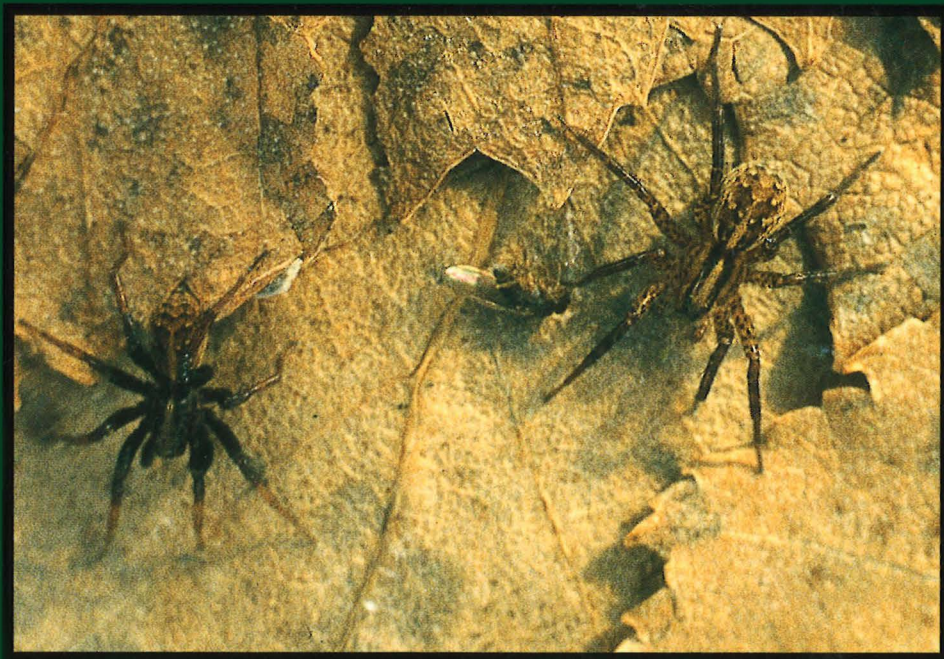


Laura Vertainen

Variation in Life-History Traits and
Behaviour among Wolf Spider
(*Hygrolycosa rubrofasciata*) Populations



UNIVERSITY OF JYVÄSKYLÄ

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ABSTRACT

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Variation in Life-history Traits and Behaviour among Wolf Spider (*Hygrolycosa rubrofasciata*) Populations

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Yhteenveto: Populaatioiden väliset erot rummuttavan hämähäkin (*Hygrolycosa rubrofasciata*) kasvussa ja käyttäytymisessä
Diss.

Differences in growth and reproduction related traits between closely located populations were studied in the drumming *Hygrolycosa rubrofasciata* wolf spider. Adult body mass varied greatly among populations and years. The variation was partly explained by environmental factors, and there were genetic differences among populations in body mass. It is probable that they reflect adaptive responses to habitat differences. Males and females had divergent growth strategies when reared on different feeding levels. In laboratory, females tried to attain as large body size as possible, while growth of males among populations responded differently to the amount of food and there was no clear tendency to grow as much as possible. Males from different habitats had variable mating tactics, i.e. males from bogs and meadows allocated differently on sexual signalling and mate searching. Females had no preference between the drumming signals of males from the own and strange habitats. Drumming activity was higher in large bog populations, and the pulse rate of the drumming signal correlated strongly with population size. The linear relationship between male pulse rate and population size may indicate weaker selection on drumming signal characters in smaller populations due to the lower encounter rate with females or males. Also population density and female presence seems to affect male allocation between components of mating effort. In sum, there appears to be considerable variation in growth and sexual courtship behaviour between the different habitats and populations, and part of this variation seems to be adaptive even if the distances between populations are in the scale of a few kilometres or less.

Key words: Drumming signal; growth; *Hygrolycosa rubrofasciata*; Lycosidae; male-male interaction; male mating tactics; reaction norms; reproduction.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-V. I am the main writer in all papers and I have performed large part of the work in each paper.

- I Vertainen, L., Ahtiainen, J. J., Alatalo, R. V., Mappes, J. & Parri, S. 2001. Variation in reproductive traits among populations of the *Hygrolycosa rubrofasciata* wolf spider. Manuscript.
- II Vertainen, L., Alatalo, R. V., Mappes, J. & Parri, S. 2001. Sexual differences in growth strategies of the wolf spider *Hygrolycosa rubrofasciata*. *Evolutionary Ecology*, in press.
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- III Vertainen, L., Ahtiainen, J. J., Alatalo, R. V., Mappes, J. & Parri, S. 2001. Habitat differences in mate searching behaviour in the drumming wolf spider *Hygrolycosa rubrofasciata*. Manuscript.
- IV Vertainen, L., Ahtiainen, J. J., Alatalo, R. V., Mappes, J. & Parri, S. 2001. Inter-population variation in sexually selected characters in the drumming wolf spider *Hygrolycosa rubrofasciata*.
- V Vertainen, L., Ahtiainen, J. J. & Alatalo, R. V. 2001. Population density and male mating tactics in the drumming wolf spider *Hygrolycosa rubrofasciata*. Manuscript.

1 INTRODUCTION

There are two principal reasons to understand intra- and interpopulation (genetic) variation of traits associated with adaptation: the first concerns the explanation of past evolutionary responses to environmental change, and the second concerns our ability to predict future evolutionary responses to selection. If we are able to understand the causes and mechanisms underlying adaptive phenotypic variation that have occurred in the past, this will provide clues to how populations, communities and ecosystems may evolve in response to future environmental change (Mousseau 2000). For example, much of the latitudinal variation currently observed in north temperate arthropod life histories is genetically based and has evolved since the last glacial period (e.g. Tauber et al. 1986). Because similar patterns of life-history variation have evolved repeatedly, independently, and even on different continents, this suggests that these patterns of genetically based phenotypic variation are generally adaptive (Endler 1987) and likely to arise again in response to similar patterns of environmental change. However, genetically identical organisms reared under different conditions may display quite distinct characters. This is due to the fact that organisms display remarkable phenotypic plasticity in response to environmental variation. To separate genetic from environmental sources of phenotypic variation is central task in evolutionary ecology research and it has huge impact to conservation biology, too.

The aim of the thesis was to study genetic and ecological differences in life-history and behavioural traits among populations inhabiting different habitat types. The drumming wolf spider *Hygrolycosa rubrofasciata* (Ohlert 1865) provides a good opportunity for this kind of a study, since it is a species with limited dispersal ability and closely located populations may be isolated from each others. Population sizes and densities vary greatly, and populations inhabit two different habitat types and thus allows us to compare the local adaptations to different environmental conditions.

Ecology of spiders is generally poorly known compared to many other animal taxa. This is also the case in variation among populations except for the studies with a desert spider species (*Agelenopsis aperta*) in respect of territorial,

foraging and anti-predator behaviour (Hedrick & Riechert 1989, Riechert 1993 a, b, Riechert & Hall 2000) and long-jawed orb-weaving spider (*Tetragnatha elongata*) in respect of foraging strategy (Gillespie & Caraco 1987). The drumming wolf spider *H. rubrofasciata* is a species with extensive studies of female choice on male drumming characteristics and also sexual selection in general (Mappes et al. 1996, Kotiaho et al. 1997, Parri et al 1997, Alatalo et al. 1998, Kotiaho et al. 1998 a, b, 1999 a, b, Parri 1999, Rivero et al. 2000). Thus, in this work on inter-population variation in life history and sexual traits we had to accomplish some studies with more exploratory approach (I, IV). Male sexual behaviour was studied also with behavioural experiments (III, V), and to test whether growth differences between populations have any genetic basis we raised spiderlings in laboratory (II).

1.1 Growth and body mass

1.1.1 Reaction norms

The reaction norm concept provides a way for studying plasticity in phenotypic expression and reaction norms are defined as a set of phenotypes produced by a single genotype across a range of environmental conditions (Woltereck 1909 ref. by Stearns 1992). Norms of reaction have been intensively studied in plants (e.g. Shaw 1986, Miller & Fowler 1993, Pigliucci & Schlichting 1996, Black-Samuelsson & Andersson 1997) and in insects (Via 1984, Weis & Gorman 1990, Blankenhorn 1991, Antolin 1992, Sibly et al. 1997), especially in *Drosophila spp.* (Gupta & Lewontin 1982, Delpuech et al. 1995, De Moed et al. 1997, Morin et al. 1997, Noach et al. 1997). There are also a few studies on other organisms such as snails (Johannesson & Johannesson 1996), hard clams (Rawson & Hilbish 1991), fish (Trexler & Travis 1990) and hylid treefrogs (Blouin 1992). Some studies have concentrated on the reaction norms themselves, their slopes and other characters (e.g. Weis & Gorman 1990, Blouin 1992, Gabriel & Lynch 1992, Delpuech et al. 1995), and in other studies the reaction norms are used to reveal the absence or presence of genetic differences among populations (e.g. Via 1984, Shaw 1986, Miller & Fowler 1993, Schmitt 1993, Pigliucci & Schlichting 1995, Johannesson & Johannesson 1996) or demonstrating that observed plasticity in animal morphology or life history is adaptive (Gotthard & Nylin 1995 and references therein). Most of the former studies have been made with clonal organisms (Pigliucci & Schlichting 1995, Jokela et al. 1997) or with well known genotypes (e.g. Meerts 1992, Schmitt et al. 1992, Noach et al. 1997). However, there are not so many studies where life history reaction norms are compared between males and females within a species, and I found only one study made in a spider species (Jakob & Dingle 1990).

1.1.2 Significance of body mass in females

Body mass of arthropod females is a principal constraint on their potential fecundity (Darwin 1874, Larsson 1990, McLain et al. 1990, Honek 1993). However, body size can affect significantly on the longevity of individuals e.g. in terms of predation risk and parasitism, and thus potential fecundity may be different than the actual fecundity (Leather 1988). Across spider taxa there is a positive correlation between female size and clutch size (Marshall & Gittleman 1994, Simpson 1995). In *H. rubrofasciata*, female juveniles reared in laboratory attempt to reach as large size as possible before maturation and thus to increase their future reproductive success (Parri 1999). Furthermore, after maturation females continuously collect reserves for reproduction increasing their body mass. However, there may also be costs of attaining and maintaining large body size, especially in poor habitats (e.g. Leather 1988), since otherwise individuals are expected to grow as large as possible to maximise their life span and reproduction. These costs can be physiological or ecological costs. An important ecological cost might be food availability and costs included to finding and gathering sufficient food for the growth (Dingle 1992, Blanckenhorn et al. 1995). Indeed, the persistence of many examples of intraspecific variation in body size and growth rate in nature suggest that there may be both costs and benefits associated with fast growth and large body size e.g. in terms of predation, parasitism or overwintering (Nylin & Gotthard 1998). Thus, we expected to find adaptive variation among different environments both in female body size and in age at maturity.

1.1.3 Significance of body mass in males

In arthropods, male body mass is often correlated with mating success in males (e.g. Borgia 1982, Partridge & Farquahr 1983, Ward 1983, Simmons 1986, Fincke 1992, Andersson 1994, Carroll & Salamon 1995, Nylin & Gotthardt 1998, Savalli & Fox 1998), also in many spider species (Bushkirk 1975, Riechert 1975, Vollrath 1980). However, male body mass is not always positively correlated with its mating success (Arita & Kaneshiro 1988) and sexual selection may also favour smaller males (see references in Savalli & Fox 1998). In *H. rubrofasciata*, male body mass is a good estimate of its size, and after maturation the mass stays relatively constant (Kotiaho et al. 1996, 1999a). Male body mass has not turned out to be connected to mating success or drumming activity of *H. rubrofasciata*, the latter being the trait affecting their reproductive success by female choice (Kotiaho et al. 1996; Mappes et al. 1996; Kotiaho et al. 1998 a, 1999 a, Parri et al. 1997, Parri 1999). Body mass is not correlated with male drumming rate, and it is not under female choice (Kotiaho et al. 1996, Mappes et al. 1996,). Costs of drumming are higher for larger males, but they appear to be able to compensate these increased energy requirements since drumming is not reduced (Kotiaho et al. 1998). Larger males are better competitors in male-male interactions (Kotiaho et al. 1997, 1999 b), but this is the case only if size difference is large, and mating success is not influenced by male size (Kotiaho et al. 1996). There is no

detectable relationship between male body mass and their overwintering survival (Kotiaho *et al.* 1999 c). Since the physiological and ecological costs of large body size are present also to males, we could expect more intra- and interpopulation variation in body size among males compared to females.

1.1.4 Age and size at maturity

In life history theory, age at maturity is generally determined by a balance between the advantages of short generation time and the advantages of large size (Higgins and Rankin, 1996; Stearns and Koella, 1986). When the quality of food is reduced, the growth rate of ectotherm animals is slowed down and maturation occurs later and at a smaller size (Gebhardt and Stearns, 1988; Berrigan and Charnov, 1994; Ebert, 1994). In endotherm animals males and females may have different strategies for optimising the maturation size and age (Stearns 1992). In species with males that compete with each other for females, males mature later and in larger than females (e.g. Clutton-Brock *et al.*, 1982; Stearns and Koella, 1986). In contrast, species with no social structure, females generally mature later and in larger size (e.g. St. Clair *et al.*, 1994). *H. rubrofasciata* wolf spider has an exceptionally long development time, it typically matures during its third growing season. In poor environments and in northern Finland maturation may last as long as four years (Mappes *et al.* unpubl.). On the other hand, in good environmental conditions some individuals have been observed to mature in two years. In this thesis I compare variation in maturation among populations and between rich and poor habitats.

1.1.5 Interpopulation variation in body mass and brood size

In many species, separate populations live in a variety of habitat types. The consequences of experiencing natural selection in different environments depend on both ecological and genetic factors. In order for natural selection to lead to evolution of divergent populations, the habitats must differ in ways that affect fitness and there must be genetic variation of relevant traits. Differences among populations in adult body sizes have been reported in several studies typically related to habitat type (e.g. Brown *et al.* 1992, Cherrill & Brown 1992, Rowe 1994, Zulian *et al.* 1995, Rowe 1997). In some cases body sizes differ remarkably between habitats, but no correlation between similarity in body size and geographic distance of populations is evident (Palmer 1994). In addition to environmental and climate conditions (Honek 1986, Hugueny & Louveaux 1986, Lindeman 1996), demography (King 1989, Bruce & Hairston 1990, Zulian *et al.* 1995, Rowe 1997) and social hierarchy may cause significant differences in body sizes among populations, even in a very small scale (Gortázar *et al.* 2000).

Brood size is correlated with body mass across spider taxa (Marshall & Gittleman 1994). Tanaka (1995) has found a trade-off between clutch size and offspring size in a web building spider (*Agelena limbata*). Offspring size of this species is positively correlated with the body size of the mother, and negatively correlated with the clutch size. Among spider genera, clutch and egg sizes are

positively correlated with body mass, and there is a negative correlation between egg number and size (Simpson 1995). However, in their comparative study Marshall & Gittleman (1994) did not find empirical evidence for a size-number trade-off in allocation of resources within clutches.

Variation in clutch size and offspring size among populations, and within population allocation between them are due to variable environmental conditions or genetic differences among isolated populations (Mashiko 1990, 1992, Rowe 1994, Brown & Formanowicz 1995, Lindeman 1996). In a shrimp (*Paratya australiensis*) egg size is under strong genetic control and clutch size is influenced by the environmental conditions (Hancock et al. 1998), as is the case also in a freshwater prawn (*Macrobrachium nipponense*) (Mashiko 1992).

1.2 Interpopulation variation in behavioural traits

1.2.1 Sexual signalling

Differences in strength and direction of sexual selection among populations of one species has gained only little attention, even though such variation may have considerable effects on speciation or on adaptations to local environments. Differences in courtship signals can form effective reproductive barriers between sympatric morphs (Claridge *et al.* 1985, 1988, Wells & Henry 1992) or allopatric populations (e.g. Miller et al. 1998). Understanding the variation in sexual traits among populations will allow us to understand the specific roles of each trait in sexual selection.

In birds, habitat structure affects signal transmission, and consequently on song characteristics (Catchpole & Slater 1995, Kroodsma & Miller 1996), but there are only a few similar studies made in arthropods. For example, in the brown planthopper *Nilaparvata lugens*, in which populations differ in signal repetition frequency, mean female preference in each population closely matches the mean male signal pulse repetition frequency (Butlin 1996). Also in the cricket frog *Acris crepitans* advertisement calls vary significantly among populations and part of this variation is due to environmental selection on call structure to enhance call transmission (Ryan & Wilczynski 1991). High variability in song characters can be due to the different selection pressures, e.g. in the field cricket *Gryllus integer* females and parasitoid flies prefer male calling songs with average number of pulses per trill and this causes stabilising and disruptive selection, respectively (Gray & Cade 1999).

In *H. rubrofasciata*, female spiders prefer higher drumming rate, higher volume of the signal (Parri *et al.* 1997) and longer drumming signals (Parri 1999). Instead, the pulse rate (number of hits per second within single drumming sound) (Parri 1999), the symmetry of the signal, peak frequency or other measured signal characters have not been found to be the target variables of female choice (Rivero *et al.* 2000). Male body mass has not proved to affect male drumming rate or mating success (Kotiaho et al. 1996, 1998 a, 1999 a;

Mappes et al. 1996). There are indications of variation in male courtship behaviour among populations and between habitat types (Parri 1999, Rivero et al. 2000). I focused to understand this variation in male courtship drumming, and whether there are local differences in female preferences in male courtship drumming.

1.2.2 Male mating tactics

Interpopulation differences in courtship signalling and mating tactics has been studied e.g. in mammals (Van Parijs *et al.* 2000), in insects (Paillette et al. 1997, Miller et al. 1998 and references therein), and in some spider species (Miller et al. 1998). However, mating success between individuals from different populations has gained relatively little attention (Rupp & Woolhouse 1999). In time, differences in courtship behaviour can lead to significant mating barriers and finally speciation can occur among allopatric (Claridge et al. 1985, 1988;) or even among sympatric populations (e.g. Wells & Henry 1992). This speciation is in many cases based only on behavioural divergence with no genetic impediment for hybridisation (Claridge et al. 1985, 1988).

Differences in mating behaviour among locally adapted populations inhabiting different habitat types has been studied very little. On variable habitats environmental factors may affect differently on male courtship success, and thus the most successful courtship behaviour strategy may vary among the habitat types. Also population density or other demographic factors may affect on the importance of male courtship behaviour and thus adaptive differences among populations may arise. Theoretical models suggest that when population density increases, mate competition and mate choice increase due to the higher encounter rate of potential competitors and mates (Eshel 1979, Hubbell & Johnson 1987, Crowley et al. 1991). In empirical studies with field crickets (*Gryllus* sp.) and grasshoppers (*Liguorotettix* sp.) individual males reduced calling and increased movements when the population density increased (Greenfield & Shelly 1985, French & Cade 1989, Hissmann 1990, Cade & Cade 1992). Thus, it seems that population density may influence both the mating effort and its allocation to different activities.

Male *H. rubrofasciata* search for females and move long distances to find receptive females, and stop drumming at times during the mating season. Kotiaho *et al.* (2000) have found that males avoid areas with high sedge cover, low elevation, and low dry leaf cover. In addition, drumming rate in field is positively correlated with dry leaf cover, and in laboratory males prefer dry leaves as drumming substrate over wet leaves, moss and bare soil. Kotiaho *et al.* (2000) also conclude that in *H. rubrofasciata* male distribution and drumming rate are greatly affected by environmental factors. Thus, presence and quality of drumming substrate may have some effects on mating success, too. Males may be locally adapted to different courtship environments, and male mating strategy, whether to allocate more on searching or drumming, may depend on the habitat.

2 METHODS

2.1 Study species

Hygrolycosa rubrofasciata (Ohlert 1865) (Araneae: Lycosidae) is a ground dwelling, sexually dimorphic wolf spider species. Males produce courtship drummings by hitting their abdomen on dry leaves or other suitable substrate. These drumming signals are audible in human ear up to several meters (Kronestedt 1984, 1996, 1997). In this species drumming characters (drumming rate, drumming signal length, and volume) are under directional female choice.

Maturation of this species typically lasts three years but may be completed in two years in particularly good growth conditions (I). Both females and males mature in the autumn of their third year and they overwinter in the adult stage. Mating season is in early May lasting only about two weeks. Females carry egg sacs with them ca. one month, until the spiderlings hatch in late June. All the mature males die in the end of the mating season, but females can survive for at least one more year and thus they may reproduce several times (Parri 1999). In the highly seasonal environment of Finland, the reproduction of this species is very synchronous.

2.2 Study area and habitat

Spiders for this study were collected from Sipoo, southern Finland (60°16'N and 25°14'E) during the years 1997-2000 from populations of variable size located in an area of ca. 5 km². These populations are located within a few hundred meters to a few kilometres from each others on suitable habitat patches separated by unsuitable habitats of forest and rocky cliffs. Habitat types suitable for *H. rubrofasciata* are half open bogs with deciduous trees (*Betula spp.*, *Salix spp.*), moss (*Sphagnum spp.*), and grasses (*Carex spp.*, *Eriophorum vaginatum*), and open

meadow habitats (mainly abandoned fields) with vegetation dominated by various species of grasses and hay (e.g. *Calamagrostis* spp., *Deschampsia cespitosa*, *Epilobium angustifolium*). There is a great variation in soil moisture among meadow habitats inhabited by *H. rubrofasciata* populations.

2.3 Environmental variables (I)

Environmental characters were measured during summers 1998 and 1999 from five sample sites from each study population. The total vegetation cover in percentages was estimated by eye from these five sample sites, each 1m² in size (1998) and canopy cover percentages of trees were estimated from a circular (diameter 20 m) in year 1999. Light index was estimated using five categories: light index 1 indicating shade by trees or bushes for the whole day, and light index 5 indicating the possibility of the whole day of sun shine. Soil litter thickness was measured, and two soil samples from each of the five sample sites with a cylindrical (diameter 57 mm) soil corer. Relative soil humidity (%) was measured in one of the two samples from each sample site. Soil litter pH was measured with an electronic pH-meter adding purified water to soil litter, mixing this solution carefully, and allowing it to extract at least for two hours before the measurement.

2.4 Collecting and weighing the specimen (I-V)

Unmated females were collected by hand picking soon after snowmelt. Males were collected either by hand picking or by pitfall trapping. After collecting, spiders were placed individually in small plastic film jars with some moss (*Sphagnum* sp.) and kept in a cool temperature (ca +10°C) until they were brought to the laboratory. Spiders were weighed within a week from collecting to the nearest 0.1 mg with an analytical balance (AND HA-202M) and kept meanwhile in low temperature, ca. +5°C, to keep their metabolic rate low. Females with egg sacs were collected in June and juveniles in September by hand picking, and placed in shade until transfer to laboratory. In laboratory both juveniles and females carrying egg sacs were placed in room temperature (+ 20 – +22 °C). The body mass was used as a measurement of adult body size, since it is highly correlated with the carapace width ($r = 0.89$, $n = 484$, $p < 0.001$) and it is a highly repeatable measure (99.4 %) (Kotiaho et al. 1996)

To estimate the population density and size, a sample of spiders was collected from an area of 100 m² within each population with pitfall traps (one trap /m²). The relative estimate of population density was the number of males captured from the area of 100 m² per day. Only the first day or two first days were considered. However, in 10 minute populations in which practically all the males were captured, the population size was estimated as the total number

of males captured during the pitfall trapping season (1-2 weeks). A robust estimate of the population size for larger populations was calculated as the number of males captured per 100 m² per day multiplied with the total area inhabited by the population, and further multiplied with 1.51. This coefficient was calculated as the mean ratio of the total number of males caught from the minute population and males caught from the 100 m² within the one capture day (the particular day used in density measurement).

2.5 Laboratory rearing (II)

I studied the growth differences between the sexes and among the seven populations that located at least 1 km from each others (except two sites which situated next to each others). In autumn ca. hundred two months old spiderlings were collected, weighed to nearest 0.01 mg, and divided systematically into two treatments (low and high food level) assuring that the initial mean mass of groups was similar. Spiderlings were reared individually in small transparent plastic containers in temperature of +20 - +22 °C. During the rearing high food level juveniles got three times more food than low food level individuals. Mature spiders were weighed to the nearest 0.1 mg, and the age at maturation was counted from the beginning of the laboratory rearing to the maturation.

2.6 Male drumming rate and mobility (III-IV)

Drumming rate and mobility of males was measured individually in plastic drumming arenas which had a piece of paper glued on their bottom. and two dry birch leaves as the drumming substrate. Arenas were marked with a central line, dividing them in two halves. The experiment room was illuminated with fluorescent tubes, and additionally lamps with 40 W bulbs were placed 30 cm above the drumming arenas to give the male spiders heat and light. The air temperature under the lamps varied between +28 and +30 °C.

On the day before the beginning of the measurements males were kept in room temperature for a few hours to trigger their drumming activity for the next day. Male spiders were taken in room temperature ca. 3 hours before, and released to arenas 2 hours before the measurements. Drumming rate and mobility of each male was measured individually, and four arenas (i.e. four males) were observed at time for a two minutes period. Mobility was measured as crossings of the central line, and drumming rate as number of the drumming bouts during the observation time. Immediately after finishing the first measurement period four next males were measured. After measuring all the groups, this measurement procedure was repeated for four more times. Thus, the drumming rate and mobility of each male was measured 5 times 2 minutes

in each measurement day. Afterwards males were placed to the temperature of +3 °C, and fed with fruit flies (*Drosophila melanogaster*). A second measurement was conducted one or two days later. Thus, the total observation time for mobility and drumming rate was 20 minutes for each male.

2.7 Drumming characteristics (IV, V)

Drumming sounds of individual males were recorded with a digital recorder (Sony TCD-D7) attached to a Telinga microphone. Before recording males were kept in room temperature (ca. +20 - +22 °C) for at least 30 minutes to trigger the drumming rate. Males were placed in small plastic containers with a piece of paper on bottom as a drumming substrate. There were ten males in a circle around the microphone, each at a distance of 25 cm from it. A minimum of three individually recognised drumming sounds per each male were recorded, and after that a male was replaced with a new individual.

Drumming sounds were analysed by using sound-analysing standard programmes (Sound Edit 16, version 2.0, Macromedia; and Canary 1.2, the Cornell Bioacoustics Workstation). The first program was used to convert the sounds from digital tapes to computer files and the latter to analyse the characters of drumming sounds. The drumming bout produced by male *H. rubrofasciata* consists of a series of equally spaced pulses increasing in amplitude towards the middle and decreasing again towards the end (see Rivero et al. 2000). An oscillogram (amplitude vs. time) and a spectrogram (frequency vs. time) was created for each drumming sound, and signal length (total duration of the signal in seconds), number of pulses (hits), and volume of the sound (dB) were measured. The pulse rate (number of hits / second) was counted from these variables as follows: pulse rate = (the number of pulses -1) / signal length.

2.8 Mate searching behaviour and play-back experiment (III)

For the mate searching experiment unmated females and males were collected from a bog and a meadow population spring 1998. Males were tested in pairs twice, on two different arena habitats, and each female was used in the experiment only once.

Arenas (bottom: 33 cm x 33 cm) had a bottom of 2 cm thick soil layer, and they were divided into nine equal sized sections (3 x 3) with narrow white paper slices. On top of the soil there was either nine dry birch (*Betula* sp.) leaves per section, or three dry pieces of hay (*Calamagrostis* sp.) leaves. Experimental arenas were illuminated by a lamp with 40 W bulb to give heat and light that are essential for male drumming behaviour.

Two males were on the arena at a time, one from the bog population and the other from the meadow population, and in the latter part of the experiment also two females, one from each population. The behaviour of males (mobility, drumming rate, aggressive behaviour towards the other male) was recorded without and with females. With females the recording of male behaviour continued until one of the two females was willing to mate with a male, or if females did not respond, 30 minutes. The experiment was repeated next day on the other type of arena for this particular pair of males.

The population recognition playback experiment was done using drumming signals of ten males from bog and ten males from meadow populations. In year 1995 male signals were recorded from two Southern Finland populations (Sipoo, lat. 60° N), and in year 1996 from two northern Finland populations (Sodankylä, lat. 67° N). In both years drumming signals were recorded similarly with a digital recorder (see details in Parri 1999). One playback tape where signals from the meadow and bog population alternated was created from the southern sounds, and the other from the northern sounds. These tapes were replayed to females from the two populations within the same geographical area. The rate of the signals on the tapes was four signals per minute and the tapes were replayed three times in row, once on each three volume levels. Number of female responses to each signal was counted (only the first response by any female considered).

2.9 Male-male interactions (V)

Males similar in body mass (difference in body mass <1.0 mg) from sparse and dense populations were used in the male-male interaction experiment. Experimental arenas consisted of plastic jars with a piece of) glued on the bottom. In the middle of the arena there was an ink line and a small cage containing an unmated female of this species to induce male drumming and fighting activity. Males (one from sparse and the other from dense populations) were kept in room temperature for 1 hour before the experiment. In the first phase of the experiment both males were kept individually in drumming arenas for 15 minutes, and numbers of drumming bouts and movements over the ink line were recorded. In the second phase the two males were released in a new arena for 15 minutes, and drumming bouts, movements, and aggressive behaviour were recorded.

3 RESULTS AND DISCUSSION

3.1 Phenotypic differences among populations (I)

3.1.1 Variation in adult body mass

Differences in adult male body mass among closely located populations of *H. rubrofasciata* were studied in three successive years (1997 - 1999). There was great differences in body mass of males among ten populations and three successive years. In year 1997, there was also great variability in female body mass among the ten populations. In this species, female body mass in spring highly depends on the reproductive stage (they collect reserves for reproduction) while male body mass stays relatively constant after maturation. Differences between years are probably caused by variation in weather conditions during the growth seasons (Honek 1986). Differences in body mass between populations may also be due to the variation in microclimate and especially in temperature. Ectotherm animals tend to be larger, though not always in cooler temperatures (e.g. Atkinson 1994, Atkinson & Sibly 1997). However, studied *H. rubrofasciata* populations located only few kilometres from each others.

Inter-population differences in body mass have been found in species inhabiting several habitat types (e.g. Brown 1992, Cherrill & Brown 1992, Palmer 1994, Zulian et al. 1995). Variation in environmental factors, such as amount of food between habitats may affect considerably body mass also in *H. rubrofasciata*. However, males having more food are not necessarily larger in size since male growth is not as clearly connected to amount of food as it is in the females (II). We have also demonstrated that part of variation in male and female body mass is due to genetic differences, since variation in size among populations remains even if growing conditions have been constant (II).

Principal components analyses (PCA) extracted two major axes from the abiotic environmental factors in both of the years 1998 and 1999. The first principal component was clearly a bog-meadow axis, but the interpretation of

the second principal component was not equally clear. The two factors were used in regression analysis to explain the variation in male body mass. In both years, the first principal component significantly explained variation in male body mass, bog males being larger in size. Principal components did not explain the variation in mean body mass within the bog habitat, but within meadows a slight tendency was evident. Among meadows, the males from wet sites, resembling thus bogs, were the largest. Even though the first principal component explained some variation, a great amount of variation still remains unexplained, especially among the bog populations. There may be other environmental variables, not measured in this study behind these differences, e.g. food availability is difficult to estimate.

3.1.2 Distribution of male body mass

Male body mass is usually normally distributed within the populations. However, in the year 1997 we found in some populations that male body mass distributions did not follow a normal curve, but had instead two separate peaks. In the year 1999, male body mass of these two populations were normally distributed. Maturation of *H. rubrofasciata* lasts normally three years, and usually there is one cohort at a time as mature individuals in any spring. However, these abnormal distributions indicate that in this particular year also part of the individuals from the younger cohort had already matured. These two populations inhabit moist and rich meadows that evidently represent good habitats for *H. rubrofasciata*. In a common garden experiment *H. rubrofasciata* juveniles were raised in enclosures on wet and dry habitats. A high proportion of individuals matured already at the end of their second year and rest at end of third year (unpublished data). These enclosures situated apparently on a good habitat for this species. Thus in favourable years part of *H. rubrofasciata* juveniles may mature in two years instead of the normal three years in particular in some of the wet meadows.

3.1.3 Brood size and juvenile body mass

There was no differences in brood size or mean offspring body mass between the habitats, but variation among populations was evident especially in meadow habitat. The effects of abiotic environmental factors on variation in brood size and juvenile body mass was studied with a linear regression using the first principal component, extracted from the abiotic environmental factors, as the independent variable. This factor separated bogs and meadows, but it did not explain variation among populations in brood size or offspring body mass.

Female body mass correlated positively with brood size among bog and meadow populations, being consistent with the general relationship between body size and fecundity in insects (e.g. Larsson 1990, McLain et al. 1990, Honek 1993, but see Leather 1988) and in spiders (Marshall & Gittleman 1994, Simpson 1995). The amount of food available for *H. rubrofasciata* females is the most likely environmental factor affecting female size and thus its reproduction. In

general, the egg size or the offspring size of invertebrates are affected by the food level (e.g. Qian & Chia 1991, Trubetskova & Lampert 1995, Guisande et al. 1996).

Female body mass is positively correlated with offspring size in many taxa (Roff 1992). In *H. rubrofasciata*, female body mass is positively correlated with offspring body mass within meadows, but not within bogs. No correlation between offspring number and offspring body mass appeared among meadow populations or bog populations. Only a weak positive correlation between number of offspring and mean juvenile body mass appeared in 1997. Thus, it would seem that there is no trade-off between clutch size and offspring size in *H. rubrofasciata*, which is found in some spiders species (Simpson 1995, Tanaka 1995) but not in all (Marshall & Gittleman 1994). However, this lack of correlation does not exclude the likely possibility that trade-offs are hidden by the variation in mother condition (Bernardo 1996, Mousseau & Fox 2000). Variation in offspring body mass was great among broods of *H. rubrofasciata*, but it was not strongly linked to female body mass or brood size. The variation in hatchling body mass between habitats and populations was small compared to the variability among individual broods, and it seems not to be related to mothers condition. Thus, there is no obvious explanation for the high variability in the size at hatching.

3.2 Genetic differences in body mass

3.2.1 Reaction norms for adult body mass and maturation time (II)

In females, there was no interaction in adult body mass between the population and the food level. Populations differed significantly from each others in female body mass, and females grown on high food level were larger in size. In males, there was a significant interaction between population and food level in body mass. High food level males grew generally larger than low food level individuals, but no significant difference among populations was evident.

Both females and males had genetic differences in size among populations, but the shapes of reaction norms differed between the sexes. Females from all the populations grew larger in size on high food level while males had more variation in response to food conditions. Maternal effects and the very early growth conditions could have partly affected the final body mass (Bernardo 1996, Mousseau & Fox 2000), but it is unlikely that the variation is entirely due to differences in non-genetic factors, since juvenile body mass did not correlate positively with field collected adult male body mass, and the initial juvenile body mass was uncorrelated with the final body mass. In addition, the resemblance between offspring and sire in body mass is high ($h^2 = 0.67$) (Parri 1999) indicating that differences in adult body mass among populations have a major genetic component.

Parallel reaction norms in females indicate that females may have a strong selection pressure to grow as large as possible, since the body size is found to be

positively correlated with the fecundity of female arthropods (Roff 1992, Honek 1993, Savalli & Fox 1998), as is the case also in *H. rubrofasciata*. Instead, males were less prone to grow as large as possible, even though variability among populations was high. This may be due to the fact that maturation in nature can take place only after the second or third summer of growth. Males from some populations matured at a certain size independently of the given amount of food, which may indicate the attempt to reach certain minimum or optimal size before maturation. There is no strong relationship between male size and sexual activity (drumming rate) (Kotiaho et al. 1996; Mappes et al. 1996, Kotiaho et al. 1998, 1999 a) or mating success (Kotiaho et al. 1996), and thus even factors weakly related to survival or mating success may cause the apparently erratic male growth patterns in different populations.

Mean adult body mass in each populations was regressed against the first principal component (extracted from environmental variables with PCA) and the food level. Female body mass was explained by the amount of food, but not by the environmental factor. However, there was a tendency of females originating from meadow-type habitats (dry, higher pH and less soil litter) to grow better in laboratory conditions, even though females from bog habitats are naturally larger in size. There may be a shortage of food in these dry habitats and thus females might be forced to use the available food efficiently. This result that females from poorer habitats invest more on growth is parallel to the countergradient variation in growth along the environmental gradients as described by Conover & Schultz (1995). Male body mass was not related to either of the factors. Male body mass is not strongly correlated to any fitness measures and the optimal growth strategy for males is not easily predictable.

Variances in male and female maturation times were highly different between the food levels, and in general, variation in maturation time was greater in juveniles grown on low food level. On low food level, there were some individuals maturing relatively early, but also individuals that were unable to do so and thus forced to delay their maturation. There was no interaction between food level and population in maturation time. Populations did not differ in maturation time, but on high food level, spiders matured significantly earlier than on low food level. The results suggest that the maturation time in *H. rubrofasciata* is a plastic trait without any genetic differences among populations.

When the mean maturation time in each population was plotted against adult body mass, the individuals grown on high and low food level formed clearly distinct groups. Females grown on high food level matured earlier and grew larger in size than did females grown on low food level. Males that received more food also matured earlier than males with less food, but the final body mass was less clearly affected by the food level. Among males, the variation was higher on low food level both in maturation time and in adult body mass than it was on high food level. Females and males did not differ in maturation time, but a tendency for a difference of adult body mass between females and males was evident suggesting sexual differences in growth strategies.

3.3 Behavioural differences among populations

3.3.1 Habitat differences in male mate searching tactics (III)

Differences in male mate searching and courtship signalling were tested for two populations inhabiting different habitats. When males were on leaf arenas without females, males from the bog population drummed more than males from the meadow population. On the hay arena, the drumming rate was generally lower and there was no difference between populations. On the hay arena males from bog and meadow population won equally often male-male interactions, but on the leaf arena bog males won more often the fights.

In the second half of the experiment two unmated females (one from the bog and the other from the meadow) were added on the arena. Now, drumming rate did not differ between populations or between arenas. Males from both populations searched and followed females' silks more actively on the hay arena. On the leaf habitat females were more likely to respond to male signals than on the hay habitat, but there was no significant difference in number of responses between males from different populations. In a playback experiment, females did not prefer male signals from their own or strange populations. The drumming activity and mobility of males from ten bog populations were higher than these activities of males from ten meadow populations. Larger males were also more active in drumming and moving.

The results indicate that males from bogs invest more in drumming behaviour when singly or with other males, but the difference between habitats disappears when the males encounter receptive females. Males from the bog populations have higher mobility when individuals are singly on arenas, but together with the other male and the females, the bog and meadow males invest equally to mobility. In sum, there are some differences between populations in male mating tactics. There are also differences in male behaviour between arenas indicating that males are able to adjust their behaviour according to environmental conditions.

3.3.2 Variation in sexual signalling between bogs and meadows with variable population size (IV)

Males from bog populations had a higher drumming rate than males from meadows. Within the bog habitat larger populations had a higher drumming rate, but the average body mass did not affect the drumming activity. Among meadow populations the drumming rate with larger males was somewhat lower. Correlations between individual male body mass and drumming activity within populations was studied with a meta-analysis. Male body mass did not correlate with drumming rate within bog or meadow populations. The size of the bog populations varied from minute (with only a few individuals) to large whereas all meadow populations were large in size. Males from large bogs drummed more actively and there was also inter-population variation within

the habitats. Higher intra-sexual competition within large bog habitats may drive higher sexual activity in these populations. Another explanation for observed correlations may be that lower drumming activity in small bogs indicates inbreeding in the very small populations.

Drumming rate is directly involved in male mating success. It is selected by females (Kotiaho *et al.* 1996, 1998 b; Parri *et al.* 1997, Alatalo *et al.* 1998) and it has proved to be an honest signal of male viability (e.g. Kotiaho *et al.* 1996, 1999 a, Kotiaho 2000). More actively drumming males also win more often in male-male competition (Kotiaho *et al.* 1997), and drumming is highly energy demanding activity for male *H. rubrofasciata* (Kotiaho *et al.* 1998 a).

Males from bog populations had higher mobility than males from meadow populations this being the case also between similarly sized bogs and meadows. Population size or mean body mass did not explain the variation in mobility between populations. This result indicates that males from very small populations were not in poorer condition compared to males from larger ones. However, smaller populations are generally sparse, and the moving effort may be more essential for male mating success. Thus, despite the lower condition males may invest more energy on moving and mate searching.

There was a strong positive correlation between population size and pulse rate, both among bog and meadow populations. Large bogs and meadows had on average similar pulse rate despite the variation among populations. Male body mass correlated negatively with pulse rate among meadow populations. There was no difference in signal length or volume between the habitats. Variation in these signal characters among populations was not explained by population size or mean male body mass.

Encounter rate with conspecific individuals is greater in large populations. Males with better signal quality and more active signalling rate may be more successful in large populations. In small populations, however, each individual meets only a very few potential mates, and it is likely that females cannot afford to be particularly choosy (Parri *et al.* 1997). There are no strong preferences by females to pulse rate (Parri 1999), and thus this signal component might be easily changing if the importance of signal in sexual selection is reduced in the smallest populations.

Meadow populations with on average smaller males had a higher drumming activity, but male body mass did not affect drumming rate among bog populations. However, there was no correlations between body mass and drumming activity within each population, indicating that the relationship between these variables is weak also in the meadows. This result is consistent with the earlier studies, where male body mass has not proved to affect on male drumming rate (Kotiaho *et al.* 1996, 1998 a, 1999; Mappes *et al.* 1996).

Differences in drumming and moving activity, and in pulse rate among populations might also be due to genetic drift, especially within small populations. In *H. rubrofasciata*, females do not discriminate between drumming sounds of males from own or different population, despite the differences in drumming sound characteristics between populations (Parri 1999, III). Roff *et al.* (1999) conclude, that when females from the two sibling species do not discriminate between males of their own or sibling species, the observed

differences between the two males are most likely to result from genetic drift than from sexual selection. However, the strong linear relationship between population size and pulse rate, and population size and drumming activity indicates that there are something more than random genetic effects behind these findings.

3.3.3 Population density and male mating tactics (V)

On separate arenas males from sparse and dense populations had similar drumming rates. Mobility had a slight tendency to be higher among males from dense populations. When together on the experimental arena, males from dense populations had higher mobility, and males from sparse populations had higher drumming rate. Males from sparse populations had also a tendency to be more successful in aggressive male-male encounters, i.e. in agonistic drumming rate, fight wins and in chasing the other male.

When in the vicinity of a female, males from sparse populations drummed more actively and had tendency to behave more aggressively towards the other male. Instead, males from dense populations had higher mobility. During the short mating season males from sparse populations encounter receptive females less frequently than males from dense populations. Thus, in sparse populations the value of each encountered female is high, and males should allocate more on aggressive behaviour towards other males when in the vicinity of a female. In dense populations there are several potential mates available, and thus it may be a more successful strategy to search for another female than to fight for it.

Drumming signal volumes produced by males from dense populations were higher, and there was a tendency for these males to produce longer drumming signals, when males were on arenas without females. Pulse rate within each drumming sound was similar in sparse and dense populations. Pulse rate was similar in both populations. Females choose higher drumming activity, longer and louder signals (Mappes et al. 1996, Parri et al. 1997, Parri 1999, Rivero et al. 2000). It might be beneficial for males from dense populations to allocate more on characters of the sexual signal that are the target of female choice, even when they are not in proximity of females. In sparse populations, the benefit of allocating on signal quality might be small compared to the benefits of allocating on mate searching.

4 CONCLUSIONS

There was considerable variation in adult male body mass among populations and years (I). The differences among populations were partly explained by an environmental factor separating bog and meadow populations, but still much of the variation remains unexplained, especially among populations inhabiting bogs. There were also differences in brood size and mean offspring body mass among the populations. Female body mass correlated strongly with the number of offspring. In favourable years spiders from particular populations may mature in two years instead of the normal three years (I). Variation in adult body mass among closely located populations had a genetic component, and the investment in growth seemed to be higher in poor environments, where spiders are naturally the smallest (II).

Female growth pattern reflected the strong selection for large size at maturity (II,) which increases female fecundity (I, II). In males the growth patterns were highly variable and males did not have any clear tendency to grow as much as possible (II). The size of male is only weakly, if at all, correlated with fitness related traits and thus no unequivocal explanation for male growth pattern could be found.

There was habitat-related variation in male mating tactics, i.e. in male allocation between mate searching and sexual signalling, but this variation had no effects on female choice (III). Females from bog and meadow populations showed no preference on drumming sounds between own and strange populations. Higher drumming rate observed among bog populations may be a male adaptation to the rich availability of the suitable drumming substrate, i.e. dry leaves (IV). Lower pulse rate on smaller populations may reflect the fact that in smaller populations the selection pressure on the signal may be diminished due to the lowered encounter rate with potential mates and other males, i.e. females in small populations could not afford to be choosy. The linear relationship between pulse rate and population size in *H. rubrofasciata* may indicate local selection rather than random genetic effects acting on this drumming signal character.

Population density seemed to affect male investment to different components of mating effort (V). Males from sparse populations invested more in any eventual encounters with females, probably because of the rarity of encounters with alternative mates. On the other hand, males in dense populations did invest more energy on each drumming signal when searching for females, probably because of a higher chance of response.

Populations of this species are fairly isolated, but it is unrealistic to expect no gene flow at all between them. However, the environmental differences may maintain strong selection on certain life-history and behaviour traits leading to adaptive evolution, despite the lack of total genetic isolation. Since body size varies greatly presumably due to food availability, these differences do have evolutionary consequences. Instead, maturation time remains as a plastic trait. Male sexual behaviour varies also considerably, and while genetic differences were not tested, it is likely that they exist. For instance, pulse rate is extremely repeatable for each male and it is not condition dependent (Rivero *et al.* 2000). Interestingly, there seems to be no evolution in female mate preferences, and thus isolated populations do not provide an easy route to speciation. Indeed, *H. rubrofasciata* is the only species of its genus in Europe (Roberts 1995).

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YHTEENVETO

Populaatioiden väliset erot rummuttavan hämähäkin (*Hygrolycosa rubrofasciata*) kasvussa ja käyttäytymisessä

Rummuttava susihämähäkki *Hygrolycosa rubrofasciata* on puoliavoimilla lehtipuuvaltaisilla soilla, niityillä ja pakettipelloilla esiintyvä juoksuhamähäkkilaji (Araneae: Lycosidae). Sen esiintymisalue kattaa Pohjois- ja Keski-Euroopan. Suomessa laji esiintyy laikuittaisesti suurimmassa osassa maata puuttuen kuitenkin ilmeisesti Keski-Suomesta. Myös paikallisella tasolla rummuttava susihämähäkki esiintyy selvärajaisina populaatioina sopivissa elinympäristöissä, jotka rajoittuvat lajille soveltumattomiin metsäisiin ja kuiviin kallioisiin alueisiin. Tutkimusalueellamme Sipoossa (noin 5 km²) populaatiot sijaitsevat lähikäin, vain muutamista sadoista metreistä muutamiin kilometreihin lähimmästä naapuristaan. Osa populaatioista on yhteydessä toisiinsa ojan- ja puronvarsien välityksellä, mutta monet populaatiot ovat eristyneitä muista ja populaatioiden välisen geenivirran voidaan olettaa olevan erittäin vähäistä.

Rummuttavalla susihämähäkillä on erityinen pariutumisaikainen käyttäytymispiirre: koiras rummuttaa takaruumiillaan kuivia lehtiä tai muuta sopivaa alustaa ja houkuttelee naaraita pariutumaan. Aiemmissä tutkimuksissa on havaittu, että naaraat valitsevat pariutumiskumppaninsa tämän rummutusäänen ominaisuuksien perusteella. Erityisesti rummutussignaalin pituus, äänen voimakkuus ja rummutusten suuri määrä aikayksikköä kohden ovat naaraiden suosimia ominaisuuksia.

Tässä tutkimuksessa havaitsin, että toisiaan lähellä sijaitsevien *Hygrolycosa rubrofasciata* populaatioiden välillä on eroja aikuisten yksilöiden painoissa. Nämä erot ovat osittain geneettisiä ja osittain paikallisten ympäristö- ja kasvuolosuhteiden aikaansaamia. Myös naaraiden tuottamien jälkeläisten määrissä oli eroja eri populaatioiden välillä. Naaraan paino myös korreloi positiivisesti niiden tuottamien jälkeläisten määrän kanssa, mutta ei niinkään jälkeläisten painon kanssa. Vertailemalla koiraiden painojen jakaumia eri populaatioiden ja vuosien välillä havaitsin myös, että joinakin suotuisina vuosina rummuttavat susihämähäkit aikuistuvat jo kahden kasvukauden jälkeen normaalin kolmen vuoden sijasta.

Väitöskirjatyöni toisessa osatutkimuksessa vertailin hämähäkinpoikasten kasvua, aikuistumista ja aikuispainoa eri populaatioiden välillä kahdessa erilaisessa ravintotasossa laboratoriossa. Laboratoriossa kasvatettujen hämähäkkien koiraat ja naaraat kasvoivat eri tavalla. Kaikkien populaatioiden naaraat kasvoivat paremmin suuremmalla ravinnon määrällä, vaikka populaatioiden välillä havaittiin eroja aikuispainoissa. Enemmän ruokaa saaneet koiraat sen sijaan kasvoivat keskimäärin vain vähän paremmin kuin niukemmalla ravinnolla olleet. Muutos ravintotasossa vaikutti eri tavalla populaatioiden kasvuun: joidenkin populaatioiden koiraat painoivat aikuistuttuaan saman verran riippumatta niiden saaman ruoan määrästä, kun taas toisissa populaatioissa ravinnon määrän lisääntyminen kasvatti koiraiden painoa. Näiden tulosten perusteella

vaikuttaakin siltä, että tällä lajilla aikuispainot vaihtelevat eri populaatioiden välillä osittain geneettisistä eroista johtuen, ja tämä vaihtelu saattaa olla sopeutumisesta vallitseviin ympäristöolosuhteisiin. Hämähäkkien aikuistumisajoissa oli suurta vaihtelua yksilöiden välillä. Ravintotaso ja kasvuympäristö vaikuttivat aikuistumisaikaan, mutta populaatioiden välillä ei ollut eroa. Aikuistumiseen kuuluva aika näyttääkin määräytyvän joustavasti ympäristöolosuhteiden mukaan.

Tässä työssäni tutkin myös populaatioiden välisiä eroja seksuaalisessa käyttäytymisessä. Kolmannessa osatyössäni havaitsin eroja koirashämähäkkien kosintamenoissa ja parin etsimisen aktiivisuudessa soilla ja niityillä elävien populaatioiden välillä. Nämä erot johtuvat todennäköisesti koiraiden sopeutumisesta erilaisiin määriin rummutusalustana toimivia kuivia lehtiä, mutta näillä eroilla ei näyttänyt olevan vaikutusta siihen, minkä populaation koiraan naaraat valitsivat.

Eri kokoisten populaatioiden välillä oli myös merkittäviä eroja rummutusäänien ominaisuuksissa ja rummutusaktiivisuudessa (neljäs osatutkimus). Isojen populaatioiden koiraat rummuttivat enemmän ja niiden rummutusäänien taajuus (iskuja sekunnissa) oli selvästi suurempi. Tämä selkeä ero eri kokoisten populaatioiden välillä saattaa johtua sopeutumisesta erilaisiin kohtaamistodennäköisyyksiin samanlajisten koiraiden ja naaraiden kanssa. Pienissä populaatioissa koiras kohtaa lisääntymiskauden aikana vain muutamia naaraita ja vähäisempikin panostus rummutuksen määrään ja ominaisuuksiin riittää saavuttamaan naaraan huomion. Toisaalta myöskään naaraat eivät voi olla kovin valikoivia koiraan rummutusäänien suhteen, sillä vaihtoehtoja on niukasti, ja näin ollen valintapaine rummutuskäyttäytymisen laatuun ja määrään saattaa kaikkein pienimmissä populaatioissa olla vähentynyt.

Viidennessä osatutkimuksessa vertailin tiheiden ja harvojen suopopulaatioiden koiraiden kosintakäyttäytymistä, koiras-koiras -kilpailua ja rummutusäänien ominaisuuksia. Tiheiden populaatioiden koiraat näyttivät panostavan enemmän liikkumiseen, eli naaraan etsimiseen, ja rummutusäänien pituuteen ja äänen voimakkuuteen, jotka ovat naaraan suosimia ominaisuuksia. Harvojen populaatioiden koiraat taas osoittivat suurempaa aggressiivisuutta toisia koiraita kohtaan. Harvoissa populaatioissa koiraat siis panostavat enemmän rummutuksen määrään ja toisten koiraiden häätämiseen, ja tiheissä liikkumiseen ja rummutusäänien laatuun. Harvoissa populaatioissa mahdollisia parittelukumppaneita on vähän, ja sellaisen kohdatessaan koiraan kannattaa taistella siitä. Tiheissä populaatioissa naarailla on paljon valinnan varaa, ja koiraiden kannattaa panostaa paitsi rummutussignaalin laatuun, myös ennemmin uuden naaraan etsimiseen kuin tappelemiseen siitä koiraiden välisessä kilpailutilanteessa.

Väitöskirjatyöni osatutkimusten perusteella voidaankin todeta, että rummuttavalla *Hygrolycosa rubrofasciata* hämähäkkilajilla havaitut populaatioiden väliset erot kasvussa ja seksuaalisessa käyttäytymisessä johtuvat osaksi ympäristön vaikutuksesta, mutta myös joitakin geneettisiä eroja löytyy. Erilaiset elinympäristöt ja yksilötiheydet suosivat erilaisia kasvun ja käyttäytymisen ominaisuuksia ja ainakin osa näistä havaituista eroista näyttäisi olevan sopeutumia paikallisiin olosuhteisiin.

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Original papers

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**Variation in reproductive traits among populations of the *Hygrolycosa*
rubrofasciata wolf spider**

by

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Manuscript

Variation in reproductive traits among populations of the *Hygrolycosa rubrofasciata* wolf spider

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Abstract

Variation in life-history characteristics such as body mass, number of offspring per female, and offspring body mass was examined among populations of a drumming wolf spider *Hygrolycosa rubrofasciata*. Males from dry meadows were on average smaller than males originating from wet meadows and bogs. There was also great variation in average body mass within the habitats, particularly among the bog populations. There was no difference in brood size between the bog and meadow habitats, but brood sizes differed among meadow populations. The mean offspring body mass was similar on both habitats, even if there was some variability among the meadow populations. The number of offspring increased strongly with the female body mass but the relationships between female and offspring sizes, and between offspring number and body mass were absent or weak. Thus, there is no visible trade-off between offspring size and number. Maturation of *H. rubrofasciata* normally takes three years, but we found that in favourable years some individuals from certain populations may mature in only two years. In sum, there is significant variation in body mass and brood sizes between even very closely located populations of the drumming wolf spider *H. rubrofasciata*. The variation in adult body mass and brood size among populations is probably due to differences in amount of food, and growth conditions seem to be generally most favourable in wet meadows and bogs.

Key words: body mass, brood size, *Hygrolycosa rubrofasciata*, local populations

Introduction

Body mass of arthropod females is a principal constraint on their potential fecundity (e.g. Larsson 1990, McLain *et al.* 1990, Honek 1993) also in spiders (Marshall & Gittleman 1994, Simpson 1995). However, body size can also affect significantly on the longevity of individuals e.g. in terms of predation risk and parasitism, and thus potential fecundity may differ from the actual fecundity (Leather 1988). In a drumming wolf spider *Hygrolycosa rubrofasciata*, we have found a strong positive correlation between female body mass and number of offspring, and when reared in laboratory conditions females attempt to reach as large size as possible before maturation. (Parri 1999, Vertainen *et al.* 2001).

There is evidence that large body size is advantageous to male arthropod mating success (e.g. Bushkirk 1975, Riechert 1978, Vollrath 1980, Borgia 1982, Partridge & Farquahr 1983, Ward 1983, Simmons 1986, Fincke 1992, Savalli & Fox 1998,) or large size of males may be advantageous to female fecundity (Savalli & Fox 1998). However, male body mass is not always linked to its mating success despite the advantage of larger males in male-male encounters (Arita & Kaneshiro 1988). Sexual selection on male body mass may also vary within and between populations (Carroll & Salamon 1995). Male body mass is a good estimate of size in *H. rubrofasciata*, and after maturation it does not vary much (Kotiaho *et al.* 1996, 1999a). However, body mass is not correlated with male drumming rate and thus it is not under female choice (Kotiaho *et al.* 1996, Mappes *et al.* 1996, Parri *et al.* 1997, Parri 1999). Costs of drumming are higher for larger males, but large males appear to be able to compensate their increased energy requirements since drumming is not reduced (Kotiaho *et al.* 1998). Larger males are better competitors in male-male interactions (Kotiaho *et al.* 1997, 1999b), but this is the case only if size difference is large, and mating success is not influenced by male size (Kotiaho *et al.* 1996). There is no detectable relationship between male body mass and their overwintering survival (Kotiaho *et al.* 1999c). In this species, the effects of male body mass on reproductive success are thus minor compared to females. Indeed, unlike females, males reared in laboratory mature more or less at certain size independently of the amount of food available (Parri 1999, Vertainen *et al.* 2001).

Differences between populations in adult body sizes have been reported in several studies, and they are typically related to habitat type (e.g. Hugueny & Louveaux 1986, Brown *et al.* 1992, Cherrill & Brown 1992, Rowe 1994, 1997). In some cases there is a habitat difference but no correlation between similarity in body size and geographic distance of populations (Palmer 1994). In addition to environmental conditions, microclimate (Honek 1986), demographic (King 1989, Bruce & Hairston 1990, Rowe 1997) or social structures may cause body size differences between populations even in a very small scale (Gortázar *et al.* 2000).

Clutch size in spiders is correlated with female body mass across spider taxa (Marshall & Gittleman 1994). Tanaka (1995) has found a trade-off between clutch size and offspring size in a web building spider (*Agelena limbata*).

Offspring size of this species is positively correlated with the body size of the mother, and negatively correlated with the clutch size. Among spider genera clutch and egg sizes are positively correlated with body mass, and there is a negative correlation between egg number and size (Simpson 1995). However, in their comparative study Marshall & Gittleman (1994) did not find empirical evidence for a size-number trade-off in allocation of resources within clutches.

Inter-population variation in clutch size and offspring size, and the allocation between them may be due to environmental variability or genetic differences between isolated populations (Mashiko 1990, 1992, Rowe 1994, Lindeman 1996). In a scorpion (*Centuroides vittatus*) among-population variation in reproductive effort may result either from genetic differences or from phenotypic plasticity (Brown & Formanowicz 1995). In a shrimp *Paratya australiensis* egg size is under strong genetic control and clutch size is influenced by the environmental conditions (Hancock *et al.* 1998). In *Hygrolycosa rubrofasciata* female body mass correlates positively with brood size, and variation in brood sizes is most likely due to the variation in environmental conditions, e.g. in food amount. There may also be genetic differences between populations in brood size, since adult female body mass differs genetically among closely located populations of this species (Vertainen *et al.* 2001).

The drumming wolf spider *H. rubrofasciata* is sexually dimorphic, ground dwelling lycosid spider. Early in spring males court females with a special drumming signal, produced by hitting the abdomen on dry leaves. Spiderlings hatch in late June – early July, and in southern Finland maturation typically takes three years. Males die after the mating season while part of females can survive to the next summer and thus reproduce several times. *H. rubrofasciata* inhabits basically two different kinds of habitats, bogs with deciduous trees and meadow type habitats that are mostly abandoned fields. We have previously found genetic differences between the local populations in adult body mass (Vertainen *et al.* 2001, Mappes *et al.* unpubl.), and also differences in male mating tactics and drumming signal characteristics (Vertainen *et al.* unpubl.).

In this exploratory study, we compared male body mass between several populations from bog and meadow habitats in three successive years. In larger data sets for two years we tested whether variation in body mass is explained by abiotic environmental conditions. The body mass distributions in populations with large sample sizes were examined to study possible variation in maturation times. We compared also brood sizes and offspring body mass between bog and meadow populations, and correlations between these variables and female body mass.

Materials and methods

Spiders for this study were collected from populations that were located in an area of ca. 5 km² in Sipoo, southern Finland (60°16'N and 25°14'E). The populations of *H. rubrofasciata* are located within a few hundred meters to a few kilometres from each others, and they inhabit half open bogs and meadow

habitats (mainly abandoned fields). Populations are isolated from each others by unsuitable habitats for this species (forest and rocky habitats).

The body mass was used as a measurement of adult body size, since it is easy to obtain from living individuals and highly correlated with the carapace width ($r = 0.89$, $n = 484$, $p < 0.001$, males from 18 populations in 1999). In addition, the body mass variation is much greater between than within males and thus it is an extremely repeatable measure (99.4 %) (Kotiaho *et al.* 1996)

Year 1997

Males from ten populations were collected by pitfall trapping and hand picking soon after snowmelt between April 25th and May 19th 1997. Males were placed individually in small plastic film jars with some moss (*Sphagnum* sp.), kept in cool temperature (ca +10°C), and brought to the laboratory. Spiders were weighed within a week to the nearest 0.1 mg with analytical balance (AND HA-202M) and kept meanwhile in low temperature, ca. +5°C, to keep their metabolic rate low.

Two distinct groups of females were collected from the same ten populations where males were collected. The first group consisted mostly of unmated females collected by hand between April 24th and May 15th, and they were treated and weighed similarly than males. The second group of females was collected from these populations between June 18th and 21st. These females carrying their egg sacs were brought to laboratory, and immediately when juveniles were hatched and detached from the surface of the egg sac (between 30th June and 30th July), they were counted and weighed individually to the nearest 0.01 mg with the analytical balance.

Year 1998

Male spiders were collected from 36 populations, 10 meadows and 26 bogs, between May 9th and 29th with pitfall traps. These populations included the same populations than in year 1997, and the rest were new study sites. Males were treated similarly than in year 1997 prior to weighing them into the nearest 0.1 mg.

Environmental characters of the all 36 study areas were measured during summer 1998 from five random sample sites. Total vegetation cover percentage was estimated by eye from these five sampling sites, each 1m² in size. Light index was estimated for these sites using five categories: light index 1 indicating a sample site shaded by trees or bushes for whole day, and light index 5 indicating open area with a possibility of sun shine all the day. Soil samples were collected between July 18th and 20th 1998 with a soil corer, which takes cylindrical (diameter 57 mm) samples which can be divided into 40 mm thick layers of sub-samples. Two samples from each 5 sample sites were taken, and soil litter thickness was measured. To gain relative soil humidity (%) one of the two samples from each study site was randomly chosen. The second sample-

layer from top (i.e. depth of 40-80 mm) of this sample was weighed (wet mass), dried in temperature of 80 °C for about 72 hours and weighed again to measure the dry mass. The soil humidity percentage was calculated from the wet and dry mass. The pH was measured with an electronic pH-meter from dry soil litter mixed with purified water. To reduce the data and to account for the fact that the different environmental variables were correlated with each others the principal component analysis (PCA) was performed for the environmental variables.

Year 1999

Males from 32 bog populations and from 9 meadow populations were collected with pitfall traps between 12th May and 2nd June 1999. Populations were mostly the same than in previous year with some new sites included. Males were brought to laboratory and they were treated and weighed using similar methods as in earlier years.

Egg sac carrying females were collected from four bog populations and four meadow populations in Sipoo between 9th and 18th June 1999. Females were brought to laboratory, fed *ad lib.* with fruitflies (*Drosophila melanogaster*), and kept in room temperature ca. +20 - +22 °C until spiderlings hatched. Number of offspring was counted, and a joint body mass of three juveniles were weighed from a sample of broods to attain an estimate of the mean body mass of juveniles.

Soil samples were collected and other environmental variables were measured during June 9th – June 11th. Soil litter thickness, moisture percentage, pH, and light index were measured in a similar way than in year 1998. Instead of vegetation cover percentage, in year 1999 canopy cover percentage of trees (circular sampling site, diameter 20 m) was used. Principal component analysis (PCA) was made for these five abiotic environmental factors.

Results

I Variation in adult body mass between populations and years

There was a significant difference between ten populations and three years (1997 - 1999) in male body mass of *H. rubrofasciata*. There was also a significant interaction indicating that male body variation between years is different in separate populations (Table 1, Figure 1). There was also a significant difference in female body mass (collected soon after snow melt) between the same populations (ANOVA: $F_{7,1092} = 20.8$, $p < 0.001$). Females of this species are ca. 1.4 – 1.7 times larger than males (Table 2), and this difference is even greater later in the mating season since females collect reserves for reproduction.

II Effects of environmental factors on male body mass

Principal component analyses (PCA) extracted two major components in both years 1998 and 1999 (Table 3). The factors were used in a regression analysis to explain the variation in male body mass in the respective year. In 1998, the first principal component, separating bogs (higher moisture, lower pH) and meadows, explained significantly variation in male body mass, and the second principal component (related to vegetation cover) had a tendency for that (Linear regression: $R^2=0.22$, $F_{2,33}=4.55$, $p=0.018$; factor 1 $\beta=0.36$, $t=2.33$, $p=0.026$; factor 2 $\beta=0.30$, $t=1.92$, $p=0.064$) (Figure 2 a). The trend was that males in the bogs were somewhat larger in size. Principal components did not explain the variation within the bog habitat (Linear regression: $R^2=0.06$, $F_{2,23}=0.69$, $p=0.512$; factor 1 $\beta=0.09$, $t=0.44$, $p=0.662$; factor 2 $\beta=0.24$, $t=1.14$, $p=0.262$). Within meadows there was a slight tendency for principal components to explain variation in body mass (Linear regression: $R^2=0.53$, $F_{2,27}=3.90$, $p=0.073$; factor 1 $\beta=0.46$, $t=1.61$, $p=0.153$; factor 2 $\beta=0.40$, $t=1.38$, $p=0.210$), the tendency being again that the more bog-like meadows with high moisture had larger males.

Also in 1999, the first principal component, separating bogs (higher moisture and lower pH) and meadows, explained significantly variation in male body mass, but the second principal component (related to light index and soil litter thickness) did not (Linear regression: $R^2=0.19$, $F_{2,38}=4.43$, $p=0.019$; factor 1 $\beta=0.42$, $t=2.86$, $p=0.007$; factor 2 $\beta=-0.12$, $t=-0.85$, $p=0.404$) (Figure 2 b). Principal components did not explain variation in male body mass within bog populations (Linear regression: $R^2=0.09$, $F_{2,29}=1.40$, $p=0.263$; factor 1 $\beta=0.21$, $t=1.18$, $p=0.244$; factor 2 $\beta=-0.18$, $t=-1.02$, $p=0.318$). Within the meadow populations, there was a slight tendency for the first principal component to explain male body mass variation (Linear regression: $R^2=0.40$, $F_{2,6}=2.01$, $p=0.214$; factor 1 $\beta=0.68$, $t=2.00$, $p=0.092$; factor 2 $\beta=0.22$, $t=0.64$, $p=0.544$).

III Body mass distributions

Male body masses are generally normally distributed within populations (see table 4 for parameters and tests). Maturation of *H. rubrofasciata* lasts normally three years, and usually there are mature males only from one cohort in each spring. However, in year 1997 we found in some populations that male body mass distributions did not follow a normal curve, but had two separate peaks (populations 12 and 14 in figure 3). This indicates, that in this particular year a part of the younger cohort has already matured after two years of growth, instead of the normal three years. In year 1999 male body masses of these two populations were normally distributed.

IV Differences among populations in brood sizes and juvenile body mass

There was no difference between the four meadow and the four bog populations in the number of offspring hatched (brood size) in 1999, but populations differed from each others within the habitats (nested ANOVA: habitat $F_{1,6} = 2.51$, $p = 0.164$, population nested within habitat $F_{6,383} = 7.47$, $p < 0.001$). There was no difference in mean offspring body mass between habitats or among populations within the habitats (nested ANOVA: habitat $F_{1,6} = 0.25$, $p = 0.633$, population nested within habitat $F_{6,77} = 1.91$, $p = 0.089$). When tested within habitats, meadow populations differed from each others in brood size (ANOVA: $F_{3,180} = 14.69$, $p < 0.001$) and in mean offspring body mass (ANOVA: $F_{3,42} = 2.96$, $p = 0.043$). There was no difference in brood size (ANOVA: $F_{3,203} = 1.43$, $p = 0.235$) or in mean offspring body mass (ANOVA: $F_{3,35} = 0.71$, $p = 0.555$) within the populations from the bog.

There was a positive correlation between female body mass and the number of offspring hatched both among bog (Pearson correlation: $r = 0.44$, $n = 207$, $p < 0.001$) and meadow populations ($r = 0.40$, $n = 184$, $p < 0.001$) (Figure 4 a). Also a meta-analysis considering correlations within each of the eight populations showed a highly positive correlation between female body mass and number of offspring (Meta-analysis, Schmidt-Hunter method after z-transformation: sample size weighted mean $r = 0.42$, $n = 391$, $p < 0.001$; Hedges & Olkin 1985).

Female body mass correlated positively with mean offspring body mass within meadows ($r = 0.40$, $n = 46$, $p = 0.006$), but not within bogs ($r = 0.08$, $n = 39$, $p = 0.643$) (Figure 4 b). However, a meta-analysis combining the relationships from the eight populations revealed no significant correlation between female body mass and offspring body mass (Meta-analysis, Schmidt-Hunter method: weighted mean $r = 0.20$, $n = 85$, $p = 0.064$).

There was no correlation between the number of offspring per brood and mean offspring body mass among meadow ($r = 0.17$, $n = 46$, $p = 0.264$) or bog populations ($r = -0.12$, $n = 39$, $p = 0.468$) (Figure 4c). Neither was there any correlations within populations (Meta-analysis, Schmidt-Hunter method: weighted mean $r = -0.07$, $n = 85$, $p = 0.543$).

To study the effects of abiotic environmental factors on brood size and juvenile body mass, the principal component analysis (PCA) was conducted for five environmental factors. PCA extracted one principal component that explained 76 % of total variance, and correlated positively with soil moisture ($r = 0.93$), soil litter thickness ($r = 0.88$), and canopy cover ($r = 0.76$), and negatively with light index ($r = -0.87$) and ln-transformed pH ($r = -0.92$). The first principal component separated bogs and meadows, but it did not explain variation between populations (Linear regressions; number of offspring: $R^2 = 0.25$, $F_{1,6} = 1.96$, $p = 0.211$; $\beta = -0.50$, $t = -1.40$, $p = 0.211$; offspring body mass: $R^2 = 0.00$, $F_{1,6} = 0.003$, $p = 0.961$; $\beta = 0.02$, $t = 0.15$, $p = 0.961$) (Figure 5).

In year 1997, there was no difference between bogs, wet meadows and dry meadows in brood size, but populations differed from each others within the habitat types (nested ANOVA: habitat $F_{2,7} = 0.77$, $p = 0.496$, population nested within habitat $F_{7,242} = 4.18$, $p < 0.001$). Mean offspring body mass was similar on

the three habitat types but within the habitats there were differences in offspring body mass between populations (nested ANOVA: habitat $F_{2,7} = 0.03$, $p = 0.973$ population nested within habitat $F_{7,211} = 2.98$, $p = 0.005$). There was a weak positive correlation between the number of offspring and mean juvenile body mass (Pearson correlation: $r = 0.19$, $n = 221$, $p = 0.006$).

Discussion

There were significant differences between populations and years in male body mass. A significant interaction between these variables indicated that mean population body mass varies differently in different years. Differences between years are probably caused by variation in weather conditions during the growth seasons (Honek 1986). Environmental factors, such as amount of food available may affect considerably the body mass in *H. rubrofasciata*, even though males having more food are not necessarily larger in size (Vertainen *et al.* 2001). Our common garden experiments in laboratory and field enclosures suggest that part of this variation in adult body mass, both in females and males, has a genetic basis (Vertainen *et al.* 2001, Mappes *et al.* unpubl.).

Inter-population differences in body mass have been found in species inhabiting several habitat types (e.g. Brown 1992, Cherrill & Brown 1992, Palmer 1994). In *H. rubrofasciata* the first principal component extracted from abiotic environmental variables separated clearly bogs and meadows, but did not explain all the variation in male body mass among the populations. Among meadows the males from populations most resembling bogs had a tendency to be larger in size. This may be due the different amounts of food available for the spider. Also differences in microclimate and especially in temperature may affect the body size of the spiders. Ectotherm animals tend to be larger in cooler temperatures (e.g. Atkinson 1994, Atkinson & Sibly 1997). However, in this study populations of *H. rubrofasciata* located only few kilometres from each others and thus the variation in temperature is not very great.

The distributions of adult male body mass generally follow normal distributions, but in year 1997 there were clear exceptions to the rule. In two populations (both wet meadows) the male body mass distribution had two separate peaks. In year 1999 body mass distributions of these two populations followed normal curve. In a common garden study we raised *H. rubrofasciata* in enclosures built on wet and dry habitats. Most of the individuals matured at the end of their second year and the rest at the end of the third year (Mappes *et al.* unpublished). The enclosures were in central Finland, about 300 kilometres north from Sipoo, and they apparently provided a very good growing habitat for this species. Thus, it seems that in some favourable years *H. rubrofasciata* may mature in two years also in natural habitats, but only in some of the wet meadows. The evidence for three years being the normal maturation time in Sipoo, is the fact that in spring (and autumn) there are two distinct size groups among the spiderlings in addition to the mature individuals (unpublished data).

Females from meadows produced on average as many offspring than females from bogs in 1999. The four meadow populations differed from each others in brood sizes, but bog populations did not. Mean body mass of offspring was similar in bogs and meadows, and there was variation in mean offspring body mass only among meadow populations. In 1997, there were no differences in number of offspring per female or in mean offspring body mass between populations from dry meadows, wet meadows, or bogs, but there is variability in these traits among populations within the habitats. Hancock *et al.* (1998) have found in a reciprocal translocation experiment that the egg size of common shrimp (*Paratya australiensis*) is under strong genetic control, but the clutch size is affected by environmental conditions. Also in a freshwater prawn (*Macrobrachium nipponense*) the genetic control on egg size is strict while that on clutch size is weak (Mashiko 1992). In *H. rubrofasciata* both brood size and mean offspring body mass were highly variable traits, but neither of them was significantly explained by abiotic environmental factors extracted from PCA.

The most probable environmental variable affecting the variation in brood size is the amount of food available for females to gather resources for reproduction. Unfortunately, we have not managed to measure directly the availability of food. However, the habitats occupied by the populations of *H. rubrofasciata* vary greatly in respect of environmental characters (e.g. soil moisture, pH, litter thickness, vegetation, soil fauna) (unpublished data) and thus there is probably differences also in amount and availability of food. In invertebrates, it is common that egg size or offspring size are affected by the food level (e.g. Qian & Chia 1991, Trubetskova & Lampert 1995, Guisande *et al.* 1996). Also female body mass is positively correlated with offspring size in many taxa (Roff 1992). Variation in offspring body mass was great within *H. rubrofasciata* populations, but it was not strongly linked to female body mass or brood size. There was no difference in hatchling body mass between habitats. and differences only among meadow populations. Thus, the optimal size at hatching seems not to be related to mother's condition, and we have no obvious explanation for the high variability among broods.

There was a strong positive correlation between female body mass and number of offspring produced, both within bog and meadow populations. The result is consistent with the general relationship between body size and fecundity in insects (Larsson 1990, McLain *et al.* 1990, Honek 1993, but see Leather 1988) and in spiders (Marshall & Gittleman 1994, Simpson 1995). Female body mass did not correlate with mean body mass of offspring within bogs, but in meadow habitats there was a slight positive correlation. Offspring number and body mass did not correlate on either habitat. In 1997 there was a weak positive correlation between offspring number and size. Thus, it would seem that there is no trade-off between brood size and offspring size in *H. rubrofasciata*, which is found in some spiders species (Simpson 1995, Tanaka 1995) but not in all (Marshall & Gittleman 1994). However, the lack of correlation in non-experimental data sets does not exclude the possibility of trade-offs hidden by variation in mother condition.

To conclude, populations differed greatly in adult male body mass with an additional yearly variation, individuals from the dry meadow habitats being

the smallest. Population variation was partly explained by an environmental factor separating bog and meadow populations, but still much of the variation remains unexplained, especially among populations inhabiting bogs. There were also differences in brood size between populations, but not between bogs and meadows. Female body mass correlated strongly with the number of offspring, and in meadows slightly with offspring body mass. However, offspring number and body mass were only weakly, if at all, correlated with each others. There was no visible trade-off between offspring number and size, even though the existence of them cannot be excluded. It is likely, that much of the variation in adult body mass among populations is due to the availability of food, and this also has an influence on brood sizes.

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TABLE 1 Variance table for 2-way ANOVA of differences between populations and years in male body mass.

Source	Df	MS	F	p
Population	9	184.64	27.83	<0.001
Year	2	166.44	25.09	<0.001
Population x year	18	59.91	9.03	<0.001
Error	3001	6.64	—	—

TABLE 2 Female body mass from 10 populations and female /male body mass ratio from each populations (year 1997).

Population number	Habitat type	n	Mean body mass (mg)	S.D.	Female / male mass ratio
2	Dry meadow	67	23.6	5.45	1.59
3	Dry meadow	42	23.2	5.67	1.58
4	Dry meadow	224	21.0	4.96	1.43
11	Dry meadow	13	23.2	4.91	1.60
7	Wet meadow	346	21.9	3.56	1.49
12	Wet meadow	44	24.6	7.07	1.55
14	Wet meadow	222	25.6	5.77	1.42
15	Wet meadow	102	24.4	4.19	1.70
1	Bog	53	25.1	4.12	1.49
8	Bog	28	26.2	4.31	1.50

TABLE 3 Correlations between principal components and environmental variables a) Year 1998 b) Year 1999.

a)

Variable	PC 1	PC 2
% of total variance explained	61.9	22.2
Vegetation cover %	0.42	0.85
Light index	-0.78	-0.42
Soil litter thickness	0.84	-0.33
Moisture %	0.90	-0.22
Ln (pH)	-0.90	0.23

b)

Variable	PC 1	PC 2
% of total variance explained	63.8	21.1
Canopy cover %	0.83	-0.40
Light index	-0.64	0.70
Soil litter thickness	0.72	0.55
Moisture %	0.86	0.27
Ln (pH)	-0.91	-0.16

TABLE 4 Parameters and normality test (Kolmogorov-Smirnov) of male body mass distributions from four bog populations in years 1997 and 1999.

a) Year 1997

Population	1	12	14	15
Mean	17.0	15.9	18.1	14.3
S.D.	2.29	4.28	3.93	2.2
Min	9.9	10.0	9.2	9.0
Max	22.9	26.1	25.7	21.7
Skewness	-0.19	0.46	-0.26	0.46
Kurtosis	-0.09	-1.16	-1.11	0.62
Statistics	0.04	0.19	0.11	0.05
Df	283	117	197	201
P	0.200	<0.001	<0.001	0.200

b) Year 1999

Population	1	12	14	15
Mean	15.1	16.1	16.8	15.5
S.D.	2.67	2.08	2.41	5.26
Min	8.6	11.0	11.7	10.0
Max	21.4	21.7	22.6	23.0
Skewness	0.08	0.13	0.09	0.53
Kurtosis	-0.28	0.35	-0.10	0.54
Statistics	0.05	0.07	0.08	0.06
Df	121	72	45	284
P	0.200	0.200	0.200	0.012

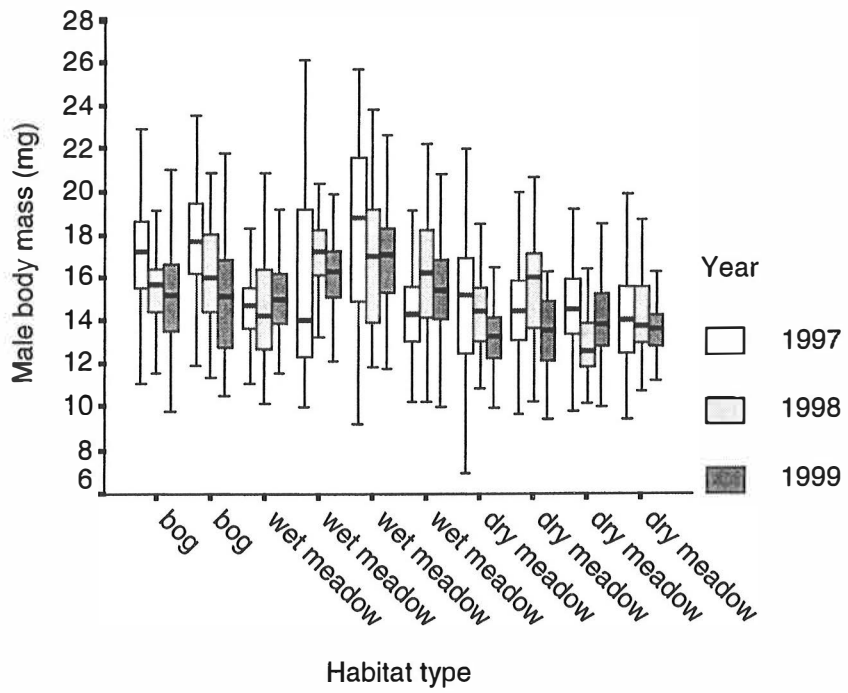


FIGURE 1 Male body mass from ten populations in years 1997 - 1999. Boxplots representing minimum, Q_1 (25 % observations), median, Q_3 (75 % observations), and maximum.

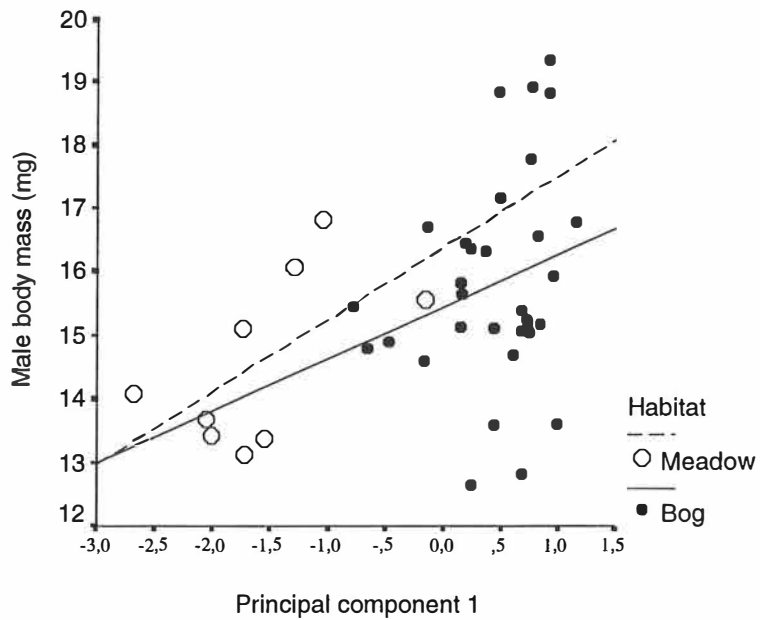
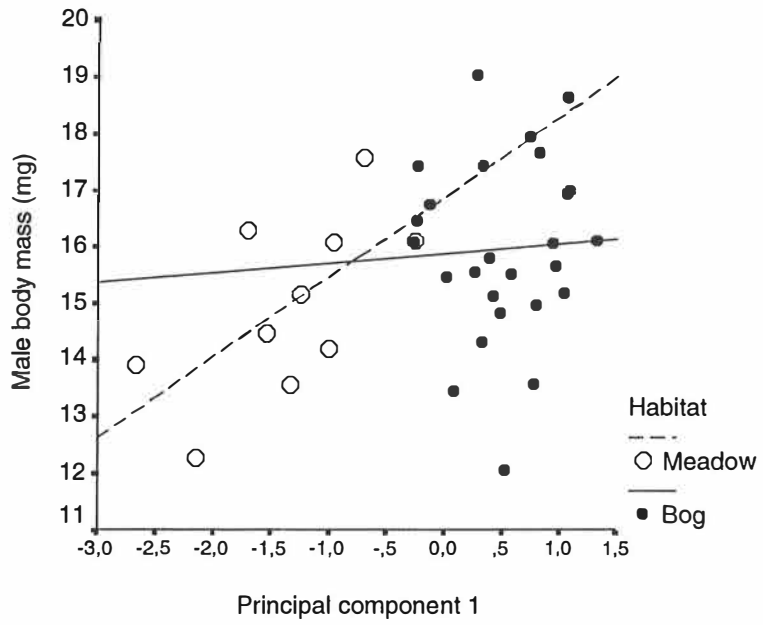
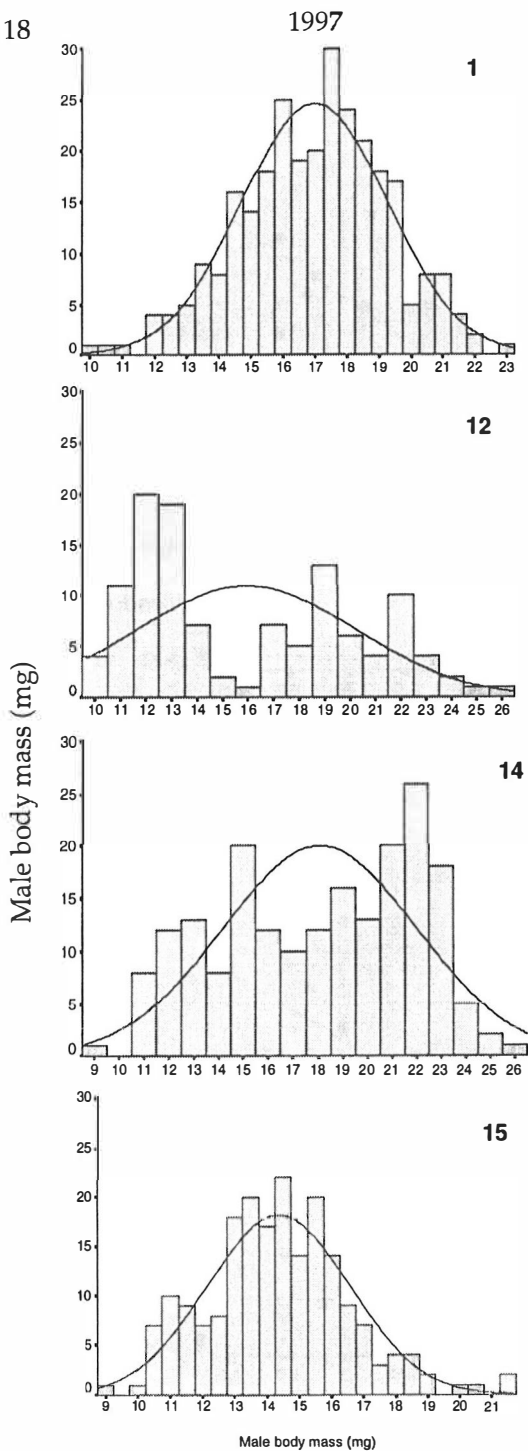


FIGURE 2 Male body mass from bog and meadow habitats plotted against the first principal component extracted from abiotic environmental factors. Each dot represents one population. a) year 1998, b) year 1999.

18



1999

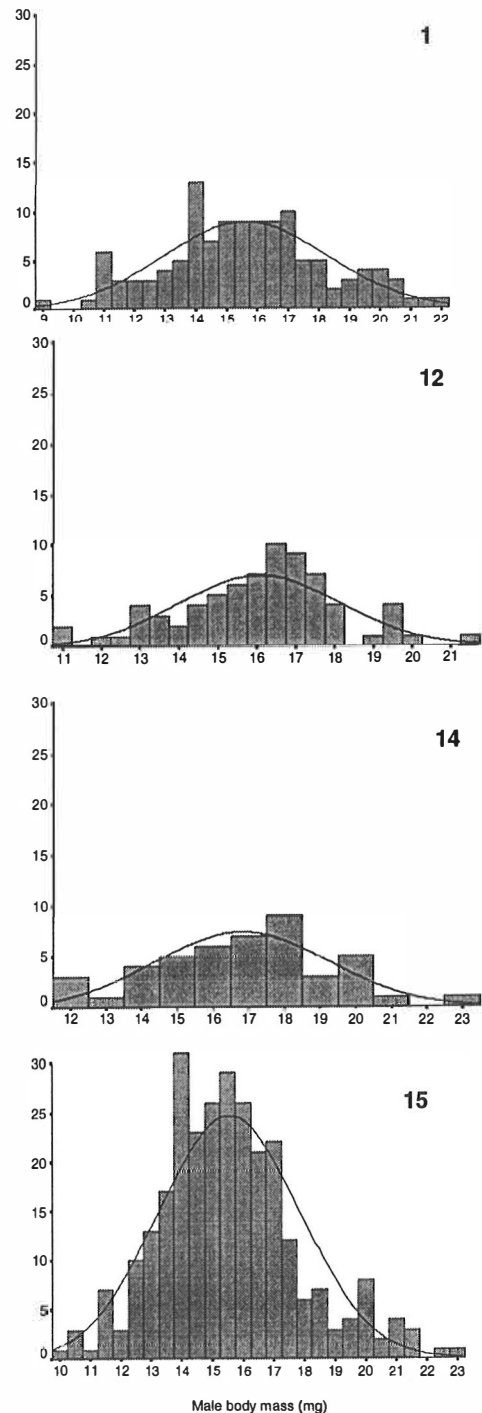
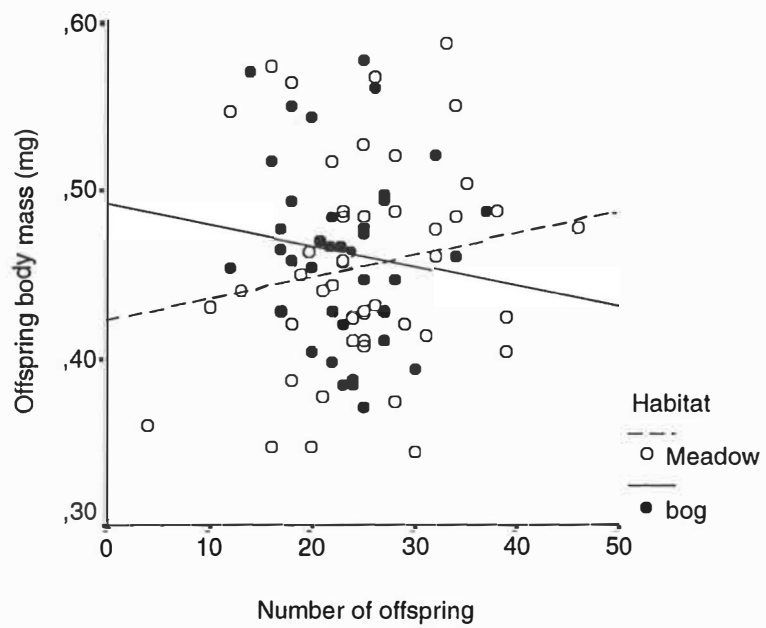
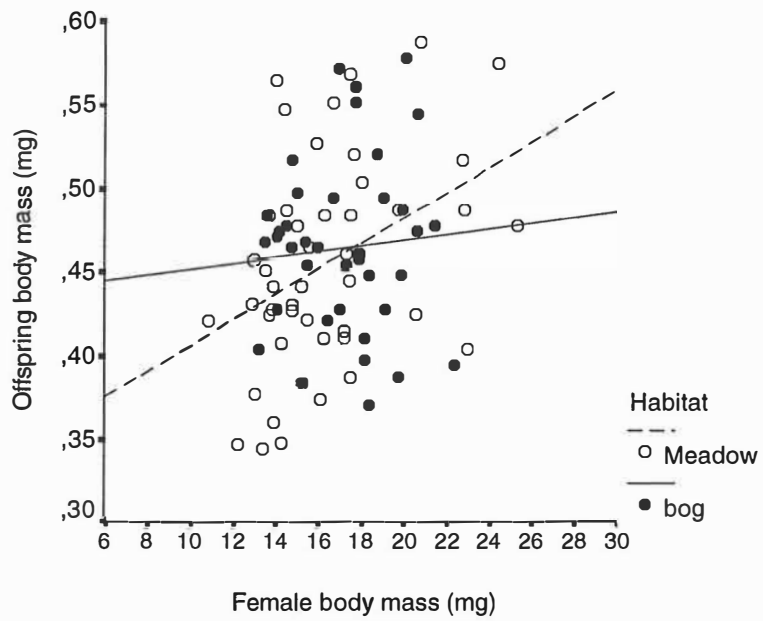


FIGURE 3 Male body mass distributions with normal curve from four populations in two years, 1997 and 1999. Population number 1 represents typical body mass distribution. Male body mass in populations 12 and 14 are not normally distributed in year 1997, having two distinct peaks. The distribution of population 15 seems to be slightly abnormal in year 1999 (Table 4).



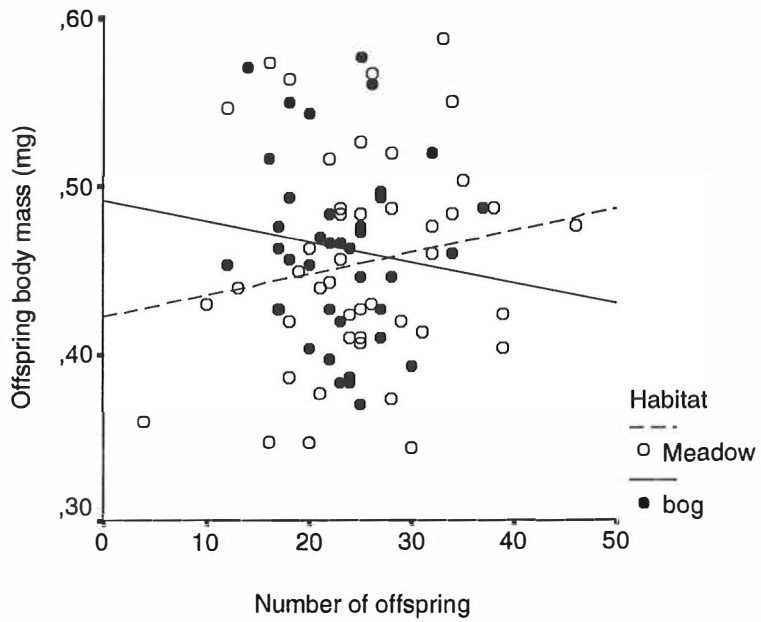


FIGURE 4 Correlations between a) female body mass, b) number of offspring and c) offspring body mass in four bog and four meadow habitats in year 1999.

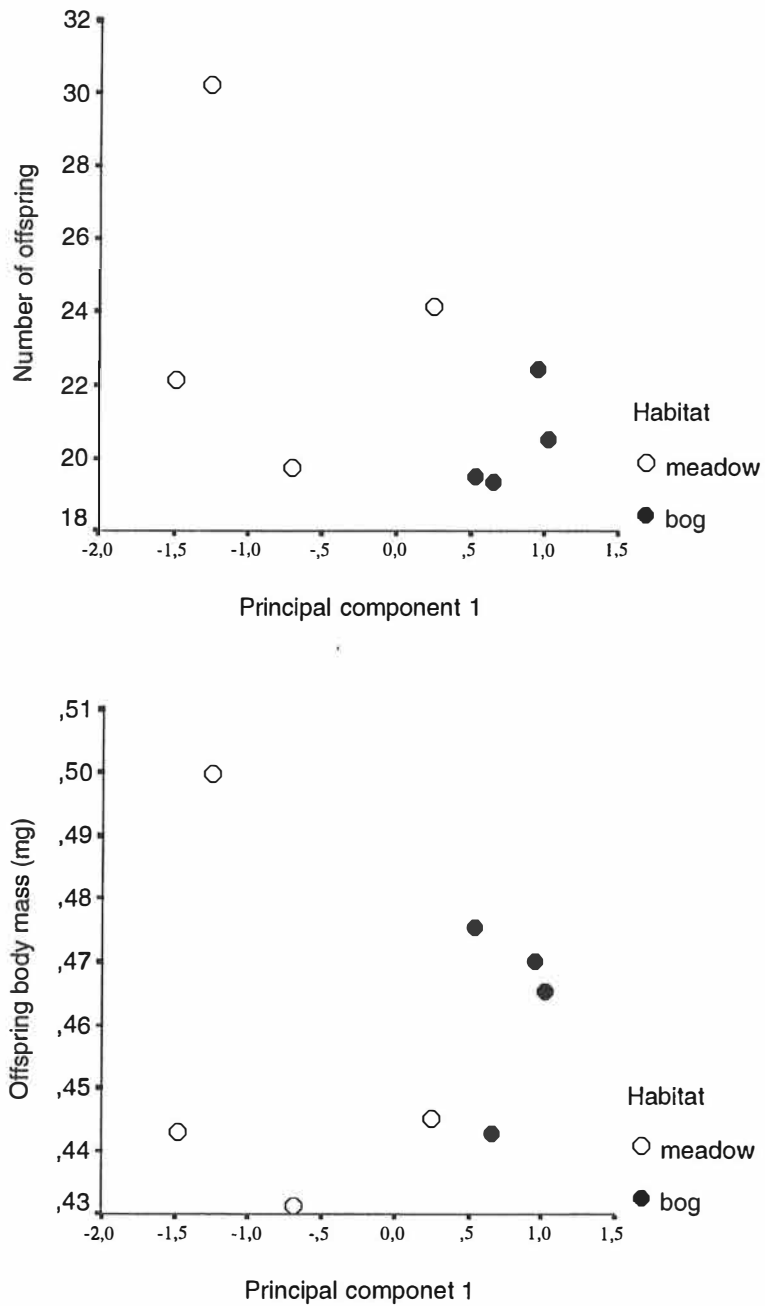


FIGURE 5 a) Mean number of offspring per female and b) offspring body mass from bog and meadow habitats plotted against the first principal component extracted from the abiotic environmental factors. Each dot represents one population.

II

**Sexual differences in growth strategies of the wolf spider *Hygrolycosa
rubrofasciata***

by

Laura Vertainen, Rauno V. Alatalo, Johanna Mappes and Silja Parri, 2001

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III

**Habitat differences in mate searching behaviour in the drumming wolf
spider *Hygrolycosa rubrofasciata***

by

Laura Vertainen, Jari J. Ahtiainen, Rauno V. Alatalo, Johanna Mappes and
Silja Parri

Manuscript

Habitat differences in mate searching behaviour in the drumming wolf spider *Hygrolycosa rubrofasciata*

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Abstract

The drumming wolf spider *Hygrolycosa rubrofasciata* inhabits meadow and bog habitats differing in vegetation type and soil quality. During the mating season, early spring bogs are dominated by fallen leaves and moss, while meadows have dry hay on the ground. Male *H. rubrofasciata* call for females by hitting their abdomen on suitable drumming substrate and females use this drumming sound in mate choice. Males search for receptive females by moving around and stopping to drum at times. We tested, whether males living on a bog covered with the optimal drumming substrate and males living on a meadow with limited availability of drumming substrate do have different mating strategies. Firstly, one male from a bog and the other from a meadow were allowed to compete for females on two arenas, containing either dry leaves or pieces of dry hay, with and without females. Despite their source population, males had a higher drumming rate on the leaf arena, but they invested more to moving on the hay arena. When females were absent, males from the bog habitat had a higher drumming rate on the leaf arena. In female presence, males from the bog population won more often male-male interactions on the hay arena, and had a tendency for that on the leaf arena. Males gained female responses more frequently on the leaf arena, but the male origin did not significantly affect female preferences. In the second part of the experiment males from ten bog populations (tested singly) had higher drumming and moving activities than males from ten meadow populations. Within habitats populations differed from each others in drumming activity. Males from bogs were larger than males from meadows and male body mass correlated positively both with drumming activity and mobility, but explained only partially the difference between habitats. In the third part of the experiment male drumming sounds were played to females from bogs and meadows. Females showed no preference for the drumming sounds of the males from their own or the strange population. The results indicate that while female choice was not affected by the difference in male signalling, there were differences in male mating tactics and mate searching strategies between the two populations originating from bog and meadow habitats.

Key words: mate searching, mating tactics, sexual signalling, wolf spider

Introduction

Interpopulation differences in courtship signalling and mating behaviour and tactics have been studied e.g. in mammals (Parijs *et al.* 2000), in insects (Paillette *et al.* 1997, Miller *et al.* 1998 and references therein), in fish (e.g. Endler 1987, Magurran & Seghers 1990, Shaw *et al.* 1994, Endler & Houde 1995, Godin 1995) and in some spider species (Miller *et al.* 1998). Mating success between individuals from different populations has gained relatively little attention (Rupp & Woolhouse 1999). In time, differences in courtship behaviour can lead to significant mating barriers and finally speciation can occur among allopatric (Claridge *et al.* 1985, 1988) or even among sympatric populations (e.g. Wells & Henry 1992). In many cases speciation is based only on behavioural divergence, and genetic impediment for hybridisation is not evident (Claridge *et al.* 1985, 1988).

Another very little studied question is the possibility of differences in mating behaviour between locally adapted populations inhabiting different habitat types. On different habitats environmental factors may affect differently on male courtship success, and thus the most successful courtship behaviour strategy may vary considerably among the habitat types. The question is, whether differences in mating tactics are local adaptations to current environmental conditions, e.g. are males most successful in their own environments. Also population density or other demographic factors may affect the male courtship behaviour and induce adaptive differences between populations.

Hygrolycosa rubrofasciata is a drumming wolf spider (Araneae: Lycosidae) inhabiting meadows and half open bogs with deciduous trees. Bogs and meadows, including also abandoned fields, differ from each others considerably, e.g. in soil pH, litter thickness, vegetation cover, and soil fauna. During the mating season of *H. rubrofasciata*, in spring soon after snow melt, bogs are dominated by fallen birch leaves, sedges and moss (*Sphagnum* spp.), whereas meadows are dominated mainly by dry grasses and hay. Male spiders court females by hitting their abdomen on dry leaves, and females use these drumming sounds in mate choice (Kronstedt 1984, Koehler 1987, Kronstedt 1996, Kotiaho *et al.* 1996, Parri 1999). Males search for females and move long distances to find receptive females, and stop for drumming at times (Kotiaho *et al.* 1999). Kotiaho *et al.* (2000) have found that males avoid areas with high sedge cover, low elevation, and low dry leaf cover. In addition, drumming rate is positively correlated with the dry leaf cover, and in laboratory males prefer dry leaves as the drumming substrate over the wet leaves, moss and bare soil. Kotiaho *et al.* (2000) also conclude, that in *H. rubrofasciata* male distribution and drumming rate are greatly affected by the environmental factors. Thus, presence and quality of the drumming substrate may have some effects on mating success, too. Males may be locally adapted to different courtship environments and male mating strategy, whether to allocate more on searching or drumming, may depend on habitat. Our earlier studies indicate adaptive

genetical differences in adult body mass among closely located populations (Vertainen *et al.* 2001, Mappes *et al.* unpubl.).

We tested differences between the bog and meadow populations of the spider populations in mate searching and courtship behaviour. Firstly, two male spiders, one from a bog and the other from a meadow population, were let to drum and search for females on hay and leaf covered arenas, and we also measured male fights and mating success. We estimated also individually drumming rate and mobility of males from 10 bog and 10 meadow populations. In the third part of the experiment drumming sounds of males from the bog and meadow populations were recorded and played to females from the same and different populations, and female responses to different sounds were analysed.

Materials and methods

I Male courtship behaviour

Adult wolf spiders (*Hygrolycosa rubrofasciata*) were collected from Sipoo, southern Finland between April 22nd and 24th 1998 from two different areas representing two typical habitats for this species. One of them, Stormossa, was a half open bog with deciduous trees (*Betula pubescens*, *Salix* spp.), and the other, Stenberg, was a grass dominated meadow (abandoned field). The floor of the bog habitat consists mostly of moss (*Sphagnum* spp.) and *Eriophorum vaginatum* growing hummocks, and there is a rich cover of dry leaves on the ground. The meadow habitat is dominated by grasses, e.g. *Calamagrostis* sp. and there is only a few dry leaves of willow (*Salix* sp.) and aspen (*Populus tremulus*). In this habitat type the wide leaves of *Calamagrostis* sp. probably serve as the main substrate for drumming.

After collecting the spiders, 78 virgin females and 39 males from the bog habitat and 78 virgin females and 39 males from the meadow habitat, were weighed to the nearest 0.1 mg, fed with fruit flies, and kept in a temperature of +3 °C. Spiders were kept individually in plastic containers, with a bottom cast of a mixture of plaster and active carbon. In laboratory, spiders were fed *ad lib.* with fruit flies (*Drosophila melanogaster*). Before the experiment (June 5th to June 7th 1998) all the spiders were marked with a dot of paint on their abdomen. To trigger the sexual activity, females were taken into room temperature (ca. +22 °C) 48 hours before the beginning of the experiment and males 2-4 hours before. Each female was used only once, but males were tested on two successive days. After the first trial males were kept in the temperature of +3 °C for a night and they were tested again the next day following a similar procedure than on the first day, but with novel females.

The experiment was conducted in plastic rectangular arenas (bottom: 33 cm x 33 cm, height 11 cm). The bottoms of the arenas were covered with a 2 cm thick soil layer of peat and sand, and they were divided into nine equal sized sections (3 x 3) with narrow (ca. 2 mm) white paper slices. On the top of the soil

there were either nine dry birch (*Betula sp.*) leaves per section or three dry pieces of hay (*Calamagrostis sp.*) leaves (ca. 0.7 cm x 7 cm in size). Before the experiment water was sprayed on the arenas to give some humidity to the spiders. Between each experiment the birch leaves were sprayed wet, shook up and let to dry. The pieces of hay were wiped up with a wet cloth and the soil of both types of experimental arenas were sprayed with water and patted with hands. These procedures were made to ensure that female silks with odours were wiped out before the next trial.

Experimental arenas were illuminated by a lamp with a 40 W bulb (ca. 18 cm above the arena bottom) to give heat and light essential for the male drumming behaviour. During each experiment there were two males on the arena, one from the bog population (Stormossa) and the other from the meadow population (Stenberg), and two females (one from each population). Males were released to the centre of the arena and they were let to run freely and get accustomed to the habitat for about 15 - 30 minutes. After the habituation period the experiment started and for the next 15 minutes the behaviour of males was recorded. Females were released to the arena immediately before the second part of the experiment. The experiment continued until one of the two females was willing to mate with one of the males. If females were not responding, the experiment was finished after 30 minutes. When female *H. rubrofasciata* is willing to mate, she responds to a male she has chosen by shaking her abdomen towards leaves or soil in a manner similar to male drumming, but with a weaker volume and pulse rate (Kronestedt 1996). Both males were returned into +3° C and the experiment was repeated next day on the other arena type for the same pair of males. Each female was used only once, so each pair of males encountered two pairs of females.

The movements of males were measured as the number of crossings of the block borders. Male drummings were also recorded and they were categorised as sexual drummings directed to females and as agonistic drummings that are short repeated pulses directed to other males (Kronestedt 1996). Male silk searching behaviour (tapping and touching the ground with pedipalps and mouthparts, and following the silk), any direct fights between males, and female responses to males (drumming like body shaking of a much weaker volume and pulse rate than male drums) were recorded. Since the female responses only when she accepts the male to mate with her, we considered that the particular male drumming just before female response would also have the mating (see Kotiaho *et al.* 1996).

II Drumming rate and mobility

To compare the drumming and moving activities of male *H. rubrofasciata* between bog and meadow populations, 283 male spiders were collected from 10 meadows and 267 male spiders from 10 bogs in the beginning of May 1999 from Sipoo, southern Finland. After collecting, the spiders were weighed to the nearest 0.1 mg, fed with fruit flies (*Drosophila melanogaster*) and kept in a temperature of +3 °C. For measurements of drumming rate and mobility, males

were placed individually in plastic drumming arenas (125 mm x 88 mm wide and 110 mm high), which had a piece of paper on the bottom (80 mm x 43 mm in size) and two dry birch leaves as the drumming substrate. Arenas had been marked with a central line. The experimental room was illuminated with fluorescent tubes, and in addition lamps with 40 W bulbs were placed 30 cm above the floors of the drumming arenas to give the male spiders extra heat and light. The ambient air temperature under the lamps was measured before and after each drumming trial and it varied between +28 and +30 °C.

The drumming rate and mobility of each male *H. rubrofasciata* were measured in two separate days between May 26th and 29th. On the day before the first trial males were kept in temperature of +30 °C for 5 hours to trigger their drumming activity for the next day. The daily procedure in measurements was as follows. Male spiders were placed in the room temperature about 3 hours before, and released to arenas 2 hours before the measurements started. Each male was observed in groups of four males, for two minutes. Number of drumming bouts (drumming rate) and crossings of the central line (mobility) were counted. Immediately after the first male group was measured, the measurement of the next four males was started. After measurements of all the groups the procedure was repeated for four more times. Thus, the drumming rate and mobility of each male was measured 5 times 2 minutes per one measurement day. After measurements males were replaced to temperature of +3 °C. The second measurement was two days later, and thus the total observation time was 20 minutes for each male.

III Playback experiment on population recognition

We constructed a playback experiment to find out whether females recognise the drumming sounds of the males that originate from the same habitat type from the males that originate from a different habitat type than their own. We used signals of ten males from each of the two southern Finland populations (lat. 60° N) (same as in male courtship behaviour part, Stormossa and Stenberg) recorded in 1995, and of ten males from two northern Finland populations (lat. 67° N), Sattanen (a bog) and Jeesiö (meadow). The northern sounds were recorded in 1996 with a similar procedure than the southern ones in year 1995 (with a digital recorder Sony TCD-D7, see details in Parri 1999). By combining signals from all the ten males per population we created one playback tape from the southern and one from the northern sounds on which the signals from the two populations were alternating. The tape of southern signals was replayed to females from the two southern populations and the northern tape to the females of the two northern populations. We used 240 females from Stormossa and 119 females from Stenberg (south), and 120 from Jeesiö and 140 females from Sattanen (north) each in groups of 20 females. The rate of the signals on these tapes was four signals per minute and the tapes were replayed three times. The volume of the signal sets was increased after each series of sounds to enhance female responsiveness. Using the playback set-up described earlier, we recorded how many females responded to each signal (Only the first response by any female considered).

Female preference for the populations within the southern and the northern tapes was compared using a preference index for each signal. This index was calculated by first subtracting the number of first responses given to each signal by females of different origin from the number of responses given by the females of same origin. This value was then divided with the mean number of responses given to signals in total. Thus positive values of this index indicates that the signal has received more responses from the females of the same population, and negative values that the signal of the different population has gained more responses. Female preference was analysed by comparing these values against zero, which indicates no preference.

Results

I Male courtship behaviour

Females absent

On the leaf arena, males from the bog population drummed more than males from the meadow population (paired samples t-test for logarithm transformed variables: $t = 3.12$, $df = 37$, $p = 0.003$) (Fig. 1 a). On the hay arena, there was no difference between populations in drumming rate (paired samples t-test for logarithm transformed variable: $t = 0.20$, $df = 37$, $p = 0.842$). When the drumming rate of the same males was compared between the two different arena types, we found that males from the bog population drummed more actively on the leaf covered arena (paired samples t-test for logarithm transformed variable: $t = -4.91$, $df = 37$, $p < 0.001$). Also males from the meadows had a slight tendency to drum more on the leaf arena, but this difference was not quite significant (paired samples t-test for logarithm transformed variable: $t = -1.84$, $df = 37$, $p = 0.074$).

There was no difference in mobility between populations on either arena habitat (paired samples t-tests for logarithm transformed variables; hay: $t = -0.72$, $df = 37$, $p = 0.475$; leaves: $t = -0.55$, $df = 37$, $p = 0.588$). Males from both populations moved more actively on the hay arena than on the leaf arena (paired samples t-tests for logarithm transformed variables; bog population: $t = 4.51$, $df = 37$, $p < 0.001$; meadow population: $t = 5.16$, $df = 37$, $p < 0.001$) (Fig. 1 b).

There was no difference in the rate of agonistic drummings between populations on either arena (paired samples t-tests for logarithm transformed variables; hay: $t = 0.13$, $df = 37$, $p = 0.900$; leaves: $t = 1.21$, $df = 37$, $p = 0.236$) (Table 1 a). Also, the arenas did not differ from each others in the rate of male agonistic drummings (paired samples t-tests for logarithm transformed variables; males from bog population: $t = -0.21$, $df = 37$, $p = 0.837$; males from meadow population: $t = 0.73$, $df = 37$, $p = 0.469$).

On the hay arena males from the bog and the meadow population won equally often male-male interactions (Wilcoxon signed rank test: $t = 0.62$, $n = 38$, $p = 0.538$). On the leaf arena males from the bog won more often male-male

interactions (Wilcoxon signed rank test: $t = 2.00$, $n = 38$, $p = 0.046$). There was no difference in the winning ability of a given male between arena types either among bog or meadow populations (Wilcoxon signed rank tests; bog population: $t = -0.04$, $n = 38$, $p = 0.969$; meadow population: $t = 1.29$, $n = 38$, $p = 0.197$).

Females present

In the second half of the experiment two unmated females (one from the bog and the other from the meadow) were added on the arena. The experimental time varied from 2 to 30 minutes depending on female responses, and thus all the behavioural variables are presented as mean values per minute (Table 1 b). Drumming rate did not differ between populations either on the hay arena (Paired samples t-test for logarithm transformed variable: $t = -1.61$, $df = 37$, $p = 0.116$) or on the leaf arena (Paired samples t-test for logarithm transformed variable: $t = -0.27$, $df = 37$, $p = 0.787$). Males from both the bog and the meadow populations had no difference in drumming rate between the two arena types (Paired samples t-tests for logarithm transformed variable; bog population: $t = 1.05$, $df = 37$, $p < 0.299$; meadow population: $t = 0.78$, $df = 37$, $p = 0.443$).

There was no significant difference in male mobility between populations on either arena type (paired samples t-tests for logarithm transformed variable; hay: $t = -1.61$, $df = 37$, $p = 0.116$; leaf: $t = -0.27$, $df = 37$, $p = 0.787$). Both the bog and meadow males moved much more actively on the hay arena (paired samples t-tests for logarithm transformed variables; bog: $t = 4.82$, $df = 37$, $p < 0.001$; meadow: $t = 5.70$, $df = 37$, $p < 0.001$).

Males from the two populations had no differences in the rate of agonistic drummings on either arena habitats (paired samples t-tests for logarithm transformed variable; hay: $t = 0.24$, $df = 37$, $p = 0.816$; leaf: $t = 1.50$, $df = 37$, $p = 0.142$). Arena habitat (hay or leaves) did not influence agonistic drumming rate of males (paired samples t-tests for logarithm transformed variable; bog population: $t = 0.25$, $df = 37$, $p = 0.806$; meadow population: $t = 1.43$, $df = 37$, $p = 0.162$).

There was no difference between populations in activity of searching female silk on either arena habitats (Wilcoxon signed rank tests; hay arena: $t = 0.22$, $df = 37$, $p = 0.823$; leaf arena: $t = 0.19$, $df = 37$, $p = 0.850$). Males from both populations searched more actively on the hay arena (Wilcoxon signed rank tests; bog population: $t = 2.00$, $df = 37$, $p = 0.046$; meadow population: $t = 2.05$, $df = 37$, $p = 0.040$).

Males from bogs had a tendency to win more often on the hay arena (Wilcoxon signed rank test: $t = 1.70$, $df = 37$, $p = 0.089$) and on the leaf arena (Wilcoxon signed rank test: $t = 1.38$, $df = 37$, $p = 0.167$). Winning male-male interactions was not affected by arena habitat type (Wilcoxon signed rank tests; bog population: $t = 1.19$, $df = 37$, $p = 0.235$; meadow population: $t = 0.56$, $df = 37$, $p = 0.575$).

Female *H. rubrofasciata* respond to male signals by shaking their abdomen when willing to mate. On the leaf habitat females were more likely to respond than on the hay habitat (Yates corrected $\chi^2 = 13.95$, $df = 1$, $p < 0.001$) (Fig. 2), but

there was no difference in the number of responses towards males from different populations (Yates corrected $\chi^2 = 0.15$, $df = 1$, $p = 0.701$).

II Drumming rate and mobility

Males from the bog habitats were, on average, similar in size with males from the meadows (mean body mass on bogs = 15.3 mg, mean body mass on meadows = 14.7 mg) but male body mass varied greatly among populations within the habitats (nested ANOVA: habitat $F_{1,18} = 0.93$, $p = 0.348$, population within habitat $F_{18,532} = 10.78$, $p < 0.001$). The drumming rate was higher among males from the bog habitats, and populations differed among each others within the two habitats. Male body mass was tested as a covariate and it correlated positively with the drumming rate (nested ANOVA for logarithm transformed drumming rate: habitat $F_{1,18} = 8.98$, $p = 0.008$, population within habitat $F_{18,529} = 2.54$, $p < 0.001$, body mass $F_{1,529} = 5.06$, $p = 0.025$) (Fig. 3 a). Males from the bog habitats had also a higher mobility than males from the meadows, but populations did not differ from each significantly within the habitats. Male body mass as a covariate correlated positively with the male mobility (nested ANOVA for logarithm transformed mobility: habitat $F_{1,18} = 22.58$, $p < 0.001$, population within habitat $F_{18,529} = 1.49$, $p = 0.087$, body mass $F_{1,529} = 17.45$, $p < 0.001$) (Fig. 3 b).

III Playback on population recognition

There were no differences in the female preference for the signals when the index of female preference was tested against zero (for the south Wilcoxon $U = 73.5$, $n = 20$, $p = 0.886$, Fig. 4 a, and for north $U = 51.0$, $n = 17$, $p = 0.609$, Fig. 4 b) (There were three signals in the northern population tape that did not receive any responses and thus the sample size for northern tape is 17 instead of 20). Thus, females did not prefer male signals between their own and strange populations.

Discussion

When two males, one from the bog population and the other from the meadow population were together on the arena, they generally drummed more on the leaf arena that contained a greater amount of suitable drumming substrate. Males from the bog population had higher drumming activity than males from the meadow population on leaf arena, but not on the hay arena. However, when males encountered receptive females, there was no difference in drumming rate between males from the bogs and meadows, or between the

different arenas. Also, there was no difference in mobility between meadow and bog populations in absence of females. Males from both populations moved more actively on the hay arena throughout the experiment. When males from ten bogs and ten meadows were observed singly, males from the bog habitats had higher drumming rate and mobility than males from the meadow habitats. Within the habitats populations differed from each other in drumming activity, but not significantly in mobility. Males from the bogs were about 0.6 mg heavier than males from the meadows, and body mass correlated positively with both the drumming rate and the mobility. However, the differences between populations and habitats remained even after the effect of body mass was excluded. In a earlier studies with this species male body mass has not affected their drumming activity (Kotiaho *et al.* 1996, 1999).

The results on male drumming rate indicate, that males from bogs invest generally more in the drumming behaviour, but the experiment comparing two populations, one from bogs and the other from meadows reveals that difference between habitats disappears when males encounter receptive females. This means that males from the meadow population were able to compensate their lower initial drumming activity when contacted with receptive females. Males originating from the ten meadow populations had higher mobility when individuals were singly on arenas, but when two populations were compared and males encountered other males and receptive females, there were no differences in mobility between populations. Earlier studies with this species indicate that male from populations of different densities may allocate their energy on different components of mating behaviour. Males also adjust their mating behaviour according to their encounters with conspecific males and females (Vertainen *et al.* unpublished).

Males from the bog population won more often fights on leaf covered arena, when males had not yet encountered females. There were no other differences between the bog and meadow populations or between the arena habitats (hay or leaf) in male aggressive behaviour or winning probability.

Males searched for females silks with odours to locate females. Searching activity did not differ between males from different populations, but it was more frequent on the hay arena. This indicates allocation to different components of male mating tactics. The hay arena contains considerably less drumming substrate than the leaf arena, and thus males allocate on moving and searching for females on the hay arena and drumming on the leaf arena. Females responded for males more often on the leaf arena, probably due to the higher drumming activity of males. There was no difference between males from bog and meadow in gaining female responses, indicating that females do not prefer males from either of the two populations. Admittedly, the sample size is limited and power of the test is weak. However, females showed similar preference for drumming signals of males from the same and different population. Thus, no sexual discrimination between populations inhabiting different habitats was evident.

To conclude, together with the other male, bog males drummed and won fights more frequently (on leaf arenas), and when two receptive females were added, most differences in male behaviour disappeared. There was no

difference between populations in gaining female responses, and females had no preference on drumming signals of their own or strange populations. Males from bog populations had higher drumming rate and mobility, when tested singly. Consequently, there were differences in male mating tactics between the bog and the meadow populations, but they had no effects on female choice. Thus, structural differences in habitats with respect to the availability of suitable drumming substrate seems to influence male mate searching tactics. However, absence of any female preference between the males suggests that isolated populations on different habitat types do not allow any easy route to speciation. Indeed, *H. rubrofasciata* is the only species of its genus in Europe (Roberts 1995).

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TABLE 1 Means (\pm S.D.) of male behavioural traits in male courtship behaviour experiment, all variables measured per minute.

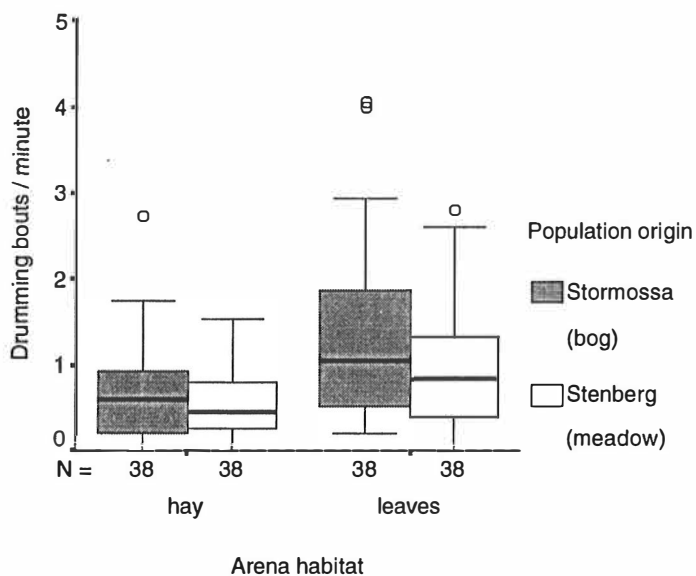
a) females absent

Measured variable	Hay arena		Leaf arena	
	Bog	Meadow	Bog	Meadow
Drumming rate	0.70 \pm 0.64	0.57 \pm 0.44	1.34 \pm 0.99	0.91 \pm 0.70
Mobility	3.19 \pm 1.20	3.35 \pm 1.22	2.13 \pm 1.33	2.17 \pm 1.19
Agonistic drumming	0.049 \pm 0.10	0.084 \pm 0.33	0.047 \pm 0.083	0.035 \pm 0.11
Fight wins	0.046 \pm 0.07	0.047 \pm 0.11	0.030 \pm 0.055	0.018 \pm 0.048

b) females present

Measured variable	Hay arena		Leaf arena	
	Bog	Meadow	Bog	Meadow
Drumming rate	0.86 \pm 0.54	0.83 \pm 0.48	1.56 \pm 0.95	1.47 \pm 0.83
Mobility	2.35 \pm 0.99	2.67 \pm 1.16	1.52 \pm 1.01	1.52 \pm 0.86
Agonistic drumming	0.070 \pm 0.097	0.11 \pm 0.33	0.15 \pm 0.26	0.086 \pm 0.16
Fight wins	0.037 \pm 0.052	0.018 \pm 0.043	0.040 \pm 0.081	0.011 \pm 0.034
Silk searching	0.057 \pm 0.12	0.061 \pm 0.095	0.049 \pm 0.10	0.049 \pm 0.088
Female response	0.18 \pm 0.39	0.18 \pm 0.39	0.50 \pm 0.51	0.32 \pm 0.47

a)



b)

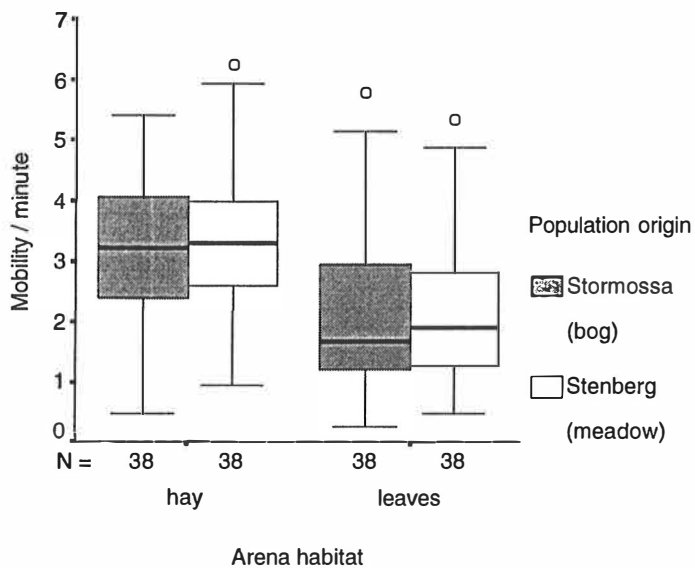


FIGURE 1 Females not present on experimental arena. a) Drumming rate of males from bog and meadow populations (drumming bouts per minute) on hay and leaf arena. b) Mobility of males from bog and meadow populations (movements over central line per minute) on hay and leaf arena.

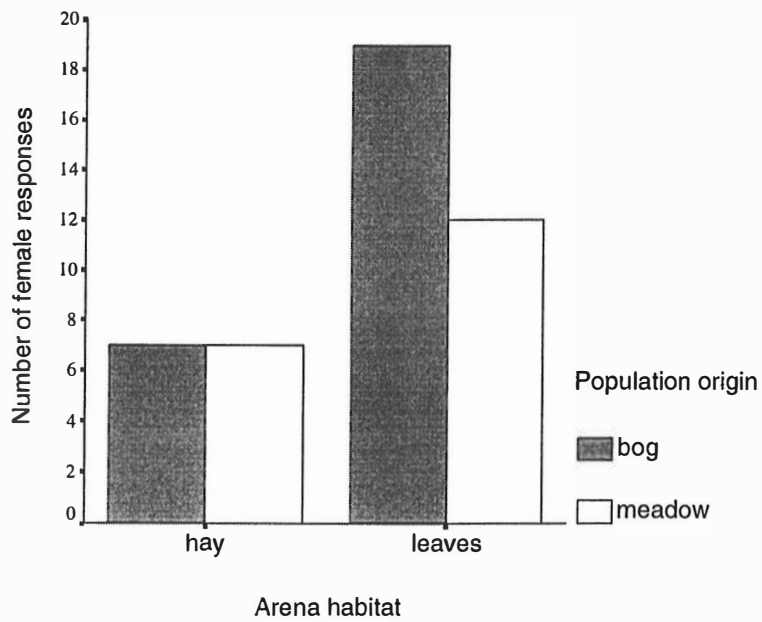
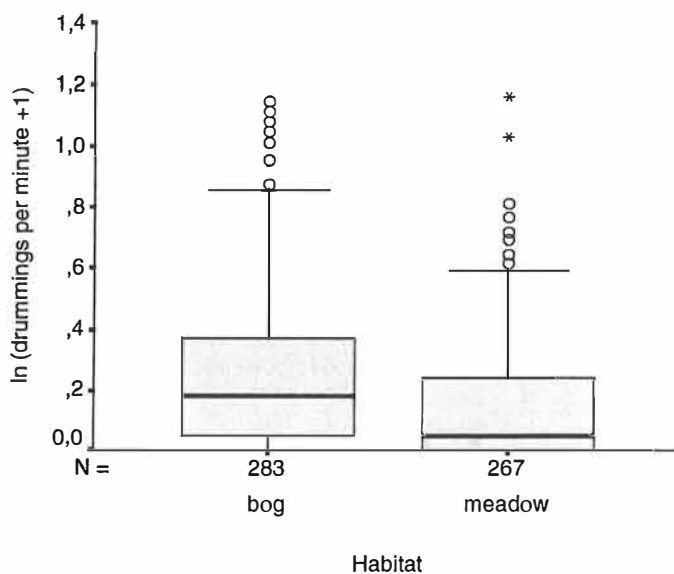


FIGURE 2 Number of female responses to males from bog and meadow habitat on hay and leaf arena.

a)



b)

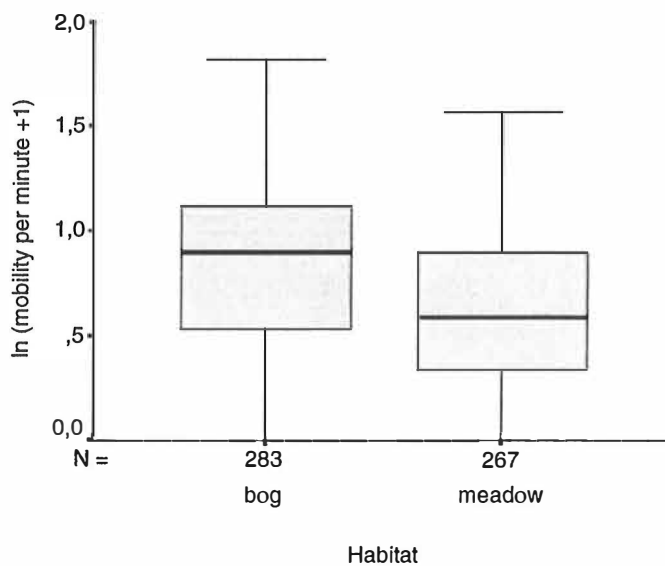


FIGURE 3 a) Drumming rate of males (drumming bouts per minute, logarithm transformed variable) from 10 bog and 10 meadow populations. Dots and asterisks represent most extreme data points. b) Mobility of males (movements over central line per minute, logarithm transformed variable) from 10 bog and 10 meadow populations.

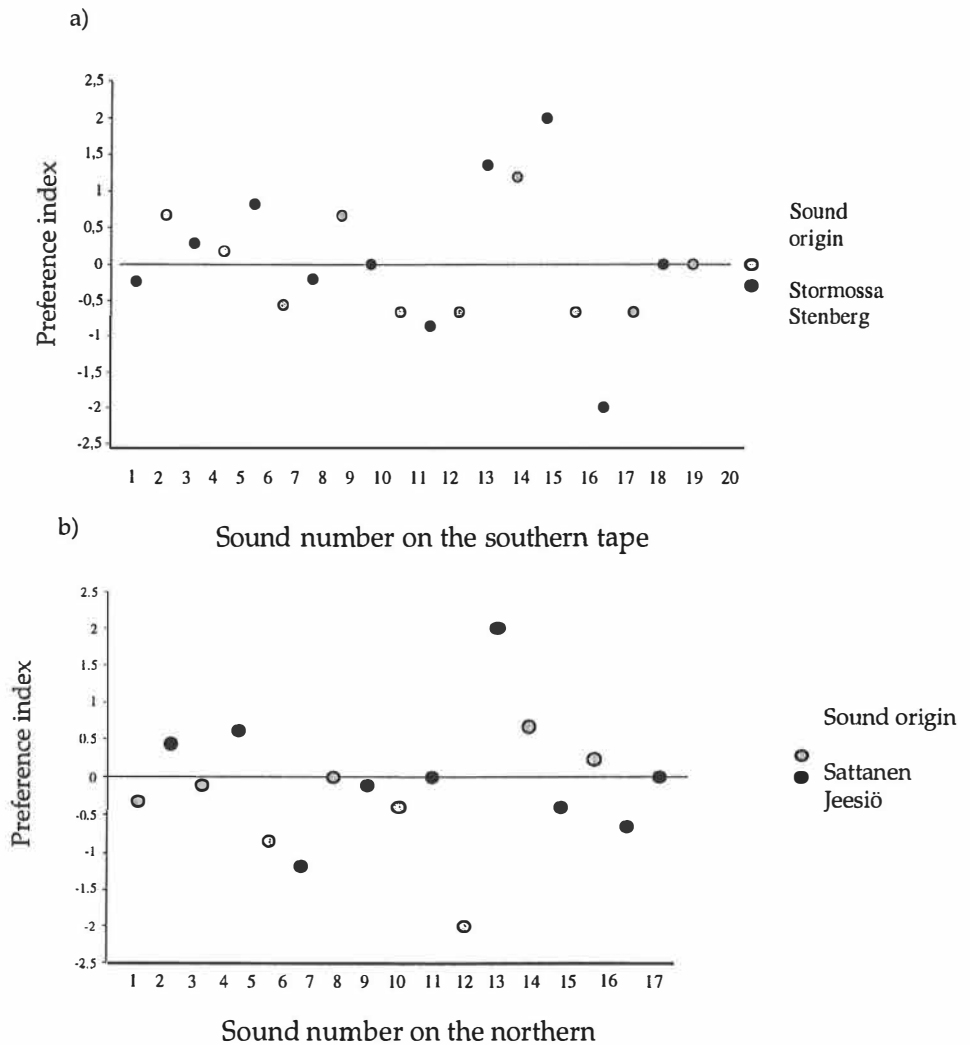


FIGURE 4 Female preference for the signals originating from their own versus different population. Positive index values indicate that the specific signal has been favoured by females from the same population, and negative values that the signal has received more responses from the females of different population. Figure a) for female preferences within the southern populations, and Figure b) within the northern populations.

IV

**Inter-population variation in sexually selected characters in the drumming
wolf spider *Hygrolycosa rubrofasciata***

by

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Manuscript

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Abstract

Variation in the strength or direction of sexual selection acting on different populations has received only little attention despite the fact that generality of results obtained from studies on single populations may be questionable. We examined covariation among sexually selected traits and environmental characters in the drumming wolf spider *Hygrolycosa rubrofasciata* on two different habitat types (26 bogs and 10 meadows). The species is exceptional in that males have a clearly audible drumming signal. Females prefer longer drumming signals, higher drumming rate and higher volume of the signal, i.e. signalling that requires more energy. Drumming rate, mobility, and pulse rate were generally higher among bog populations in comparison to meadow populations. On bogs, population size had a positive relationship with the average drumming and pulse (hits/second) rates. Within bog populations the drumming rate of males did not correlate with their body mass, but smaller males had higher pulse rates. In meadows, populations with smaller male body mass had a higher mean drumming rate. Within meadow populations, male pulse rate correlated negatively with body mass, but there was no correlation between drumming rate and male size. Male mobility, drumming signal length or volume did not differ between the two habitat types or between populations of different size. The higher drumming rate in bog populations may be a male adaptation to the rich availability of greater amount of suitable drumming substrate, i.e. dry leaves, on the forested bogs. The clearly lower pulse rate in smaller populations may reflect the fact that the selection pressure on the signal may be diminished due to the lowered encounter rate with potential mates and competing males, or it may result from inbreeding depression. In small populations females might not afford to be choosy because of the rarity of encounters.

Keywords: *Hygrolycosa rubrofasciata*, acoustic sexual signalling, inter-population differences, local adaptations, pulse rate

Introduction

Variation in the strength and direction of sexual selection acting on different populations has gained only little attention, even though such variation may have considerable effects on speciation or on adaptations to local environments. However, there are several studies concerning differences in male mating behaviour and female choice among populations with different predation risk different (e.g. Magurran & Seghes 1990, Luyten & Liley 1991, Endler & Houde 1995, Godin 1995, Differences in courtship signals can form effective reproductive barriers between sympatric morphs (Claridge *et al.* 1985, 1988, Wells & Henry 1992) or allopatric populations (e.g. Miller *et al.* 1998). Local adaptation may be more valuable than new genetic material acquired by outcrossing between differentiated populations (Rupp & Woolhouse 1999). Understanding the variation in sexual traits between populations will allow us to understand the specific roles that each trait has in sexual selection.

In birds, it is well known that habitat structure affects signal transmission, and consequently on song characteristics (Catchpole & Slater 1995, Kroodsma & Miller 1996). In arthropods there are only a few studies of this question. For example, in the brown planthopper *Nilaparvata lugens*, in which populations differ in signal repetition frequency, mean female preference in each population closely matches the mean male signal pulse repetition frequency (Butlin 1996). In the cricket frog *Acris crepitans* advertisement calls vary significantly among populations and part of this variation is due to environmental selection on call structure to enhance call transmission (Ryan & Wilczynski 1991). High variability in song characters can be due to the different selection pressures, e.g. in the field cricket *Gryllus integer*, females and parasitoid flies prefer male calling songs with average number of pulses per trill and this causes stabilising and disruptive selection, respectively (Gray & Cade 1999).

The drumming wolf spider *H. rubrofasciata* inhabits bogs and meadows patchily in Finland and Northern Europe. Most populations can easily be distinguished, since suitable habitat patches, bogs and meadows are isolated from each other by unsuitable forests and rocky cliffs. Gene flow between populations, even those located within a few hundred meters from each other, is likely to be highly restricted, and we have observed adaptive genetic differences between populations in a very small geographical scale (Vertainen *et al.* 2001). *H. rubrofasciata* inhabits two kinds of habitats; abandoned fields and other meadow-like habitats, and half open bogs with deciduous trees. These habitats differ from each others in many ecological characters, e.g. in soil moisture, pH, vegetation, soil fauna, and soil litter. On the forested bog habitats the amount of dry leaf litter is considerably higher than on the more open meadow habitats that are dominated by grass vegetation. Dry leaves are used by male *H. rubrofasciata* as substrate for drumming signalling early in the spring, and thus there is possibility for adaptive differences in male drumming and in other components of male courtship behaviour between bogs and meadows. Another aspect for this study is the huge variation in population size, from under ten individuals to several tens of thousands, the smallest

populations being common in the bogs. Larger populations of *H. rubrofasciata* are generally denser than smaller ones, and the importance of the drumming signal may differ owing to the density and the number of individuals within population. Thus, there is a possibility for differences in the use of male courtship signal between habitats and populations of different sizes.

During the mating season (in late April – early May) male *H. rubrofasciata* produce audible drumming signals by hitting their abdomen on dry leaves or other suitable substrate to court females. Females receive these signals both as audible signals via the air and as vibrations via the drumming substrate. From previous studies we can conclude that female spiders prefer higher drumming rate, higher volume of the signal (Parri *et al.* 1997) and longer drumming signals (Parri 1999). Instead, the pulse rate (number of hits per second within a single drumming bout) (Parri 1999), the symmetry of the signal, peak frequency or other measured signal characters have not been found to be target variables for female choice (Rivero *et al.* 2000). Male body mass has not turned out to affect male drumming rate or mating success (Kotiaho *et al.* 1996, 1998 a, 1999; Mappes *et al.* 1996).

We studied the variation in the activity of drumming and the structure of the drumming signals among different sized populations of *H. rubrofasciata* living on bog and meadow habitats. We recorded and analysed drumming sounds of males from 10 meadow and 26 bog populations, and measured male mobility, drumming rate, the length of drumming signal, mean volume of the drumming bout, and pulse rate. In particular, we examined whether population size or mean male body mass explain differences in these sexual characters among bog and meadow populations.

Methods

We collected 430 males (up to 30 per population) of *H. rubrofasciata* spiders from 36 differently sized populations (26 bogs and 10 meadows) 9th and 29th of May 1998 from Sipoo, southern Finland. Spiders were collected from an area of 100 m² within each population with pitfall traps (one trap /m²). The relative estimate of population density was the number of males captured from the area of 100 m² per day. Only the first day or two first days of capture were considered. However, in 10 minute populations in which practically all males were eventually captured, and the population size was estimated as the total number of males captured during the pitfall trapping season. In these sites 20 - 100 additional traps were used to cover all the suitable habitat of the spiders. A robust estimate of the total population size for larger populations was calculated as number of males captured per 100 m² per day multiplied with the total area inhabited by the population, and further multiplied with 1.51. The latter coefficient was calculated as the mean ratio of the total number of males caught from the minute populations and the males caught from the 100 m² within the first capture day. Population size (ln-transformed) correlated highly

with the ln-transformed population density ($r = 0.90$, $n = 36$, $p < 0.001$), and thus only population size was used in analyses.

After collecting the spiders were placed individually in small plastic jars (diameter 34 mm, height 50 mm) containing some *Sphagnum sp.* moss to control for the humidity suitable for spiders. The temperature was kept as low as possible during transportation to laboratory where they were kept in temperature of +3 °C. The spiders were weighed to the nearest 0.1 mg and fed with fruit flies.

The drumming and mobility rates of males were measured in laboratory between 20th and 26th May 1998. Males were placed individually in open plastic containers as drumming arenas (125 mm x 88 mm as bottom dimensions) with a piece of paper (80 mm x 43 mm) glued on the bottoms. Drumming rate was measured once with 3-4 dry birch leaves (*Betula pendula*) on the bottom of the jars and once without leaves (pooled data used here). The drumming rate of each male was measured in observation periods of 2 minutes, and the periods were repeated 5 times per one measurement day. Male movements were measured only when arenas did not have the leaves. The drumming arena was divided into two halves by an ink line and one movement was counted each time a male crossed the line. The experiment room was illuminated with ordinary fluorescent lamps and the natural sunlight was blocked with curtains to ensure even light and temperature conditions for all the spiders. Lamps with 40 W bulbs were placed 30 cm above the drumming arenas to produce the male spiders extra heat and light. The ambient air temperature under the lamps was measured before and after each drumming trial and it varied between +27.8 °C and +31.6 °C.

After drumming rate measurements the drumming sounds of individual males were recorded between May 25th and June 11th 1998 with a digital recorder Sony TCD-D7 attached to a Telinga microphone. Before recording males were kept in room temperature (ca. +20 - +22 °C) for at least 30 minutes to trigger the drumming behaviour. For drumming recordings the males were placed in small plastic containers (diameter 60 mm, height 40 mm) with a piece of paper on bottom as a drumming substrate. There was about ten males around a circle at a time and the microphone was in the middle of this circle at a distance of 25 cm from each male. Males were kept in these drumming arenas until a minimum of three individually recognised drumming sounds were recorded, and after that males were replaced with new ones.

Drumming signals were analysed by using sound-analysing programmes (Sound Edit 16, version 2.0, Macromedia; Canary 1.2, the Cornell Bioacoustics Workstation). The first program was used to convert the sounds from digital tapes to computer files and the latter to analyse the characters of drumming sounds. The drummings of male *H. rubrofasciata* consist of a series of equally spaced pulses increasing in amplitude towards the middle and decreasing again towards the end (see Rivero et al. 2000). An oscillogram (amplitude vs. time) and a spectrogram (frequency vs. time) were produced for each drumming sound and signal length (total duration of the signal in seconds), number of pulses (hits), and volume of the sound (dB) were measured. The

pulse rate (number of hits / second) was counted from these variables as follows: pulse rate = (the number of pulses - 1) / signal length (s).

Results

Drumming rate

Males from bog populations drummed more actively than males from meadow populations ($t=2.54$, $df=34$, $p=0.016$) (Figure 1 a). We used population means in analyses to weight large and small populations similarly, since only very few individuals can be obtained from the small populations. Males from large bog populations had a higher drumming rate than males from smaller bog populations, but the average male body mass had no effect on the average drumming rate (linear regression: $R^2 = 0.28$, $F_{2,23}=4.36$, $p=0.025$; body mass $\beta=0.14$, $t=0.73$, $p=0.473$; \ln population size $\beta=0.56$, $t=2.93$, $p=0.008$). However, on meadow habitats the drumming rate in populations with larger males was reduced. All the meadow populations were large in size, and population size did not affect the drumming rate (linear regression: $R^2 = 0.62$, $F_{2,27}=5.76$, $p=0.033$; body mass $\beta=-0.79$, $t=-3.39$, $p=0.012$; \ln population size $\beta=-0.03$, $t=-0.14$, $p=0.895$) (Figure 1 b). To combine the correlations between individual male body mass and drumming activity from the populations, the meta-analysis using the Schmidt-Hunter method (z-transformed correlations pooled weighting populations with the sample size) was used (Hedges & Olkin 1985). Male body mass did not correlate with drumming rate within bog populations (weighted mean $r = -0.002$, $n = 275$, $p=0.486$) or within meadow populations (weighted mean $r = 0.08$, $n = 145$, $p=0.163$). There was only a weak negative correlation between population size and population mean body mass among bog populations ($r = -0.37$, $n = 26$, $p=0.066$) and no correlation among meadow populations ($r = 0.02$, $n = 10$, $p= 0.968$).

Since all meadow populations were large in size, we also compared the drumming activity of meadows and large bogs. The smallest bog populations with estimated population size under 150 individuals, which is approximately the estimated population size of the smallest meadow population were excluded. Males from bogs drummed more and populations differed from each others within the habitat (nested ANOVA: habitat $F_{1,22} = 11.15$, $p = 0.003$, population nested within habitat $F_{22,297} = 1.98$, $p = 0.006$)

Mobility

Males from bog populations had a higher moving activity than males from meadow populations ($t=3.29$, $df=34$, $p=0.002$). Also males from large bogs had higher mobility than males from the meadows (nested ANOVA: habitat $F_{1,22} = 6.72$, $p = 0.017$, population nested within habitat $F_{22,297} = 2.29$, $p = 0.001$). Mobility was not significantly explained by the population size or male body

mass, either on the bog habitat (linear regression: $R^2=0.13$, $F_{2,23}=1.77$, $p=0.193$; body mass $\beta=0.19$, $t=0.90$, $p=0.376$; ln population size $\beta=0.39$, $t=1.87$, $p=0.075$) or on the meadow habitat (linear regression $R^2=0.40$, $F_{2,7}=2.36$, $p=0.165$; body mass $\beta=-0.61$, $t=-2.08$, $p=0.076$; ln population size $\beta=0.19$, $t=0.66$, $p=0.533$) (Figure 2).

Signal quality

There was no significant difference in population mean signal length between the habitats ($t=1.47$, $df=34$, $p=0.152$). The drumming signals were also similar among individuals from large bogs and from meadows (nested ANOVA: habitat $F_{1,22}=2.54$, $p=0.125$, population nested within habitat $F_{22,301}=2.12$, $p=0.003$). Signal length was not explained by the estimated population size or mean male body mass on the bogs (linear regression: $R^2=0.07$, $F_{2,23}=0.92$, $p=0.415$; body mass: $\beta=0.15$, $t=0.68$, $p=0.501$; ln population size $\beta=-0.18$, $t=-0.84$, $p=0.412$) or on the meadows (linear regression: $R^2=0.24$, $F_{2,7}=1.12$, $p=0.378$; body mass $\beta=-0.49$, $t=-1.49$, $p=0.180$; ln population size $\beta=-0.04$, $t=-0.13$, $p=0.901$) (Figure 3).

The volume of the drumming signal was similar on bog and meadow habitats (population means: $t=1.31$, $df=34$, $p=0.199$) and there was no difference between individuals from large bogs and meadows either (nested ANOVA: habitat $F_{1,22}=1.81$, $p=0.192$, population nested within habitat $F_{22,301}=0.52$, $p=0.964$). Signal volume was not affected by the measured variables among bog populations (linear regression: $R^2=0.03$, $F_{2,23}=0.36$, $p=0.703$; body mass $\beta=-0.08$, $t=-0.36$, $p=0.723$; ln population size $\beta=-0.19$, $t=-0.85$, $p=0.407$). Population size or male body mass neither influenced the volume of the drumming sound on meadow habitat (linear regression: $R^2=0.06$, $F_{2,7}=0.23$, $p=0.803$; body mass $\beta=-0.24$, $t=-0.65$, $p=0.535$; ln population size $\beta=0.06$, $t=0.17$, $p=0.868$) (Figure 4).

The average pulse rate was higher on the meadow populations than on the bog populations (population means: $t=-2.10$, $df=34$, $p=0.044$). There was no difference between individuals from large bog and meadow populations even though pulse rate varied among populations (nested ANOVA: habitat $F_{1,22}=0.23$, $p=0.638$, population nested within habitat $F_{22,301}=3.12$, $p<0.001$). Among bogs, the larger populations had a clearly higher pulse rate, but the body mass had no effect on this variable (linear regression: $R^2=0.56$, $F_{2,23}=14.69$, $p<0.001$; body mass $\beta=-0.21$, $t=-1.38$, $p=0.180$; ln population size $\beta=0.65$, $t=4.37$, $p<0.001$) (Figure 5 a). In the meadow populations, heavier males had a lower pulse rate, but the population size had no significant effect on pulse rate (linear regression: $R^2=0.71$, $F_{2,7}=8.74$, $p=0.013$; body mass $\beta=-0.78$, $t=-3.88$, $p=0.006$; ln population size $\beta=0.33$, $t=1.62$, $p=0.149$) (Figure 5 b). In fact, there was no difference in pulse rate between males from meadow populations (all of which are large in size) and large bog populations. The meta-analysis (as above) revealed, that the individual male body mass correlated negatively with the pulse rate within the bog populations (weighted mean $r = -0.26$, $n = 280$, $p<0.001$) and within the meadow populations (weighted mean $r = -0.22$, $n = 146$, $p=0.004$).

Discussion

In previous studies we have shown (Vertainen *et al.* 2001, Mappes *et al.*, unpublished) that in the drumming wolf spider, morphological and life-history characteristics vary between environments and they reflect adaptive genetic variation between local environmental conditions. Our results show that this covariation can be found also from sexually selected traits.

Males from bog populations drummed and moved more actively than males from meadow populations. Among bogs the larger populations had a higher drumming rate, and among meadow populations the smaller males drummed more actively. Drumming rate is directly involved in male mating success: it is directionally selected by females (Kotiaho *et al.* 1996, 1998 b; Parri *et al.* 1997) and it has proven to be an honest signal of male viability (e.g. Kotiaho *et al.* 1996, 1999, Kotiaho 2000). The more actively drumming males also win more often in male-male competition (Kotiaho *et al.* 1997). Drumming is a highly energy demanding activity for male *H. rubrofasciata* and the costs are higher for larger males (Kotiaho *et al.* 1998 a). The higher drumming rate of males from bog populations can be due to an adaptation to an environment containing more of the suitable substrate (dry leaf litter) to perform drumming sounds. Kotiaho *et al.* (2000) have found that during the mating season males prefer habitat patches covered with dry leaves as a drumming substrate.

In small populations, the drumming rate is remarkably lower than in larger populations. A possible explanation for the observed pattern is that higher intrasexual competition in large bog habitats drives higher sexual activity in these populations. Another explanation may be that lower drumming activity in small bogs indicates reduced genetic quality in the very small populations. However, male mobility was not affected by the population size or male body mass. This result indicates that males from the very small populations were not in poorer condition compared to males from larger ones during this experiment. However, in smaller populations the moving effort may be essential for male mating success, and thus males despite the lower condition may invest relatively more on moving and mate searching. In another study Ahtiainen *et al.* (unpubl.) have shown that in extremely minute populations (under 10 males) males have clearly lowered drumming rate, even if mobility is not significantly lowered. However, also survival was reduced in the smallest populations suggesting that these males were of poor quality. Thus, inbreeding may partly contribute to the reduced drumming rates in the very small populations.

Pulse rate was generally higher among meadow populations than among bog populations. On bogs, the population size correlated positively with pulse rate, and on meadows the male body mass correlated negatively with this variable. However, there was no difference in pulse rate between large bogs and meadows, since all meadow populations are large. All the large populations of this species are also dense. Variation in pulse rate within different sized bog populations may be due to male adaptations to population density. Encounter rate with conspecific males and females is greater in large

populations, and more actively drumming males with higher pulse rate may be more successful in dense and large populations. In sparse and small populations, however, each individual encounters only a few potential mates, and it is likely that females can not afford to be too choosy. There are no strong preferences by females to pulse rate (Parri 1999), and thus this character might evolve easily to reduce the costs of signals if the importance of the signal in sexual selection is reduced in the smallest populations.

Looking at the population means, the meadow populations with smaller males had a higher drumming activity, while male body mass had no effect on drumming activity on bog populations. However, the meta-analysis considering correlations within populations revealed no relationship between individual male body mass and drumming rate on either habitat. These results suggest, that the relationship between these variables is weak also in meadows, and thus male body mass has no major influence on drumming activity. This is consistent with the earlier studies, where male body mass has not proved to affect on male drumming rate (Kotiaho *et al.* 1996, 1998 a, 1999; Mappes *et al.* 1996). Male body mass did not influence male mobility, drumming signal length, or drumming signal volume. Pulse rate of smaller males (population mean) was higher on meadow populations, and on bogs the relationship between mean male body mass and pulse rate was also negative, but not significant. The meta-analysis considering correlations within populations revealed significant, negative correlations between male body mass and pulse rate both among bog and meadow habitats. So, the result that pulse rate of smaller males is greater is relevant at both the individual and the population level. This may be related to the higher energetic costs of drumming in the large males (see Kotiaho *et al.* 1998), if large males tend to avoid excessive costs by the reduced pulse rate.

Differences in drumming and moving activity, and in pulse rate between populations could also be due to genetic drift, especially within small populations. In *H. rubrofasciata*, females do not discriminate between drumming sounds of males from own or different population, despite the differences in drumming sound characteristics between populations (Parri 1999). Roff *et al.* (1999) conclude, that when females from the two sibling species do not discriminate between males of their own or sibling species, the observed differences between the two males are more likely to result from genetic drift than from sexual selection. However, the relationship between pulse rate and population size in *H. rubrofasciata* is linear, and thus we expect that there is local selection rather than random genetic effects acting on this drumming signal.

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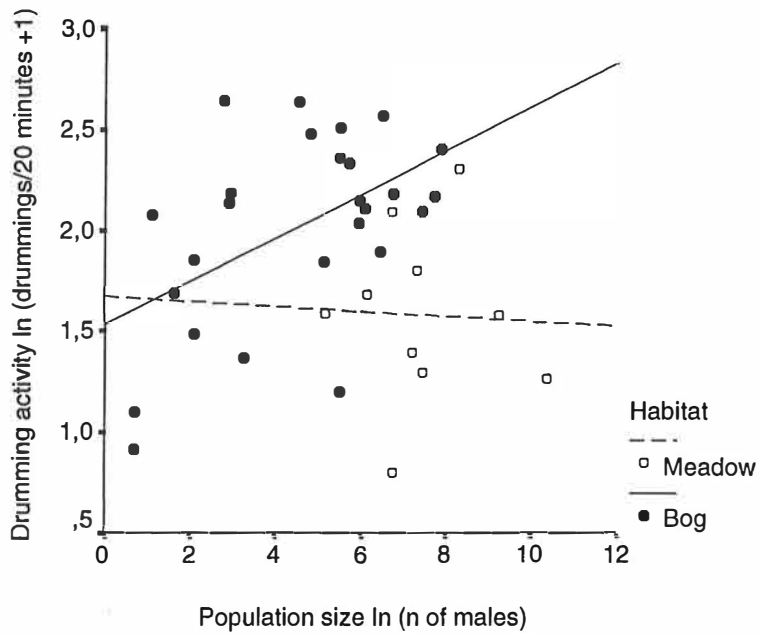
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a)



b)

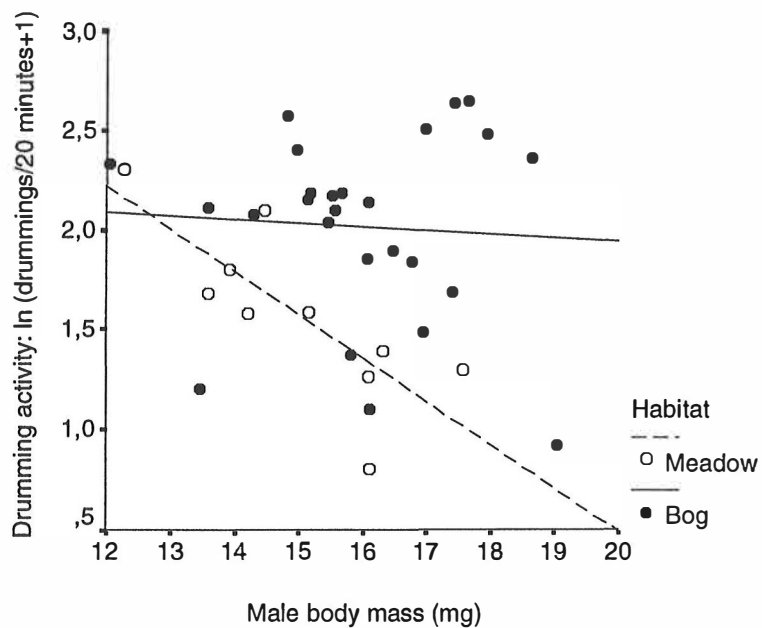


FIGURE 1 The drumming rate of male spiders (logarithm transformed) from bog and meadow populations a) plotted against estimated population size (logarithm transformed), and b) plotted against population means of male body mass. Each dot represents one population.



FIGURE 2 The moving activity of male spiders (logarithm transformed) from bog and meadow populations of different size (logarithm transformed). Each dot represents one population.

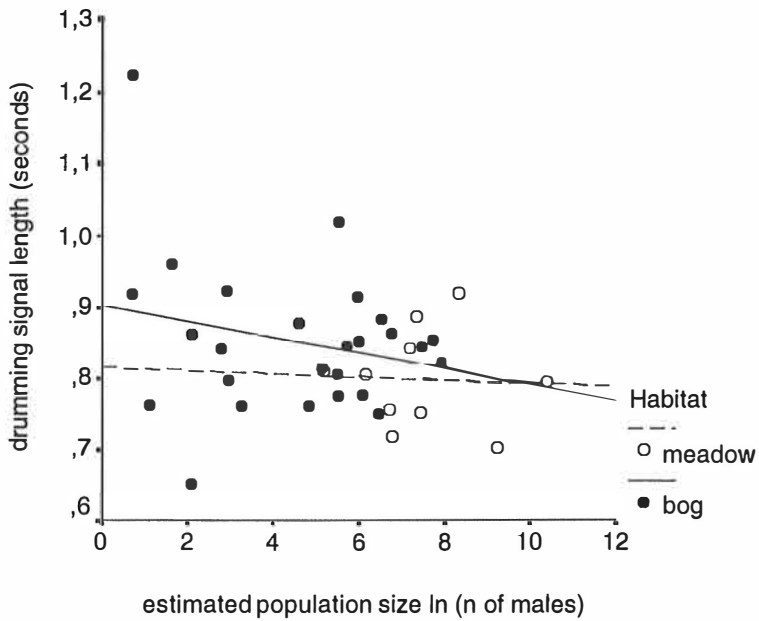
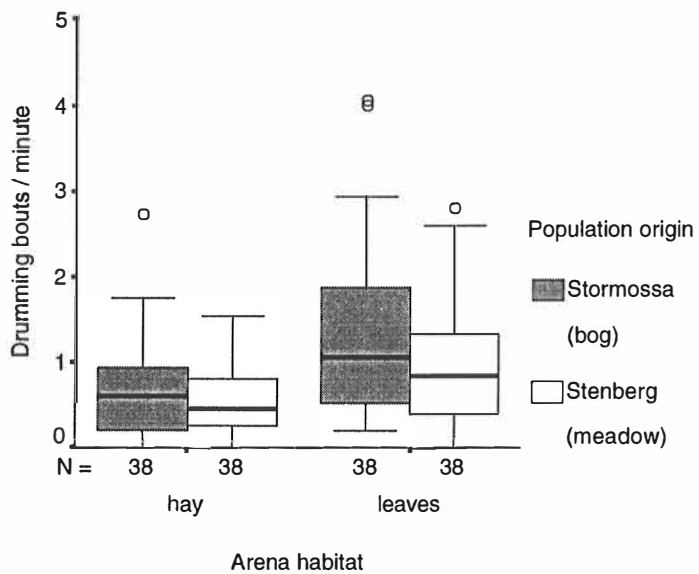


FIGURE 3 The lengths of drumming signals (seconds) of males from different sized bog and meadow populations (logarithm transformed). Each dot represents one population.

a)



b)

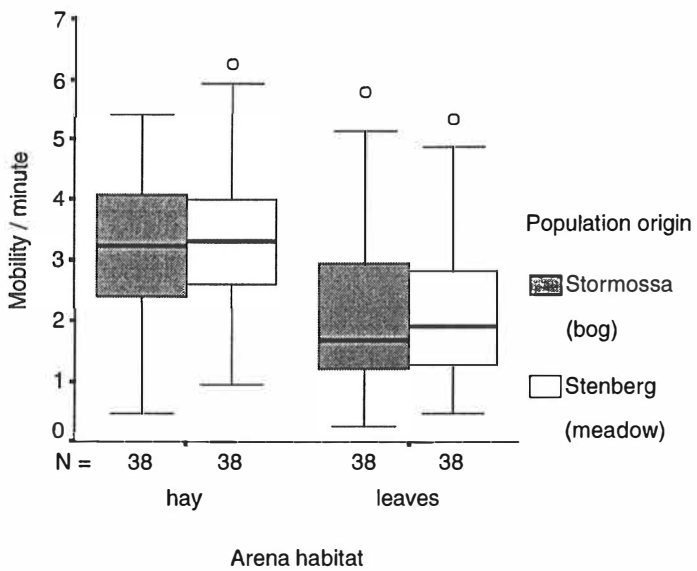
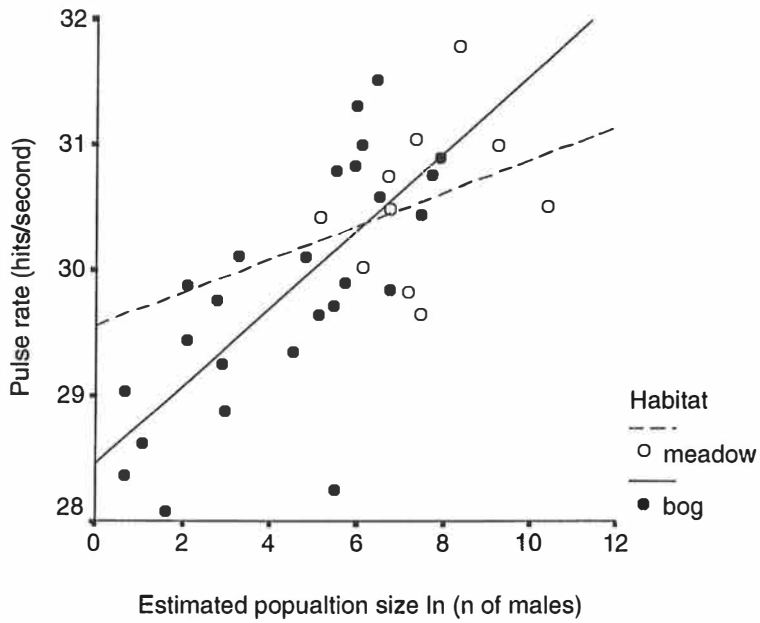


FIGURE 1 Females not present on experimental arena. a) Drumming rate of males from bog and meadow populations (drumming bouts per minute) on hay and leaf arena. b) Mobility of males from bog and meadow populations (movements over central line per minute) on hay and leaf arena.

a)



b)

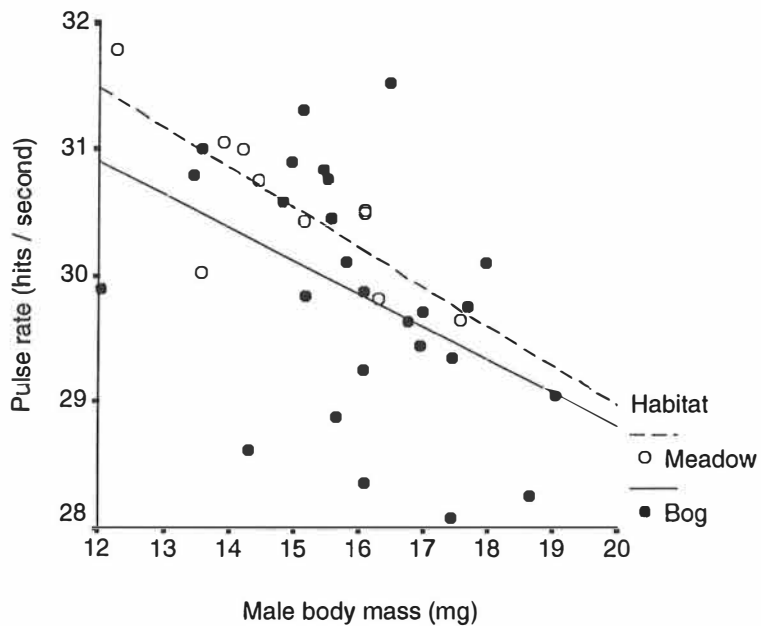


FIGURE 5 The pulse rates of male drumming sounds from bog and meadow populations a) plotted against the estimated population size (logarithm transformed), and b) plotted against the population means of male body mass. Each dot represents one population.

V

Population density and male mating tactics in the drumming wolf spider
Hygrolycosa rubrofasciata

by

Laura Vertainen, Jari J. Ahtiainen and Rauno V. Alatalo

Manuscript

Population density and male mating tactics in the drumming wolf spider *Hygrolycosa rubrofasciata*

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Abstract:

The drumming wolf spider *Hygrolycosa rubrofasciata* lives patchily on suitable bog and meadow habitats. Males court females by producing audible drumming sounds, and females prefer males with higher drumming activity, longer drumming sound, and higher volume. Male courtship includes also moving and searching for females, and aggressive male-male interactions are also frequent. There is great variability in population density between the populations of *H. rubrofasciata* and thus in encounter rate with other individuals. We studied how population density affects male-male interactions, male courtship behaviour and signalling. Male fighting ability, drumming and moving activity, and drumming sound characters were compared between males from sparse and dense populations. When placed in vicinity of females, males from sparse populations had a higher courtship drumming rate and a tendency to be more aggressive towards other males. On the other hand, males from dense populations had higher mobility. When females were not present, males from dense populations had longer and louder drumming signals, i.e. their signals contained more energy. These results indicate, that population density may affect male mating tactics. In sparse populations, with reduced encounter rate between males and females, males allocate heavily on sexual signalling and aggressive behaviour once encountering a female. In dense populations, with several potential mates males allocate more on moving around and searching for receptive females, and on signal quality even if no female has yet been observed. In sum, population density seems to affect male investment to different components of male mating effort.

Key words: aggressiveness, *Hygrolycosa rubrofasciata*, male-male interaction, mating tactic, population density, sexual signalling

Introduction

Theoretical models suggest that when population density increases, mate competition and mate choice increase due to the higher encounter rate of potential competitors and mates (Eshel 1979, Hubbell & Johnson 1987, Crowley *et al.* 1991). However, most empirical studies show that aggressiveness of individual males decreases when population density increases (reviewed in Jirotkul 1998, 1999). Furthermore, empirical studies in field crickets (*Gryllus* sp.) and grasshoppers (*Liguorotettix* sp.) have shown that individual males reduce calling and increase movements when population density increases (Greenfield & Shelly 1985, French & Cade 1989, Hissmann 1990, Cade & Cade 1992). Thus, it seems that population density may influence both the mating effort and its allocation to different activities.

The drumming wolf spider *Hygrolycosa rubrofasciata* inhabits bogs with deciduous trees and abandoned fields patchily across Northern Europe. Males produce audible drumming signals by hitting their abdomen on dry leaves. There are two types of drumming signals: courtship and agonistic drummings. Courtship drummings are longer while agonistic drummings that are used in male-male competition are shorter and more intense, and thus clearly distinguishable from courtship drummings (Kronstedt 1984, 1996).

Females of *H. rubrofasciata* prefer males with higher drumming rate, and drumming is costly for males in terms of energy demand, predation risk, and mortality. Drumming is seemingly an honest indicator of male quality (Mappes *et al.* 1996, Alatalo *et al.* 1998, Kotiaho *et al.* 1998, Kotiaho 2000). Females also choose males with higher signal volume and longer drumming bouts (Parri *et al.* 1997, Rivero *et al.* 2000). Female preference for other drumming signal characters, such as peak frequency, symmetry of signal, or pulse rate (number of hits / second within a drumming sound) has not been found (Parri 1999, Rivero *et al.* 2000).

Male body mass is not correlated with courtship drumming activity (Kotiaho *et al.* 1996), but both large body mass and high drumming activity independently increase the probability of winning in male-male competition (Kotiaho *et al.* 1997). Agonistic drumming rate is an honest signal of male fighting ability, but it is not an indicator of body size (Kotiaho *et al.* 1999). Pulse rate has been found to be higher in denser and larger populations (Vertainen *et al.*, unpublished data).

We studied male mating effort and its allocation to different components in sparse and dense populations of *H. rubrofasciata*. We compared male mobility, drumming rate, fighting ability, and drumming sound characters of similarly sized males from dense and sparse populations.

Materials and methods

We collected 78 males of *H. rubrofasciata* from 11 bog populations between 29th of April and 11th of May 2000 from Sipoo, southern Finland. Populations were from bog habitats and they were either sparse or dense (Table 1). Dense populations of this species are typically also larger in population size. After collecting, spiders were weighed, fed and placed in temperature of +3 °C. To compare the fighting ability and drumming sound characters between sparse and dense populations pairs of males were selected from these populations. Differences in drumming characters between different sized populations living in different habitats have been found in *H. rubrofasciata* (Vertainen *et al.*, unpublished data). Male pairs were similar in body mass (difference in body mass <1.0 mg). Males were marked individually with a spot of paint on abdomen.

Experimental arenas consisted of plastic containers (bottom: 125 mm x 88 mm, height 110 mm) with a piece of paper (80 mm x 43 mm in size) glued on the bottom. In the middle of the arena there was a cylindrical cage (diameter 3 cm) containing an unmated female to induce male drumming and fighting activity. Arenas were illuminated with a lamp with 60 W bulb placed 30 cm over the arenas. Each pair of males was kept in room temperature (+23 °C) for 1 hour before the beginning of the interaction experiment. In the first phase, both males were kept individually in the drumming arena for 15 minutes and drumming bouts and movements over a central line (marking two equal halves of the arena) were recorded. In the second phase, pairs of males from sparse and dense populations were put together in a new arena for 15 minutes, and drumming bouts, movements, and aggressive behaviour were recorded. The measure of aggressiveness was counted as a sum of agonistic drummings, chases, and fights. Agonistic drummings differ clearly from courtship drummings being shorter, more intense, and rapidly repeated signals directed towards other males. A chase was recorded when the male run after the other male and chased it away. Fights are intense confrontations where males roll around the arena and attempt to bite each others. However, we have not noticed males having any fatal injuries during fights.

Drumming sounds of the males were recorded in 18th – 29th of May 2000 by using a digital recorder Sony TCD-D7 attached to a Telinga microphone. Display is costly to males, and there was some mortality of males reducing the sample size here. For recordings males were placed in small plastic containers (diameter 60 mm, height 40 mm), with a piece of paper on the bottom to serve as a drumming substrate. There was about ten males around a circle at a time and the microphone was in the middle of this circle, at a distance of 30 cm from each male. Males were kept in containers until a minimum of three individually recognised drumming sounds were recorded for each male.

Drumming signals were analysed using sound-analysing computer programmes (Sound Edit 16, version 2.0, Macromedia, and Canary 1.2, the Cornell Bioacoustics Workstation). The first program was used to convert the sounds from digital tapes to computer files and the latter to analyse the

characters of drumming sounds. Each drumming bout of male *H. rubrofasciata* consists of a series of equally spaced pulses (hits) (see Rivero et al. 2000). An oscillogram (amplitude vs. time) and a spectrogram (frequency vs. time) were created for each drumming bout and drumming signal length (seconds), number of hits per drumming bout, and volume of the sound (dB) were measured. The pulse rate (number of hits / second) was counted from these variables as follows: pulse rate = (the number of pulses - 1) / signal length (s).

Results

When males were on separate arenas there was no difference in drumming activity between males from sparse (mean \pm S.D. = 20.08 \pm 26.95 drummings / 15 minutes) and dense (mean \pm S.D = 16.77 \pm 25.93 drummings / 15 minutes) populations (Wilcoxon signed rank test: $t = 0.57$, $n = 39$, $p = 0.572$). Moving activity had a slight tendency to be higher among males from dense populations (sparse: mean 26.87 \pm 14.70; dense: mean 31.92 \pm 15.40) (Paired samples t-test: $t = -1.73$, $df = 38$, $p = 0.093$). On separate arenas males did not produce any agonistic drummings.

When the two males were together on the experimental arena, males from dense populations had a higher moving activity (Paired samples t-test: $t = -2.09$, $df = 38$, $p = 0.044$) (Fig. 1). Males from sparse populations had a higher drumming activity than males from dense populations (Wilcoxon signed rank test: $t = 2.13$, $n = 39$, $p = 0.033$) (Fig. 2). Males from sparse populations had also a tendency to be more successful in male-male encounters in terms of aggressiveness score (sum of the number of agonistic drummings, fight wins, and chases) (Wilcoxon signed rank test: $t = 1.94$, $n = 39$, $p = 0.053$) (Fig. 3).

There was a tendency for males from dense populations to produce longer drumming bouts (Paired samples t-test: $t = -1.74$, $df = 54$, $p = 0.088$) (Fig. 4), and volumes of the drumming sounds were higher on dense populations (Paired samples t-test: $t = -2.39$, $df = 54$, $p = 0.020$) (Fig.5). Pulse rate (hits/ second) within each drumming sound was similar in sparse (31.36 \pm 1.05) and dense (31.40 \pm 1.44) populations (Paired samples t-test: $t = -0.13$, $df = 54$, $p = 0.900$).

Discussion

When there was a female on the arena, males from sparse populations had a higher drumming activity and these males had also a tendency to behave more aggressively towards the other male. Instead, males from dense populations had a higher moving activity. During the short mating season males from sparse populations encounter receptive females less frequently than males from dense populations. Thus, in sparse populations the value of each encountered

female is high and males may allocate more on aggressive behaviour towards other males when in the vicinity of a female. In dense populations there are several potential mates available, and thus it may be a more successful strategy to search for another female than to invest on continuous fighting for a single female.

Based on studies with guppies (*Poecilia reticulata*) Jirotkul (1999) suggests that opportunity for sexual selection is smaller at high density due to the decrease in courtship displays and the increase in interference. However, in sparse populations of *H. rubrofasciata* there are only a few potential mates available during the short mating season and the possibilities for female choice are limited.

Males from dense populations produced louder and slightly longer drumming bouts, when they were on arenas without females. Pulse rate was similar in both populations. In dense populations males encounter several females during the mating season, and it is probable that females compare several males before their choice (Parri *et al.* 1997, Alatalo *et al.* 1998). Females choose higher drumming activity, longer and louder signals (Kotiaho *et al.* 1996, Parri *et al.* 1997, Parri 1999, Rivero *et al.* 2000). It might be beneficial for males from dense populations to allocate more on characters of the sexual signal that are the target of female choice, even when they are not aware of any females close by. In sparse populations, the benefit of allocating on signal quality might be small compared to the benefits of allocating on finding any of the few females, and because of the rarity of encounters these females could not afford to be very choosy on signal characteristics.

Males from sparse populations had a higher drumming rate, but only when they were on arena together with the other male. This may indicate that differences in male mate searching tactics are present only when they encounter other males or females. An alternative explanation for differences in mate searching tactics would be different growth conditions and consequent differences in male condition. Sparse populations are either small or they live on marginal habitats while dense populations are usually large and inhabit more suitable habitats. However, our results are contradictory to the condition explanation. Males from sparse populations are more active in drumming and aggressiveness towards other males, even if males from dense populations move more actively and have longer and louder drumming signals. Thus, it is unlikely that dense and sparse populations would differ systematically in male condition between populations. Therefore, there may be differences between populations in allocating the energy on different components of male mating strategy. Populations of *H. rubrofasciata* are isolated from each other and genetic differences between closely located populations have been found in adult body masses (Vertainen *et al.*, 2001). It is possible that males from these different kinds of populations have adapted to different environmental and demographic conditions, and thus have population level differences also in male mating strategy.

In sum, population density seems to affect the male mating effort. Males from sparse populations invest more in any eventual encounters with females, probably because of the rarity of encounters with alternative mates. On the

other hand, males in dense populations do invest more energy on each drumming signal when searching for females, probably because of a higher chance of response.

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TABLE 1 Soil humidity, litter thickness, number of individuals in experiment, population mean of body mass, area inhabited by population, and estimated population densities. Soil parameters and population densities are measured in 1998. Population density was measured between 9th and 17th May 1998 placing 100 pitfall traps on area of 10x10 m² for 1-2 days. The estimated population density in this experiment was calculated as males from pitfalls per 100 m² per trapping day.

	Soil Moisture %	Soil litter thickness (cm)	N	Male body mass \pm S.D. (mg)	Population area (m ²)	Estimated density (males /100 m ² /day)
a) dense populations						
Rotkosuo	74.2	1.4	8	19.33 \pm 1.58	1220	20.7
Näresuo	91.2	1.7	1	15.63 \pm 1.37	7560	23.5
J-suo	91.7	1.7	9	16.49 \pm 3.37	9370	16.0
Krokot pohjoinen	85.9	1.7	13	15.95 \pm 2.48	950	27.8
Velttosuo	90.0	1.3	7	16.33 \pm 2.79	2900	14.3
b) sparse populations						
Minimossa	69.4	1.3	1	15.95 \pm 1.06	230	4.1
Takaperhonen	89.2	1.8	10	16.90 \pm 2.55	240	4.2
Mutkasuo	87.7	2.0	7	17.74 \pm 2.25	800	0.2
Hökkelisuo	89.0	1.7	3	21.83 \pm 4.96	1010	2.0
Pitkulasuo	87.9	1.5	14	18.06 \pm 2.64	1400	0.5
Munuaissuo	88.0	1.6	4	14.43 \pm 1.33	220	1.2

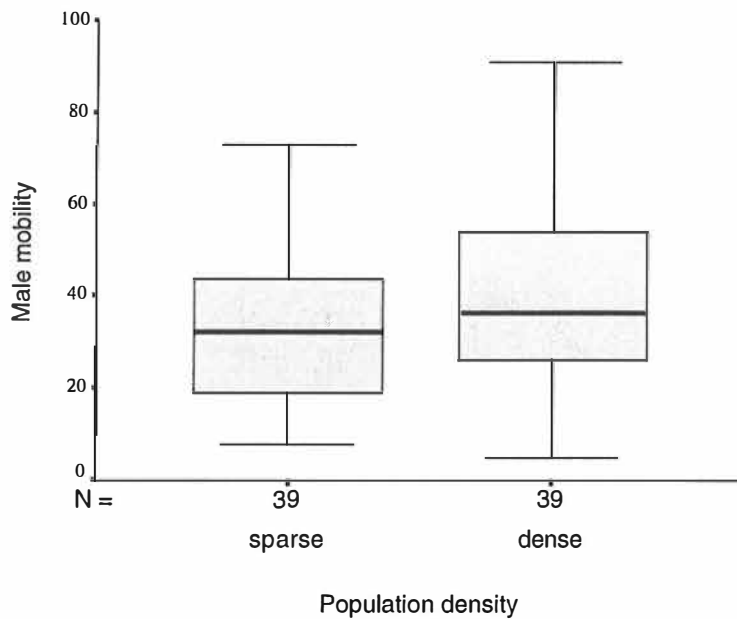


FIGURE 1 The moving activity of males during the 15-minutes measurement. Males together on the experimental arena.

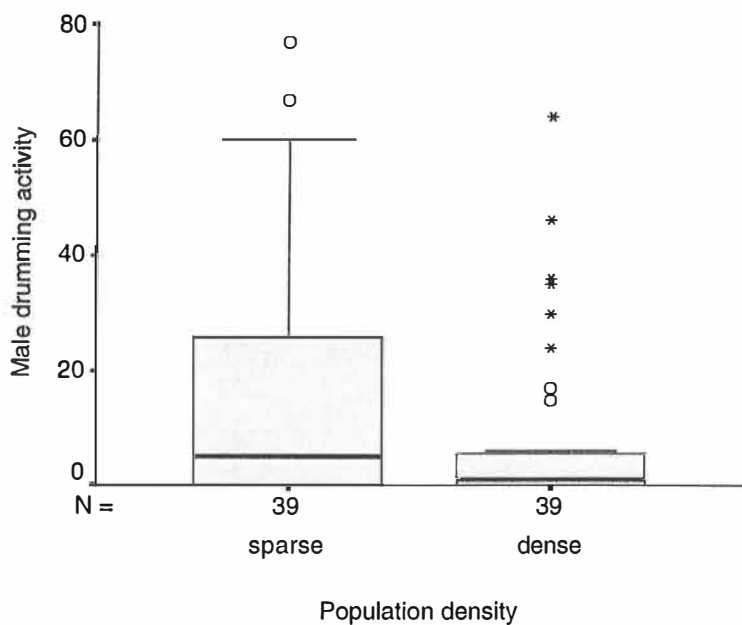


FIGURE 2 The number of drummings during the 15 minutes trial. Males from the sparse and the dense populations together on the experimental arena. Circles and asterisks represent the most extreme data points.

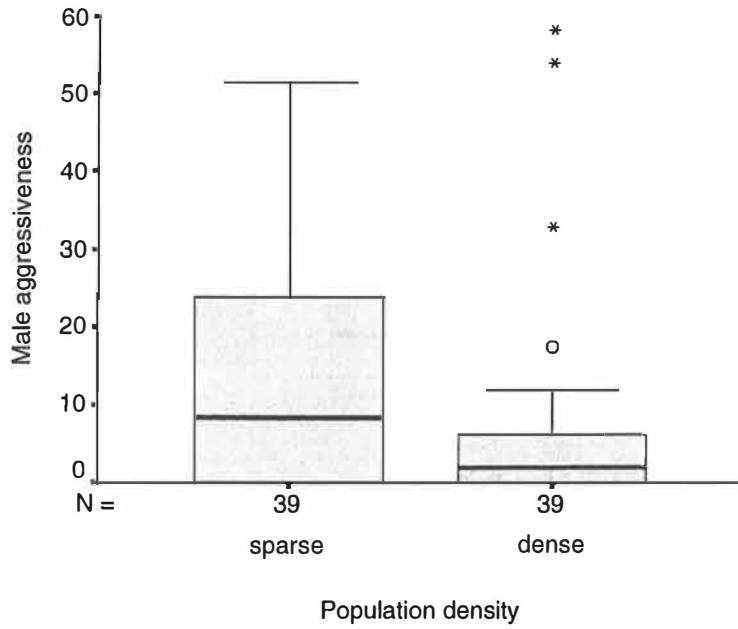


FIGURE 3 The male aggressiveness towards the other male measured as the sum of agonistic drummings, the number of fight wins, and the number of chases.

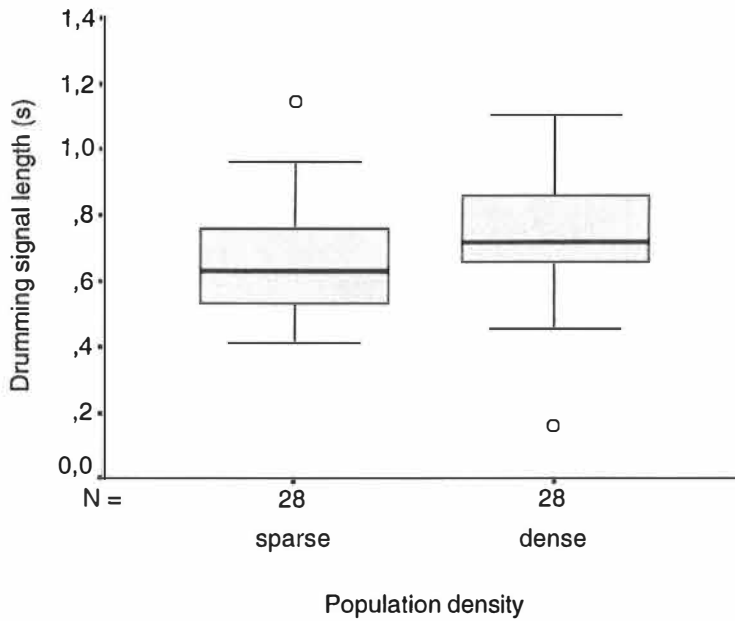


FIGURE 4 The length of drumming signal (seconds) produced by males from the sparse and the dense populations.

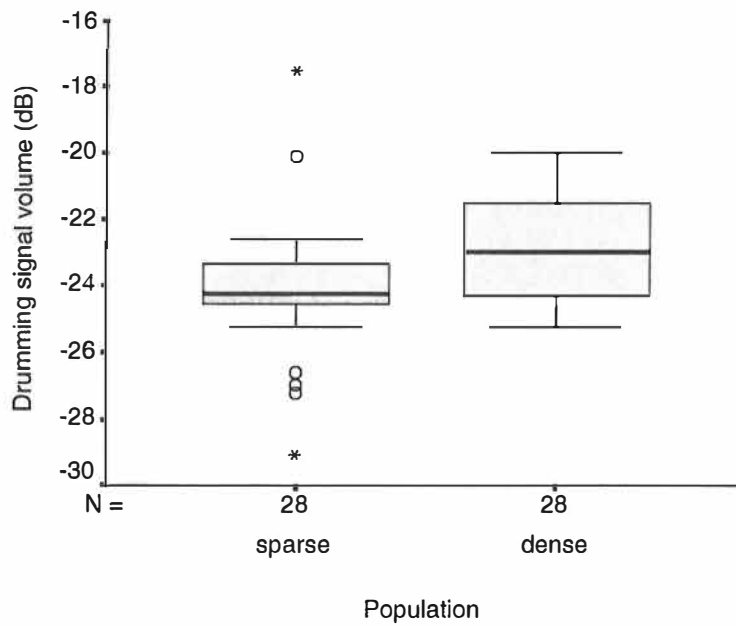


FIGURE 5 The volume of the drumming signal (dB) produced by males from the sparse and the dense populations.