

**Pro Gradu –tutkielma**

**Health parameters and sexual signalling in yearling  
black grouse males  
(*Tetrao tetrix*)**

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### TIIVISTELMÄ

Useilla lajeilla koiraan lisääntymismenestys määräytyy naaraan valinnan ja koiras-koiraskilpailun kautta. Näiden tekijöiden ohella myös investointi seksuaalisignalointiin vaihtelee koiraiden välillä yksilöiden laatueroista johtuen. Tässä tutkimuksessa tavoitteenani oli selvittää yksilön kunnan ja lisääntymispanostuksen välistä yhteyttä nuorilla teerikoirailla (*Tetrao tetrax*). Tutkiakseni lisääntymispanostukseen vaikuttavia koiraan ominaisuuksia, tarkastelin 191 nuoren teerikoiraan morfologisia ominaisuuksia, ornamentointia ja kuntoa. Lisäksi koiraiden lisääntymismenestys sekä käyttäytyminen soitimella havainnoitiin. Koiraat, jotka olivat läsnä soitimella, erosivat poissaolleista yksilöistä plasman immunoglobuliinien määrän, hematokriitin, microfilaria-loisten määrän sekä pyrstön asymmetrian suhteen. Soitimelle osallistuneiden koiraiden välillä ilmeni eroja soidinaktiivisuudessa, mutta ainoastaan hematokriitti selitti tätä vaihtelua. Aktiivisuus paransi paritumismenestystä nuorilla koirilla, ja lisäksi aktiivisten yksilöiden reviirit olivat suuntaa-antavasti lähempänä soitimen keskustaa kuin passiivisemmilla yksilöillä. Aktiivisten ja passiivisten koiraiden loistaakassa, immunokompetenssissa tai ornamentoinnissa ei ollut eroja. Tulosteni mukaan kuntotekijöillä on merkitystä siihen, ovatko nuoret koiraat lainkaan läsnä soitimella, sillä huonokuntoiset yksilöt ovat todennäköisesti kuolleet tai dispersoineet muille alueille ennen kevään soidinta. Sen sijaan soitimella olleiden nuorten koiraiden välillä ei havaittu eroja kuntotekijöissä soidinaktiivisuuden suhteen tässä kohortissa.

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HÄSÄ LAURA, M.: Health parameters and sexual signalling in yearling black grouse males (*Tetrao tetrix*)

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## ABSTRACT

In many species, male mating success is determined through female choice and male-male competition over females. Besides these factors, there are also differences in individual male investment to sexual signalling due to quality differences between males. In this study I investigated the effect of male condition on reproductive effort in yearling black grouse males (*Tetrao tetrix*). To discover male traits affecting reproductive effort, morphological traits, ornamentation and condition of 191 young black grouse males were studied and males' mating success and lekking behaviour were recorded. Males, which were present in leks, differed from absent individuals in plasma immunoglobulin concentration, hematocrit, the number of microfilaria parasites and lyre asymmetry. Lek activity varied between males that attended the lek, but only hematocrit explained these differences. Activity on the lek improved mating success in yearling males and active males tended to achieve more central territories than passive males. Active and passive juveniles did not differ in the number of parasites, immunocompetence or ornamentation. My results indicate, that health parameters affects whether yearling males are present in lek or not, since males in poor condition are dead or dispersed to other areas before the lekking season in spring. Instead, there were no significant differences among lekking yearling males in health factors in relation their lekking activity in this cohort.

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## 1. INTRODUCTION

In many species females choose their mate, which leads to male-male competition over females (Darwin 1871, Andersson 1994). The basis for the female choice may be direct benefits, like high-quality territory or good parental care. Benefits could also be indirect, when female gains better viability for her offspring via genetic contribution from male (reviewed by Kirkpatrick & Ryan 1991, Andersson 1994). In the case of indirect benefits, mate choice is often based on certain morphological or behavioural traits, which may act as cues for female of male condition (reviewed in Andersson 1994). According to Zahavi's handicap principle, male ornamentation may indicate genetic quality of individual, when it is honest because of being a costly signal (Zahavi 1975, 1977).

Males can increase their reproductive success by increasing the number of matings, whereas females benefit more from high quality of mates. In consequence, females usually are choosier than males, what leads to a great variation in mating success among males (Andersson 1994). But there are also differences in individual male investment in sexual advertisement due to quality differences between males and thus, individuals investing most have higher reproductive success. However, sexual advertisement is also a subject to a life-history trade-offs and as life-history theory argues, resources allocated in one activity will lead to reduced expenditure to other activities (Williams 1966, Levins 1968). Therefore producing and maintaining costly sexually selected traits will reduce investment in other life-history variables or decrease elaboration of ornamentation and hence mating success in subsequent years (Kokko 1998).

As mentioned above, the theory of sexual selection proposes that sexual traits which increase male fitness, also incur some costs or trade-offs (Zahavi 1977). Costs represent an essential part of honest handicap signalling, because high-quality males should pay lower costs for sexual advertising than males of low quality (Grafen 1990). Importantly, individuals maximize their fitness by allocating resources according to their condition or quality with as low costs as possible (Höglund & Sheldon 1998). While fitness benefits of high reproductive investment have been detected in numerous studies, the costs of signalling are quite poorly known. However, it is widely accepted that sexual advertisement decreases male viability because of increased mortality. Conspicuous sexual traits may expose individual to predators or parasites, because predators could exploit sexual traits in locating their prey (e.g. Kotiaho et al. 1998, Huhta et al. 2003). Reproduction costs may also arise as increased energy expenditure or mass loss during the production or display of sexual traits (eg. Vehrencamp et al. 1989, Mappes et al. 1996, Kotiaho 2001) or decreased immune function because of allocation to reproduction (Folstad & Karter 1992, Sheldon & Verhulst 1996, Westneat & Birkhead 1998, Kilpimaa et al. 2004). When evaluating the indicator mechanism of sexual selection, also differential costs of traits have to be taken into account although empirical evidence is scarce. In barn swallow (*Hirundo rustica*) males with elongated tail feathers had lower survival than males with naturally long tail (Møller & de Lope 1994). Also a study by Kotiaho (2000) revealed differential costs of sexual signalling in a drumming wolf spider (*Hygrolycosa rubrofasciata*). Both sexual signalling rate and condition of males were manipulated and males manipulated to be in good condition survived better the increased signalling rate than males manipulated to be in poor condition.

General life-history patterns predict that reproductive effort should increase as life expectancy decreases (Clutton-Brock 1984, Roff 1992, Stearns 1992). Individuals with low

prospect of survival may invest more in present reproduction than individuals with higher residual reproductive value and thus low-quality males may have at some stage higher trait expression than high-quality males. High-quality males can still allocate anytime as much resources in certain sexually selected trait than low-quality males do in their best strategy (Kokko 1997). The empirical evidence of the effects of decreased survival prospects on reproductive investment is still defective. In three-spined stickleback, males in poor condition increased their sexual signalling effort by producing more intensive nuptial-coloration than males in good condition (Candolin 1999, Candolin 2000) and similarly, experimentally parasitized *Drosophila* males with the high risk of mortality elevated their courtship activity (Polak & Starmer 1998). Javois & Tammaru (2004) also found that oviposition rate increased when life expectancy decreased in a moth *Scotopteryx chenopodiata*. However, effect of a residual reproductive value on reproductive effort may decrease the honesty of a signal if the most intensively displaying males are not the most viable ones (Johnstone & Grafen 1993, Kokko 1997). Evidently, honest signalling still remains if the frequency of cheaters is sufficiently small (Johnstone & Grafen 1993) or if females can discriminate cheaters by using multiple male sexual traits (Møller & Pomiankowski 1993, Alatalo et al. 2006).

Black grouse (*Tetrao tetrix*) is a relatively well-studied lek species with strong sexual selection (Alatalo et al. 1991). Females are very choosy although they are not known to get any direct benefits from the males. The female choice is based on multiple morphological, behavioural and territorial male traits, which ensure the copulation with one of the most viable males (Alatalo et al. 2006, unpubl.). In consequence, mating success among males is highly skewed with top males attaining the majority of the copulations on the lek (Alatalo et al. 1992). Briefly, the most important traits affecting male mating success are the fighting ability over central territories on the lek and male status compared to other males.

In black grouse, age is an honest signal of male viability and lifetime mating success is strongly related to male age, top males being in general three to five years old (Alatalo et al. 2006, unpubl.). Young males seldom have chance to mate and additionally, their mortality in the first year is proportionally high. However, there are large differences among juvenile males in behaviour on the leks, because some of the birds display actively already in their first possible breeding season. Alatalo et al. (2006, unpubl.) found out that active, dominant juveniles had better mating success as a yearling but lower lifetime mating success than passive juvenile males. This is probably due to the shorter life expectancy of the active yearlings and consequently, they behave to invest in sexual activity during the first year. Recent studies have also shown that experimental increase in testosterone levels with hormone implants improved sexual activity in young males, but deteriorated ornamentation next year (Siitari et al. 2006). Additionally, Kilpimaa et al. (2006, unpubl.) discovered that yearling black grouse males with high immune defence could invest more to ornamentation than weaker individuals.

The main objective of this thesis was to investigate the effect of male quality on investment in sexual advertisement in yearling black grouse males. I aimed to study, which factors are related to the fact, whether yearling males are present in the lek or not, since a large proportion of individuals dies or disperses before the lekking season in spring. Additionally, I aimed to detect whether there is variation among lekking yearling males in sexual activity and to survey, which factors are related to this variation. In particular I concentrated on significance of the health factors, such as blood parasites, immunocompetence and carotenoid levels, since active juveniles may not be as viable as the more passive individuals.

## 2. METHODS

### 2.1 The study species

The black grouse is a polygamous species and mating takes place on leks. Dimorphism between sexes is very obvious in both colour and size males being larger than females and their coloration being almost black compared to cryptic brown coloration of females. Males also have several sexual ornaments, such as an extravagant tail, blue-coloured breast and red eye combs (Alatalo et al. 2006, unpubl.). Males gather on lek sites during the mating season between late April and mid May with the purpose to persuade females to mate. Males compete for central territories in lekking arena and the most dominant and central males are usually favoured by females.

### 2.2 Field work

This study was conducted in 2005 during January-May in Central Finland. We used eight study sites, which were located in Multia (Saarisuo, Kummunsuo and Utusuo), Keuruu (Lehtosuo and Pirttilampi), Petäjävesi (Valkeissuo and Teerisuo) and Jämsänkoski (Pohjansuo). For this study we captured 191 yearling black grouse males by using walk-in traps baited with oats, from winter feeding sites located on their natural lekking sites. Birds were aged as yearlings or adults on the basis of the wing coloration and the shape of primaries (Helminen 1963) and ringed with aluminium rings and with individual colour ring combinations. The coloration of secondary and tertiary wing coverts was evaluated in a scale from zero to five (0 = black, 5 = female-like brown). Birds were weighed to the nearest 10 g with a Pesola spring balance and a blood sample (1-2 ml) was taken from brachial vein. The length of the lyre was measured as the length of the longest outer tail feathers (measured to the nearest 1.0 mm) and asymmetry was calculated as a difference between the outermost tail feathers from each side. If either of the outermost tail feathers was missing, the individual was excluded from the asymmetry analysis. A feather sample of two to five feathers was plucked from the central parts of blue breast area and preserved in a paper envelope for colour measurements. Blood samples were centrifuged (12000 rpm, 5 min) in laboratory and the plasma and red blood cells were separated. Plasma was stored in  $-20^{\circ}\text{C}$  and blood cells in  $7^{\circ}\text{C}$ . To assess the hematocrit value and the number of microfilaria parasites, two 75  $\mu\text{l}$  capillary tubes were filled with blood and centrifuged as above.

### 2.3 Eye comb measurements

In black grouse males' red eye combs are conspicuous sexual signals, and size and redness vary greatly among males. The colour of eye combs was measured in field with an Ocean Optics USB2000 spectrophotometer from 320 to 700 nm. The measurement was done by placing a fiber-optic cable with six illuminating fibers on 60 degrees angle against the eye comb. Each eye comb was measured twice and the measuring spot was changed every time. The measurement was standardized with a 99% white standard (LabSphere<sup>TM</sup>) before the first measurement. The hue of the eye combs (redness) was estimated with the spectral location  $\{\lambda (R_{50})\}$  that is the wavelength at which reflectance is halfway between its minimum ( $R_{\min}$ ) and its maximum ( $R_{\max}$ ) (Pryke & Andersson 2003).

The eye combs of the males were also recorded with a digital video camera in field to assess the size of the combs. Each eye comb was recorded from about a one-meter distance and a scale of a millimeter paper was held behind the bird's head to standardize the size of combs. The area of the eye combs (cm<sup>2</sup>) was calculated from video shots by using ImagePro-program.

## 2.4 Behavioural observations

Birds were observed every morning during the lekking time in late April and early May (between 27 April and 3 May 2005) from the hide using binoculars and telescopes. Observations were started when birds arrived at dawn (2.00-4.00) and ended after male departure (8.00-9.00). The mating success of the young males was recorded as the number of copulations per male. The attendance and activity of males were observed using a scan sampling scheme approximately every 5 minutes. At the same time the positions of the males were mapped at the accuracy of 1 m with the aid of a 10 m \* 10 m grid marked with sticks on the lekking arena. The centre of the lek was defined as the median of x- and y-axes for all males observed on arena during the lekking period. The centre of an individual male territory was similarly calculated as a median for all observations of a particular male and the centrality of the male was then defined as the distance from territory centre to the lek centre. The attendance of a male was calculated as a proportion of presence compared to the male that attended the lek most often.

Fighting rate, vocal activity and tail posture of males were also observed. Fighting rate was defined as proportion of scans where male was in close contact with any of its neighbours. The proportion of scans where male emitted "hissing" or "rookooing" sounds was used to define vocal activity. Tail posture was used to reflect the general activity of a male, because males erect their tails when active and lower it when inactive. Males' tail posture was evaluated in a scale from one to three (1 = tail folded, 2 = tail semi-folded, 3 = tail erected) and the mean of all scans was used.

All leks within five kilometres around the main leks were also monitored during the lekking time once or a few times and the attendance, activity and the mating success of ringed juvenile males were recorded. When a lek was monitored only once, ringed males were excluded from the behavioural analysis.

## 2.5 Colour measurements

In many birds, structural feather colouration can function as a sexual signal. In black grouse, males are bluish in the breast, neck and the rump. The coloration of blue breast feather samples was measured in laboratory using a fiber optic spectrometer (Avantes AvaSpec-2048) from 320 to 700 nm in steps of 5 nm. A feather sample was placed on black velvet, which has low reflectance in all wavelengths to remove possible background reflection. The sample was illuminated at 60° to the measuring surface by an Avantes AvaLight-DHS deuterium-halogen light source and reflected light was collected with a fibre optic probe (Avantes UV200-2-1.5x40) connected to an angled fiber (Avantes AFH-15) at 60° (90° to illumination). Measurements were performed randomly from the blue area on the tip of the feather and the measured spot was changed between scans. Every sample was measured three times and the scans were assessed in relation to calibrated 98% white reflectance standard (Avantes WS-2). For the analysis the mean of the three measurements was used to calculate blue chroma ( $R_{400-480\text{ nm}}/R_{320-700\text{ nm}}$ ) (Siitari et al. 2006). Blue chroma was used in the analysis because it is previously detected that blue chroma is positively related to male mating success in the black grouse (Siitari et al. 2006).



## 2.6 Parasite measurements

Microfilaria parasites were assessed from the capillary blood samples under a light microscope at 400 times magnification. After centrifuging, microfilaria and other extracellular parasites are found at the interface of erythrocytes and plasma and they are easy to detect. All the visible microfilaria parasites were counted from two samples per bird by rotating capillar on 360°.

The presence of the three most common intracellular avian parasites *Plasmodium* spp., *Haemoproteus* spp. and *Leucocytozoon* spp. were assessed with the nested-PCR technique (Hellgren *et al.* 2004). These parasites are protozoans of the phylum Apicomplexa and the method is based on cytochrome *b* gene of mitochondrial genome, which has been found to have conserved regions for construction of primer sites. Between the conserved regions there are variable sections of DNA, which enables detection of *Plasmodium* and *Haemoproteus* lineages. The method enables simultaneous typing of all three species and is highly repeatable and reliable. Briefly, in a first PCR step, parasite DNA of all three genera was amplified and after that, two primer pairs were chosen to amplify either *Leucocytozoon* spp. singly or *Plasmodium* spp. and *Haemoproteus* spp. together. Finally, *Plasmodium* and *Haemoproteus* infections were separated by determining base sequences of samples and comparing these sequences to known base sequences of *Plasmodium* and *Haemoproteus*. Sequences were analysed with three computer programs, since sequences were revised with SeqScape-program, phylogenetic tree was constructed with ClustalX-program and phylogenetic trees were analysed with TreeView.

## 2.7 Measurement of plasma carotenoid concentration

The carotenoid concentration of the plasma samples was measured by spectrophotometry (see Tella *et al.* 1998). Variable volumes (20 \* 40 µl) of plasma were mixed with acetone to obtain the sample volume of 800 µl. The dilution ratio varied between 1:19 and 1:39. The samples were vortexed and centrifuged (13 000 x g, 5 min) in order to precipitate the flocculent plasma protein. Supernatants were then read in a spectrophotometer (Beckman DU® 640) determining the light absorbance of the samples at 470 nm, the absorption maximum for lutein, a common type of carotenoids. The assay was calibrated using a series of samples with known concentration of lutein (Sigma Co.) as standards, and their absorbances were used to generate a regression line between concentration and absorbance. Derived equation was then used to convert the absorbance value of the plasma samples into carotenoid concentration applying the dilution ratios of each sample.

## 2.8 Immunoglobulin analysis

Total plasma immunoglobulin concentration was analysed using an indirect enzyme-linked immunosorbent assay (ELISA) with commercial anti-chicken antibodies (Sigma C-6409). The anti-chicken IgG antibody binds to the light chain of the IgM and IgG and therefore, IgM and IgG responses cannot be distinguished in the analysis (Müller *et al.* 2005). Primary immune response consists mainly of IgM and hence, it is probable that the measurement consists a great extent of IgM.

To assess antibody concentrations, a standard of pooled plasma of all individuals was used and an arbitrary concentration of 10<sup>6</sup> was assigned for the standard. All values were subsequently expressed relative to these standards and the measured concentrations of immunoglobulins were expressed as units per millilitre (U/ml). Briefly, ELISA-plates

(Nunc™ Immunoplate) were coated with anti-chicken IgG (Sigma C-6409) and the plates were incubated at +4°C overnight. After washing the plates, they were masked with 1% BSA-PBS (Roche Diagnostics) for 1 hour and then washed three times with 200µl PBS-Tween. Samples and standards (50µl/well) were diluted with 1% BSA-PBS and added to the wells. For each plate, a standard (mixture of plasma of all individuals measured, an arbitrary concentration of  $10^6$ ) with different dilutions (50 µl/well; diluted also with 1% BSA-PBS) was added. The plates were incubated for 3 h at room temperature, and then washed three times with 200 µl PBS-Tween. Subsequently, an alkaline phosphatase conjugated secondary antibody (Sigma© A-9171 anti-chicken IgG, 1: 15 000 diluted with 1% BSA-PBS) was added to the wells and incubated overnight at +4°C. Finally, after washing the wells (3 x 400µ with PBS-Tween), an alkaline phosphatase substrate PNPP (p-nitrophenyl phosphate, Sigma 104® phosphatase substrate) in 1M diethanol amine buffer (1 mg/ml) was applied (50µl/well). The absorbance of the wells was measured with an ELISA reader at wavelength 405 nm for up to 1 hour (or until the highest standard reached the absorbance 2.0). Dilutions used in analysis were 1:20 000 and 1:50 000, and the conjugated antibody was diluted with the ratio of 1:20 000. Applied standard points were 250, 100, 50, 25, 10, 5, 2.5, and 0 U/ml.

## 2.9 Statistical analysis

To examine the differences between the males that were present and absent in the lekking period, and to determine differences in behaviour between lekking yearlings, we used binary logistic regression. For the analysis yearlings were divided in two groups according to their presence or absence on the lek and additionally, lekking yearlings were divided in active and passive individuals according to their lek activity. Normality and the homogeneity of variances were tested before statistical analyses and when the normality assumptions were not fulfilled, non-parametric statistics were applied. Carotenoid concentrations, microfilaria and hematocrit values were ln-transformed to obtain the normality. Mann-Whitney U-test was used as a nonparametric test.

Data for eye comb size and redness was corrected with the capturing date using linear regression, since redness decreases and size increases towards the lekking season (Halme et al., unpubl.). Correction was done also for microfilaria and hematocrit values, which both increase linearly with the date (R. Alatalo, unpubl). Statistical analyses were conducted using SPSS for Windows, versions 11.1-13.0. All probability values are two-tailed unless otherwise stated.

## 3. RESULTS

### 3.1 Presence on the lek

77 males of total 191 (40.3%) captured young birds were observed at least once on the leks. We studied whether presence on the lek (0 = absent; dead or dispersed over 5 km, 1 = present) was affected by immunoglobulin concentration, the number of microfilaria parasites, hematocrit value, plasma carotenoid concentration or lyre asymmetry by using multiple binary logistic regression. In our model there was a significant positive relationship between lek presence and immunoglobulin concentration (Binary logistics regression;  $\beta = 0.991$ ,  $P = 0.024$ ). The number of microfilaria parasites correlated negatively with lek presence ( $\beta = -0.381$ ,  $P = 0.032$ ) as well as the lyre asymmetry ( $\beta = -1.467$ ,  $P = 0.033$ ). Hematocrit value was positively related to lek presence ( $\beta = 18.472$ ,  $P =$

0.032), whereas plasma carotenoid concentration did not explain male presence on the lek ( $\beta = 0.258$ ,  $P = 0.723$ )(Table 1.).

### 3.2 Lek activity

For determining the possible differences in lekking behaviour between individuals, males were divided in two categories according to their attendance (1 = active, attendance > 0.2; 0 = passive, attendance < 0.2). Mean attendance for active birds was 0.697 (SD = 0.271, N = 31), whereas for passive birds it was 0.045 (SD = 0.06, N = 41). The effect of immunoglobulin concentration, the number of microfilaria parasites, hematocrit value, plasma carotenoid concentration and lyre asymmetry on attendance was studied using multiple binary logistic regression. Active and passive males differed significantly only in the hematocrit value ( $\beta = 53.284$ ,  $P = 0.02$ ) but there was also a positive tendency between lyre asymmetry and activity ( $\beta = 3.349$ ,  $P = 0.072$ ). There were no interactions between lek activity and microfilaria load ( $\beta = -0.325$ ,  $P = 0.315$ ), immunoglobulin concentration ( $\beta = -0.494$ ,  $P = 0.549$ ) or carotenoid concentration ( $\beta = -0.336$ ,  $P = 0.780$ ).

There was a tendency that active young males achieved a territory closer to the lek centre than more passive males (Mann-Whitney U-test;  $N_{\text{active}} = 21$ ,  $N_{\text{passive}} = 25$ ,  $Z = -1.753$ ,  $P = 0.08$ ,  $\text{mean}_{\text{active}} = 17.45$ ,  $\text{SD} = 9.12$ ,  $\text{mean}_{\text{passive}} = 20.57$ ,  $\text{SD} = 8.53$ ) and they spent more time to fighting than passive males ( $N_{\text{active}} = 21$ ,  $N_{\text{passive}} = 25$ ,  $Z = 5.118$ ,  $P < 0.001$ ,  $\text{mean}_{\text{active}} = 0.265$ ,  $\text{SD} = 0.172$ ,  $\text{mean}_{\text{passive}} = 0.043$ ,  $\text{SD} = 0.2$ ). There were also significant differences between the active and passive birds in vocal activity (Mann-Whitney U-test;  $N_{\text{active}} = 21$ ,  $N_{\text{passive}} = 25$ ,  $Z = 3.705$ ,  $P < 0.001$ ,  $\text{mean}_{\text{active}} = 0.561$ ,  $\text{SD} = 0.192$ ,  $\text{mean}_{\text{passive}} = 0.228$ ,  $\text{SD} = 0.331$ ) and in tail posture (Mann-Whitney U-test;  $N_{\text{active}} = 21$ ,  $N_{\text{passive}} = 23$ ,  $Z = 4.148$ ,  $P < 0.001$ ,  $\text{mean}_{\text{active}} = 2.87$ ,  $\text{SD} = 0.192$ ,  $\text{mean}_{\text{passive}} = 1.78$ ,  $\text{SD} = 0.761$ )(Table 2.). Activity improved mating success significantly in young males (Mann-Whitney U-test;  $N_{\text{active}} = 31$ ,  $N_{\text{passive}} = 41$ ,  $Z = -2.884$ ,  $P = 0.004$ ,  $\text{mean}_{\text{active}} = 0.125$ ,  $\text{SD} = 0.824$ ,  $\text{mean}_{\text{passive}} = 0$ ,  $\text{SD} = 0$ ).

### 3.3 Parasite measurements

Microfilaria was the most prevalent blood parasite since it was found from 83.2 % of juvenile males (N = 191 in every case). Other parasites were less common since leucocytozoon occurred in 29.8 % of males, plasmodium in 6.8 % and haemoproteus in 5.8 % of males.

There were no differences in the prevalence of blood parasites between males present and absent on leks ( $N_{\text{present}} = 77$ ,  $N_{\text{absent}} = 114$  in all comparisons) (Mann-Whitney U-test; plasmodium:  $Z = -1.309$ ,  $P = 0.191$ ,  $\text{mean}_{\text{present}} = 0.04$ ,  $\text{SD} = 0.195$ ,  $\text{mean}_{\text{absent}} = 0.09$ ,  $\text{SD} = 0.284$ ; haemoproteus:  $Z = -0.989$ ,  $P = 0.323$ ,  $\text{mean}_{\text{present}} = 0.08$ ,  $\text{SD} = 0.27$ ,  $\text{mean}_{\text{absent}} = 0.04$ ,  $\text{SD} = 0.206$ ; leucocytozoon:  $Z = -0.315$ ,  $P = 0.753$ ,  $\text{mean}_{\text{present}} = 0.29$ ,  $\text{SD} = 0.455$ ,  $\text{mean}_{\text{absent}} = 0.31$ ,  $\text{SD} = 0.463$ ). The prevalence of blood parasites did not relate to males' activity ( $N_{\text{active}} = 31$ ,  $N_{\text{passive}} = 41$  in all comparisons) during the lekking season either (Mann-Whitney U-test; plasmodium:  $Z = -0.345$ ,  $P = 0.73$ ,  $\text{mean}_{\text{active}} = 0.03$ ,  $\text{SD} = 0.18$ ,  $\text{mean}_{\text{passive}} = 0.05$ ,  $\text{SD} = 0.218$ ; haemoproteus:  $Z = 0.356$ ,  $P = 0.722$ ,  $\text{mean}_{\text{active}} = 0.1$ ,  $\text{SD} = 0.301$ ,  $\text{mean}_{\text{passive}} = 0.07$ ,  $\text{SD} = 0.264$ ; leucocytozoon:  $Z = -0.022$ ,  $P = 0.983$ ,  $\text{mean}_{\text{active}} = 0.29$ ,  $\text{SD} = 0.461$ ,  $\text{mean}_{\text{passive}} = 0.29$ ,  $\text{SD} = 0.461$ ).

### 3.4 Relationships between carotenoid and immunoglobulin parameters

In general there was a positive correlation between plasma carotenoid concentration and the redness of the eye comb (Spearman's correlation; N = 180,  $r_s = 0.251$ ,  $P = 0.001$ ;

Fig. 1). In addition, carotenoid concentration was positively related to the size of eye comb ( $N = 189$ ,  $r_s = 0.168$ ,  $P = 0.021$ ). Males infected with leucocytozoon parasite had lower plasma carotenoid concentration than healthy males (Mann-Whitney U-test;  $N_{\text{not infected}} = 134$ ,  $N_{\text{infected}} = 57$ ,  $Z = 1.981$ ,  $P = 0.048$ ,  $\text{mean}_{\text{not infected}} = 3.58$ ,  $\text{SD} = 0.26$ ,  $\text{mean}_{\text{infected}} = 3.49$ ,  $\text{SD} = 0.27$ ).

Immunoglobulin concentration significantly positively correlated with fighting rate (Spearman's correlation;  $N = 46$ ,  $r_s = 0.322$ ,  $P = 0.029$ ). Yearling males infected with plasmodium parasite had higher plasma immunoglobulin levels than males not infected with plasmodium (Mann-Whitney U-test;  $N_{\text{not infected}} = 178$ ,  $N_{\text{infected}} = 13$ ,  $Z = -2.534$ ,  $P = 0.011$ ,  $\text{mean}_{\text{not infected}} = 13.56$ ,  $\text{SD} = 0.42$ ,  $\text{mean}_{\text{infected}} = 13.99$ ,  $\text{SD} = 0.6$ ; Fig. 2).

### 3.5 Eye combs

In black grouse red eye combs are important sexual signals. The size and the redness of combs are strongly related to each other (Spearman's correlation;  $N = 185$ ,  $r_s = 0.241$ ,  $P = 0.001$ ). However, the redness of eye combs was not related to lek presence in young males (Mann-Whitney U-test;  $N_{\text{present}} = 72$ ,  $N_{\text{absent}} = 108$ ,  $Z = -0.984$ ,  $P = 0.325$ ,  $\text{mean}_{\text{present}} = 596.81$ ,  $\text{SD} = 3.23$ ,  $\text{mean}_{\text{absent}} = 597.05$ ,  $\text{SD} = 3.38$ ) nor did the size of the combs ( $N_{\text{present}} = 77$ ,  $N_{\text{absent}} = 112$ ,  $Z = -0.062$ ,  $P = 0.95$ ,  $\text{mean}_{\text{present}} = 0.79$ ,  $\text{SD} = 0.33$ ,  $\text{mean}_{\text{absent}} = 0.85$ ,  $\text{SD} = 0.28$ ). Among males that were present on the lekking period, active and passive birds did not differ in the redness of the eye combs (Mann-Whitney U-test;  $N_{\text{active}} = 28$ ,  $N_{\text{passive}} = 39$ ,  $Z = 1.386$ ,  $P = 0.166$ ,  $\text{mean}_{\text{active}} = 597.51$ ,  $\text{SD} = 3.42$ ,  $\text{mean}_{\text{passive}} = 596.18$ ,  $\text{SD} = 3.15$ ), nor in the size of eye combs ( $N_{\text{active}} = 31$ ,  $N_{\text{passive}} = 41$ ,  $Z = 0.472$ ,  $P = 0.637$ ,  $\text{mean}_{\text{active}} = 0.81$ ,  $\text{SD} = 0.37$ ,  $\text{mean}_{\text{passive}} = 0.76$ ,  $\text{SD} = 0.34$ ). However, redness of eye combs positively correlated with fighting rate (Spearman's correlation;  $N = 43$ ,  $r_s = 0.425$ ,  $P = 0.004$ ; Fig. 3).

### 3.6 Breast feather coloration

Males present and absent on the lekking time did not differ in the blue chroma of the breast feathers (Mann-Whitney U-test;  $N_{\text{present}} = 75$ ,  $N_{\text{absent}} = 113$ ,  $Z = -0.721$ ,  $P = 0.471$ ,  $\text{mean}_{\text{present}} = 0.26$ ,  $\text{SD} = 0.024$ ,  $\text{mean}_{\text{absent}} = 0.261$ ,  $\text{SD} = 0.029$ ). Among lekking birds, there were no differences between active and passive males either ( $N_{\text{active}} = 31$ ,  $N_{\text{passive}} = 39$ ,  $Z = 1.531$ ,  $P = 0.126$ ,  $\text{mean}_{\text{active}} = 0.265$ ,  $\text{SD} = 0.212$ ,  $\text{mean}_{\text{passive}} = 0.254$ ,  $\text{SD} = 0.027$ ). Blue chroma correlated positively with the wing coloration in young males (Spearman's correlation;  $N = 187$ ,  $r_s = 0.170$ ,  $P = 0.02$ ) since more black winged males also had higher blue chroma than brownish ones. Blue chroma was positively related to plasma carotenoid concentration (Spearman's correlation;  $N = 188$ ,  $r_s = 0.175$ ,  $P = 0.016$ ) and also to hematocrit value ( $N = 186$ ,  $r_s = 0.151$ ,  $P = 0.04$ ). Interestingly, males with more asymmetric tails also had lower blue chroma ( $N = 145$ ,  $r_s = -0.180$ ,  $P = 0.03$ ; Fig. 4). Among behavioural variables only the vocal activity correlated with blue chroma ( $N = 45$ ,  $r_s = 0.370$ ,  $P = 0.012$ ).

## 4. DISCUSSION

According to my results, there are differences in both lek presence and lekking behaviour between yearling males. Lek presence represents also yearling males' survival from winter to the spring and it seems to be related to health factors. Immunoglobulin concentration, hematocrit value, the number of microfilaria parasites and lyre asymmetry explained young males' presence in the leks, whereas only hematocrit was related to the lekking activity. Activity improved yearling males' status on the leks, since active males

tended to achieve territories closer to the lek centre than passive males and they also got more copulations.

Activity improved mating success in yearling males and besides, the number of copulations for yearlings was relatively high. Typically the first year males do not have opportunities to copulate and they can not usually hold territories (Alatalo et al. 1991, Höglund & Alatalo 1995). Previous studies have also shown, that the centrality of territory strongly affects male mating success in the black grouse (Hovi et al. 1994, Höglund & Alatalo 1995, Rintamäki et al. 1995). In my study, active yearlings tended to achieve territories closer to the lek centre, which improves chances to get copulations. To achieve a central territory, a male has to lek actively for several years (Kokko et al. 1998) and consequently, territory centrality of a male might act as a reliable signal of male quality. Behavioural observations also revealed that male attendance was strongly positively related to fighting rate, tail posture and vocal activity. Besides reflecting the overall activity of males, these variables may also reveal male condition. In the black grouse, males with high fighting rate also had higher survival (Alatalo et al. 1991) and thus, fighting rate might be an honest signal of quality, too. Hematocrit levels were positively related to both lek presence and the lekking activity. Hematocrit is a percentage of red blood cells of total blood volume and therefore, it could be used to evaluate the overall health of individuals.

Plasma carotenoid concentration positively correlated with the redness of the eye comb, which indicates that red colouration is carotenoid-based. This is in parallel to earlier studies, which have shown positive correlations between ornament colour and plasma carotenoid level (Bortolotti et al. 1996, McGraw et al. 2003, Peters et al. 2004). Generally, carotenoid concentrations are also assumed to reveal an individual condition, since carotenoids are acquired from food and their concentration in plasma may reflect foraging efficiency of individuals (Hill et al. 2002). Furthermore, carotenoids are antioxidants and immunostimulants (Bendich 1989, Chew 1996) and hence, there might be a trade off in carotenoid allocation between immune function and ornamentation. Many studies have shown that females prefer males with brighter carotenoid-dependent colours (Hill 1991, Hill et al. 1999), since only males in good condition are able to invest in immune function and ornamentation. In a study by Blount et al. (2003), increase of dietary carotenoid supply improved immune function and sexual attractiveness in zebra finches (*Taeniopygia guttata*), which supports the hypothesis that carotenoid-based ornamentation may act as a reliable cue of individual health. In my study, I found a positive correlation between blue chroma and plasma carotenoid concentration. However, I did not detect any relationship between plasma carotenoid concentrations and immunoglobulin levels. In addition, higher carotenoid concentrations or redder eye combs did not improve mating success in juvenile males. This may be due to poor mating success among juveniles, since they are not able to invest heavily to sexual ornamentation. It is possible that the trade off in carotenoid allocation between immune function and ornamentation arises in older males, which compete stronger over females. Besides, there is also a lot of evidence that parasites may inhibit expression of carotenoid-pigmentation (eg. Thompson et al. 1997, McGraw & Hill 2000). My results support these results, since I found a negative relationship between plasma carotenoid concentration and a *Leucocytozoon* parasite.

Immunoglobulin concentrations measured during the winter were significantly higher in males that were present in the lekking period. Immunoglobulins are proteins produced by plasma cells and they function as antibodies antigens. Thus, the amount of immunoglobulins in blood reflects individuals' ability to cope with diseases and parasites and their amount might also be related to survival. On the other hand, immunoglobulin

levels increase also when immune system is activated. In another study on black grouse, juvenile males with high immune response were more likely to survive to the next breeding season than males with low immune defence (Kilpimaa et al. 2006, unpubl.). On the other hand, immune defence may also be traded off against other life-history traits, like reproduction and hence, individuals balancing best the costs and benefits of investing resources in the immune system might have the highest fitness. In my study, males with high Ig levels fought more, which may reveal better condition of these males. Nevertheless, I detected a strong positive correlation between immunoglobulin levels and *Plasmodium* parasite. My results suggest that high Ig levels improve young male survival and fitness, except when high Ig levels are due to infections. However, immunoglobulin levels are individual-specific and hence, it is difficult to separate when high Ig levels reflect healthy and when infections.

*Microfilaria* was the most prevalent parasite and it was also related to the lek presence of males. *Microfilaria* is highly prevalent through the year although its prevalence reaches a maximum in summer. Also *Haemoproteus*, *Leucocytozoon* and *Plasmodium* are most abundant during the summer, when their vectors are most active (Siitari et al. 2006). Former studies on black grouse have shown that parasites affect the expression of black grouse ornaments. Höglund et al. (1992) found that males with fewer parasites have longer lyre and additionally, blue chroma on the male breast has been shown to be negatively related to *Microfilaria* abundance (Alatalo et al. 2006, unpubl.).

Structural coloration within the short wavelengths (400-480 nm) is known to be an important factor in sexual selection in black grouse. Blue chroma positively correlates with mating success in old males and thus, it might indicate male quality (Siitari et al. 2006). Likewise my results suggest, in yearlings blue coloration is related to individual quality, since I found positive correlations between blue chroma and both plasma carotenoid levels and hematocrit values. Young males with more asymmetric lyres also had lower blue chroma than in more symmetric individuals. In black grouse, yearlings do not achieve their eventual coloration until their second summer. However, in my study blacker birds had higher blue chroma than brownish ones already as yearlings and hence, blue coloration might be a reliable signal in young males in spite of delayed plumage maturation.

Lyre asymmetry was positively related to male presence on the lek during the mating season. Fluctuating asymmetry is the random deviation from perfect bilateral symmetry in morphological traits, which arises due to stress during the development of symmetric traits (Van Valen 1962, Palmer & Strobeck 1986). Hence, levels of asymmetry might also reflect individual condition (Møller & Pomiankowski 1993). In a previous study on black grouse, lyre asymmetry was not related to males' territorial centrality or male mating success (Rintamäki et al. 1997). This is probably because lyre asymmetry is related to lyre length, which is not under strong sexual selection. My results indicate that symmetrical males are possibly more viable than asymmetrical males, since their survival to spring was higher and they tended to lek more actively. Additionally, yearlings with more symmetrical lyres also had higher blue chroma, which is known to be an important ornament detecting male mating success in old males (Alatalo et al. 2006). However, it is possible that females cannot use lyre asymmetry as a signal of male quality, because differences in tail feather length might be hard to detect when males display and lift their tails. On the other hand, in male-male contest tail is often damaged, since males try to tear feathers off from each others' tails which in turn increases asymmetry. Hence, tail condition might be more reliable cue of male viability for females than asymmetry but asymmetry may still reflect male condition.

According to my results, male condition might have an effect on survival and dispersal behaviour in yearling black grouse. Males that were present in the lekking time had higher hematocrit and Ig levels and also had less microfilaria during the winter, which all indicates better physical condition of these males. Instead, males in poorer condition have probably been dead or dispersed to other areas, where competition between males is not so strong. In my thesis, I studied mainly large, central leks, where the number of males is relatively high. Females have been shown to prefer larger leks, which leads to a higher average male mating success in large leks (Alatalo et al. 1992). However, the mating success of yearling males does not usually improve with lek size, since on larger leks females prefer adult males. Therefore it might be a better strategy for young males to disperse to smaller leks. In addition, the cohort I studied was very large, which may have lead to stronger competition among males and consequently to higher dispersal rates.

To conclude, male quality has an effect on reproductive effort and sexual advertisement to some extent. Especially health parameters reflecting male condition affects whether males are present in the lekking time or not and additionally, at least some of the ornaments seem to be condition-dependent. Nevertheless, health parameters were not strongly related to male lekking activity in this cohort, since only hematocrit explained variation in the activity. However, factors affecting reproductive effort in yearling males might be hard to detect, since besides male quality there might be other, external factors, too. Presumably it would require a long-term study to detect the ultimate causes for differences in sexual advertisement.

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Table 1. Factors explaining lek presence in yearling males (N = 143) analysed with Multiple binary logistic regression.

| Variables       | $\beta$ | SE    | df | P     |
|-----------------|---------|-------|----|-------|
| Immunoglobulins | 0.991   | 0.438 | 1  | 0.024 |
| Microfilaria    | -0.381  | 0.178 | 1  | 0.032 |
| Hematocrit      | 18.472  | 8.623 | 1  | 0.032 |
| Lyre asymmetry  | -1.467  | 0.69  | 1  | 0.033 |
| Carotenoids     | 0.258   | 0.726 | 1  | 0.723 |

Table 2. Differences between active and passive yearlings in lekking behaviour. Territory centrality refers to territory distance from the lek centre. All the variables were analysed with Mann-Whitney U-test.

|                      | Active            |    | Passive           |    | Z      | P      |
|----------------------|-------------------|----|-------------------|----|--------|--------|
|                      | Mean $\pm$ SD     | N  | Mean $\pm$ SD     | N  |        |        |
| Territory centrality | 17.45 $\pm$ 9.12  | 21 | 20.57 $\pm$ 8.53  | 25 | -1.753 | 0.08   |
| Fighting rate        | 0.265 $\pm$ 0.172 | 21 | 0.043 $\pm$ 0.2   | 25 | 5.118  | <0.001 |
| Vocal activity       | 0.561 $\pm$ 0.192 | 21 | 0.228 $\pm$ 0.331 | 25 | 3.705  | <0.001 |
| Tail posture         | 2.87 $\pm$ 0.192  | 21 | 1.78 $\pm$ 0.761  | 23 | 4.148  | <0.001 |

## FIGURE LEGENDS

Figure 1. Relation between plasma carotenoid levels and eye comb redness.

Figure 2. Plasma immunoglobulin levels in yearling males infected and not infected with *Plasmodium* parasite. The presented values of immunoglobulin concentrations are natural logarithms of the original data.

Figure 3. Relation between fighting rate and the redness of eye comb in yearling males.

Figure 4. Relation between lyre asymmetry and blue chroma.

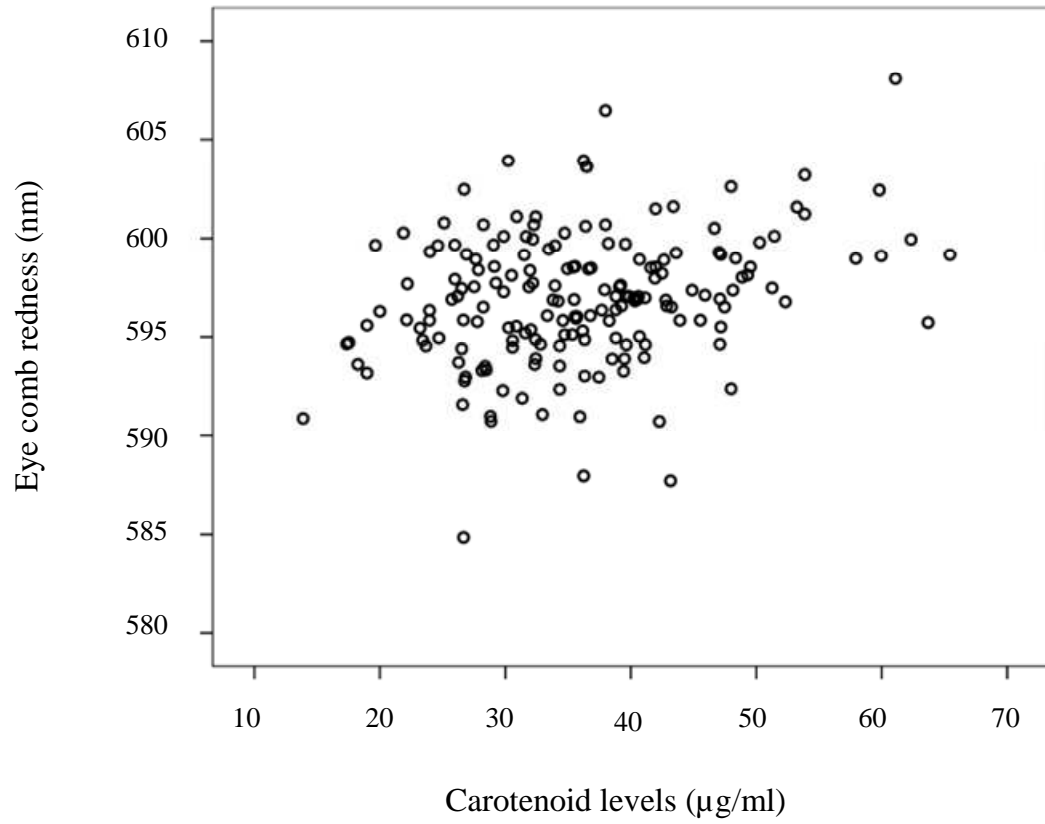


Figure 1. Häsä 2006

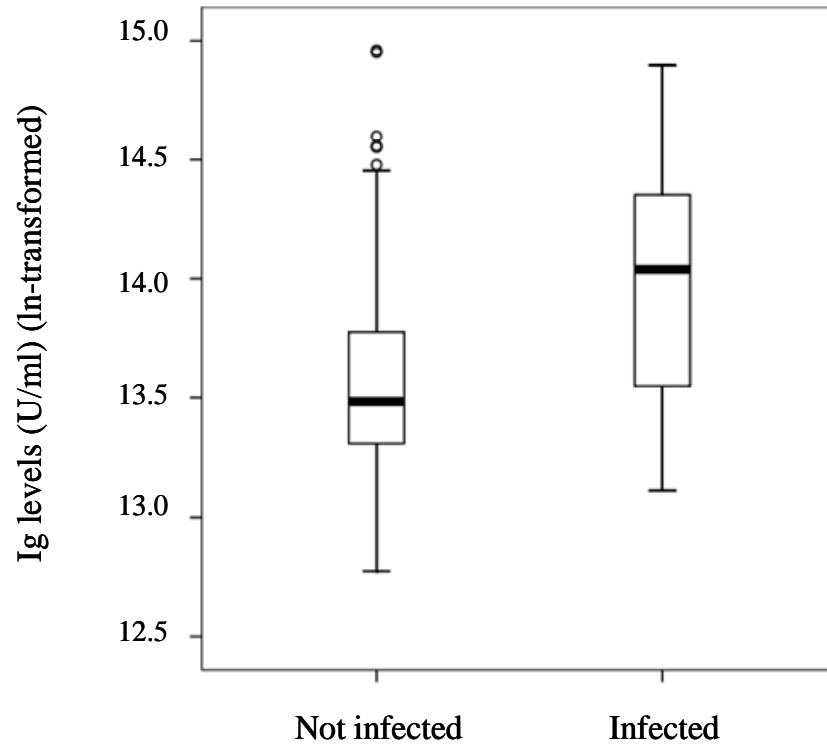


Figure 2. Häsä 2006

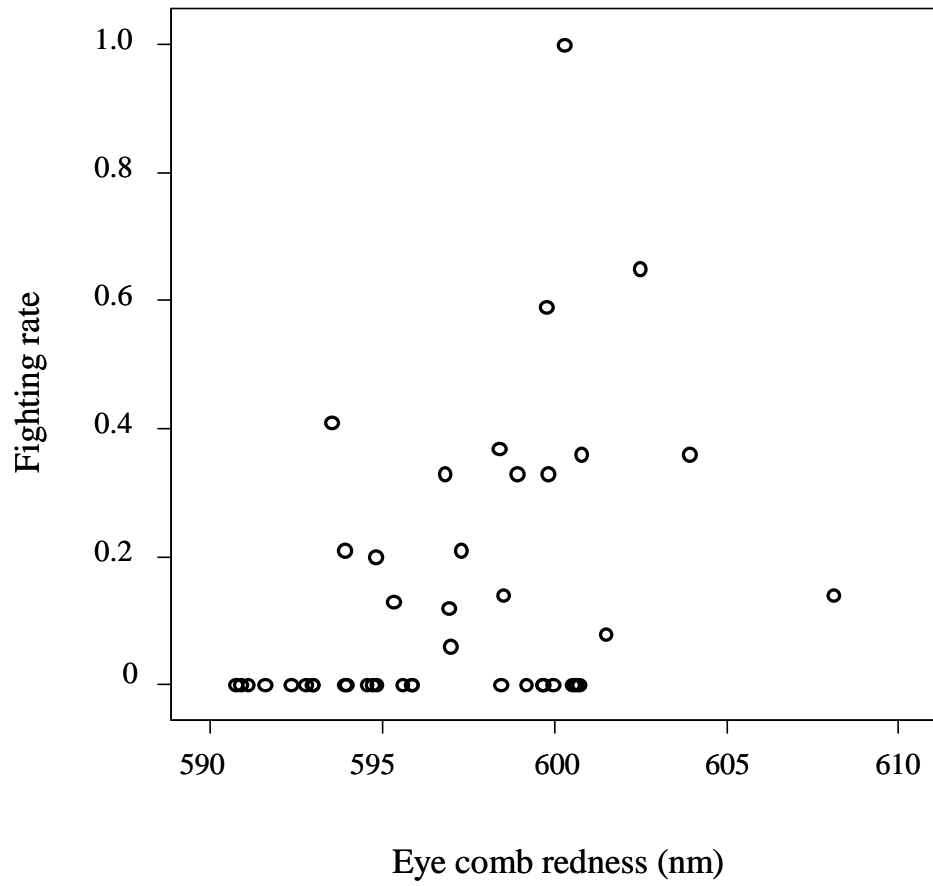


Figure 3. Häsä 2006

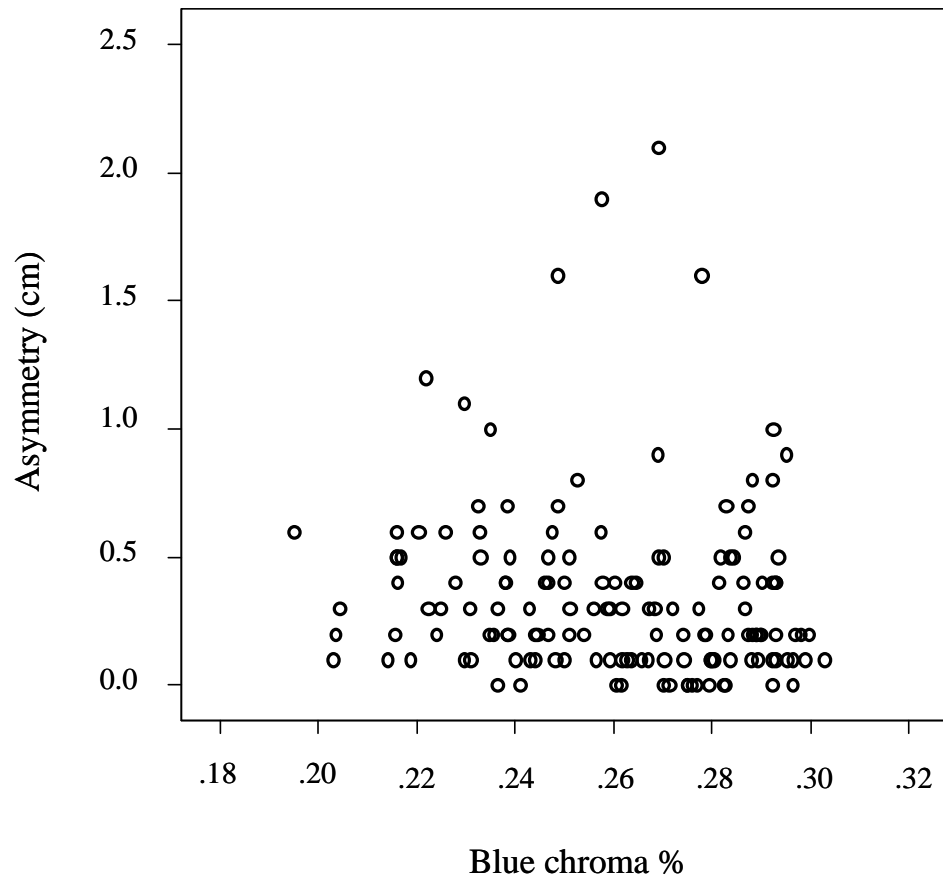


Figure 4. Häsä 2006