Title: Male body size and breeding tubercles are both linked to intra-sexual dominance and reproductive success in the minnow
Authors: Jacob A., Evanno G., Renai E., Sermier R., Wedekind C.

Journal: Animal Behaviour
Year: 2009

Volume: 77(4)

Pages: 823-829

DOI: 10.1016/j.anbehav.2008.12.006

Male body size and breeding tubercles are both linked to intra-sexual dominance and reproductive success in the European minnow (*Phoxinus phoxinus*)

ALAIN JACOB[1°], GUILLAUME EVANNO[1,2,3°], EMANUELA RENAI[1°], ROBERTO SERMIER[1] & CLAUS WEDEKIND[1]

°Equally contributing authors.

[1]Department of Ecology and Evolution, University of Lausanne

[2]INRA, Ecology and Health of Ecosystems, Rennes.

[3]Agrocampus Ouest, Rennes.

Abstract

Male dominance hierarchies are usually linked to relative body size and to weapon size, i.e. to determinants of fighting ability. Secondary sexual characters that are not directly used as weapons could still be linked to dominance if they reveal determination or overall health and vigour and hence, indirectly, fighting ability. We studied the mating behaviour of the minnow (*Phoxinus phoxinus*), a cyprinid fish in which males develop breeding tubercles during the spawning season. The function of these breeding tubercles is still not clear. Using microsatellites markers we determined the male reproductive success under controlled conditions. We found that the minnows were territorial and that they quickly established a dominance hierarchy at the beginning of the spawning season. Dominance was strongly and positively linked to fertilisation success. Although body size and number of breeding tubercles were not significantly correlated in our sample, both large males and males with a higher number of breeding tubercles were more dominant and achieved a higher fertilization success than small males or males with few tubercles. We found multi-male fertilization in most clutches, suggesting that sperm competition is important in this species. Females showed behaviour that may be linked to spawning decision, i.e. male dominance might not be the only determinant of male reproductive success in minnows.

Key-words: lek; male-male competition; perl organs; reproductive behaviour; secondary sexual character; sexual selection

Antagonistic encounters between males are often costly (Smith 1974) and dominance hierarchies may be established to reduce the intensity of such interactions (Collias 1943). Dominant males usually have larger and higher-quality territories (Foote 1990; Andersson et al. 2002; Candolin and Voigt 2001), better access to females (Fleming and Gross 1994; Quinn and Foote 1994; Creighton 2001; Wong and Candolin 2005) and they often get a higher fertilisation success (Andersson 1994; Dewsbury 1982; Wiley 1973; Esteve 2005).

Across various taxa, dominance is usually well indicated by body size and weight (Andersson 1994; Qvarnstrom and Forsgren 1998), but often also by secondary sexual characters that are not directly used as weapons (Kortet et al. 2004; Kortet and Taskinen 2004; Berglund et al. 1996; Cluttonbrock et al. 1980). Sometimes, male dominance seems to be closer linked to body size than to secondary sexual characters (Hudman and Gotelli 2007; Zucker and Murray 1996), and sometimes secondary sexual characters may be the better indicators of male dominance (Kortet et al. 2004; Kitchen et al. 2003; Setchell et al. 2006; Stuart-Fox et al. 2006), especially so if secondary sexual characters indicate good health and vigour. Indeed, high resistance or tolerance to pathogens have been found in dominant males (Ahtiainen et al. 2006; Rantala and Kortet 2004) and in males with elaborated secondary sexual characters (Kortet and Taskinen 2004; Wedekind 1992; Milinski and Bakker 1990; Taskinen and Kortet 2002; Ezenwa and Jolles 2008). Secondary sexual characters can therefore be important not only in female choice but also in male-male competition (Andersson 1994).

We studied male reproductive success with regard to dominance and secondary sexual characters in the European minnow (*Phoxinus phoxinus*), a cyprinid whose mating system has so far only been qualitatively described as "communal spawning" (Breder & Rosen 1966, (Bless 1992). Mature males seem to establish a dominance hierarchy and to defend territories before females begin to spawn (Bless 1992). Males often display secondary sexual characters during the reproductive period. These characters can include conspicuous skin colours (e.g. melanin-based patterns and/or red colours – the latter are usually most pronounced around the mouth and the pectoral and pelvic fins) and breeding tubercles that are mostly located on the head. Breeding tubercles are little colourless and horny epidermal structures that are common in many fish species. Their functional significance is not fully understood yet (see discussion in Wiley & Collette (1970) and Wedekind et al. (2008)). In the case of the minnow, breeding tubercles may simply facilitate the maintenance of body contact between the mating partners, be used as weapons during male fights, or act as signals that provide information about male genetic quality and parasitic load through visual or sensory hydrodynamic signals (Wiley and Collette 1970; Müller and Ward 1995).

Here, we provide a description and a quantitative analysis of the spawning behaviour of minnows in a controlled semi-natural set up. We then test whether male body size and/or breeding tubercles are linked to dominance and to male reproductive success as confirmed by microsatellite typings of parents and offspring.

METHODS

Recording of Spawning Behaviour

Minnows were caught by electric fishing from a natural population in the catchment of the river Venoge (Vaud canton, Switzerland) in April 2007, i.e. some weeks before spawning season. Males were narcotised (with Aqui-S (Aqui-S New Zealand LTD); 0.04 ml / l) and individually marked with different black and white combinations on a nylon filament that was fixed to the dorsal fin by penetrating its basal part from one side with a small needle and attaching a perforated plastic globule (diameter 2mm) on the other side. The fish were then introduced into two aquaria (50 x 50 x 100 cm, 8 males with 2 females per aquarium) in a climate chamber.

Individuals were fed twice a day with dry fish food (Tetra Min, BioActive formula), and with live zooplankton on the weekends. Four perforated metallic boxes were filled with gravel of 2-3 cm diameter, a substrate known to be preferred by minnows for spawning (Bless 1992). These filled metallic boxes fitted into four plexiglas boxes that had been put into each aquarium before the fish were introduced. The gravel and the perforated metallic boxes allowed the spawned eggs to fall through the gravel down to a 2 cm gap between the plexiglas and the metallic box. This way, the fish were prevented from eating eggs. In each tank, an area without gravel was left for potential use as resting zone.

In order to describe male dominance hierarchy and their spawning behaviour, the aquaria were monitored with 8 surveillance cameras (CCD cam 1/3'' SONY Super HAD, lens angle 78°, minimum illumination 0.05 Lux, Profiline, 2 cameras per side and per aquarium), which were linked to a MultiCam GV-1000 System (Ecoline) (see Jacob et al. (2007) for further description). We recorded all behaviour between 10 May 2007 and 1 June 2007. A seasonal change was simulated by increasing the water temperature from 7°C to 14°C (1°C every 2 days) and by changing the light cycle from 8 to 13 hours of light per day. Observations during the first days indicated that fish activities depended on the light regime with low activities in darkness and increased activities when the light was switched on in the morning. The cameras were therefore programmed to film the aquaria from 0800 hours to 2100 hours. Boxes were controlled every morning for the presence of eggs. Eggs were collected and individually distributed to 24-well multiwell plates (BD Falcon; non-treated polystyrene, flat bottom). Each well had been filled before with 2 ml of temperated water that was chemically standardised according to the OECD guidelines (OECD 1992). The isolated embryos were then incubated at 10,7°C until hatching (no water exchange in between).

On 14 June 2007, all males were narcotised for biometry. Digital photos were taken of their foreheads to later count the breeding tubercles. The diameters of individual breeding tubercle were also measured with the open-access software IMAGEJ (http://rsb.info.nih.gov/ij/). For this measurements we first sampled the four largest tubercles of the anterior part of the forehead, which are situated more or less rectangularly to each other between the nostrils, two on the left and two on the right side of the mesial sagittal line (see Fig. 1 in Frost (1943)). We also measured four randomly picked tubercles of the forehead that were situated posterior to the eyes (the tubercles were numbered on both sides of the mesial sagittal line and then selected for measurements using a random number generator).

We described the dominance hierarchy based on the antagonistic behaviour during three different kinds of observation periods. The first covered 2 hours shortly before female spawning activity started (p_{before}), the second covered one hour from the moment female spawning activity had started (p_{during}), and the third (p_{end}) covered 30 minutes starting one hour after the end of the second period. An antagonistic act was defined as an interaction between two males that ended by one male swimming away being followed or chased by the other male. The total number of recorded antagonistic interactions were in aquarium 1: $n_1 = 445$ (60 in p_{before}; 254 in p_{during} ; 131 in p_{end}), and in aquarium 2: $n_2 = 978$ (428 in p_{before}; 382 in p_{during} ; 168 in p_{end}). We assigned a winner and a loser for each of these interactions and calculated dominance hierarchies for each aquarium using the David's score method. This method takes the relative strength of the opponents into account (De Vries et al. 2006; Gammell et al. 2003) and results in continuous scores (instead of ranks). We calculated an overall dominance hierarchy per aquarium. We also determined dominance hierarchies for each of the three observation periods per aquaria.

In order to identity male territories, we used the same video sequences as for the calculations of the dominance scores. The gravel area in each aquarium was divided into 16 sections of the same size. The position of each male was recorded every five minutes but only if

no female showed any spawning activities on the spawning area. Otherwise, we skipped to the next observation point five minutes later. The size of a male territory was estimate using a score s_{ij} for male i in section j, calculated as

$$s_{ij} = 1/n_j$$

where n_j is the number of males in section j at the time of observation, i.e. a male's score is weighted for the presence of other males in a given section. This procedure was followed for all observations separately to produce a sum of scores for each male in each section. We then computed a relative score for each male per section by dividing a male's score by the sum of all scores for the given section. We summed these relative scores for each male over all sections to obtain the overall territoriality per male. This way, we obtained an index that is weighted by the presence of other males in each section of the potential spawning area. After data collection the fish were used in another study on sperm motility (manuscript in preparation) and then killed with a lethal dose of Aqui-S for gonad measurements.

Genetic analyses

We used microsatellite markers to genotype all adults and a random sample of offspring (that had been killed with a lethal dose of Aqui-S at the hatchling stage). To estimate male fertilisation success per clutch c we genotyped the following hatchling numbers: $n_{c1} = 40$; $n_{c2} = 63$; $n_{c3} = 80$; $n_{c4} = 28$ for aquarium 1 and $n_{c5} = 32$; $n_{c6} = 33$; $n_{c7} = 30$; $n_{c8} = 6$ for aquarium 2; i.e. a total of 211 individuals were analysed for the aquarium 1 and 101 individuals for the aquarium 2.

Genomic DNA was extracted from tissue samples using the QIAgen DNeasy™ Kit (QIAgen), following the manufacturer protocol. We used five microsatellite loci (Ca 1, Ca 12, Ca3, Ca 5, Ppro 126) previously developed in other cyprinids (Bessert & Orti 2003; Dimsoski et al. 2000). PCR amplification was carried out separately for Ca 5 in 10 µl final volume containing: 100-250 ng DNA, 1.5mM MgCl2, 0.5-µM of each primers, 0.2-mM of dNTPs, and 0.5 U of QIAgen Taq polymerase. All other loci were multiplexed in 8 µl final volume containing: 2.5 µl of Qiagen® Multiplex PCR Kit, 0.28-µM of Ca 1primers, 0.12-µM of Ca12 primers and 0.06-µM of Ca 3 and Ppro 126 primers. The PCR profile for Ca 5 was: (i) 94°C for 3 min, (ii) 94°C for 30 sec, (iii) 51°C annealing for 30 sec, (iv) 72°C for 30 sec, (v) return to step (ii) for 30 cycles, (vi) 72°C for 10 min. The PCR profile for the multiplexed loci was: (i) 95°C for 15 min, (ii) 94°C for 30 sec, (iii) 58°C annealing for 90 sec, (iv) 72°C for 60 sec, (v) return to step (ii) for 35 cycles, (vi) 60°C for 30 min. The forward primers were labelled with a fluorescent dye (HEX, FAM or NED) on the 5'end. PCR products were run on a ABI 3100 Automated Sequencer (Applied Biosystems) and analysed with the GENEMAPPER software (Applied Biosystems).

The assignment of the hatchlings to their parents was done by simple exclusion since all potential parents were known. The number of hatchlings sired by a male seemed to directly reveal the number of eggs that the male fertilised, because total hatching rate was high (96.1% in aquarium 1 and 95.7% in aquarium 2).

Statistical Analyses

We used a randomization test on the Kendall's coefficient of concordance (W) to test if the dominance hierarchies of the three different periods are in agreement to each other. We therefore randomized the order of the dominance scores for each of the three different periods and calculated W to get a null-expectancy (i.e. a distribution of expected W based on 10,000 runs each) to which the observed W could be compared.

We fitted linear mixed effect models with overall dominance score per male as the response variable and one or two fixed effects as explanatory variable (i.e. male size, number of tubercles per male and male weight). To control for potential differences between the two aquaria we added a random aquarium effect to each model. The aquarium effect was tested by randomizing the aquarium origin between the males and recording the quality of fit (log-likelihood) of the model. The procedure was repeated 10,000 times to obtain an empirical distribution of log-likelihoods for the aquarium affiliation per male. We then tested a potential effect of aquaria on this empirical distribution. The aquarium effect never explained a significant part of the variance (all respective p-values > 0.2). For further analyses we thus excluded this effect and pooled the data of both aquaria. We then calculated Pearson correlations (r_p), or Spearman correlations (r_s) when graphical inspection of the data suggested a significant deviation from normality. We also fitted a linear multiple regression model with dominance as a function of male size and number of breeding tubercles (the same was done for male weight instead of male size).

During a study on sperm motility (manuscript in preparation) that directly followed the present one, we found that all males except one from aquarium 1 had well developed gonads. This one non-mature individual was excluded from all present analyses. The individual reproductive success of the other males was estimated for every clutch by multiplying the proportion of eggs the male had fertilised with the total number of eggs of the given clutch. These numbers were then summed up to compute the total individual reproductive success, which was log-transformed and included in linear mixed effect models as the response variable. A random aquarium effect was introduced in each model and fixed effects included the dominance score, the number of tubercles, male size and territory size. The significance of the fixed effects was tested with likelihood ratio tests in separate models (see Jacob et al. (2007) for further explanations), but the effects of the number of tubercles and male size were also tested together in a single model. The aquarium effect on male reproductive success was significant in two out of five models (based on randomisation tests as described above), so we kept this random effect in all five models. Analyses were done with the R software (R Development Core Team 2007). We used the lme4 package for the mixed effect models analyses (Bates 2007).

Ethical note

The electro fishing was performed by professional fishery managers who routinely use this technique to monitor wild fish populations. We could not observe any significant adverse effects on the fish or the other wildlife in the river. The filament marking method was chosen because it does not seem to significantly affect the fish (Jacob et al. 2007). The filament is fixed with a single puncture in the dorsal fin. We believe this marking procedure results in less disturbance to the fish as compared to other marking methods that are usually used for individual visual recognition (e.g. branding or tag fixation with a wire perforating the body cavity). We indeed observed no difference in behaviour between marked and unmarked fish and the degree of stress was classified as 0 (“minimal degree of severity”) by the veterinary office of the Vaud canton (the authority that gave the permission for our experiments). During dominance fights, bites were never observed and actual physical contacts were rare, i.e. in the majority of cases the antagonistic interactions were chases. During the spawning events males were close to each other so that physical contact usually occurred (see video 1 & 2 in electronic supplementary material). Throughout the study the fish were able to retract to a resting area, i.e. they were able to avoid antagonistic encounters. The study conforms to Swiss laws and was done with permissions of the *Centre de conservation de la faune et de la nature* and of the *Service Vétérinaire* of the Vaud

canton (permission number 1994.0). The fish were used in a follow-up study on sperm physiology and were euthanized at the end of these studies (this was a condition of the permits).

RESULTS

The males displayed antagonistic behaviours soon after release into the aquaria and long before the first spawning event (see video 1 in electronic supplementary material). Males seemed to defend territories by trying to keep their respective whereabouts and chasing away other males. Figure 1 illustrates the male whereabouts at the spawning ground in relation to their breeding tubercles, their body size, and the females' spawning behaviour.

The dominance hierarchies from the different observation periods (before, during, and at the end of spawning) appeared to be consistent over time when combining the two tests for both aquaria (Fig. 2; Fisher combination test: $\chi^2_4 = 18.2$, $p < 0.01$; the concordance in aquarium 1: $p = 0.11$, in aquarium 2: $p < 0.001$;).

The scores for male territoriality were positively linked to the overall dominance scores ($r_p = 0.65$; $n = 15$; $p < 0.01$). Male dominance was also positively linked to male size (Figure 3a; $r_p = 0.69$, $n = 15$, $p < 0.01$) and weight ($r_p = 0.74$, $p < 0.01$). During the observational period, all mature males developed breeding tubercles on their forehead. The number of breeding tubercles was not significantly correlated to male size ($r_p = 0.39$, $n = 15$, $p = 0.15$) or weight ($r_p = 0.43$, $p = 0.11$). Male dominance was, however, strongly and positively related to the number of breeding tubercles (Figure 3b; $n = 15$, $r_p = 0.71$, $p < 0.01$). Neither the mean diameter of the low forehead tubercles ($r_p = 0.25$, $n = 15$, $p = 0.37$) nor the mean diameter of the high forehead tubercles ($n = 14$, $r_p = 0.21$, $p = 0.48$) was significantly correlated with an individual's number of tubercles. Our measures of tubercle size were also not significantly correlated to male dominance (anterior forehead: $r_p = 0.39$, $n = 15$, $p = 0.16$; posterior forehead: $r_p = 0.42$, $n = 14$, $p = 0.13$, one male was excluded as posterior forehead tubercles were not present).

About 70 % of the variance in dominance could be explained in a linear model that includes body size and number of tubercles ($n = 15$; body size: $t = 2.87$, $p < 0.014$; tubercle number: $t = 3.02$, $p < 0.010$, respectively). The interaction term was not significant ($n = 15$, $t = 0.63$, $p = 0.54$) and was thus removed from the models. We found analogous results when male size was replaced by male weight (data not shown).

At the beginning of the experiment, females tend to be discrete and to approach males only rarely. Later in the spawning season, females frequently swam close to the gravel, sometimes touching it. They were then usually closely followed by most of the males. Females who spawned batches of eggs into the gravel were always closely accompanied by all or almost all the males of an aquarium (see video 2 in electronic supplementary material).

We found a total of 707 eggs in aquarium 1 and 805 in aquarium 2. Twelve of the 15 males could be confirmed by later genetic analyses to have sired offspring (7 out of 7 males in Aquarium 1 and 5 out of 8 males in Aquarium 2). The number of sires per batch of eggs ranged from 1 to 6 (mean ± s.d.: 4.5 ± 2.4) in aquarium 1 and from 3 to 4 (mean ± s.d.: 3.25 ± 0.5) in aquarium 2. Male reproductive success ranged from 11 to 199 embryos (mean ± s.d.: 101.1 ± 62.6) in aquarium 1 and from 0 to 488 (mean ± s.d.: 100.6 ± 176.3) in aquarium 2. Male reproductive success differed between aquaria (10'000 permutations of model 2 in table 1, $p = 0.04$), a random aquarium effect was thus included in the respective models (Table 1). Male dominance was positively linked to reproductive success (Figure 3, Table 1). Tubercle number, body size, and overall territoriality were also positively related to male reproductive success (Table 1). When male tubercle number and body size were entered together in a single model (AIC = 63.076) the effect of male tubercle number was significant (LRT: $\chi^2_1 = 4.82$, $p < 0.03$) but

not the one of male body size (LRT: $\chi^2_1 = 1.13$, $p = 0.29$). The model with the best AIC was the one including dominance (Table 1).

DISCUSSION

The minnows in our study showed a lek-like breeding system with males defending territories at the spawning ground. The males quickly established dominance hierarchies, i.e. long before the beginning of female spawning activities. These dominance hierarchies seem to play a significant role in the breeding system of minnows: dominance is positively linked to territory size and to reproductive success. However, our behavioural observations and our genetic analyses of the offspring revealed that multi-male fertilizations was common. This suggests that the spawning territories could usually not be defended to allow for pair-wise spawning, confirming previous observations on European minnows in the wild (Breder & Rosen 1966).

As expected from findings in other fish, male body size turned out to be a reliable indicator of dominance rank. But what about the breeding tubercles? Male size and tubercle number were not significantly correlated in our sample (in contrast to other samples, see Müller and Ward (1995)). However, dominance status and the induction of breeding tubercles are based on similar physiological pathways, i.e. both dominance (Cardwell et al. 1996; Fitzpatrick et al. 2008) and breeding tubercles (Kortet et al. 2003) can be positively linked to androgen concentration (mainly to 11-ketotestosterone but also to testosterone). Indeed, both male size and tubercle number turned out to be reliable indicators of dominance rank. This latter result contrasts with findings on roach (*Rutilus rutilus*) where tubercle size but not number was linked to dominance (Kortet et al. 2004), and with findings on fathead minnow where tubercle numbers was also not linked to dominance (Hudman and Gotelli 2007). In our study, male size and tubercle number seemed to capture different aspects of dominance, as both are significant when included simultaneously in a single model. We found no significant correlation between tubercle size and tubercle number or male dominance.

Our results confirm the assumption made by other authors that the European minnow is a group spawning species (Bless 1992; Stockley et al. 1997). Such a mating system is expected to make female choice difficult, but females may still be able to increase the relative fertilisation success of some males. During the spawning period the females performed up- and downward movements often at the same spot and in high frequency. This was often but not always followed by the spawning of a batch of eggs. i.e. the possibility exists that females may only release eggs, or more eggs than usual, when an attractive male is close and thus more likely to fertilise a larger share of the eggs. The number of breeding tubercles could act as a stimulus that would trigger the egg release by the female. In line with this hypothesis, breeding tubercles are significantly linked to reproductive success and when entered in a model together with male size, they still significantly predict reproductive success, while male size does not. It is still unclear whether there is any female choice in minnows, but if so, females may gain twofold by choosing dominant males with elaborate secondary sexual ornamentation. On one hand they may get high quality males with possibly better surviving offspring (Wedekind et al. 2008; Wedekind et al. 2001). On the other hand, if tubercle number and preference for it is heritable, their sons may have a higher fertilisation success (Fisher 1930). Alternatively, female choice could be based on the quality of male territories. In some fish it was shown that better territories are occupied by more dominant males (Foote 1990; Dijkstra et al. 2008). Accordingly, we found in our sample that more dominant (and more ornamented) males have larger territories. Last but not least, females may also profit from spawning their eggs in well-defended territories if egg feeding rates in territories of dominant males are lower due to limited access of other males.

We could not observe any behaviour that would indicate that breeding tubercles serve as weapons as proposed by Müller and Ward (1995). In the pre-spawning period, most male-male interactions ended after some male display and without any direct physical contact. Direct contact between males usually occurred only shortly before and during spawning when several males tried to get close to a female. We therefore believe that breeding tubercles are signals that indicate a male's determination to fight for its territory and/or signals that are used in female choice.

ACKNOWLEDGEMENTS

We thank A. Cavin and C. Moresi for access to the fish, U. Gutmann, F. Chalard, C. Bornand and J. Notari for assistance, and J. Greeff, B. von Siebenthal, S. Nusslé, N. Perrin, and two anonymous reviewers for valuable comments on the manuscript. The project was supported by the Swiss National Science Foundation.

REFERENCES

- SORT

- CHECK FOR MISSING REFS (e.g. Breden and Rosen 1966)

- CHECK FOR SPELLING, ITALICS, ETC (EXAMPLES GIVEN)

Smith, J. M. 1974. Theory of games and evolution of animal conflicts. *Journal of Theoretical Biology*, 47, 209-221.

Collias, N. E. 1943. Statistical analysis of factors which make for success in initial encounters between hens. *American Naturalist*, 77, 519-538.

Foote, C. J. 1990. An experimental comparison of male and female spawning territoriality in a Pacific salmon. pp. 283-314.

Andersson, S., Pryke, S. R., Ornborg, J., Lawes, M. J. & Andersson, M. 2002. Multiple receivers, multiple ornaments, and a trade-off between agonistic and epigamic signaling in a widowbird. *American Naturalist*, 160, 683-691.

Candolin, L. & Voigt, H. R. 2001. Correlation between male size and territory quality: consequence of male competition or predation susceptibility? *Oikos*, 95, 225-230.

Fleming, I. A. & Gross, M. R. 1994. Breeding competition in a Pacific salmon (Coho: *Oncorhynchus kisutch*) - measures of natural and sexual selection. *Evolution*, 48, 637-657.

Quinn, T. P. & Foote, C. J. 1994. The effects of body-size and sexual dimorphism on the reproductive behavior of Sockeye-salmon, *Oncorhynchus nerka*. *Animal Behaviour*, 48, 751-761.

Creighton, E. 2001. Mate acquisition in the European blackbird and its implications for sexual strategies. *Ethology Ecology & Evolution*, 13, 247-260.

Wong, B. B. M. & Candolin, U. 2005. How is female mate choice affected by male competition? *Biological Reviews of the Cambridge Philosophical Society*, 80, 559-571.

Andersson, M. B. 1994. *Sexual Selection*. New Jersey: Princeton University Press.

Dewsbury, D. A. 1982. Dominance rank, copulatory-behavior, and differential reproduction. *Quarterly Review of Biology*, 57, 135-159.

Wiley, R. H. 1973. Territotiality and non-random mating in the sage grouse *Centrocercus urophasiasus*. *Animal Behaviour Monograph*, 6, 87-169.

Esteve, M. 2005. Observations of spawning behaviour in Salmoninae: *Salmo*, *Oncorhynchus* and *Salvelinus*. *Reviews in Fish Biology and Fisheries*, 15, 1-21.

Qvarnstrom, A. & Forsgren, E. 1998. Should females prefer dominant males? *Trends in Ecology & Evolution*, 13, 498-501.

Kortet, R., Taskinen, J., Vainikka, A. & Ylonen, H. 2004. Breeding tubercles, papillomatosis and dominance behaviour of male roach (Rutilus rutilus) during the spawning period. *Ethology*, 110, 591-601.

Kortet, R. & Taskinen, J. 2004. Parasitism, condition and number of front head breeding tubercles in roach (*Rutilus rutilus* L.). *Ecology of Freshwater Fish*, 13, 119-124.

Berglund, A., Bisazza, A. & Pilastro, A. 1996. Armaments and ornaments: An evolutionary explanation of traits of dual utility. *Biological Journal of the Linnean Society*, 58, 385-399.

Cluttonbrock, T. H., Albon, S. D. & Harvey, P. H. 1980. Antlers, Body Size and Breeding Group-Size in the Cervidae. *Nature*, 285, 565-567.

Hudman, S. P. & Gotelli, N. J. 2007. Intra- and intersexual selection on male body size are complimentary in the fathead minnow (*Pimephales promelas*). *Behaviour*, 144, 1065-1086.

Zucker, N. & Murray, L. 1996. Determinants of dominance in the tree lizard Urosaurus ornatus: The relative importance of mass, previous experience and coloration. *Ethology*, 102, 812-825.

Kitchen, D. M., Seyfarth, R. M., Fischer, J. & Cheney, D. L. 2003. Loud calls as indicators of dominance in male baboons (*Papio cynocephalus ursinus*). *Behavioral Ecology and Sociobiology*, 53, 374-384.
Setchell, J. M., Wickings, E. J. & Knapp, L. A. 2006. Life history in male mandrills (*Mandrillus sphinx*): Physical development, dominance rank, and group association. *American Journal of Physical Anthropology*, 131, 498-510.
Stuart-Fox, D. M., Firth, D., Moussalli, A. & Whiting, M. J. 2006. Multiple signals in chameleon contests: designing and analysing animal contests as a tournament. *Animal Behaviour*, 71, 1263-1271.
Ahtiainen, J. J., Alatalo, R. V., Kortet, R. & Rantala, M. J. 2006. Immune function, dominance and mating success in drumming male wolf spiders Hygrolycosa rubrofasciata. *Behavioral Ecology and Sociobiology*, 60, 826-832.
Rantala, M. J. & Kortet, R. 2004. Male dominance and immunocompetence in a field cricket. *Behavioral Ecology*, 15, 187-191.
Wedekind, C. 1992. Detailed information about parasites revealed by sexual ornamentation. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 247, 169-174.
Milinski, M. & Bakker, T. C. M. 1990. Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature*, 344, 330-333.
Taskinen, J. & Kortet, R. 2002. Dead and alive parasites: sexual ornaments signal resistance in the male fish, *Rutilus rutilus*. *Evolutionary Ecology Research*, 4, 919-929.
Ezenwa, V. O. & Jolles, A. E. 2008. Horns honestly advertise parasite infection in male and female African buffalo. *Animal Behaviour*, 75, 2013-2021.
Bless, R. 1992. Einsichten in die Ökologie der Elritze *Phoxinus phoxinus* (L.); praktische Grundlagen zum Schutz einer gefährdeten Art. *Schriftenreihe für Landschaftspflege und Naturschutz*, 35, 1-57.
Wiley, M. L. & Collette, B. B. 1970. Breeding tubercles and contact organs in fishes : their occurrence, structure, and significance. *Bulletin of the American Museum of Natural History*, 143, 145-216.
Wedekind, C., Evanno, G., Urbach, D., Jacob, A. & Müller, R. 2008. 'Good-genes' and 'compatible-genes' effects in an Alpine whitefish and the information content of breeding tubercles over the course of the spawning season. *Genetica*, 132, 199-208.
Müller, G. & Ward, P. I. 1995. Parasitism and heterozygosity influence the secondary sexual characters of the european minnow, *Phoxinus phoxinus* (L) (Cyprinidae). *Ethology*, 100, 309-319.
Jacob, A., Nusslé, S., Britschgi, A., Evanno, G., Müller, R. & Wedekind, C. 2007. Male dominance linked to size and age, but not to 'good genes' in brown trout (Salmo trutta). *BMC Evolutionary Biology*, 7, 207.
OECD. 1992. Guideline 203: fish, accute toxicity test.: Organisation for Economic Co-operation and Development. .
Frost, W. E. 1943. The natural history of the minnow, *Phoxinus phoxinus*. *Journal of Animal Ecology*, 12.
De Vries, H., Stevens, J. M. G. & Vervaecke, H. 2006. Measuring and testing the steepness of dominance hierarchies. *Animal Behaviour*, 71, 585-592.
Gammell, M. P., De Vries, H., Jennings, D. J., Carlin, C. M. & Hayden, T. J. 2003. David's score: a more appropriate dominance ranking method than Clutton-Brock et al.'s index. *Animal Behaviour*, 66, 601-605.
R Development Core Team. 2007. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Bates, D. 2007. lme4: Linear mixed-effects models using S4 classes., R package version 0.99875-99879.

Cardwell, J. R., Sorensen, P. W., VanDerKraak, G. J. & Liley, N. R. 1996. Effect of dominance status on sex hormone levels in laboratory and wild-spawning male trout. *General and Comparative Endocrinology*, 101, 333-341.

Fitzpatrick, J. L., Desjardins, J. K., Milligan, N., Stiver, K. A., Montgomerie, R. & Balshine, S. 2008. Female-mediated causes and consequences of status change in a social fish. *Proceedings of the Royal Society B-Biological Sciences*, 275, 929-936.

Kortet, R., Vainikka, A., Rantala, M. J., Jokinen, I. & Taskinen, J. 2003. Sexual ornamentation, androgens and papillomatosis in male roach (*Rutilus rutilus*). *Evolutionary Ecology Research*, 5, 411-419.

Stockley, P., Gage, M. J. G., Parker, G. A. & Møller, A. P. 1997. Sperm competition in fishes: The evolution of testis size and ejaculate characteristics. *American Naturalist*, 149, 933-954.

Petrie, M. 1994. Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature*, 371, 598-599.

Petrie, M. & Halliday, T. 1994. Experimental and natural changes in the peacocks (*Pavo cristatus*) train can affect mating success. *Behavioral Ecology and Sociobiology*, 35, 213-217.

Uller, T., Eklöf, J. & Andersson, S. 2005. Female egg investment in relation to male sexual traits and the potential for transgenerational effects in sexual selection. *Behavioral Ecology and Sociobiology*, 57, 584-590.

Wedekind, C., Müller, R. & Spicher, H. 2001. Potential genetic benefits of mate selection in whitefish. *Journal of Evolutionary Biology*, 14, 980-986.

Fisher, R. A. 1930. *The Genetical Theory of Natrual Selection*. Oxford: Clarendon Press.

Dijkstra, P. D., Van der Zee, E. M. & Groothuis, T. G. G. 2008. Territory quality affects female preference in a Lake Victoria cichlid fish. *Behavioral Ecology and Sociobiology*, 62, 747-755.

Table 1

Results from linear mixed effect models testing the effects of male dominance, male size, number of breeding tubercles, and overall territoriality on male reproductive success. Effects are tested by comparing each model against the reference model 1. Such likelihood ratio tests are based on a χ^2 distribution with one degree of freedom. The AICs describe the quality of fit of each model. The table also gives the Pearson's correlation coefficients (r_p) between male traits and male reproductive success.

						r_p	
Model	Fixed effect tested	Random effect	AIC	χ^2	p	Aquarium 1	Aquarium 2
1		Aquarium	69.11				
2	Dominance	Aquarium	60.12	10.98	0.0009	0.85	0.80
3	Size	Aquarium	65.90	5.21	0.023	0.65	0.57
4	Tubercle number	Aquarium	62.21	8.90	0.003	0.69	0.72
5	Territoriality	Aquarium	67.29	3.81	0.05	0.43	0.55

Figure 1

Male whereabouts at the spawning ground in relation to their breeding tubercles, their body size, and the females' spawning behaviour. Every figure panel shows an aquarium with its 16 sections. The bar plots in each section represent the territoriality of each male in this section. In panels (a) (aquarium 1) and (b) (aquarium 2), the males are sorted based on their body size with the largest male on the left and the smallest one on the right. In panels (c) (aquarium 1) and (d) (aquarium 2), the males are sorted by decreasing tubercle number from left to right. The same bar colours are used for the same males in panels (a) and (c), or (b) and (d), respectively. Sections in which a female laid eggs are marked with a grey background.

Figure 2

Male dominance scores based on antagonistic encounters over three different kinds of observation periods for aquarium 1 (a) and aquarium 2 (b). Dashed lines connect male dominance scores measured before female spawning activity, dotted lines during spawning activity and straight lines towards the end of spawning activity. The filled symbols show the overall dominance scores that include all antagonistic interactions. Males are ordered by decreasing overall dominance from left to right.

Figure 3

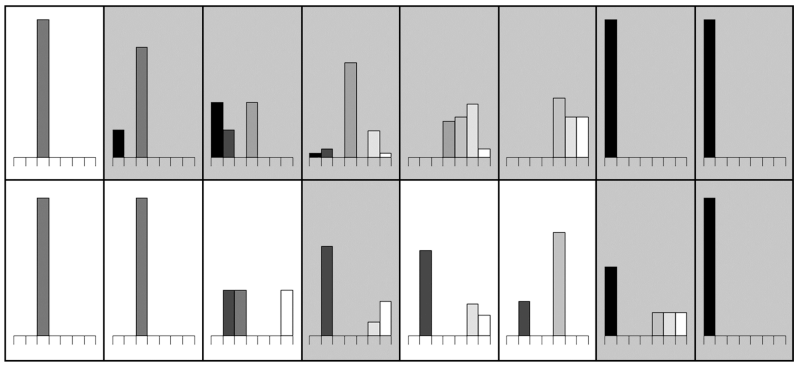
Relationship between male dominance status and (a) male body size or (b) number of breeding tubercles. Individuals from aquarium 1 and 2 are represented by open and solid symbols, respectively. Regression lines are given to illustrate the trends. See text for statistics.

Figure 4

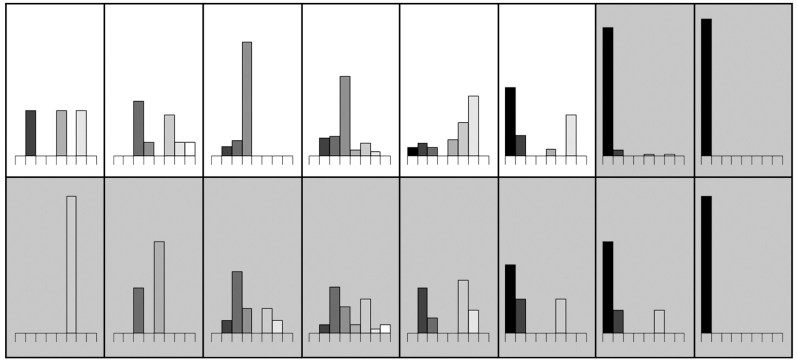
Relationship between male dominance and reproductive success (see text for statistics; line fitted according to model 2 in table 1). Males from aquarium 1 and 2 are represented by open and solid circles, respectively.

Figure 1

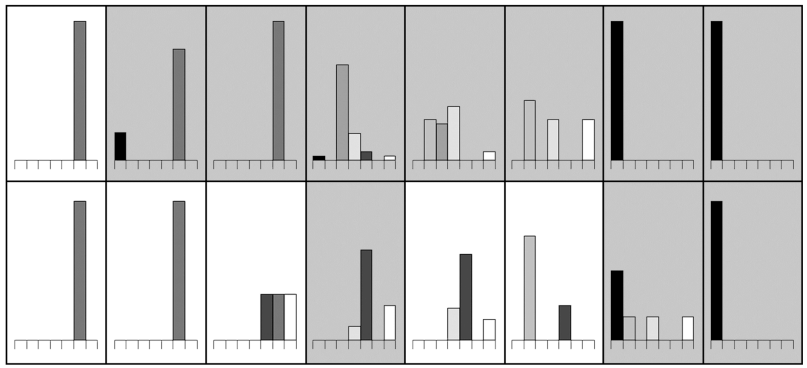
a)



b)



c)



d)

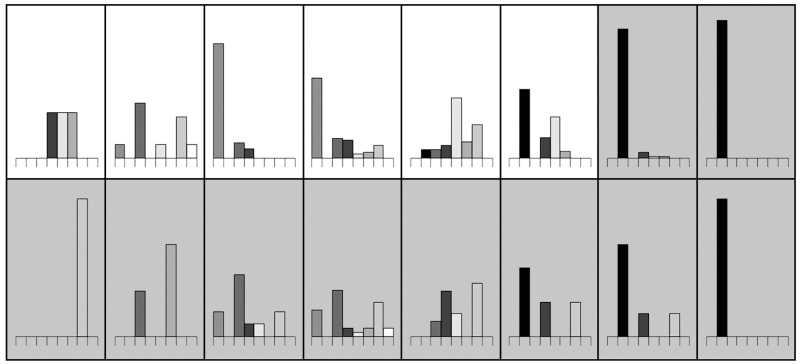


Figure 2

a) b)

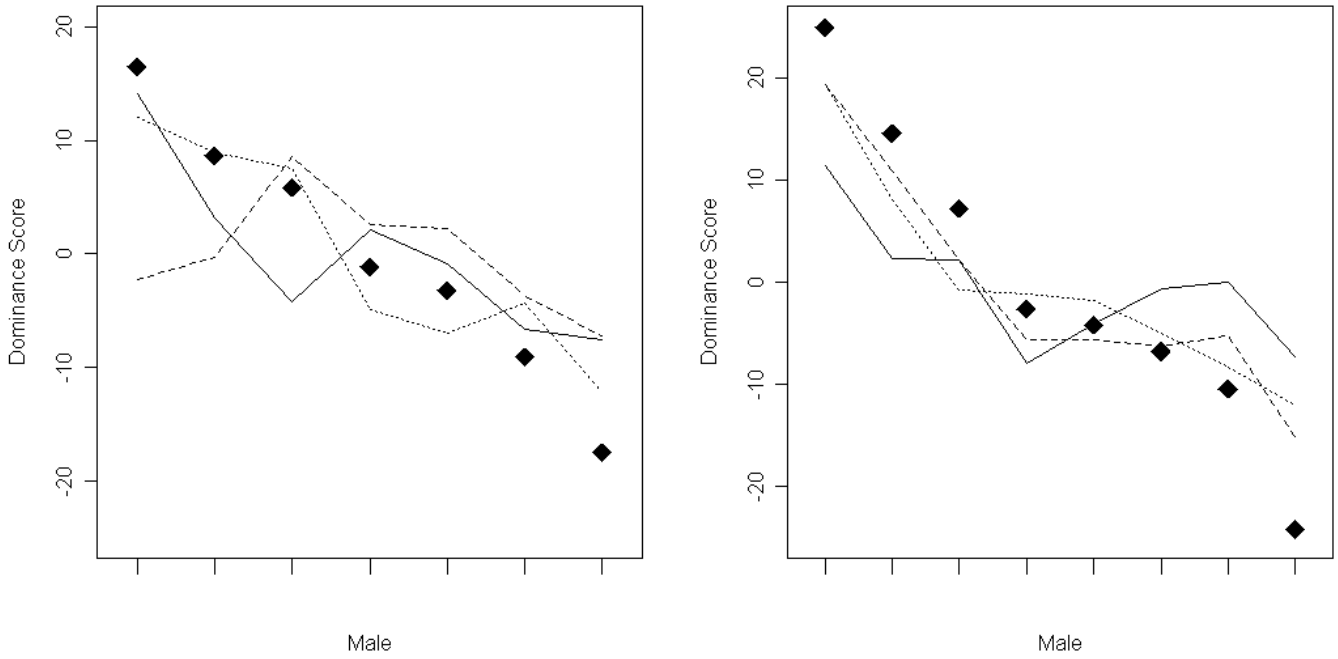

Dominance Score
20
10
0
-10
-20
Male
Dominance Score
20
10
0
-10
-20
Male

Figure 3

(a) (b)

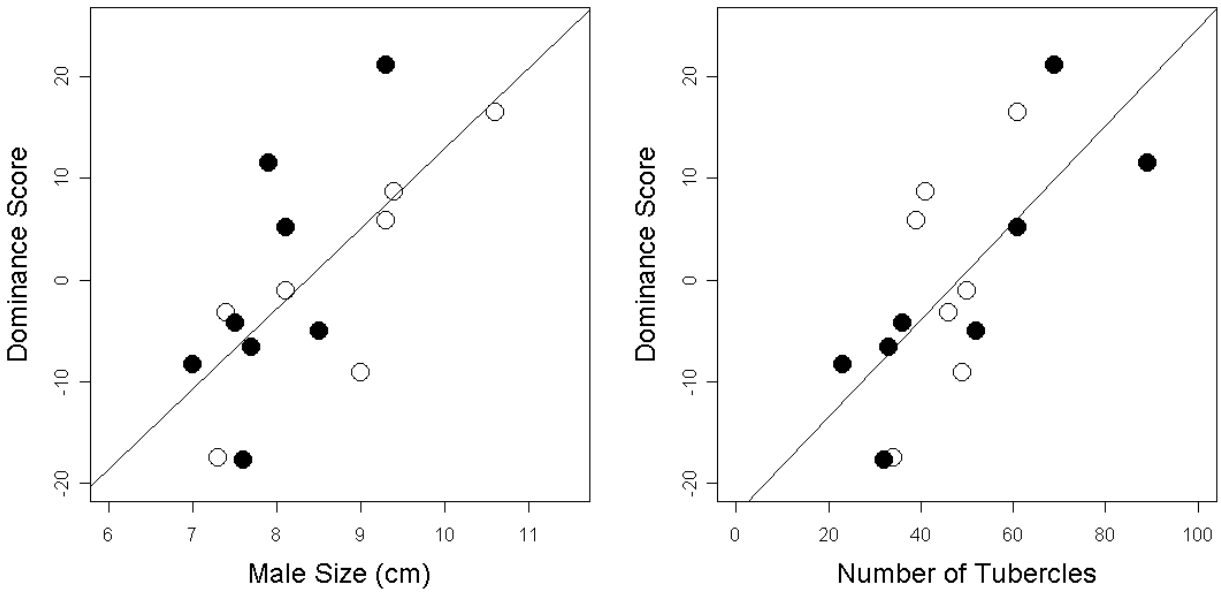
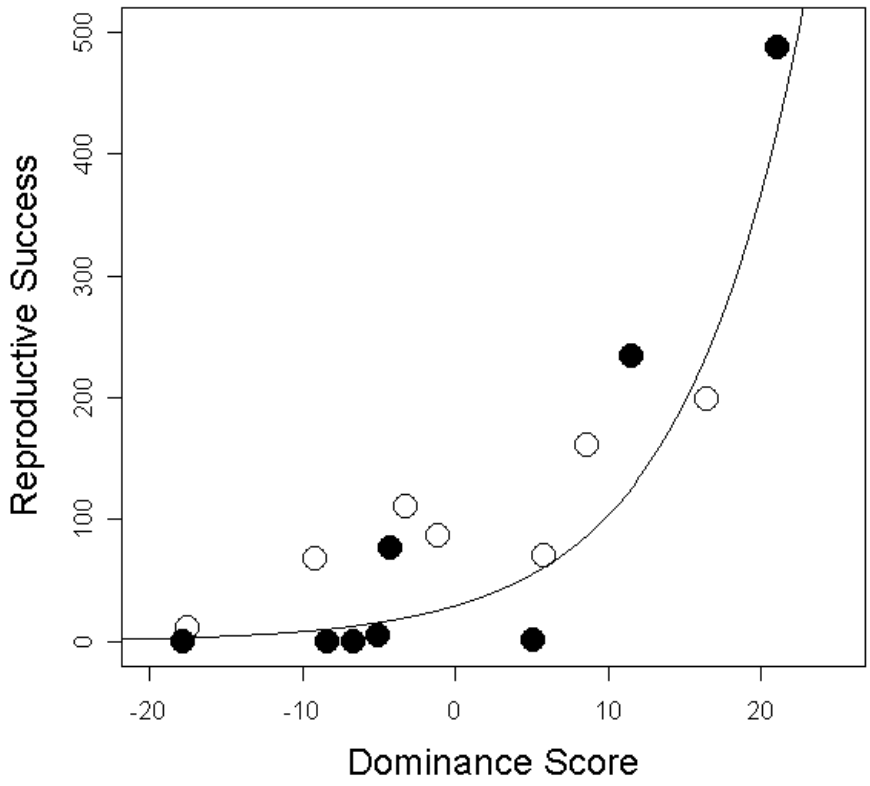

Dominance Score
Male Size (cm)
Dominance Score
Number of Tubercles

Figure 4


Reproductive Success
0
100
200
300
400
500
-20
-10
0
10
20
Dominance Score

Electronic Supplementary Material

Video 1

Antagonistic behaviour and territory defence among males. Videos were taken in the period before female spawning activity started.

Video 2

Spawning behaviour. During spawning activity females were always closely accompanied by all or almost all the males. The female in the video is rather large more brightly coloured than most of the males and is usually swimming in front of the shoal.