera: Panorpidae). Males and females of this species are polygamous, resulting in a high level of sperm competition (Sauer et al. 1990, 1997, 1998, 1999, Sindern 1996). While males produce salivary secretions on which females feed during copulation, females adjust mating duration to the number of nuptial gifts they receive (Sauer et al. 1998, Sindern 1996). Since sperm transfer rate is continuous during copulation and fertilisation of eggs follows the fair raffle principle (Parker et al. 1990), males have an interest in maximising mating duration (Sauer et al. 1990, 1997, 1999).

Previous studies indicated that the males' capability of producing salivary masses depends on their nutritional status (Bockwinkel & Sauer 1994, Sauer et al. 1997, 1998). The aim of the present study was to examine whether and in which way salivary gland development in male *P. vulgaris* is influenced by the amount of food available during adulthood. Since saliva secretion is essential for males to obtain long copulations, the amount of saliva males are capable of investing in matings is the most prominent factor determining male reproductive success. To get further information on the function of salivary masses as a Zahavian quality indicator (Zahavi 1975) in the scorpionfly *P. vulgaris* (Sauer 1996, Sauer et al. 1998), I investigated if nuptial gift production implies differential marginal costs for males of various qualities. Furthermore, I looked for evidence of cryptic male choice and under which circumstances males may invest their mating resources strategically according to female quality.

Methods

Breeding

Animals used in the experiments were offspring of the second annual generation of 2002 and of the first generation of 2003 of *P. vulgaris*. Adults were collected at a field site near Freiburg, south-west Germany and taken to Bonn for breeding. They were held pairwise in plastic boxes (10 cm x 10 cm x 7 cm) containing moist tissue paper, ad libitum food and a small Petri dish filled with moist peat for oviposition. F_1-larvae of the spring generation of 2003 were reared at an 18 h light : 6 h dark cycle at 18° C on moist tissue paper with ad libitum food at a maximum density of 20 larvae per Petri dish (12 cm diameter). Third instar larvae were transferred into soil-filled, open-bottomed plastic cylinders (40 cm diameter) placed outdoors in the ground, where they finished their diapause-free development. F_1-larvae of the summer generation of 2002 were reared under the same conditions, but at a light : dark cycle of 12 h light and 12 h dark. Moreover, after the 2nd larval moulting they were kept singly in small Petri dishes. After 20 days they were transferred into plastic tubes filled with moist peat and entered diapause in October 2002.

Nutritional status and mating success of males (F_1-offspring of the first generation 2003)

Experimental treatment and recorded parameters

Hatching adults were kept singly in plastic tubes (8 cm x 3.5 cm) containing moist tissue paper. Males were assigned to either a high or a low nutrient treatment. They received food on the day of emergence (= day zero) and five days after emergence (= day 5). Well-nourished males were fed with two segments of *Tenebrio molitor* larvae, whereas poorly-nourished males received only one segment per feeding. Females were provided with two segments every fourth day. On day nine all males were mated to a female. I measured copulation duration and the number of salivary masses a male produced. Some randomly chosen males were mated a second and some of them a third time without receiving food in the meantime. Only virgin females were used for copulations. A total of 84 males could be included in the analyses.

Nutritional status and male investment (F_1-offspring of the second generation 2002)

Experimental treatment and recorded parameters

Hatching animals were kept singly in plastic tubes (8 cm x 3.5 cm) containing moist tissue paper. Males were separated into three groups exposed to different food availability. Males of one group received food on days zero (= hatching date), four, eight and 12 (well-nourished males), a second group of males on days zero, seven and 14 (medium-nourished males), the last group of males on days zero and 10 (poorly-nourished males). Each feeding consisted of one segment of *T. molitor* larvae. Females were provided with two segments on days zero and eight.

A total of 151 males could be included in the analyses of which 76 were used as control animals whose salivary glands were dissected on day 15. Therefore, I was able to determine mean salivary gland size having developed under different controlled food availability. On day 15 the remaining 75 males were mated to females. Recorded parameters were again duration of copulation and the number of salivary masses produced. After copulation the salivary glands of males were dissected.

The body weight of all males was measured on the day of emergence and on day 15 (in case of the mated males prior to copulation) using a balance of Sartorius. Female body weight was measured on day 15 prior to copulation.

Measuring weights of salivary masses

During some of the copulations I removed the second salivary mass that was produced. This was done using small tweezers and was generally possible without disturbing the copulating pair. I collected a total of 51 salivary masses. The secretions were transferred into Eppendorff reaction tubes and were dried at 20° C until weight constancy. Dry weights of secretions (individual weight of salivary mass = IWSM) were measured with a precision balance of Sartorius (2004 MP).

Dissection and weighing of salivary glands

On day 15 males were anaesthetised using CO_2 and subsequently were killed by drowning them in 70% ethanol. After approximately 5 minutes they were transferred into a water filled preparation dish and placed under a binocular magnifier (Leica WILD M3B). Bodies were laterally opened by cutting the integument with a small dissection scissor starting at the end of the abdomen. Afterwards the insects were put back into 70% ethanol where they remained for at least 24 h. This was necessary to harden the secretion inside the salivary gland. The salivary gland is a very soft tissue which is almost impossible to dissect properly without this treatment. After dissection the glands were placed on a piece of aluminium foil inside a small Petri dish (5 cm diameter) and were dried at 30° C until weight constancy. The salivary gland dry weight was measured using a precision balance of Sartorius (2004 MP).

Calculated values

I calculated male investment as the amount of saliva secretion males invested in a copulation: S salivary masses x IWSM (see above). Accordingly, for a sample size of 51 males I estimated salivary gland dry weight before copulation as follows: gland dry weight after copulation + S salivary masses x IWSM.

Statistics

Statistical analyses were performed using SPSS 11.0. All tests were two-tailed and the level of significance was determined $\alpha = 0.05$ for all cases. Mean values are given as mean ± standard error (SE). To avoid non-random samples I removed individuals that failed at

initiating a second or third mating from analyses concerning saliva production and copulation duration in successive matings (Figs. 1 and 2). Therefore, in these cases sample sizes are slightly smaller than in other analyses based on the same data set (N = 75 instead of N = 84).

Results

To measure the effect of food availability on male mating performance I exposed males to different food regimes and recorded different parameters linked to male mating success: beside the number of nuptial gifts produced in successive copulations I determined the weight of the salivary gland dependent on food availability and to what extent saliva resources were invested in a single copulation. Moreover, I tested whether males invested their mating effort strategically depending on female quality.

Nutritional status and mating success of males

By establishing two distinct food regimes I generated a variance among males with respect to their nutritional status. First, I regard all males as a single group (Figs. 1 and 2) before comparing the individuals of both nourishment groups (Fig. 3).

The results clearly show that males are strongly limited in salivary mass production. The number of produced salivary secretions differed significantly when comparing males in first, second and third copulations that had been without any chance to feed in between the matings (Fig. 1; first copulation: 5.9 ± 0.45 salivary masses, N = 34; second copulation: 4.3 ± 0.55 salivary masses, N = 25; third copulation: 1.9 ± 0.56 salivary masses, N = 16; ANOVA: $F_{2,72}$ = 13.074, $p < 0.001$, Post-Hoc LSD: copulation 1 vs. 2: $p = 0.025$, copulation 1 vs. 3: $p < 0.001$, copulation 2 vs. 3: $p = 0.004$). Accordingly, the duration of copulation declines with increasing mating frequency (Fig. 2; first copulation: 239.47 ± 11.64 min, N = 34; second copulation: 172.80 ± 20.13 min, N = 25; third copulation: 102.88 ± 19.23 min, N = 16; ANOVA: $F_{2,72}$ = 15.762, $p < 0.001$, Post-Hoc LSD: copulation 1 vs. 2: $p = 0.003$, copulation 1 vs. 3: $p < 0.001$, copulation 2 vs. 3: $p = 0.01$).

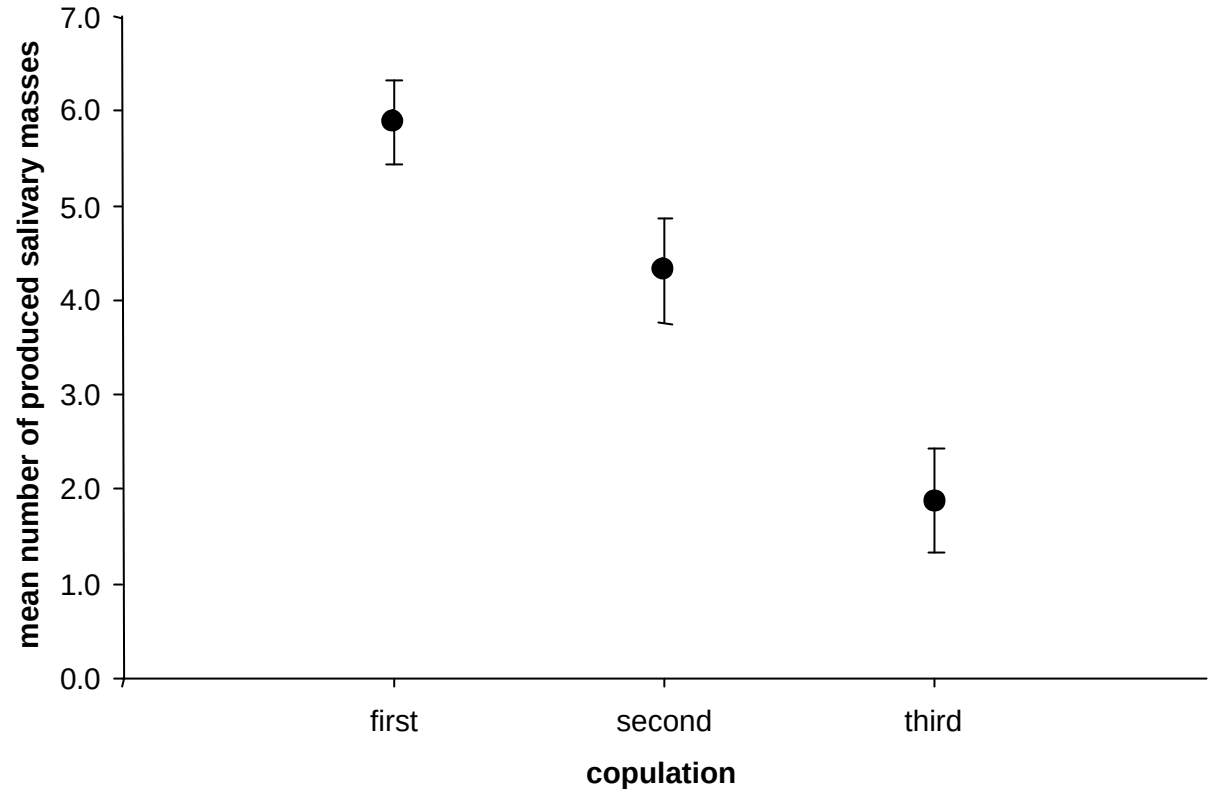

Figure 1: Comparison of the mean number ± SE of salivary masses produced during a male's first, second and third copulation.

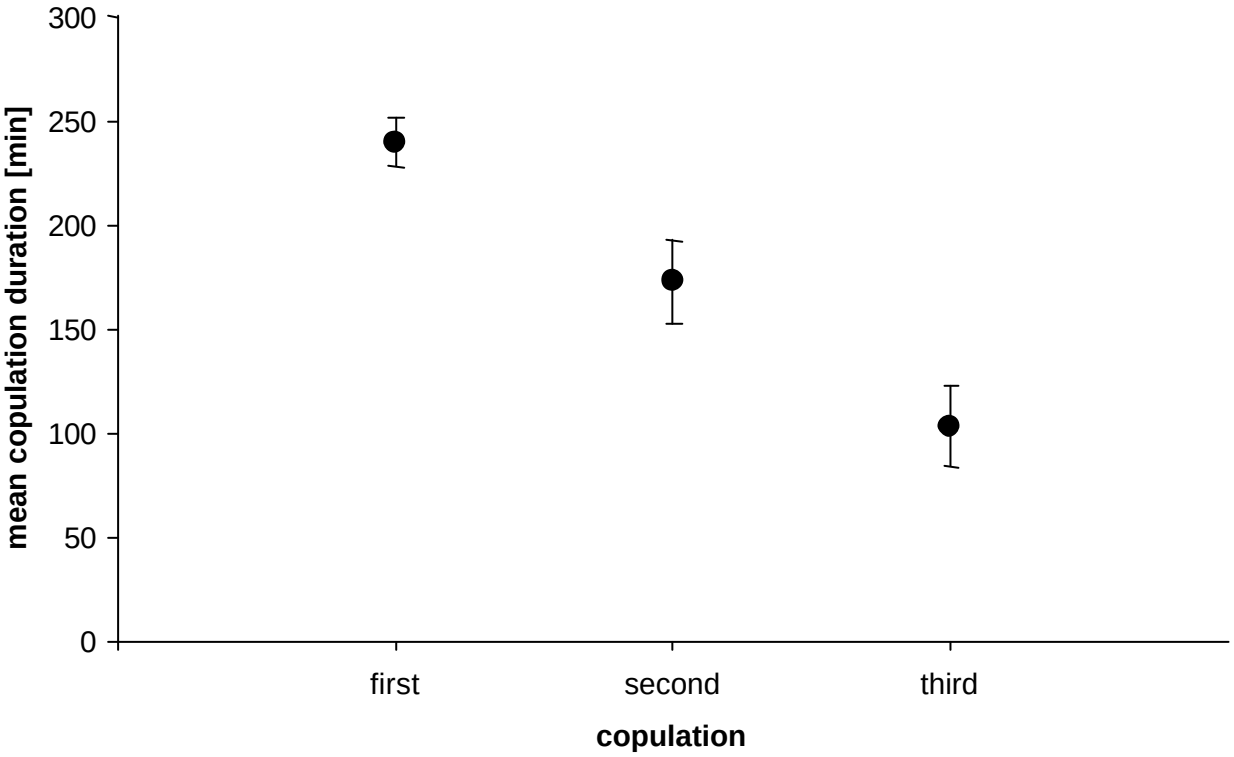

Figure 2: Comparison of the mean copulation duration ± SE of a male's first, second and third copulation.

Male body weight determined immediately before the first copulation correlated with the number of salivary masses a male provided to the female as well as with the duration of copulation in any of the three copulations (Tab. 1). This relation becomes stronger in successive copulations, showing that the importance of body weight increases with mating frequency. Therefore, male body weight could be a measure of the amount of available saliva.

Table 1: Spearman rank correlation between male body weight at copulation and the number of salivary masses produced and copulation duration of a first, second and third copulation

	first copulation		**second copulation**		**third copulation**	
	no. of salivary masses	copulation duration	no. of salivary masses	copulation duration	no. of salivary masses	copulation duration
body weight at copulation	r_S = 0.405 P < 0.001 N = 84	r_S = 0.420 P < 0.001 N = 84	r_S = 0.650 P < 0.001 N = 44	r_S = 0.618 P < 0.001 N = 44	r_S = 0.877 P < 0.001 N = 16	r_S = 0.715 P = 0.002 N = 16

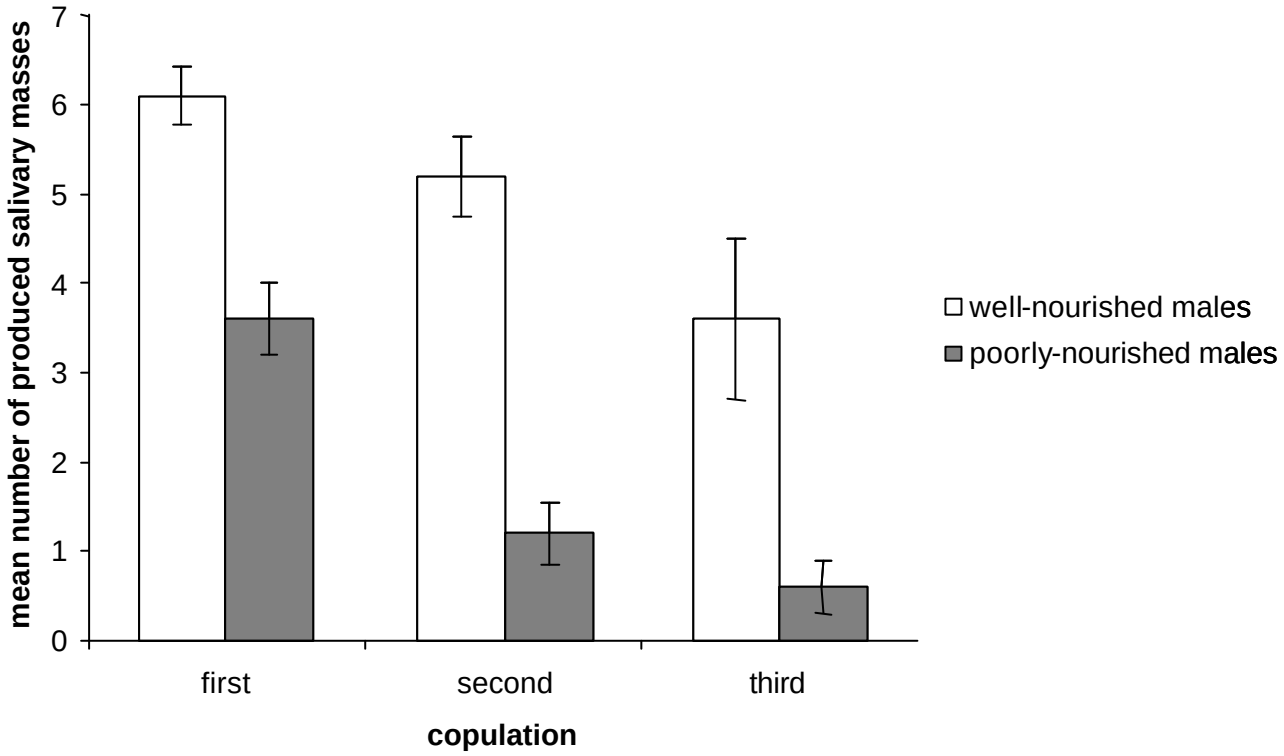

Figure 3: Comparison of the mean number ± SE of produced salivary masses between males of nutritionally high and low status during a first, second and third copulation.

Different food availability resulted in males of significantly different nutritional status (males of nutritionally high status: 46.18 ± 0.84 mg; males of nutritionally low status: 34.36 ± 0.92 mg; ANOVA: $F_{1,82}$ = 88.14, p < 0.001). Accordingly, males of nutritionally high status produced significantly more salivary secretions in any of the copulations (Fig. 3; first copulation: males of nutritionally high status: 6.1 ± 0.33, males of nutritionally low status: 3.6

± 0.40, Mann-Whitney U Test: U = 371.5, N_1 = 49, N_2 = 35, p < 0.001; second copulation: males of nutritionally high status: 5.2 ± 0.45, males of nutritionally low status: 1.2 ± 0.34, U = 47.5, N_1 = 23, N_2 = 21, p < 0.001; third copulation: males of nutritionally high status: 3.6 ± 0.90, males of nutritionally low status: 0.6 ± 0.29, U = 4.5, N_1 = 7, N_2 = 9, p = 0.003) and also achieved longer copulations (ANOVA: first copulation: males of nutritionally high status: 225.43 ± 11.17 min, males of nutritionally low status: 145.63 ± 14.05 min, $F_{1,82}$ = 20.181, p < 0.001; second copulation: males of nutritionally high status: 202.22 ± 18.59 min, males of nutritionally low status: 78.14 ± 14.23 min, $F_{1,42}$ = 27.302, p < 0.001; third copulation: males of nutritionally high status: 164.86 ± 25.99 min, males of nutritionally low status: 54.67 ± 13.19 min, $F_{1,14}$ = 16.365, p = 0.001). Considering the ability of saliva secretion of differently nourished males separately reveals its dependence on food availability more conspicuously. Salivary mass production of males of both nourishment groups declined in successive matings. However, in well nourished males only the number of salivary masses produced during matings decreased with increasing mating frequency, while all individuals, with only one exception (during second copulation), were able to deliver salivary secretions. Contrary, in poorly-nourished males the number of salivary masses produced declined, whereas the percentage of males not providing any salivary masses increased dramatically with increasing mating frequency (first copulation: 2.86%; second copulation: 42.86%; third copulation: 66.67%). These results impressively show the strong selective pressure on males of *P. vulgaris* to find as much food as possible to improve their mating success.

Nutritional status and male investment

There is a positive correlation between male body weight and the dry weight of the salivary gland (Fig. 4; Spearman rank correlation: r_s = 0.659, N = 76, p < 0.001). Moreover, I found significant differences in salivary gland weights between males of nutritionally high, medium and low status (ANOVA: males of nutritionally high status: 2.70 ± 0.16 mg; males of nutritionally medium status: 1.84 ± 0.12 mg; males of nutritionally low status: 1.13 ± 0.13 mg; $F_{2,73}$ = 27.41, p < 0.001). Of 51 males I measured the dry weight of a salivary mass and the salivary gland after copulation. The mean dry weight of a salivary mass was 0.15 ± 0.01 mg (N = 51), the calculated mean dry weight of the salivary gland prior to copulation was 2.34 ± 0.15 mg (N = 51). Male investment in a copulation (= S salivary masses x IWSM) correlated with the estimated salivary gland weight before copulation (Fig. 5; Spearman rank correlation: r_s = 0.507, N = 51, p < 0.001). Thus, with increasing salivary gland size males

produce more salivary masses. But the slope of the regression line is smaller than unity (slope b = 0.294 ± 0.071; ANOVA: $F_{1,98}$ = 99.163, p < 0.001) and the intercept larger than zero (intercept = 0.452 ± 0.182), indicating that relative male investment decreases with increasing salivary gland size, and hence with increasing male condition.

No relation exists between female quality (body weight) at copulation and the number of salivary masses produced by males of nutritionally high and medium status (Spearman rank correlation: males of nutritionally high status: r_s = -0.12, N = 30, p = 0.528; males of nutritionally medium status: r_s = 0.027, N = 27, p = 0.894). Although not statistically significant, such a relationship seems to exist for males of nutritionally low status (Fig. 6; Spearman rank correlation: r_s= 0.431, N = 18, p = 0.074).

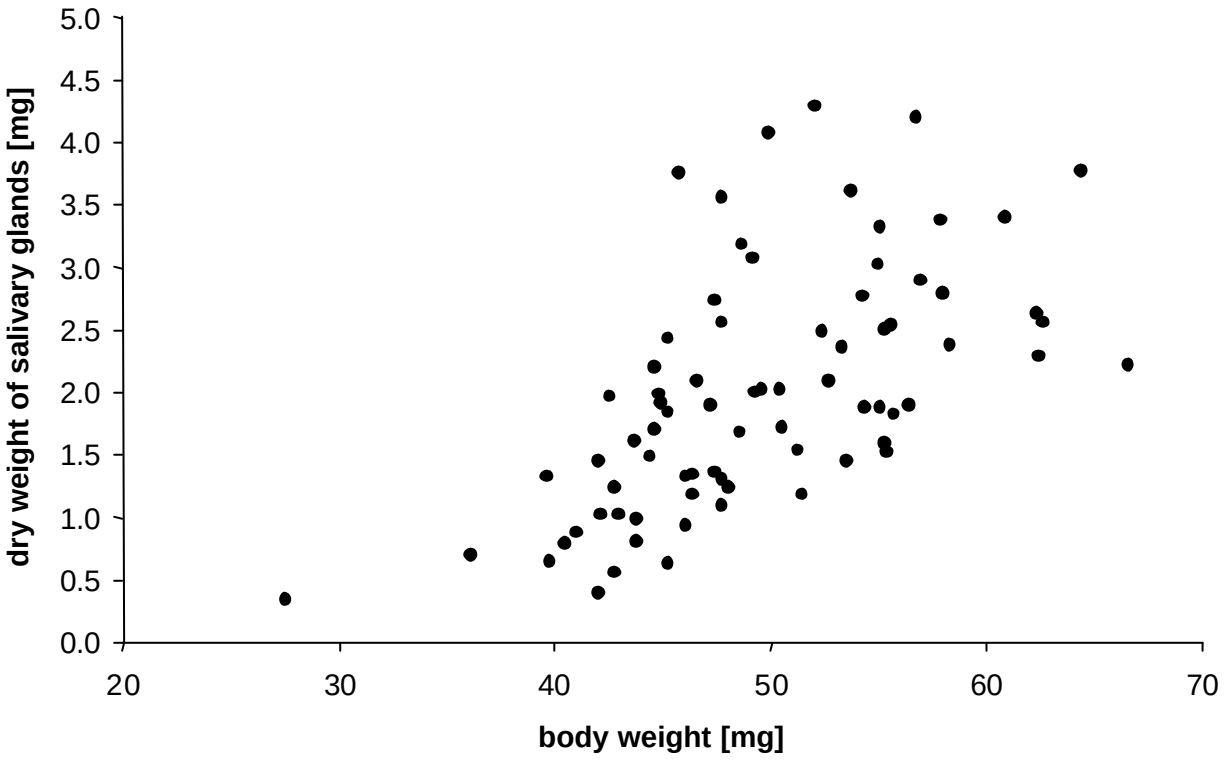

Figure 4: Relationship between male body weight and dry weight of the salivary gland.

Figure 5: Relationship between the estimated dry weight of the salivary gland prior to copulation and the overall dry weight of salivary masses produced during this copulation. The dashed line indicates the maximum possible male investment.

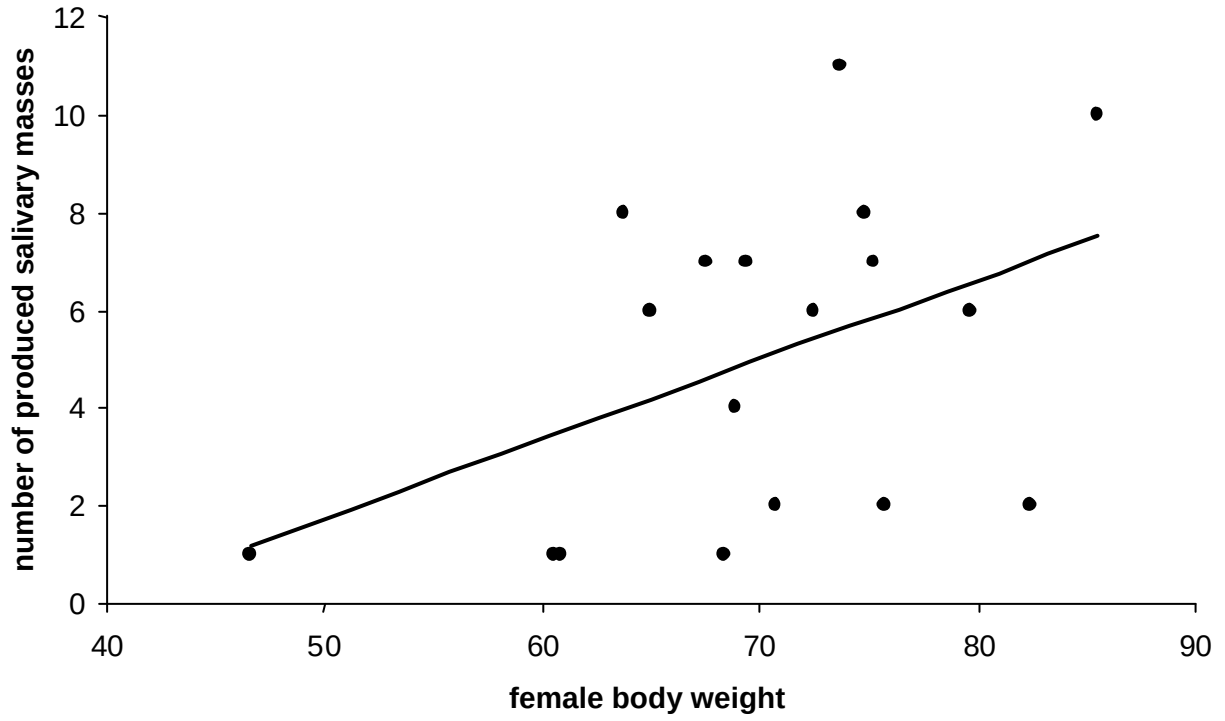

Figure 6: Relation between female body weight at copulation and the number of produced salivary masses for males of nutritionally low status.

Discussion

The present study demonstrates experimentally that salivary gland development and saliva secretion in male *P. vulgaris* are resource dependent traits heavily influenced by the availability of food. The degree of male mating effort measured as the number of salivary masses offered per copulation decreases in successive matings, if males are not allowed to feed in between. Males of nutritionally high status produce more salivary masses than those of nutritionally low status. Furthermore, while all males of nutritionally high status are capable of producing salivary masses in any of three successive copulations, the proportion of non-producers within the group of males of nutritionally low status increases dramatically with mating frequency. This is due to a correlation between male body weight and salivary gland size, with salivary gland size being a measure of the availability of saliva.

Since sperm transfer is continuous during copulation (Sauer et al. 1997), the number of sperm transferred is controlled by copulation duration which in turn depends on the number of salivary masses a male is capable to invest (Sauer et al. 1998). This has again been demonstrated in this study. Since fertilisation of eggs in the scorpionfly *P. vulgaris* follows the fair raffle principle (Parker et al. 1990, Sauer et al. 1990, 1998, Sindern 1996), saliva secretion can be referred to as mating effort (Sauer et al. 1998, Sauer 2002).

It has previously been suggested that nuptial gift production in *P. vulgaris* is dependent on food availability (Bockwinkel & Sauer 1994, Sauer et al. 1997, 1998). However, this is the first experimental approach showing the direct interaction between food availability, salivary gland development, and saliva allocation in *P. vulgaris*. The results strengthen the importance of traits like foraging ability and fighting prowess as basic requirements for a successful participation in the game of reproduction. While copulation duration can be viewed as the most prominent proximate fitness determinant (Sauer et al. 1997, 1998, 1999), food acquisition is fundamental for the development of males' salivary glands. Since the availability of saliva determines males' lifetime copulation duration, foraging ability plays an important role for males in terms of achieving mating durations long enough to be competitive in sperm competition. Therefore, foraging ability strongly influences males' lifetime reproductive success. Previous studies have stressed the importance of male fighting prowess for reproductive success and could show that offspring of superior fighters were able to win more fights over food and sons of good fighters were more successful in obtaining matings when compared to offspring of inferior males (Thornhill & Sauer 1992). So far, the importance of fighting prowess in connection with gaining resources in *P. vulgaris* may have

been overestimated to some extent. Even a good fighter has nothing to win when there is nothing to fight for. Due to the temporally and spatially unpredictable distribution of food, the capability of detecting food items should be at least of equal if not of higher relevance in order to develop or replenish the salivary glands.

Males of *P. vulgaris* are forced to adjust their mating effort to the size of their salivary glands, but relative investment decreases with increasing salivary gland size and hence with male condition. Sauer (1996) and Sauer et al. (1998) demonstrated that saliva secretion in *P. vulgaris* must be considered as a Zahavian handicap that can serve females as a quality indicator (Zahavi 1975, see also e.g. Andersson 1982, 1986, 1994, Maynard Smith 1991). Sauer (1996) and Sauer et al. (1998) showed that nuptial gift production in *P. vulgaris* fulfils four of the main predictions of the indicator models. One of these assumptions implies that quality indicators must be honest signals that are more costly for low- than for high-quality individuals. While Sauer et al. (1998) provided only indirect evidence, in the present study I have been able to present direct evidence for relative male investment to decrease with male quality. Concluding from this, the marginal cost of advertising (Maynard Smith 1991) is higher for males in poor condition. These results support the idea of the nuptial gift's role being a Zahavian quality indicator serving as a basis for cryptic female choice (Sauer et al. 1998).

My results seem to confirm the hypothesis that male *P. vulgaris* choose females cryptically by preferring those in good condition i.e. those of high fecundity. This is not surprising, since Sindern et al. (1994, 1995) and Sauer (1996) already demonstrated the existence of male choice in *P. vulgaris*. However, unlike former experiments, in the present study male choice was exhibited only by poorly-nourished males and therefore, was related to relative mating effort. As already emphasised the marginal costs of display are less for males of high quality so that in general, mating costs are relatively higher for poorly than for well nourished males. As a consequence, males of low nutritional status seem to be choosy, whereas males of high quality exhibited no discrimination among females concerning their fecundity. These results are consistent with those of Engqvist & Sauer (2001) for the scorpionfly *P. cognata.* Male mating effort in *P. vulgaris* seems, at least in part, to depend on female quality. The result that only males of low quality are choosy in terms of allocating their saliva resources, leads to the conclusion that they have to be economical in order to save some of their resources for future matings. Food availability is spatially and temporally highly unpredictable and the point of time when the saliva secretion can be replenished remains highly uncertain. Therefore, it seems to be advantageous to reduce the investment in a single copulation, suffering a lower

sperm competition capacity, but keeping resources for future matings. Engqvist & Sauer (2001) demonstrated for *P. cognata* that high quality males "invest a relatively small fraction of their available resources, whereas males in poor condition invest a considerable portion of their limited mating resources. Consequently, the importance of female quality in any single mating will differ between males.". This is also true for *P. vulgaris*. For males in good condition there is no need to restrict their mating investment, since they will be able to produce a sufficiently high number of salivary masses in several successive copulations anyway.

Conclusions

The present study points out the importance of adult feeding history on salivary gland development in *P. vulgaris*. Since salivary gland size determines a male's capacity of saliva secretion, the ability to gain food, either through finding or fighting, is crucial for its reproductive success. Only males that invest a relatively high proportion of their available mating resources discriminate between females of high and low fecundity which can be referred to as cryptic male choice. Our results show that mating costs are different for males of various quality. This, in connection with results of previous studies (Sauer 1996, Sauer et al. 1998), supports the view that nuptial gifts in *P. vulgaris* represent honest signals for male genetic quality in the sense of Zahavi's (1975) indicator models of sexual selection.

Chapter III: Does the nutritional status of larvae of the scorpionfly *Panorpa vulgaris* influence adult male mating performance?

Abstract

The basic requirement for selection to take effect is the variation in fitness relevant traits among individuals of a population. The present study is concerned with the question whether environmental conditions met during an early phase of life history that is dominated by the natural component of selection will affect traits and behaviour in a sexual selection context later on in life. I examined the effects of nutrition as a proximate factor responsible for intrasexual phenotypic variation in the mating performance of male *Panorpa vulgaris* (Mecoptera: Panorpidae). For this purpose I manipulated food availability during larval development as well as during adulthood. To obtain matings males must secrete salivary masses which are then consumed by the females during copulation. The duration of copulation, and thus the amount of transferred sperm increases with the number of nuptial gifts provided to a female. Since sperm utilisation follows the fair raffle principle, saliva secretion determines male reproductive success. My results are consistent with those of previous studies demonstrating a strong effect of nutrition during adulthood on various fitness relevant traits (salivary gland development, saliva investment in copulations, etc.). But moreover, I could show that food availability during larval development affected male body weight, salivary gland weight before and after a copulation and the proportion of saliva resources that was invested in a mating. Therefore, larval feeding history implies considerable long time consequences and affects several traits that become important in a sexual selection context later on in male life history.

Introduction

Fitness differences between individuals of a species emerge from variation in fitness relevant traits. Life history theory distinguishes two sources of variation that influence total fitness: i) variation in resource acquisition, and ii) variation in the allocation of resources among fitness components (Stearns 1992). Those traits contributing to an individual's fitness are favoured by the process of selection (Darwin 1859). However, there are two distinguishable components of selection that are of different importance in the course of an individual's life history (ontogeny s. l.). Variation in resource acquisition first of all results in non random differential survival and/or viability. Therefore, differential foraging ability is subject to natural selection (Sauer 2001). Variation in resource allocation among fitness components of course implies reproductive effort and leads to non random differential mate acquisition and as a result to non random differential reproduction. Therefore, in this phase of life history when individuals start to reproduce the sexual component of selection is effective. Generally, natural selection is dominant in the pre-mature phase of life history followed by a growing impact of sexual selection as soon as an individual reaches sexual maturity or begins developing sexual ornaments.

Since in the majority of cases mate choice is performed by females, males are typically the ones that enhance reproductive success by developing costly sexual ornaments (Andersson 1994, Trivers 1972). These sexually dimorphic traits can concern various body structures or certain behaviours (e.g. Andersson 1994). Increasing investment in a trait selected by the sexual component of selection is often only possible at the expense of investment in traits promoting survival and viability. Therefore, traits selected in the context of sexual selection sometimes cause considerable costs to one or more fitness components of the trait bearer. Moreover, mating success usually increases with the size and therefore with the costs of the sexually selected ornament (Andersson 1994, Darwin 1871). Darwin (1871) already was aware of such sexually dimorphic structures and knew about their significant importance in the context of mate choice. Nevertheless, he was not able to explain how these traits could evolve. It had to be a mechanism contradictory to his theory of natural selection (Darwin 1859). The most established theories today to explain such sexually selected characters are the Fisherian self-reinforcing theory (Fisher 1930, Maynard Smith 1991) and the indicator mechanism theory (Andersson 1982, 1986, Maynard Smith 1991, Zahavi 1975).

However, there are not only differences between the sexes. Especially sexually selected traits are subject to intrasexual phenotypic variation which sometimes enables the opposite sex to

perform mate choice based on genetic quality (see Andersson 1986, Zahavi 1975). Variation can be continuous as the colouration and hair length of male lions' manes (West & Packer 2002) or discontinuous like the horn length in the scarab beetle *Onthophagus taurus* (Moczek & Emlen 1999) reflected by two or more discrete forms of the trait. Generally, such variation can result from genetic differences or from environmental conditions. As diverse as the potentially acting environmental factors are the consequences for an animal's morphology or behaviour. For example factors like larval density, temperature or nutritional stress can potentially affect body size, secondary sex traits or mating performance in different insect taxa (e.g. Gage 1995, Gage & Cook 1994, He & Tsubaki 1992, Hellriegel & Blanckenhorn 2002, Moczek & Emlen 1999, Stockley & Seal 2001, Tomkins 1999, Wagner & Hoback 1999). Especially dietary composition as well as the amount of food available have considerable influence on an individual's development and viability (Chapman 1971, Wigglesworth 1972, 1974).

In the present study I used scorpionflies of *Panorpa vulgaris* (Imhof & Labram 1836) as a model system to investigate the proximate environmental factors affecting a sexually selected trait in males. *P. vulgaris* exhibits sexually dimorphic developed salivary glands. While males develop strongly enlarged glands, those of the females are almost degenerate and remain virtually unused (Grell 1938, Kaltenbach 1978). This is due to an evolutionary change in their functional significance. They have been detached from the context of nutrition and now play a central role in male mating performance. *P. vulgaris* males produce saliva secretions during copulation and feed them to females who in turn adjust copulation duration to the number of nuptial gifts they receive (Sauer et al. 1998, Sindern 1996). Due to sperm mixing and sperm utilisation in accordance to their numerical representation in the spermatheca, transferring a maximum number of sperm per copulation is beneficial for males (Sauer et al. 1990, 1997, 1999). Since sperm transfer is continuous, the duration of copulation and therewith salivary mass production is the main fitness determining factor for males (Sauer et al. 1990, 1997, 1998, 1999).

In this study I investigated nutrition as a proximate factor responsible for intrasexual phenotypic variation in the mating performance of *P. vulgaris* males. While effects of nutrition during adulthood on the mating performance and salivary gland development in *P. vulgaris* have already been studied (Engels & Sauer in prep.b, Sindern 1996), the present experiment comprises the entire life history (ontogeny s. l.). Although saliva storage starts only after adults have hatched, I examined to what extend different food availability during the larval stage as well as during adulthood affected saliva production in matings and the

development of the salivary gland. Therefore, I was able to distinguish the influence of food intake during these different stages of development on body weight, salivary gland weight before and after a copulation and the number and weight of salivary masses produced by male *P. vulgaris*. Through this experimental design it was possible to detect if food availability in an early life history phase (ontogenetic stage) where resource allocation is thought to be restricted to viability can nevertheless affect different components of male mating performance.

Methods

Breeding and experimental treatment

I used offspring of *P. vulgaris'* second annual generation of 2002. Adults were collected from a field population near Freiburg i. Br., south-west Germany and taken to Bonn for breeding. They were held pairwise in plastic boxes (10 cm x 10 cm x 7 cm) containing moist tissue paper, food ad libitum and a small Petri dish filled with moist peat to permit oviposition. Larvae were reared on a 12 h light : 12 h dark cycle at 18° C on moist tissue paper with food ad libitum at a maximum density of 20 larvae per Petri dish (12 cm diameter). Third instar larvae (10 days of age) were individually isolated in small Petri dishes (5 cm diameter) containing moist tissue paper and were randomly separated into three groups exposed to different food availability. Larvae were provided with food on days one (= day of isolation), nine and 17. Group 1 received four (nutritionally high larvae = NHL), group 2 two (nutritionally medium larvae = NML) and group 3 one (nutritionally low larvae = NLL) segment piece of last instar *Tenebrio molitor* larvae per feeding.

Individuals of each group were numbered consecutively. Each group consisted of 266 individuals. On day 20 (= larvae 30 days old) the larvae's body weight was measured and animals were transferred into plastic tubes (8 cm x 3.5 cm) filled with moist peat. Temperature was lowered gradually by 1° C per day until reaching 4° C. Animals were kept throughout the winter under these conditions and entered diapause. The peat was moistened regularly. In April 2003 temperature was raised gradually until reaching 17° C. Then the animals were transferred to a laboratory room where they finished their development at 18-20° C and where they remained for the time of the experiment.

Hatching adults were kept singly in plastic tubes (8 cm x 3.5 cm) containing moist tissue paper. Males of each larval group (NHL, NML and NLL) were separated into three groups exposed to different food availability. One group received food on days zero (= hatching

date), four, eight and twelve (nutritionally high adults = NHA), the second group on days zero, seven and 14 (nutritionally medium adults = NMA), the third group on days zero and ten (nutritionally low adults = NLA). Each feeding consisted of one segment piece of *T. molitor* larvae. I therefore made up three male groups with different food supply during larval development as well as three groups during adulthood.
Hatching success for NLL was very low and only 4 males survived until the end of the study. Therefore I had to exclude this group from statistical analyses. Consequently, I got a total of six groups that were kept under dissimilar food availability:

NHL-NHA, NHL-NMA, NHL-NLA,
NML-NHA, NML-NMA, NML-NLA.

All females were provided with two segments on days zero and eight.
The salivary glands of 76 males were dissected on day 15, while another 75 males had the chance to mate with a female. I measured duration of copulation and the number of salivary masses provided to the female. Following copulation the salivary glands of the mated males were dissected as well.
The body weight of all males was measured on the day of emergence and on day 15 (in case of the mated males prior to copulation) using a balance of Sartorius.

Measuring weights of salivary masses

During some of the copulations I removed the second produced salivary mass. This was done for each of the six nutrition groups. The secretions were transferred into Eppendorf reaction tubes and were dried at 20° C until weight constancy. Dry weights were measured with a precision balance of Sartorius (2004 MP).

Dissection and weighing of salivary glands

Males were anaesthetised using CO_2 and subsequently transferred into 70 % ethanol. After a few minutes they were placed into a water filled preparation dish and were dissected under a binocular magnifier (Leica WILD M3B). Bodies were laterally opened by cutting the integument with a small dissection scissor starting at the end of the abdomen. Afterwards the bodies were put back into 70 % ethanol where they remained for at least 24 h. This was necessary to harden the secretion inside the salivary gland which is essential for a proper dissection. After dissection the glands were placed on a piece of aluminium foil inside a small

Petri dish (5 cm diameter) and were dried at 30° C until weight constancy. The dry weight of salivary glands was measured using a precision balance of Sartorius (2004 MP).

Calculated values

I calculated male investment as the amount of saliva secretion males invested in a copulation: S salivary masses x mean weight of salivary masses. Accordingly, I estimated salivary gland weight prior to copulation for each mated male as follows: gland weight after copulation + S salivary masses x mean weight of salivary masses.

Mean weights of salivary masses were different for NHA, NMA and NLA. Therefore, calculations of male investment and estimated salivary gland weight prior to copulation for these groups were based on different values for salivary mass weight.

Statistics

Statistical analyses were performed using SPSS 11.0. All tests were two-tailed and the level of significance was determined $\alpha = 0.05$ for all cases. Mean values are given as mean ± standard error (SE). Measured values for male investment in a copulation and salivary gland weight after copulation conformed to normality after square root transformation. To report means of these variables I transformed the values back.

Results

Different food availability during the larval stage resulted in different body weights of 30 days old larvae (mean body weight NHL: 56.82 ± 0.72 mg, mean body weight NML: 49.85 ± 0.53 mg; ANOVA: $F_{1,149} = 56.67$, $p < 0.001$) as well as in differences in hatching weight after diapause (mean hatching weight NHL: 39.82 ± 0.58 mg, mean hatching weight NML: 32.26 ± 0.46 mg; ANOVA: $F_{1,149} = 98.97$, $p < 0.001$).

Body weight of 15 days old male adults was affected by larval as well as by adult nourishment (Fig. 1; two-way ANOVA: larval group membership: $F_{1,145} = 42.39$, $p < 0.001$, adult group membership: $F_{2,145} = 19.59$, $p < 0.001$).

A two-way ANOVA revealed an effect of food availability during adulthood on the size of the salivary gland (Fig. 2; $F_{2,70} = 26.8$, $p < 0.001$, Post-Hoc LSD test: NHA and NMA: $N_1 = 30$, $N_2 = 27$, $p < 0.001$, NHA and NLA: $N_1 = 30$, $N_3 = 19$, $p < 0.001$, NMA and NLA: $N_2 = 27$, $N_3 = 19$, $p = 0.001$), whereas nutrition during the larval stage does not seem to play a major role ($F_{1,70} = 1.92$, $p = 0.171$). The effect of an interaction between the two factors remained

slightly above the level of significance ($F_{2,70}$ = 2.59, p = 0.082). When only considering NMA a comparison of NHL and NML shows that these groups developed salivary glands of different weight (Fig. 2; ANOVA: $F_{1,25}$ = 14.86, p = 0.001). This effect was absent in NHA as well as in NLA (see Fig.2).

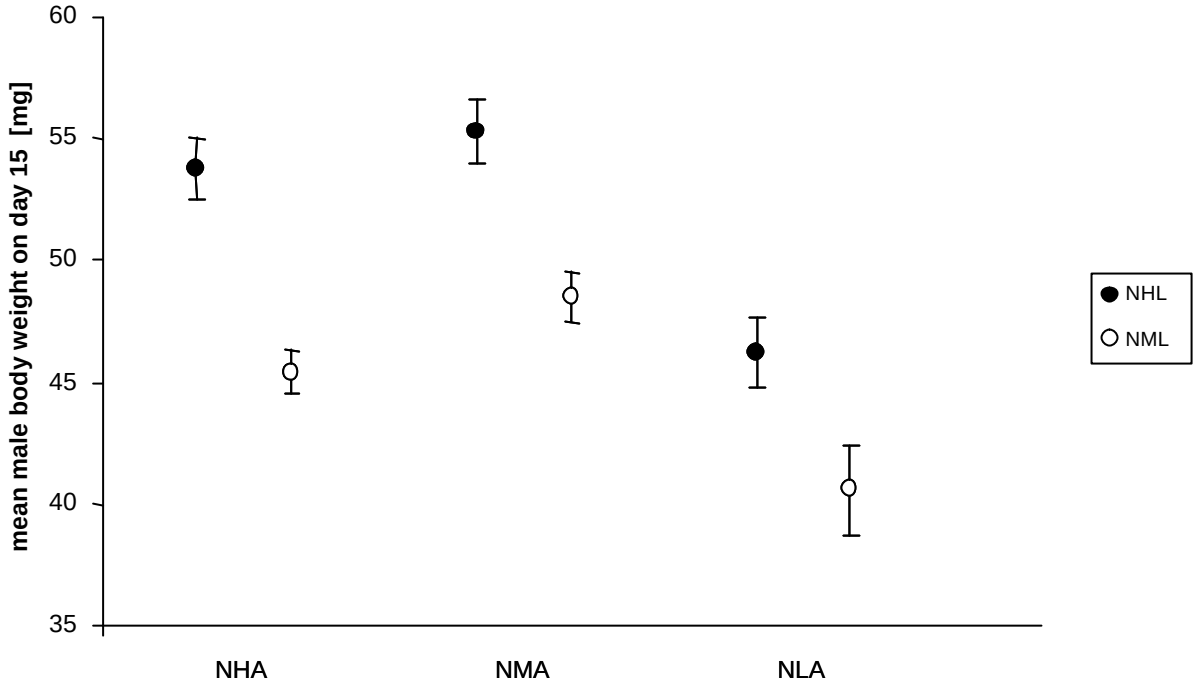

Figure 1: Comparison of the mean male body weight (± SE) between the different nourishment groups.

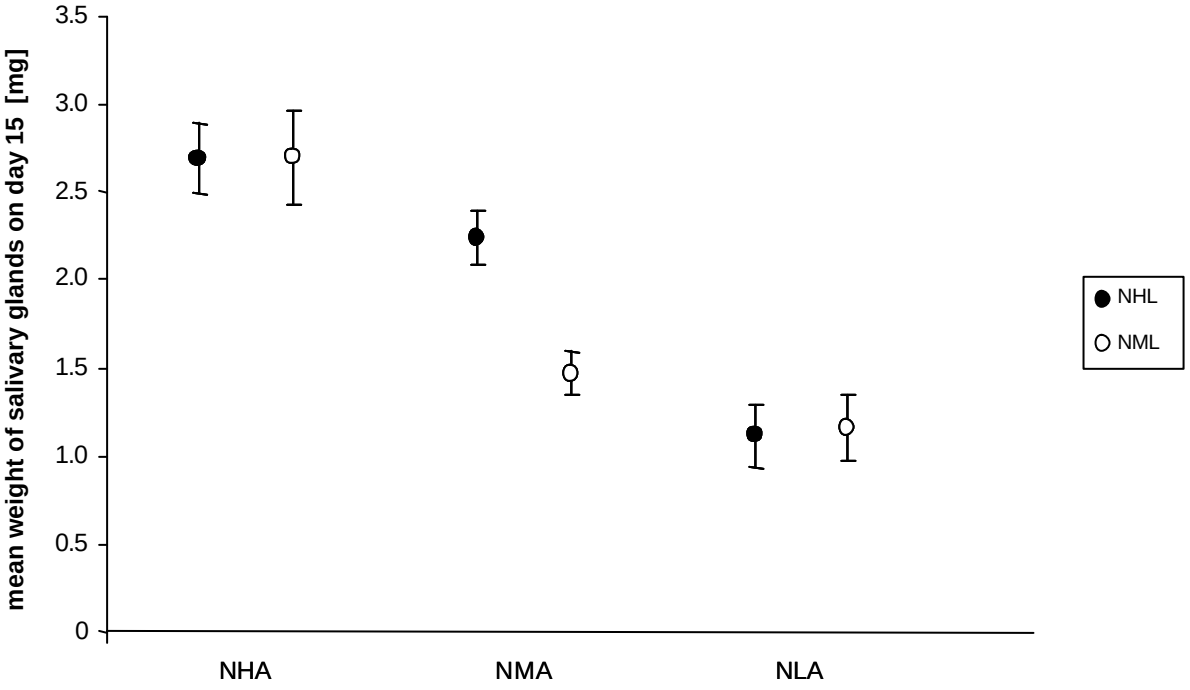

Figure 2: Comparison of the mean weight of males' salivary glands (± SE) at 15 days of age.

There was no effect of nourishment during adulthood or during the larval stage on the number of produced salivary masses during copulations. Copulation duration was unaffected as well. However, NHA males produced significantly heavier salivary secretions than males of the other adult groups (Fig. 3; ANOVA: $F_{2,49} = 24.61$, $p < 0.001$, Post-Hoc LSD test: NHA and NMA: $N_1 = 24$, $N_2 = 17$, $p < 0.001$, NHA and NLA: $N_1 = 24$, $N_3 = 10$, $p < 0.001$, NMA and NLA: $N_2 = 17$, $N_3 = 10$, $p = 0.407$). The same result was obtained when considering NHL (Tab. 1; ANOVA: $F_{2,26} = 11.26$, $p < 0.001$, Post-Hoc LSD test: NHA and NMA: $N_1 = 12$, $N_2 = 12$, $p < 0.001$, NHA and NLA: $N_1 = 12$, $N_3 = 5$, $p = 0.001$, NMA and NLA: $N_2 = 12$, $N_3 = 5$, $p = 0.637$) and NML separately (Tab. 1; ANOVA: $F_{2,19} = 11.31$, $p = 0.001$, Post-Hoc LSD test: NHA and NMA: $N_1 = 12$, $N_2 = 5$, $p = 0.002$, NHA and NLA: $N_1 = 12$, $N_3 = 5$, $p = 0.001$, NMA and NLA: $N_2 = 5$, $N_3 = 5$, $p = 0.6$). Larval nourishment had no effect on salivary mass weight.

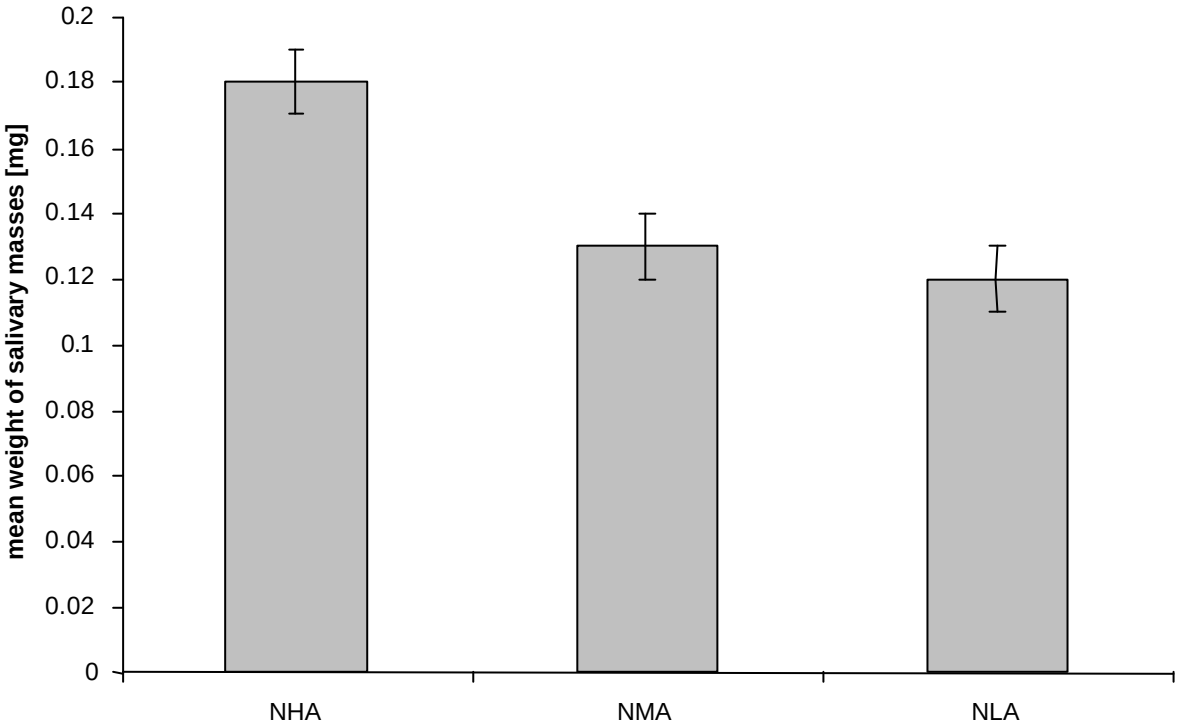

Figure 3: Mean weight of saliva secretions (± SE) produced during copulations compared between the different adult nutrition groups (each adult group comprises both larval nutrition groups).

Table 1: Mean weight of salivary masses [mg] separated for the different nourishment groups.

	NHA	NMA	NLA
NHL	0.18 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
NML	0.19 ± 0.01	0.13 ± 0.02	0.12 ± 0.01

Male investment in a copulation, measured as the total mass of saliva secretion, was only affected by food availability during adulthood (Fig. 4; two-way ANOVA: $F_{2,69} = 10.01$, $p < 0.001$, Post-Hoc LSD test: NHA and NMA: $N_1 = 30$, $N_2 = 27$, $p = 0.001$, NHA and NLA: $N_1 = 30$, $N_3 = 18$, $p < 0.001$, NMA and NLA: $N_2 = 27$, $N_3 = 18$, $p = 0.223$). I obtained the same result when expressing male investment as the percentage of body weight (Tab. 2; two-way ANOVA: adult group membership: $F_{2,69} = 10.29$, $p < 0.001$, Post-Hoc LSD test: NHA and NMA: $N_1 = 30$, $N_2 = 27$, $p < 0.001$, NHA and NLA: $N_1 = 30$, $N_3 = 18$, $p < 0.001$, NMA and NLA: $N_2 = 27$, $N_3 = 18$, $p = 0.791$).

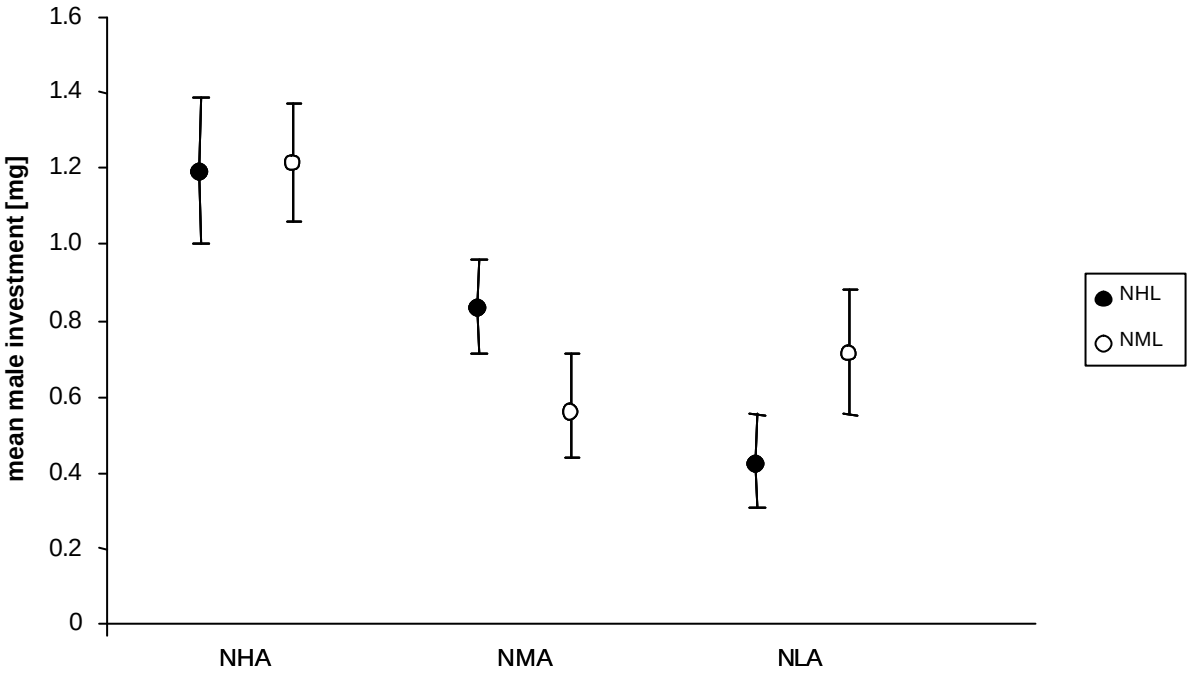

Figure 4: Mean male investment in a copulation (± SE) dependent on nutrition group membership.

Table 2: Mean male investment in a copulation in relation to body weight [%] for the different nutrition treatments.

	NHA	NMA	NLA
NHL	2.46 ± 0.35	1.66 ± 0.24	1.13 ± 0.27
NML	2.91 ± 0.33	1.34 ± 0.27	1.87 ± 0.32

Regarding male investment as percentage of the estimated salivary gland weight before copulation reveals an almost significant influence of larval nourishment as well as an almost significant effect of an interaction between larval and adult nutrition (Fig. 5; two-way

ANOVA: larval group membership: $F_{1,69} = 3.52$, $p = 0.065$, interaction between larval group membership and adult group membership: $F_{2,69} = 2.91$, $p = 0.061$). When having a closer look it becomes clear that this result is provoked by a difference of relative investment of mating resources between NHL and NML only within NLA (see Fig 5; ANOVA: $F_{1,16} = 4.43$, $p = 0.051$). Adult group membership had no effect ($F_{2,69} = 0.31$, $p = 0.731$).

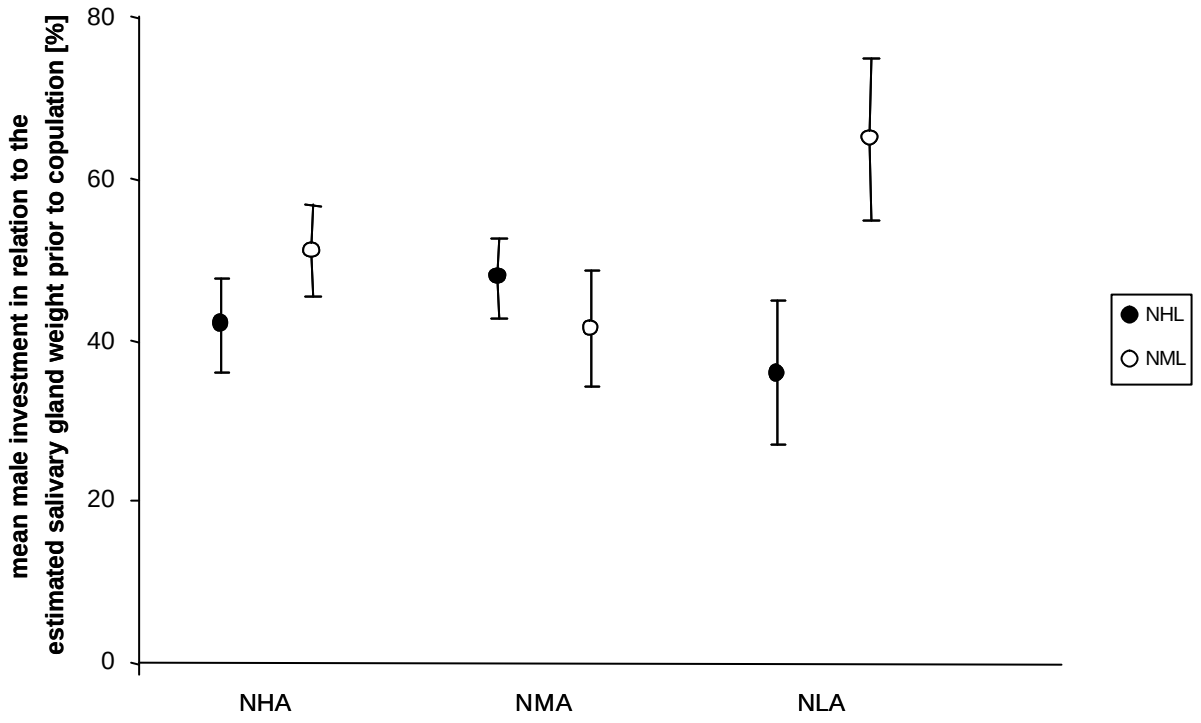

Figure 5: Mean male investment (± SE) in relation to the estimated salivary gland weight prior to copulation separated for the different nourishment treatments.

The remaining salivary gland weight of mated males that had produced a certain amount of salivary secretions was influenced by food availability during the larval stage as well as during adulthood (Fig.6; two-way ANOVA: larval group membership: $F_{1,69} = 9.25$, $p = 0.003$, adult group membership: $F_{2,69} = 10.12$, $p < 0.001$, Post-Hoc LSD test: NHA and NMA: $N_1 = 30$, $N_2 = 27$, $p = 0.003$, NHA and NLA: $N_1 = 30$, $N_3 = 18$, $p < 0.001$, NMA and NLA: $N_2 = 27$, $N_3 = 18$, $p = 0.282$). When only considering NHA or NLA, respectively, NHL had significant heavier glands after copulation than NML (see Fig. 6; ANOVA: for NHA: $F_{1,28} = 4.36$, $p = 0.046$; for NLA: $F_{1,16} = 6.16$, $p = 0.025$). This is not the case for NMA.

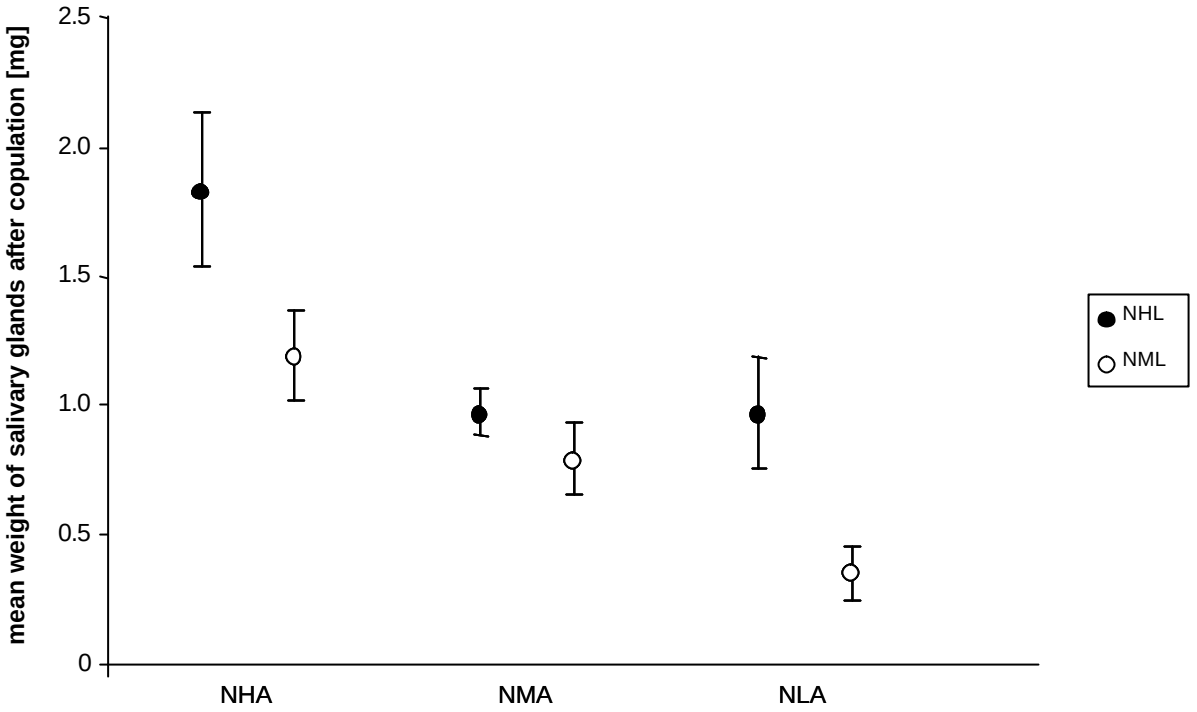

Figure 6: Mean weight of males' salivary glands (± SE) after copulation.

Discussion

In the present study I focused on the effect of food availability on different aspects concerned with male mating performance and therefore male mating success in *P. vulgaris.* Nutritional condition in insects is thought to first of all affect egg production, and therefore female reproductive success (Wigglesworth 1972, 1974). This is due to the necessity to receive a sufficient amount of substances (e.g. protein) that is needed to build up eggs. In *P. vulgaris* males must be capable of producing protein rich nuptial gifts to maximise their mating and reproductive success (Fleck 1997, Sauer et al. 1998). Therefore, it seems plausible that male mating performance might be affected by food availability during larval development and/or during adulthood. While the effects of nutrition during adulthood have already been studied (Engels & Sauer in prep. b), this time I was able to control food supply throughout the animals' entire life history, including larval development. Therefore, it was possible to distinguish between the impact of nutrition during larval stage and adulthood and to detect effects of nutritional condition established during the pre-mature phase on several components of male mating performance.

Effects of nutrition during larval development

Food availability affected body weight of 30 days old larvae. This matched my expectations, since relative higher food availability is supposed to result in relative better condition during the larval stage. Furthermore, NHL were able to keep up this conditional advantage throughout their diapause, resulting in higher adult hatching weights than recorded for NML. The ability to store the resources gained as larvae and to transfer them into the adult stage which is reflected by different hatching weights of NML and NHL, stresses the importance of foraging ability during larval development. This is further confirmed by the result that male body weight on day 15 after hatching was also influenced by larval nutrition history. NHL reached higher body weights than NML no matter which adult nutrition group they belonged to. Thus, food availability during larval development is an important factor determining the level of condition or body weight a male *P. vulgaris* can achieve as an adult. Previous studies showed that body weight correlates with salivary gland weight and in turn salivary gland weight correlates with the number of nuptial gifts a male can produce during copulation (Engels & Sauer in prep. b). Since the number of produced salivary masses determines copulation duration and increases female fecundity (Sauer et al. 1998, Engels & Sauer in prep. a), male body weight and therefore larval nourishment would become a major factor affecting male fitness. However, these correlations cannot sufficiently explain the importance of larval nourishment in detail, because consequences are more complex than this. Indeed it is necessary to have a closer look. At first sight the present study gives the impression that larval nourishment has no effect on male salivary gland weight. In fact the implication of larval food history is different depending on food availability during adulthood. It is not relevant for NHA and NLA but has an effect on NMA. This leads to the conclusion that extreme food conditions during adulthood have such strong effects on the animals that larval history has no chance to break through. In case of NHA food supply is sufficient for NML to compensate for their larval disadvantages whereas NHL within NLA can take no advantage of their luxurious larval life, because food was too scarce during adulthood. Only when facing medium food conditions during adult life, which is probably the most natural-like situation, the amount of food intake as larva has a considerable effect on the development and therefore on the weight and size of the salivary gland. Thus, larval nourishment influences different traits which can be regarded as requirements for male mating/reproductive success. But do males in fact realise their faculties and incapacities provoked by larval nutrition in their mating performance? Surprisingly, almost all parameters directly linked to mating, such as the number of produced salivary masses, copulation duration, salivary mass weight, absolute

saliva investment and investment as percentage of body weight are not linked to food availability during the larval stage. Furthermore, animals of all groups seem to invest the same relative amount of their mating resources (percentage of estimated salivary gland weight prior to mating). Only NML within NLA invest a higher proportion of their saliva resources. Although I could not find a significant influence of larval nourishment on absolute saliva investment, it appears to have an effect on salivary gland weight after copulation. While larval nourishment influenced salivary gland weight before a copulation only in a medium food environment during adult stage (Fig. 2), it is now the other way round. NMA are not affected whereas under extreme food conditions (high and low) during adulthood NHL remain with more resources left than NML after a mating (Fig. 6). Of course this result seems strange at first sight. If all individuals invest the same amount of saliva, then the relation of salivary gland weights between the groups should stay the same before and after a copulation. However, the lack of statistical significance does not always mean that there are no differences. When having a closer look at mean male investment (Fig. 4) it becomes clear that NHL within NMA invested 48.21 % more saliva than NML. This is obviously not a marginal difference and cannot be ignored. Within NLA the NML invested even 69.05 % more than NHL. These differences in investment are not statistically significant, but result in and explain the change of the pattern of salivary gland weight before and after a mating (Fig. 2 and 6). But how can I explain the different investment behaviour of NHL and NML depending on which adult nutrition group they belong to (see Fig. 4)? When adult *P. vulgaris* face an environment providing a lot of food as for NHA, they can find enough resources to produce a large quantity of saliva independently of food availability during the larval stage. But as food supply decreases larval feeding history becomes more important. Within NMA NHL develop heavier glands (Fig. 2) and therefore are in the condition to produce more saliva than NML (Fig. 4). Despite their higher investment they still have enough resources left for further matings. In a nutritionally low environment one would expect more than ever that NHL either could invest more than NML or that the lack of food has such a heavy impact on the animals' condition that both groups can only invest very few saliva. Surprisingly, NML-NLA invest more saliva than NHL-NLA. I can give two associated explanations for this behaviour. First of all male *P. vulgaris* of low nutritional status are known to perform mate choice investing higher amounts of saliva in heavy females (Engels & Sauer in prep. b). Since NML-NLA are lighter than NHL-NLA and females in all matings were of equal condition, the relative quality of females was higher for NML-NLA than for NHL-NLA. Therefore, NML-NLA are expected to increase their investment. Furthermore, there is a trade-off between current and

future reproduction (Stearns 1992). If survivorship is low, as expected for NML-NLA because of their low nutritional status, it may pay for them to invest surplus energy in current reproductive behaviour ensuring a high fertilisation success in a present mating, rather than storing it for future use (Stearns 1976). NHL-NLA instead, expect a longer life span and have better chances to obtain further copulations so they should keep some of their mating resources for future matings.

Effects of nutrition during adulthood

Expectedly, body weight of 15 days old adults was influenced by the amount of food they received after hatching. Surprisingly, those animals exposed to medium food availability reached higher weights than NHA ($p = 0,066$). To explain this result one has to notice that despite this difference in body weight NHA developed heavier salivary glands. Thus, higher food availability enables male *P. vulgaris* to build up more saliva secretion which is certainly the most relevant trait to maximise reproductive success. In general, there are two alternative patterns of energy allocation for males. Energy that exceeds the animals' basic maintenance requirements can either be used to maximise mating success or to enhance survival and viability. While in some species males invest any energy surplus in mate attraction as demonstrated for *Gryllus lineaticeps* (Wagner & Hoback 1999), it seems that *P. vulgaris* males do so only when experiencing high food availability. NHA and NMA both surely received more energy than needed for pure survival, but utilised the excess nutrients differently. When facing medium food conditions males stored the energy to enhance their viability and achieved higher body weights. In contrast, NHA used their energy surplus to increase their mating resources and developed bigger salivary glands. Thus, male *P. vulgaris* appear to adjust the pattern of energy allocation to environmental conditions. Contrary to previous studies (e.g. Engels & Sauer in prep. b, Sauer et al. 1998) adult nutrition had no effect on the number of produced salivary masses. Instead, NHA produced heavier secretions than the other groups resulting in a higher investment per copulation (absolute and relative to body weight) but not in an extended mating duration. This is quite astonishing since one would expect that it will take a female longer to consume a large than a small salivary mass. But obviously the high nutrition group did not draw advantages from their better condition in terms of transferring more sperm via prolonged copulation durations. So why should they then invest more saliva? First of all copulation duration is not coercively the only determinant of how many sperm are transferred. Vermeulen & Sauer (in prep.) recently showed that females are able to counteract sperm transfer by muscle contraction. If females reduced the

counter pressure in accordance to the mass of saliva they receive, thereby allowing high quality males to transfer more sperm, then these males would benefit from their higher investment without achieving longer mating durations. Moreover, Engels & Sauer (in prep. a) showed that females receiving more saliva significantly increase their reproductive output. Therefore, males providing females with more secretion increase the number of eggs a given female can produce and therewith the absolute number of eggs these males can fertilise. In this context, the fact that mating duration is not prolonged despite of the higher investment, might support the interpretation of the salivary mass in *P. vulgaris* as paternal investment (in addition to mating effort) as given by Engels & Sauer (in prep. a).

Nutrition had no effect on the proportion of invested mating resources. Since all males invested more or less the same percentage of their unequal mating resources, NHA still had more resources left after a mating despite of their higher absolute investment. Therefore, the costs imposed by nuptial feeding in *P. vulgaris* are not the same for any individual. Dependent on an individual's condition, the marginal cost of saliva production varies greatly between males. It has already been demonstrated (Engels & Sauer in prep. b, Sauer 1996, Sauer et al. 1998,) that saliva secretion in *P. vulgaris* must be regarded as an honest quality indicator in the sense of Zahavi (1975, see also Andersson 1994, Maynard Smith 1991). One of the indicator model predictions is that the marginal cost of advertising must be higher for low than for high quality males. The present study could once again show that salivary mass production in *P. vulgaris* fulfils this prediction.

Conclusions

The present study points out again the far reaching importance of nutrition during adulthood for various traits and behaviours linked to male mating success in *P. vulgaris*. Moreover, I could show that food availability during larval development is also of great importance. Several traits influencing male mating performance and success were heavily affected by the amount of food animals could access as larva. The absence or presence of such larval feeding history effects was sometimes dependent on the amount of food available during adulthood. However, my results show that environmental conditions faced during an early life history phase where all resources are thought to be invested in ensuring survival and enhancing viability can nevertheless affect characters which become important during mating and are subject to sexual selection. Therefore, successful food acquisition as larva is the key to become competitive in reproduction later on in life history particularly if food availability

during adulthood is limited. Thus, larval nutrition not only affects larval survivorship and viability, but also bears long time consequences for a male's reproductive success.

General Discussion

One goal of this thesis was to investigate the function of nuptial feeding in the scorpionfly *P. vulgaris*. While its function as mating effort has been demonstrated several times, I was concerned with the question if it also works as a form of paternal investment. Furthermore, I manipulated food availability during different phases of life history to detect consequences for salivary gland development and nuptial gift production, and therefore for male mating performance and success. In this context I additionally investigated the relative costs of nuptial gift production for males of different nutritional condition (quality). While all results are discussed in detail in the corresponding chapters, here I give some brief general conclusions on the ultimate as well as on the proximate level on nuptial feeding in *P. vulgaris* drawn from the results of this work.

Salivary mass production as paternal investment

The results presented in chapter I show that salivary mass consumption affects egg production in female *P. vulgaris*. Correlations between gift consumption and egg production as well as comparisons between groups of females that received different numbers of salivary masses demonstrate that females receiving more gifts will significantly increase their reproductive output. According to Simmons & Parker (1989) male nuptial feeding represents a form of paternal investment if the female's reproductive output is increased and as a result the fitness of the male's progeny as a whole gains benefits by an increased number of offspring. Problems arising from females mating with different males before oviposition, which could lead to pseudo-parental investment (Wickler 1985), are discussed in detail in chapter I. Due to the pattern of sperm utilisation pseudo-parental investment can be ruled out in *P. vulgaris*. Therefore, I conclude that salivary mass production represents a form of true paternal investment. The only uncertainty arising from methodical problems concerns the location of the fecundity increasing substances. From the experimental design I cannot clearly distinguish between effects of nuptial gift consumption and effects of the ejaculate. Furthermore, it is not clear if the increase of egg production is caused by an incorporation of additional nutrients or by allohormones. Again, chapter I gives a more detailed discussion on these aspects.

Food dependency and varying marginal costs

Salivary mass production is one of three mating tactics males of *P. vulgaris* can employ to obtain copulations. In the second chapter I am able to show that the capability of producing saliva is strongly dependent on food availability. The number of produced salivary masses declines in successive matings if males are not able to feed in between the copulations. Moreover, males that were assigned to a high nutrition treatment produced a higher number of gifts in any of three successive copulations than males of a low nutrition treatment. Correspondingly, I found a correlation between male body weight and the weight of the salivary gland. Therefore, the amount of saliva a male can produce is determined by its capability to find food and by food availability. The absolute saliva investment in a copulation increases with the size of the salivary gland, as the relative investment decreases. Therefore, I conclude that the marginal costs of saliva production differ for males of various nutritional status. This, in addition with results of previous studies (Sauer 1996, Sauer et al. 1998), supports the view of the nuptial gifts in *P. vulgaris* as indicators for male genetic quality in the sense of Zahavi (1975).

However, not only females are likely to perform mate choice. Although not statistically significant, from the results given in chapter II one may conclude that males in a bad nutritional condition perform mate choice by adjusting the number of salivary masses produced during mating to female body weight. Since female body weight in insects is known to determine fecundity, these males discriminate against low fecundity females since their own mating resources (amount of saliva) are strongly limited.

Effects of nutrition during the larval phase and during adulthood

While the results presented in chapter II clearly show effects of nutrition during adulthood on several parameters of male mating performance, in chapter III I present results from an experiment where I controlled food supply throughout the animals' entire life history. Therefore, it was possible not only to detect effects of food availability, but to distinguish between effects arising from the larval or adult phase. During the pre-mature phase of life history resources are thought to be invested in viability. Therefore, traits involved in mating should not be affected by food restriction, since investment in secondary sex traits that help to obtain mating partners should only start when these traits begin developing. In case of *P. vulgaris* saliva storage starts after adult animals have hatched. However, I found that the

amount of food animals could access as larva heavily affected several traits influencing male mating success including salivary gland development. In some cases the absence or presence of larval feeding history effects was determined by food availability during adulthood. Therefore, the results show that environmental conditions during larval development can affect characters which become important during mating and are subject to sexual selection. Concluding, successful food acquisition as larva is the key for male *P. vulgaris* to become competitive in reproduction and bears long time consequences for a male's reproductive success.

Summary

In my thesis I was concerned with ultimate as well as with proximate aspects of nuptial feeding in the scorpionfly *Panorpa vulgaris* (Mecoptera: Panorpidae). Chapter I begins with the attempt to verify the ultimate function of nuptial gifts in this species. Nuptial feeding is a common strategy shown by males of many insect species. These gifts presented during or after courtship and/or copulation are mostly considered to represent a form of mating effort. During copulations males of *P. vulgaris* produce salivary secretions which are then consumed by the females. Since females adjust copulation duration and thereby the number of received sperm to the number of salivary masses they receive from a male, the gifts' function as mating effort is unquestionable. Here I present data which indicate that nuptial feeding in *P. vulgaris* also represents paternal investment. Receiving a high number of salivary masses causes females to lay significantly more eggs compared to females receiving few or no salivary secretions. Thus, in *P. vulgaris* the nuptial gift increases the reproductive output of females and hence must not only be considered as mating effort but also as paternal investment. However, I cannot decide whether the increase in egg production is caused by an incorporation of additional nutrients or by allohormones.

In chapter II I leave the ultimate level to deal with proximate factors affecting salivary gland development and nuptial gift production during matings. In mating systems that are characterised by resource dependent male behaviour like nuptial feeding, food limitation obviously plays a major role in male performance. In *P. vulgaris* the ability to produce nuptial gifts, however, implies major fitness consequences, as the number of gifts decides about copulation duration. Since the number of transferred sperm increases with ongoing mating duration and sperm of different males are utilised according to the fair raffle principle, nuptial gift production determines male mating success. The results presented in chapter II show that males of *P. vulgaris* are limited in their production of salivary secretions. The number of saliva secretions males are able to produce declines in successive matings. Moreover, males of nutritionally high status produce more gifts than those of nutritionally low status. The degree of male mating effort corresponds to the size of the salivary gland, yet while absolute investment increases with gland size, the relative investment decreases. Thus, the marginal costs of saliva production are differential for males of different nutritional status. This result provides further evidence of the nuptial gift's function as a Zahavian quality indicator. Furthermore, I found evidence that males of low nutritional status seem to allocate their

mating effort strategically according to the female's fecundity. Therefore, cryptic male choice may exist in *P. vulgaris*, but only below a certain quality threshold of males.

In the last experiment, presented in chapter III, I manipulated food availability throughout the animals' entire life history. Here I am concerned with the question whether environmental conditions met during an early phase of life history that is dominated by the natural component of selection will affect traits and behaviour in a sexual selection context later on in life. I once again found strong effects of nutrition during adulthood on various fitness relevant traits (e.g. salivary gland development, saliva investment in copulations). But moreover, I am able to show that food availability during larval development affects male body weight, salivary gland weight before and after a copulation and the proportion of saliva resources that is invested in a mating. Therefore, larval feeding history implies considerable long time consequences and affects several traits that become important in a sexual selection context later on in male life history.

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