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Jari Juhani Ahtiainen

Condition-Dependence of Male Sexual
Signalling in the Drumming Wolf Spider
Hygrolycosa rubrofasciata



UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2003

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Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Ambiotica-rakennuksen salissa (YAA303)
marraskuun 21. päivänä 2003 kello 11.

Academic dissertation to be publicly discussed, by permission of
the the Faculty of Mathematics and Science of the University of Jyväskylä,
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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2003

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JYVÄSKYLÄ STUDIES BIOLOGICAL AND ENVIRONMENTAL SCIENCE 123

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JYVÄSKYLÄ 2003

ABSTRACT

Ahtiainen, Jari Juhani

Condition-dependence of male sexual signalling in the drumming wolf spider *Hygrolycosa rubrofasciata*

Jyväskylä: University of Jyväskylä, 2003, 31 p.

(Jyväskylä Studies in Biological and Environmental Science,

ISSN 1456-9701; 123)

ISBN 951-39-1645-6

Yhteenveto: Koiraan seksuaalisen signaloinnin kuntoriippuvuus rummuttavalla susihämähäkillä *Hygrolycosa rubrofasciata*

Diss.

The 'good genes' or 'handicap' model of sexual selection focuses on the evolution of female preferences for male sexual traits by using condition-dependence as a mediating mechanism. As the 'size' of a preferred sexual trait increases, male fitness increases in terms of mating success, but this is traded-off with viability which decreases as the size of a sexual trait increases. Males in good condition are better in bearing the viability costs of developing and maintaining a sexual trait than males in poor condition. Ultimately, females that base their choice on the size of a condition-dependent male sexual trait benefit in terms of increased offspring viability (i.e. 'good genes'). In the wolf spider *Hygrolycosa rubrofasciata*, males have a costly and condition-dependent acoustic signal, courtship drumming, which is the target of female choice. Males with higher drumming rate have considerably higher viability than males with lower drumming rate, and females that mate with the more actively drumming males gain genetic benefits in terms of increased offspring viability. Also, male mobility is positively associated with male mating success. In this thesis, I tested if higher immunocompetence level can partly explain the better viability of males with high sexual performance. I also investigated if males with high sexual activity can compensate costs of increased risk of predation. Further, I examined if fluctuating symmetry can be used as a short-cut measure of male quality. Finally, I studied if sexual selection for viable males has any population-level consequences. My results show that males with higher drumming rate had higher encapsulation rate than males with lower drumming rate, and males with higher mobility had higher lytic activity than males with lower mobility. I also found that males with increased investment in drumming rate had lower lytic enzyme activity than control males. However, males with increased investment in drumming rate had higher encapsulation rate than control males. This might be due to males investing their immune effort to components of the body where it is likely to be most useful. My results suggest that conspicuous signalling increases the risk of predation, but males with high sexual activity might be better in escaping from predation than males with low sexual activity. My results indicate that fluctuating asymmetry was negatively, but weakly related to male sexual performance at the population level. Finally, my results show that male survival and drumming rate were substantially reduced only in the smallest wolf spider populations. This suggests that the indirect benefits of female choice in terms of 'good genes' might cover the reduction in viability in small populations. The results of this thesis suggest that, in the drumming wolf spider *H. rubrofasciata*, sexual traits are related to several measures of individual quality through 'good genes', and that sexual selection may have significant consequences at the population level.

Key words: Araneae; developmental stability; fitness in small populations; honest signalling; *Hygrolycosa rubrofasciata*; immunity; Lycosidae; male quality; predator-prey interactions; sexual selection; wolf spider.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by Roman numerals I – V. I have personally written papers I, IV and V and performed most of the work. I have performed a significant proportion of the work in papers II and III.

- I Ahtiainen, J. J., Alatalo, R. V., Kortet, R. & Rantala, M. J. Sexual advertisement and immune function in an arachnid species (Lycosidae). *Behav. Ecol.*, in press.
- II Rantala, M. J., Ahtiainen, J. J., Alatalo, R. V. & Kortet, R. Does increased sexual signalling lead to the immune redistribution in a drumming wolf spider? Submitted.
- III Lindström, L., Ahtiainen, J. J., Alatalo, R. V., Kotiaho, J. S., Lyytinen, A. & Mappes, J. Predation costs of condition-dependent sexual signalling in a drumming wolf spider. Manuscript.
- IV Ahtiainen, J. J., Alatalo, R. V., Mappes, J. & Vertainen, L. 2003. Fluctuating asymmetry and sexual performance in the drumming wolf spider *Hygrolycosa rubrofasciata*. *Ann. Zool. Fennici* 40, 281-292.
- V Ahtiainen, J. J., Alatalo, R. V., Mappes, J. & Vertainen, L. Sexual behavior reveals reduced viability in small populations of the drumming wolf spider *Hygrolycosa rubrofasciata*. Submitted.

1 INTRODUCTION

1.1 Models of sexual selection

Although Charles Darwin pointed out the importance of sexual selection already in 1871 in his *The Descent of Man and Selection in Relation to Sex*, it took over a century for scientists to ultimately discover its power in explaining the diversity and function of secondary sexual characters. Sexual selection can be divided into two components: intra- and intersexual selection (Andersson 1994). Intrasexual selection relates to the interactions between individuals of the same sex, and it is manifested as, for example, weapons or status signals. In intersexual selection the choosier sex, usually the female, is choosing either resources or secondary sexual traits of the opposite sex. While resource-based female choice is easy to understand due to the direct effects on female reproductive success, indirect selection affecting through offspring performance has been debated.

There are three main ideas of intersexual selection, the 'sensory bias' hypothesis, the 'Fisherian self-reinforcing' hypothesis, and the 'good genes' hypothesis, that all focus on the evolution of sexual preferences for secondary sexual traits. The 'sensory bias' hypothesis argues that there are pre-existing sexual preferences ("sensory bias") for certain male secondary sexual traits that females inherit from their ancestors (Ryan 1985). The preferred secondary sexual traits will spread in the population by simply utilizing this pre-existing sensory bias. The 'Fisherian self-reinforcing' hypothesis is based on the idea that when females prefer males with higher life-time reproductive success, their preference genes will be united in their offspring with the male's genes for higher attractiveness (Kirkpatrick 1982, Pomiankowski & Iwasa 1993). In this way, both the female preference genes and the male attractiveness genes will then spread in the population. The preferred male secondary sexual traits can be neutral or even harmful for male viability, but these traits must be carried by males with higher fitness. According to the 'good genes' or 'handicap' hypothesis, females should prefer males displaying honest, costly secondary

sexual traits that signal genes for survival (Andersson 1986, 1994, Johnstone 1995). The 'good genes' hypothesis states that as the size of a secondary sexual trait increases, male fitness increases in terms of mating success, but this is traded-off with viability which decreases as ornament size increases. Males vary in their ability to withstand the costs of secondary sexual characters so that males in poor condition have lower optimum level of the ornament expression than males in good condition (but see Candolin 1999, 2000). Thus, males in good condition have better mating success and fitness, and females mating with them will enjoy increased viability for their offspring inherited from their fathers.

In literature, female choice for attractive males that sire more viable offspring has traditionally been interpreted as support for 'good genes'. Therefore, 'good genes' requires that there is heritable variation in sire viability that is mediated to offspring through sire attractiveness. If offspring do not survive better than their sire, but instead inherit the sire attractiveness, results are typically interpreted to favour the 'Fisherian' process. Recently, Kokko et al. (2002) have presented a general model of female choice for indirect benefits that unites the 'Fisherian' and 'good-genes' models. According to their model, a single process favours female choice for males who sire offspring of high reproductive values. The 'Fisherian' and 'good-genes' models are endpoints of a continuum, and the exact point in this continuum depends on the intensity of sexual selection (see also Kokko 2001, Kokko et al. 2003).

There are three conditions that must be met before a signal can be classified as an honest condition-dependent signal: (i) there must be within-individual repeatability and among-individual variability in the signal, which must have an effect on mate choice; (ii) there must be substantial costs of signalling in terms of increased mortality or reduced subsequent mating success; (iii) there must be condition-dependence of signalling in a way that there are differential costs between individuals in different condition (see Kotiaho 2001). Then, in a given signalling level individuals in poor condition pay higher costs than individuals in better condition.

1.2 Immune function and sexual selection (I and II)

The immunocompetence handicap hypothesis (ICHH) provides a mechanistic explanation for the costs of the ornamentation through the positive within-individual relationship between the size of a sexual trait and susceptibility to parasites (Folstad & Karter 1992). ICHH also proposes two different mechanisms by which parasites can specifically influence the development and maintenance of sexual traits. First, in cases where the acquisition of important metabolites for ornamental development (e.g. carotenoids) results in increased exposure to pathogens, ornaments may reveal an individual's ability to tolerate parasite exposure (Folstad et al. 1994). Second, because development and

maintenance of sexual traits may impose costs to immunity, they may honestly signal parasite resistance (Folstad & Karter 1992, see also Wedekind 1994). For example, if resources must be diverted away from immune function in order to maximise the expression of a trait, males may suffer increased susceptibility to pathogens (Folstad & Karter 1992, Sheldon & Verhulst 1996). Thus, only males with good condition or high immunocompetence, or both, can afford to allocate resources for large ornaments. By choosing males with larger sexual traits females may gain benefits either directly or indirectly. The size of the sexual trait may directly indicate parasite burden of the mate (e.g. Saino & Møller 1994, Hurst et al. 1995), or indirectly the heritable component of the ability to resist various pathogens (e.g. Møller 1990a, Kurtz & Sauer 1999, Johnsen et al. 2000).

1.3 Forms of immune function in invertebrates (I and II)

The term immunocompetence is often used to refer to the ability of the individual's immune system to resist and control pathogens and parasites (see Norris & Evans 2000). Encapsulation is a cellular immune response through which arthropods defend themselves against endoparasitoid wasps and flies (Salt 1970). During the encapsulation process, haemocytes recognize invading particles as non-self and cause other haemocytes to aggregate and form capsules around particles. A cascade of reactions involving the tyrosine-phenyloxidase pathway causes melanization of the capsule, which results in the death of the parasite by asphyxiation (Fisher 1963), or through the production of necrotising compounds (Nappi et al. 1995). The humoral immune system of invertebrates is comprised of a myriad of soluble proteins and enzyme cascades, which act in recognizing, signaling and attacking foreign targets (Leonard et al. 1985), and probably in coordinating cellular responses (Pech & Strand 1995).

1.4 Predation risk and sexual selection (III)

Natural and sexual selection together determine how strong a sexual signal could develop (Endler 1982, Andersson 1994). In other words, females prefer males with a stronger sexual signal, but at the same time the viability costs of producing a stronger sexual signal are likely to increase. This is due to a fact that sexual signals have, by definition, developed to make signalling individuals conspicuous. The more conspicuous the animal is, the more likely it is to become predated. Thus, selection by predation is considered to increase with enhanced sexual signalling (Zuk & Kolluru 1998). It is well documented that acoustic sexual signals are frequently exploited by predators (see

Magnhagen 1991, and references therein). Moreover, there is some empirical evidence that signalling males may alter their behaviour when predators are present (e.g. Candolin 1997, 1998, Hedrick 2000). Therefore, when predators are in vicinity, the prey's signalling modulations might compensate the viability costs of increased sexual signalling. In fact, prey individuals in good condition might be better in escaping from predators' attacks as condition is likely to influence the prey's vigilance and ability to escape (but see Candolin 1999). Many sexual signals are condition-dependent, where they have evolved to honestly indicate mate quality (review in Møller & Alatalo 1999). Therefore, the use of condition-dependent sexual signals can offer a convenient way to test the significance of mate quality on predation risk.

1.5 Fluctuating asymmetry and sexual selection (IV)

Fluctuating asymmetry (FA) of a bilateral trait is defined as subtle, random deviations from genetically controlled trajectory towards optimal phenotype, i.e. perfect symmetry (Van Valen 1962, see also Palmer & Strobeck 1986). FA has recently been proposed to indicate individual quality (review in Møller & Swaddle 1997, Møller & Thornhill 1998, Thornhill et al. 1999, but see Palmer 1999, Simmons et al. 1999). This proposal is based on the assumption that FA is a sensitive measure of environmental and genetic stress, and has led to the prediction that FA covaries negatively with condition (Møller 1990b, Møller & Pomiankowski 1993, Watson & Thornhill 1994). It also has been predicted that there is condition-dependence in the expression of sexual traits: Individuals in good condition may be better able to bear costs of sexual traits, and therefore may produce larger sexual traits than individuals in poor condition (review in Jennions et al. 2001, Kotiaho 2001). Therefore, through condition dependence of both the size of the sexual trait and FA, size may be negatively related to FA at the population level.

1.6 Population viability and sexual selection (V)

Sexual selection can have many contradictory effects on population viability (see Kokko & Brooks 2003). Intense sexual selection may increase the risk of population extinction by increasing costs of sexual traits (Tanaka 1996, Møller 2000, 2003 and references therein, Houle & Kondrashov 2002). For example, introduction probability to islands has been shown to be considerably smaller in dichromatic than in monochromatic bird species (McLain et al. 1995, Sorci et al. 1998). Plumage dichromatism is supposed to have evolved under sexual selection pressures, which probably makes dichromatic species more vulnerable to different extinction risks than monochromatic species. Sexual selection can

also increase the probability of population extinction through effects on population structure. For example, sexual selection can generate mating skew, which may reduce effective population sizes and increase the loss of genetic diversity in small and isolated populations (Anthony & Blumstein 2000, Gosling & Sutherland 2000, see also Westemeier et al. 1998, Höglund et al. 2002). However, sexual selection can also have positive effects on viability in small populations. First, a theoretical model predicts that, in small populations, sexual selection may act to reduce the fixation probability of deleterious new mutations and increase the probability of fixing new beneficial mutations (Whitlock 2000). Second, females may avoid costs of inbreeding by multiple mating (Tregenza & Wedell 2002), or by avoiding mating with inbred males (e.g. Reusch et al. 2002). Costly male sexual traits are good candidates for indicators of population viability, since they may be linked to viability through condition-dependence (see Møller 2000). Moreover, condition-dependent sexual traits may be sensitive indicators of genetic and environmental fluctuations. These fluctuations are likely to be more frequent and stronger in small populations.

1.7 Aims of the study

In this thesis, I studied whether immune function, predation risk, fluctuating asymmetry (FA), and population viability are related to the size of two sexual traits (drumming rate and mobility) in the drumming wolf spider *Hygrolycosa rubrofasciata*. In study I, I investigated the relationship between male sexual advertisement and immune function by using encapsulation rate and lytic activity as measures of immune function. In study II, I experimentally tested whether increased investment in costly drumming rate is manifested in reduced immune function (encapsulation rate and lytic activity) of male wolf spiders. I induced males to increase their drumming rate by presenting females in proximity. In study III, I investigated whether an audible sexual signal, drumming rate, is costly in terms of increased predation risk, and whether male sexual performance affects the male's ability to escape predator attacks. In study IV, I examined with a large sample size whether pedipalp FA could be used as a short-cut measure of male quality. This is done by estimating how strongly FA correlates with male sexual performance. In study V, I examined the effect of population size on male drumming rate, mobility and survival in 39 natural populations of *H. rubrofasciata*.

2 STUDY SPECIES

Hygrolycosa rubrofasciata is a ground-dwelling wolf spider (Araneae: Lycosidae), which can be found in patchily located populations widely distributed over northern Europe. It is found along coastal regions as well as inland in south-eastern and northern parts of Finland. Gene flow between populations of *H. rubrofasciata*, even those located within a few hundred meters from each other, is likely to be highly restricted, as there is adaptive genetic differences between populations in a very small geographical scale (Vertainen et al. 2000). *H. rubrofasciata* inhabits two kinds of habitats: abandoned fields and other meadow habitats and half-open bogs with deciduous trees (*Betula* spp., *Salix* spp.). These two habitats differ from each other in many ecological characters, e.g. in soil moisture, pH, vegetation, soil fauna, and soil litter thickness. In my thesis, I collected spiders only from half-open bogs. *H. rubrofasciata* seems to have rather specific habitat requirements in half-open bogs, which include *Sphagnum*-mosses and two grasses (*Carex* spp., *Eriophorum vaginatum*). Although there may be tens of *Hygrolycosa rubrofasciata* in a square meter in the high-density habitat, they are not readily visible, as they are used to hide under leaf litter.

In southern Finland, most matings of *H. rubrofasciata* take place during sunny days immediately after the snow has melted in late April or early May. Male wolf spiders of *H. rubrofasciata* (Araneae: Lycosidae) (Ohlert 1865) have a drumming signal that is used for sexual communication (Kronstedt 1996). During the mating season, males drum while wandering around the habitat searching for receptive females, which are more stationary than males (unpublished data). Males produce drumming signals by hitting their abdomen on dry leaves or other suitable substrate to court females. One drumming consists of ca. 30-40 separate pulses, lasts ca. 1 second (Rivero et al. 2000), and is audible to the human ear up to a distance of several meters. Both the mark-recapture data and direct observations in the field and laboratory have revealed that males of *H. rubrofasciata* are not distributed randomly among the habitat: fewer males are found in areas that have high sedge cover and low elevation, and males spend more time on dry leaf substrate than on other substrates (Kotiaho et al. 2000). Also, drumming rate in the field is positively correlated

with dry leaf cover, and males clearly prefer dry leaves as a drumming substrate. Kotiaho et al. (2000) have also shown that male drumming rate and mobility are positively correlated with temperature. Therefore, males may be sexually selected to optimize their signalling habitat by active microhabitat choice.

In *H. rubrofasciata*, male courtship drumming has demonstrated to be an honest indicator of heritable viability for choosy females (review in Ahtiainen et al. 2001). Females prefer more actively drumming males as mating partners (Kotiaho et al. 1996). There is considerable within-male repeatability and among-male variability in drumming rate (Kotiaho et al. 1996). Male drumming incurs both physiological (Mappes et al. 1996, Kotiaho et al. 1998a) and predation costs (Kotiaho et al. 1998b), that eventually cause the death of all males in the end of the mating season (unpublished data). The more actively drumming males have better viability (Kotiaho et al. 1996, 1999, Mappes et al. 1996). Survival costs of male drumming are condition-dependent, being manifested in decreased viability of males in poorer condition (Mappes et al. 1996, Kotiaho 2000). By choosing males with the highest drumming rates females benefit through better offspring survival (Alatalo et al. 1998). Also, male mobility is positively associated with male mating success (Kotiaho et al. 1998b). Body mass is one of the most common sexually selected male traits among animal taxa (Andersson 1994). However, male body mass does not seem to be intersexually selected, and is not correlated with male mating success in *H. rubrofasciata* (Kotiaho et al. 1996, Mappes et al. 1996, Vertainen et al. 2000).

Shortly before and during the emittance of a drumming produced by tapping the abdomen against the substrate, males of *H. rubrofasciata* also perform oscillations of the pedipalps. The tips of pedipalps are in contact with the drumming substrate producing a weak humming sound just prior to a drumming and a weak rattling sound at the end. Spider pedipalps are used in food handling, but they also function as sexual organs in males. Male pedipalps do not actually produce the sperm, but, instead, they are used to transfer the sperm to the female, via the epigyne, for storage in the spermathecae prior to fertilization (Roberts 1996). Each pedipalp consists of six segments (beginning from the body): coxa, trochanter, femur, patella, tibia, and tarsus.

When a male encounters a female he stops and drums several times. If the female is willing to copulate with the male, she responds by vibrating her body. This response is given immediately after a male drum, and it is clearly visible like male drummings, although female percussions are usually not sufficiently intense to produce audible sounds. After an initial female response, the male and the female, while approaching each other, drum several times before the copulation begins (the so-called duetting; Kronstedt 1996). In *H. rubrofasciata*, males are polygynous, whereas females seem to copulate only once (personal observation). The major advantage in this species is that male drumming rate can be manipulated so that it minimizes the risk of manipulation itself having side effects (Mappes et al. 1996). This can be accomplished by not manipulating males themselves, but manipulating only their environment; when males are

placed in the vicinity of a virgin female they spontaneously increase their drumming rate. However, males of *H. rubrofasciata* produce drumming signals even without the presence of females.

Mated females of *H. rubrofasciata* produce an egg sac, which they carry attached to their spinnerets (silk glands in their abdomen). After approximately three to four weeks the offspring will emerge from the egg sac. The offspring usually remain on the female's abdomen or on top of the empty egg sac for a day to chitinise their exoskeleton, after which they disperse. The development of *H. rubrofasciata* to maturation typically lasts 2-3 years in Southern Finland depending on the prevailing environmental conditions. Male spiderlings resemble cryptic brownish female spiderlings, and only after the final moult does the typical blackish appearance of males emerge. The final moult of maturing individuals happens in autumn preceding the mating season in the following spring. Each adult male cohort reproduces only during one mating season, and the males die during or immediately after it. However, Alatalo et al. (unpublished data) have observed individually marked females to survive until the next mating season.

3 MATERIALS AND METHODS

Hygrolycosa rubrofasciata can easily be collected alive by pitfall traps and by hand picking. Unmated virgin females can be collected by hand picking while there is still some snow on the ground. In studies I-V, I collected *H. rubrofasciata* males from Sipoo, Southern Finland (60°16'N and 25°14'E) at the beginning of the mating season. I collected males in studies I, II and III and virgin females in study II from a single bog, which is inhabited by a large *H. rubrofasciata* population. In study IV, I collected males from 36 different-sized populations. In study V, I collected males from 39 different-sized populations. After collection, I placed spiders individually into small plastic containers (film jars) with some moist moss (*Sphagnum* spp.) in cool temperature (ca. +10 °C) until I brought them into the laboratory. Before experiments, I prevented spiders from reaching the active sexual phase by keeping them in cool temperature (+5 ± 2 °C). In the laboratory, I weighed spiders to the nearest 0.1 mg with an analytical balance. Then, I fed spiders with fruit flies (*Drosophila* spp.) *ad libitum* and kept them in moistened film jars filled with some moss (*Sphagnum* spp.) in cool temperature (+5 ± 2 °C) in darkness.

3.1 Measurement of male sexual activity (I-V)

To measure male sexual activity (drumming rate and mobility) in studies I-V, I placed *H. rubrofasciata* males individually in plastic arenas (125 mm × 88 mm × 110 mm high) in the laboratory. I covered the bottom of plastic arenas with a piece of white paper (8 cm × 4 cm) glued to the bottom. For mobility measurements, I divided each arena with a line into two equal rectangles. I placed two dry even-sized birch leaves in rectangles as drumming substrates. I illuminated the laboratory with fluorescent tubes and lamps with 40 W bulbs placed 30 cm above the floors of drumming arenas to give extra heat and light. On the day before the onset of behavioral measurements, I kept males at the laboratory temperature (31 ± 1°C) for two hours to trigger their sexual activity.

I measured drumming rate as the number of separate drumming bouts, and mobility as the number of times the male crossed the line between the rectangles. In total, I measured drumming rate and mobility five times for 2 min, and repeated this procedure on consecutive days. Between the trials, I fed males with fruit flies (*D. melanogaster*), and kept them in moistened film jars filled with some moss (*Sphagnum* spp.) at a cool temperature ($+5 \pm 2^\circ\text{C}$).

3.2 Measurement of male immune function (I and II)

In arthropods, one of the most informative ways to assay immunocompetence is to measure the magnitude of encapsulation response to a novel and standardized antigen, such as nylon monofilament (e.g. Köning & Schmid-Hempel 1995, Siva-Jothy et al. 1998, Rantala et al. 2000, 2002). In studies I and II, I inserted a single sterile 1 mm long piece of nylon monofilament (diameter 0.08 ± 0.001 mm) into the spider's haemocoel. After that, I allowed the spiders' immune system to encapsulate a microfilament for several hours. Then, I removed and photographed the implant. As a measure of the individual's encapsulation rate, I used the average grey value calculated from three video pictures. A common monitoring technique in immunoecology is to quantify lytic activity over time, which measures the status of the humoral immune system (see Ellis 1990, Rantala & Kortet 2002). In studies I and II, I pipetted a 0.5 μl haemolymph sample from a sterile puncture made on the abdomen membrane between the epigastric furrow and the spiracle. Then, I assayed the lysozyme activity of the haemolymph turbidometrically (see Ellis 1990, Rantala & Kortet 2003). Lytic activity was expressed as the change in optical density of a sample in the 10 minutes interval.

3.3 Measurement of predation costs (III)

To use as predators in study III, I captured migratory pied flycatchers (*Ficedula hypoleuca*) from the forests around Konnevesi Research Station at the time they arrived to Finland before the nesting period. These pied flycatchers were naïve predators, as there are no wolf spider populations, to my knowledge, in the nesting area from where I captured the birds. I kept birds individually in illuminated cages (65 cm \times 80 cm \times 65 cm high) on a diet that contained mealworms (*Tenebrio molitor*) *ad libitum*. After the experiment, I released birds at the same location they were captured from.

3.3.1 Cost experiment

To measure the predation cost of the drumming, I placed two containers ($\varnothing = 30$ cm), both containing a loudspeaker, in the aviary. I placed a piece of styrofoam on top of the loudspeakers to silence the spiders' own drumming. One loudspeaker was drumming three times per minute (high rate) and the other was drumming one time per minute. I gave birds a choice between two spiders, one spider in each container, with similar sexual performance (either "high sexual performance" or "low sexual performance"). I allowed birds to eat only one spider. I observed each predator-prey encounter through a one-way mirror, and I also shot each encounter from top of the containers with digital video cameras. After a week I repeated the experiment, but at that time I gave birds that experienced first "high" treatment the "low" treatment and vice versa.

3.3.2 Escape ability experiment

In the second experiment, I simultaneously presented three pairs of spiders for each bird, three males with high sexual performance and three males with low sexual performance. I placed spiders individually in six containers ($\varnothing = 23$ cm) that were placed in a circle around a perch in the same aviary as described above. I covered each container with brown paper and six dry birch leaves. I allowed birds to eat five spiders from the containers, thus enabling me to rank the order the birds took the spiders.

3.4 Measurement of fluctuating asymmetry (FA) (IV)

I used a stereomicroscope (Wild M5A) equipped with an ocular micrometer to measure the length between femur and tarsus to the nearest 0.01 mm from each pedipalp. I measured each pedipalp twice from all individuals. First, I measured the left-hand side pedipalps (L) once, followed by the measurements of the right-hand side pedipalps (R) in a random order without reference to the measurements of the left-hand side pedipalps (see Palmer & Strobeck 1986). I re-measured the same individuals with exactly the same procedure, and in a random order without reference to the first measurements. Pedipalp FA was defined as the mean unsigned length difference between left- and right-hand side pedipalps.

3.5 Measurement of male survival (V)

Following the sexual activity experiment in study V, I conducted the survival experiment for *H. rubrofasciata* males. I placed males individually in plastic vials (diameter 28 mm, height 67 mm) at $+22 \pm 1^\circ\text{C}$ with continuous light and moisture. I did not provide any food for spiders. I recorded male survival daily, and I used the number of days a male survived from the beginning of the experiment as the estimate of male survival.

4 RESULTS AND DISCUSSION

4.1 Immune function and sexual selection (I and II)

In study I, encapsulation rate was higher among males with higher drumming rates. My results indicate that females might use drumming rate also as a reliable signal for choosing males with good immunocompetence. My results also showed that males with higher mobility had higher lytic activity. As females are more likely to encounter those males that have higher mobility, this also selects males with better immune function. Encapsulation rate was partly explained by pre-manipulation time (i.e. the time animals were in warm temperature before implantation), but the partial effect of drumming rate remained significant. My results suggest that the immunocompetence handicap might work also among spiders.

In study II, treatment (i.e. introduction of a virgin female into vicinity of a male) had a strong positive effect on male drumming rate: male spiders exposed to females drummed over 7.5 times more than control males. However, treatment did not have any effect on male mobility or male mortality. Males in treatment group had lower lytic enzyme activity in their hemolymph than males in control group. Surprisingly, males in treatment group had higher encapsulation rate than control males.

In study II, I found that increased investment in drumming rate reduced lytic activity of haemolymph in male wolf spiders. This is consistent with a study in *Drosophila melanogaster* where increased sexual activity reduced male ability to clear a bacterial infection (McKean & Nunney 2001). In insects, the fat body synthesises antimicrobial peptides (e.g. Hetru et al. 1998). Thus, increased investment in drumming rate might reduce the fat body of male wolf spiders reducing males' ability to synthesise lytic enzyme.

What was surprising was the finding that increased investment in drumming rate increased male encapsulation rate. One adaptive explanation to elevation of encapsulation rate as a result of increased investment in drumming rate might be the increased vulnerability to parasitoids. Encapsulation response

is known to be the main defense against parasitoids (e.g. Gillespie et al. 1997). Selection could have favored males which both have high drumming rate and high encapsulation rate. I have empirical evidence that over 10 percent of the *H. rubrofasciata* population can be parasitized by parasitoids and about 1 percent can be parasitized by a parasitic fly (unpublished data). Therefore, natural selection could have favored those males which are able to shift immune function to components of the body where it is likely to be most useful.

4.2 Predation risk and sexual selection (III)

4.2.1 Cost experiment

In study III, the naïve birds did not show any preference difference between differently drumming males, indicating that high drumming rate did not increase the predation cost against naïve predators. However, when I repeated the same experiment with the same birds after a week, 72 % of the experienced birds attacked the more frequently drumming males. This result indicates that *Ficedula hypoleuca* learned to associate the audible sexual signal, drumming, to the spider, and increased the attack rate towards the more frequently signalling prey. In the field, sexually most active males are likely to gain most of the matings (see Ahtiainen et al. 2001). Therefore, in *H. rubrofasciata* increased sexual signalling rate benefit males through sexual selection, but at the same time natural selection seems to provoke direct balancing costs to the same trait (see also Kotiaho et al. 1998a). There is empirical evidence that, when males vary in their susceptibility to predators, predation risk may affect the mating success of competing males (Candolin 1997).

4.2.2 Escape ability experiment

As their first choice, the naïve birds attacked equally against males with low and high sexual performance. There was no correlation between the order of attack and male quality. Drumming rate measured before the experiment did not have any effect on the likelihood to escape the first predation attempt. However, there was a tendency that males with higher mobility were better in escaping from predation than males with lower mobility. Previously, Kotiaho et al. (1998a) have found that *H. rubrofasciata* males with high moving activity had significantly higher risk of being predated by a lizard than males moving less actively. Why lizards ate more likely the most actively moving *H. rubrofasciata* males, while pied flycatchers did not? One reason for these seemingly contradictory results might be that lizards predate only moving prey, thereby making them a different type of predator than pied flycatchers. Kotiaho et al. (1998a) introduced lizards to the same arena as spiders. Those spiders with high mobility were likely to be most conspicuous and therefore most easily being

predated by lizards. Also, lizards were collected from the same area as *H. rubrofasciata* males (Kotiaho et al. (1998a), which could have made them experienced wolf spider predators *a priori*. In my study, pied flycatchers were naïve predators, as I do not know any wolf spider population in the nesting area from where I captured the birds.

4.3 FA and sexual selection (IV)

In study IV, my results showed that there was only a very weak negative relationship between pedipalp FA and mobility. Because pedipalp FA explains only 0.7 % of the total variance in mobility, it is an unreliable measure of individual quality in *H. rubrofasciata*. My estimate of $r_k = -0.086$ (Kendall's partial correlation) is much smaller than the weighted mean effect size of $r = -0.24$ between single trait's FA and male mating success (in nonhuman species excluding all sexually selected traits, experimental studies and composite scores; total estimates = 54) computed by Møller & Thornhill (1998) in their meta-analysis (see also Palmer 1999), and the overall effect size of $r = -0.26$ between FA and several fitness measures computed independently by Leung & Forbes (1996). There was no significant relationship between unsigned pedipalp FA and male drumming rate. There were 17 specimens (2.1 % of the pedipalp FA data) that were classified as extreme outliers (i.e. > 3 interquartile ranges from the 75 % upper quartile). Outliers differed highly significantly from other specimens with respect to drumming rate and mobility. Extreme outliers of FA were individuals with poor sexual performance. These extreme outliers can be considered as biologically meaningful, suggesting that FA can be used as an indicator of phenodeviants (see Clarke 1993).

4.4 Population viability and sexual selection (V)

In study V, male survival, drumming rate and mobility increased in a non-linear fashion with increasing population size. Population size had a significant effect on survival and drumming rate, indicating that males in larger populations (≥ 10 males) had higher survival and drumming rate than males in the smallest populations (< 10 males). Populations did not differ in mobility. My results show that male survival was decreased only in the smallest populations (< 10 males). In *H. rubrofasciata*, females prefer males with higher than lower drumming rates as mating partners (review in Ahtiainen et al. 2001). By choosing males with the highest drumming rates, females benefit genetically through better offspring viability (i.e. 'good genes') (Alatalo et al 1998). Therefore, the indirect benefits of female choice in terms of good genes might level out the assumed viability differences in larger populations of *H.*

rubrofasciata (≥ 10 males). This suggests that, apart from in the smallest populations, sexual selection might actually compensate for the reduction in viability in small populations. As pointed out by Møller (2003), rather than choosing good genes, females can actually avoid bad genes by basing the information they gather on honest male signals. This can be particularly true in small and inbred populations, where the risk of accumulation of deleterious mutations is high (but see Whitlock 2000).

5 CONCLUSIONS

For several years, sexual selection has been one of the most extensively studied fields in evolutionary ecology (review in Andersson 1994). This research has been heavily focused on female mate choice due to the strikingly diverse forms of male sexual traits. Recently however, there has also been much interest in discovering different correlates of male mating success as short-cut measures of individual quality. In this thesis, I used several measures of individual quality, such as immune function (encapsulation rate and lytic activity), the ability to escape from predation, fluctuating asymmetry (FA), and viability in different-sized populations. I correlated these measures with two estimates of male mating success in wolf spider males *H. rubrofasciata*, i.e. drumming rate and mobility. In previous studies, male courtship drumming has demonstrated to be condition-dependent viability indicator for choosy females (see Ahtiainen et al. 2001). Male mobility is also positively related to male mating success.

In this thesis, I found that different measures of male quality, in overall, seem to follow condition-dependence. However, the relationships between estimates of male quality and mating success varied from null to moderate in magnitude, and one relationship (II) changed the sign from that expected by the initial 'good genes' models (but see Kokko 2001). Thus, when studying sexual selection, it is of utmost importance to use several measures of individual quality for understanding the different benefits and costs related to sexual traits.

Finally, sexual selection may have important consequences at the population level. The effects of sexual selection in small populations have been poorly studied, and thus theoretical expectations are somewhat speculative. The strong mating skew might critically reduce the effective population sizes causing inbreeding. However, my results suggest that strongly condition dependent sexual signals might even enhance the viability of small populations. Therefore, sexual traits can offer a new sensitive way of estimating population persistence and viability against a global background of increasing habitat loss.

Acknowledgements

I have had an honour to work several years at the Department of Biological and Environmental Science in the University of Jyväskylä. First, I like to address my gratitude to my supervisors, Rauno Alatalo and Johanna Mappes. Without them, I would be still desperately collecting spiders, filling applications for grants, trying to learn to write science, or planning still another experiment to my book. They have played a key role in making this all possible. Rauno and Jonna, we have come a long way from 1998, when I started my Master of Science. I was feeling like a schoolboy, when I made my maiden voyage to spider bogs in Sipoo. Day by day, you changed my way of thinking towards more science orientated. This growth process has sometimes been quite painful, but in the end I have never felt more confident and serene than today. Three characteristics over all others have been dominating in this “mission impossible” –project: Perseverance, ambition and endless patience. Under your progressive supervision, I have learned the importance of those words in science and life in general.

Above all, I want to point my gratitude to my loved one, Sanna. She has been in on once-in-a-life-time experience, showing exceptional understanding and support during my PhD-project. She has taught me that there are, in the end, more important things in life than working.

Obviously, this project would never have seen its end without efforts of many people, who have been helping us collecting, measuring, and observing spiders in the field and lab. Especially, (in alphabetical order) Ville Haahkala, Marko Haapakoski, Panu Halme, Antti Hannuniemi, Sonja Koistinen, Marjo Laurikainen, Jaana Suutari, Katja Tynkkynen and Hertta Yliniemi have been of enormous help. Konnevesi Research Station and the staff there, especially Helinä Nisu, provided irreplaceable help in drumming experiments. We have also been working in the facilities of Experimental Animal Unit of our Department, which deserves special thanks.

Next, I want to thank Laura Vertainen and Silja Parri, who have been doing their own theses during my PhD-project. They have been like tutors for me, showing how things work in our spicy spider team. I also want to acknowledge my colleagues in the Department, especially (in alphabetical order) Janne Kilpimaa, Raine Kortet, Janne Kotiaho, Tomi Kumpulainen, Seppo Kuukasjärvi, Leena Lindström, Tuula Oksanen, Tanja Puttonen, Mikael Puurtinen, Markus Rantala, and Heli Siitari. You have taken part with me in several unforgettable congress tours, graduate courses, and unofficial happenings. Some of you are even co-authors in this thesis, underlining the significance of co-operation in PhD-work.

Last but not least, I want to express thanks to my mom, who, as a single parent, has made a rearing work of at least two parents. I owe special gratitude also to my relatives and friends, who have been together in the same boat, sharing this wonderful time with me.

YHTEENVETO

Koiraan seksuaalisen signaloinnin kuntoriippuvuus rummuttavalla susihämähäkillä *Hygrolycosa rubrofasciata*

Väitöskirjatyössäni tutkin koiraiden seksuaalisen signaloinnin kuntoriippuvuutta käyttämällä kuntoindikaattoreina koiraiden immuunipuolustuskykyä, saaliiksi jäämisalttiutta, vaihtelevaa epäsymmetriaa ja populaation elinkykyä. Työssä keskityttiin koiraan tuottamaan kosintääneen ja koiraan liikkumiskykyyn *Hygrolycosa rubrofasciata* hämähäkillä. Rummuttava susihämähäkki *H. rubrofasciata* on puoliavoimilla lehtipuuvaltaisilla soilla, niityillä ja pakettipelloilla esiintyvä juoksuhämähäkkilaji (Araneae: Lycosidae). Laji esiintyy Suomessa paikallisena etenkin rannikolla sekä myös sisämaassa, etenkin Itä- ja Pohjois-Suomessa. Lakin kosintamenoihin liittyy olennaisena osana rummutus, jota koiraat tuottavat rummuttamalla takaruumiillaan kuivia lehtiä tai muuta vastaavaa alustaa. Ääni on myös ihmiskorvin kuultavaa, koostuen noin sekunnin kestävästä pärähdyksestä. Lisääntymiskaudella, joka ajoittuu huhtikuun ja kesäkuun välille Etelä-Suomessa, rummuttavat *H. rubrofasciata* koiraat vaeltelevat tupasvillamättäältä toiselle houkutellen naaraita paritteluun.

Aikaisemmissa tutkimuksissa on havaittu, että naaraat valitsevat parittelukumppaninsa tämän rummutusäänen ominaisuuksien perusteella. Erityisesti rummutussignaalin pituus, äänen voimakkuus ja rummutusaktiivisuus aikayksikköä kohden ovat naaraiden suosimia ominaisuuksia. Koiraan liikkumiskyvyllä on myös havaittu olevan merkitystä koiraan lisääntymismenestykseen: mitä enemmän koiras liikkuu lisääntymiskauden aikana, sitä enemmän se kohtaa parittelemaan halukkaita naaraita. Koiraan rummutusaktiivisuus on kuntoriippuvainen siten, että mitä huonommassa ravintolanteessa koiras on, sitä vähemmän se rummuttaa. Lisäksi aiemmissa tutkimuksissa on kokeellisesti osoitettu koiraan kunnan vaikuttavan siihen, kuinka hyvin koiras säilyy hengissä rummutuksesta: mitä paremmassa kunnossa koiras on, sitä suuremmalla todennäköisyydellä se säilyy hengissä rummutuksesta aiheutuvista kustannuksista

Ensimmäisessä osatutkimuksessa havaitsin koiraan rummutusaktiivisuuden korreloivan positiivisesti koiraan enkapsulaationopeuden kanssa. Enkapsulaatio on tärkeä immunologinen puolustusmekanismi parasitoideja vastaan, missä hemosyytit eristävät tunkeutuvan pieneliön muodostamalla kapselin sen ympärille. Lisäksi havaitsin koiraan liikkumiskyvyn korreloivan positiivisesti koiraan ruumiinnesteen lyyttisen aktiivisuuden kanssa. Selkärangattomien ruumiinnesteessä on vesiliukoisia proteiineja ja entsyymijärjestelmiä, jotka hyökkäävät erilaisia mikrobiologisia tunkeilijoita vastaan.

Toisessa osatutkimuksessa tutkin, aiheuttaako koiraan lisääntynyt rummutusaktiivisuus immunologisia vasteita. Tässä työssä havaitsin, että koiraan ruumiinnesteen lyyttinen aktiivisuus pieneni niillä koirilla, joiden rummutusaktiivisuutta olin luonnonmukaisesti lisännyt. Yllättävästi vastoin

yleisiä odotuksia havaitsin, että koiran enkapsulaationopeus kasvoi niillä koirilla, joiden rummutusaktiivisuutta olin luonnonmukaisesti lisännyt. Nämä erot immunologiassa vasteissa saattavat johtua lisääntymisiässä olevan koiraan erilaisesta panostuksesta erilaisiin immunologisiin puolustusmekanismeihin.

Kolmannessa osatutkimuksessa käytin kirjosioppoa (*Ficedula hypoleuca*) *H. rubrofasciata*n saalistajana ja vertasin eri aktiivisuuksilla rummuttavien koiraiden saaliiksi jäämisalttiutta. Lisäksi selvitin, olisiko paremmassa seksuaalisessa kunnossa olevilla koirilla paremmat mahdollisuudet paeta kirjosioppojen saalistusyrityksiltä. Kokeissa havaittiin, että enemmän rummuttavilla koirilla oli suurempi riski joutua kirjosiopon saaliiksi. Toisaalta seksuaalisesti aktiivisemmilla koirilla oli paremmat mahdollisuudet paeta kirjosioppojen saalistusyrityksiltä, kun hämähäkeille annettiin mahdollisuus paeta lehtien alle.

Neljännessä osatutkimuksessa selvitin, voitaisiinko vaihtelevaa epäsymmetriaa käyttää koiraan seksuaalisen kunnan indikaattorina. Yleisesti luonnossa esiintyvällä vaihtelevalla epäsymmetrialla tarkoitetaan esimerkiksi raajaparin pituuksien erotusta, jossa ihanteellinen arvo on symmetria. Tässä tutkimuksessa havaitsin koiraan liikkumiskyvyn korreloivan odotusten mukaan negatiivisesti, mutta heikosti vaihtelevan epäsymmetrian kanssa. Koska havaittu yhteys oli pieni, voidaan todeta, ettei vaihteleva epäsymmetria ole luotettava kuntoindikaattori tällä lajilla. Tätä johtopäätöstä tulee myös toinen tämän tutkimuksen tulos, jossa en havainnut korrelaatiota koiraan rummutusaktiivisuuden ja vaihtelevan epäsymmetrian välillä.

Viidennessä osatutkimuksessa vertailin yhteensä 39:n *H. rubrofasciata*n populaation koiraiden seksuaalista kuntoa ja elossa säilyvyyttä. Nämä 39 populaatiota jakaantuivat yksilömäärältään pieniin, keskisuuriin ja isoihin populaatioihin. Tässä tutkimuksessa havaitsin, että koiraiden seksuaalinen kunto ja elossa säilyvyys olivat pienentyneet ainoastaan alle kymmenen koiraan populaatioissa. Tämä viittaa siihen, että naaraanvalinnasta koituvat epäsuorat edut jälkeläisten lisääntyneen elinkyvyn muodossa saattaisivat pienentää sisäsiittoisuudesta aiheutuvia ongelmia pienissä populaatioissa.

Väitöskirjatyön osatutkimusten perusteella voidaankin todeta, että *Hygrolycosa rubrofasciata*n koiraiden seksuaalinen signaali näyttää olevan kuntoriippuvainen. Tätä ominaisuutta *H. rubrofasciata* naaraat voivat käyttää mittana arvioidessaan koiraan fenotyypistä ja geneettistä laatua. Näyttäisi myös siltä, että seksuaalivalinnalla on merkittäviä populaatiotason vaikutuksia. Tämän väitöskirjatyön perusteella eläinten seksuaaliominaisuudet voivatkin tulevaisuudessa tarjota uusia keinoja populaatioiden sukupuuttouhan ja elinkyvyn arvioimiseen.

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