Behavioural lateralisation in response to sexual signal asymmetry in the swordtail characin *(Corynopoma riisei)*

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Abstract

Different kinds of male signals play an important role in sexual selection. In mate choice these signals may provide the female with important information about male individual quality. Lateralisation is the allocation of the neurological control of different behaviours and body parts to different parts of the brain that leads to handedness in different behaviours. Such handedness is common in species with bilateral traits. To date, it is not known how lateralization in male sexual signal traits may affect signal behaviours. This is particularly interesting in species with non-perfect symmetry in such traits since signal behaviour could be influenced by fluctuating asymmetry (FA) and asymmetric males could trick females to perceive them as more attractive by showing more of their best side. To investigate this I performed an experiment on the swordtail characin, a species of fish where males carry paddle-like structures on each side that are used during courtship. I found that male swordtail characins bias their courtship displays towards the side with the longest paddle thus potentially deceiving the female to think that he is more attractive than he actually is. Further, following manipulation of the paddles where the relative lengths of the paddles were altered the males adjusted their behaviour accordingly suggesting that the males are aware of how they look. This has implications for our understanding of the origin and honesty of signals used during courtship.

Keywords: sexual signalling; behavioural lateralisation; fluctuating asymmetry; swordtail characin (*Corynopoma riisei*)

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Introduction

Sexual selection is the difference in fitness that results from competition within and mate choice between sexes (Andersson, 1994). The mechanisms behind sexual selection can be divided into two major groups: intra-sexual competition and inter-sexual mate choice. Intra-sexual competition can be rather straight forward such as males fighting each other in order to get mating advantages (Andersson, 1994). For example, red deer stags defend a good grazing area to get mating access to all females visiting that area (Carranza, 1994). Intra-sexual competition can also be less obvious at a post-copulatory stage for example through different forms of sperm competition (Parker, 1970). In inter-sexual mate choice one sex, usually the female, based on different characteristics or traits in the other sex, chooses which individual he or she wants to mate with. The reason why the female is commonly the choosy sex is still a debated issue (e.g. Kokko & Jennions, 2003). The most common explanation was provided by Trivers (1972) and is based on differences in reproductive investment between the sexes. The sexes are anisogametic: the female invests more in each offspring producing big, nutrient-rich gametes and hence should be more careful in her choice of mate than the male who produces smaller gametes containing little but his genetic material (Trivers, 1972; Andersson, 1994; reviewed by Kokko & Jennions, in press). Note however that recent theory and empirical data now suggest there may be other reasons for the classic 'female cares – males compete for females' dogma (Queller, 1997; Kokko & Jennions, in press; Gonzalez-Voyer *et al.*, in review). Through mate choice the interests of the male and the female may diverge starting at this difference in gametic investment going through to relative investment in parental care, risk taking and so on (Andersson, 1994; Arnqvist & Rowe, 2005). In this so called sexual conflict both sexes want to maximize their individual fitness even if this means costs to the other sex (Arnqvist & Rowe, 2005). Sexual selection is usually a strong evolutionary force and may exaggerate the expression of a character to the point where natural selection (i.e. reduced survival) is strong enough to stop it (Lande, 1981; Andersson, 1994). The intensity of sexual selection as well as sexual conflict varies depending on the relative parental investment and the ratio of males and females available for breeding at a given time (Andersson, 1994).

Females may base their mate choice on different qualities in males and thus receive either direct or indirect benefits (Andersson, 1994). Direct benefits, for example food or parental care, are such that the female may gain from them directly and thus will be able to provide better care for her eggs and offspring. A common situation is that the male contributes with nothing but genes to offspring fitness in which case only indirect benefits may be gained by the female in the form of genetic advantages for the offspring. Examples of indirect benefits can be increased survival of or higher mating success in the offspring. The female can choose a male based on one or several cues indicating direct and/or indirect benefits (see Candolin, 2003, for a review).

A problem for the female is that in order to make a good mate choice she must be able to assess the male correctly. For this, various kinds of signals indicating direct or indirect male quality have evolved (Andersson, 1994). A signal that is directly associated with male quality can be used by the female to accurately evaluate the benefits she can receive from each male. This kind of signal is known as a "handicap" signal because it is costly for the male to produce and maintain (Zahavi, 1975). The expression of conspicuous secondary sexual characters lowers the male's overall fitness, for example in energy expenditure and exposure to predation and the female may draw the conclusion that since the male is able to survive in spite of this handicap he must be good in other respects as well (Krebs & Davies, 1993). One example is heavy antlers in deer that are costly to produce and carry (Vanpé *et al.*, 2007). This kind of signal has been regarded as an honest indicator of fitness (Andersson, 1994). But it has also been argued that there are costs for the female to receive the signal, for example in time spent evaluating the signal when she could have been foraging or in the increased risk of predation associated with the male's conspicuous behaviour or appearance (Dawkins & Guilford, 1991). It would therefore be in the interest of both sexes to lower the costs of both signalling and receiving as much as possible even though this will potentially reduce the honesty of the signal (Dawkins & Guilford, 1991).

Another kind of signal is one that only expresses male attractiveness, for example elaborate plumage in birds. Females will choose to mate with the most "handsome" males

because this will give them indirect benefits in the form of "sexy sons" (Andersson, 1994). There is a genetic relationship between female preference and the male sexual character so that the daughters will inherit the preference and the sons will inherit their father's attractiveness and mating success (Pomiankowski *et al.*, 1991). This is known as Fisher's runaway process or the "sexy son" principle (Andersson, 1994). An extension of this principle is the sensory exploitation hypothesis where the male exploits a female preference for something not initially related to sexual selection (Ryan & Keddyhector, 1991). For example the sword in the swordtail fish has been suggested to have evolved as a result of a pre-existing female preference (Basolo, 1990). The females of the closely related but swordless platyfish were found to prefer males with an artificial sword to natural, swordless, males suggesting that the preference evolved prior to the male trait (Basolo, 1990). The female will prefer males that provide the highest amount of sensory stimulation but this does not give the female any direct benefits (Ryan & Keddyhector, 1991). Unlike Fisher's runaway process and the handicap theory the preference and the trait did not evolve together but the preference preceded the trait (Krebs & Davies, 1997). An important point to make is that the different kinds of signals are not mutually exclusive. For example a signal that originated from sensory exploitation may in time become an indicator of male quality because it is costly to produce or makes the male more vulnerable to predation (Jennions *et al.*, 2001; Kokko *et al.*, 2003).

Laterality and fluctuating asymmetry
Lateralisation refers to the organisation of the brain into two different parts that are in control of different parts of the body and different behaviours (Bisazza *et al.*, 1998). For a long time lateralisation was thought to exist only in humans (Bisazza *et al.*, 1998). However, it is now clear that lateralisation is widespread in a variety of animals and across different taxa such as mammals, birds, reptiles, amphibians, fishes and insects. Examples of behaviours showing lateralisation are hand use in chimpanzees (Hopkins *et al.*, 2003), obstacle avoidance in sheep (Versace *et al.*, 2007), feeding responses in chicks (Andrew *et al.*, 2000), predator escape in toads (Lippolis *et al.*, 2002), predator inspection in the mosquitofish (Bisazza *et al.*, 1999) and prey inspection and capture in the spitting spider (Ades & Ramires, 2002).

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In these animals the lateralised behaviours usually manifest themselves at the population and/or species level. For example in some species of toads the majority of the individuals tend to react more strongly to a predator approaching from the left than to one approaching from the right (Lippolis *et al.*, 2002). However, the fact that all individuals within a population respond in the same way to a specific stimuli, for example a predator, would not seem optimal since it makes their behaviour predictable and offers the predator a chance to learn and exploit this behaviour (Vallortigara, 2006). As there seems to be an obvious cost for the individual to react in a similar manner to the rest of the population there must be advantages linked to lateralisation that make up for this disadvantage. It has been suggested that a potential benefit of lateralisation for the individual is that the separating of different behaviours spatially in different parts of the brain allows further specialisation of the different responses and enhances the overall performance of the brain (Vallortigara, 2006). But this still does not explain why all individuals in a population are lateralised in the same direction, it would still be better if half of the population behaved in one way and the other half in the other way to avoid predictability. Lateralisation on the individual level manifests itself as all individuals of the population being lateralised but not necessarily in the same direction. This is what one would expect to find in solitary animals and it has indeed been found in for example eye use in a solitary living octopus (Byrne *et al.*, 2004). As for social animals there are advantages for the individuals to coordinate their behaviour to the rest of the group in order to for example minimise the risk of predation (Vallortigara, 2006). Such behaviours can for example be seen in shoaling fish. Vallortigara (2006) proposed that population lateralisation arose as an evolutionary stable strategy where the direction of laterality is frequency dependent: the majority of the individuals in a population are lateralised in one direction suffering from less risk of predation in the safety of numbers but pay a cost because predators can predict their reaction and exploit this. The few individuals that are lateralised in the other direction trade the security of the group with being better able to handle a predator should they meet one (Vallortigara, 2006). However, the origin of lateralisation at the population level has elicited a hot debate and still remains unclear (Vallortigara & Rogers, 2005).

Lateralisation can also be the result of morphologic variations on the individual level in the form of deviations from symmetry in bilateral traits (Bisazza *et al.*, 1998). At the population level there are three different kinds of distributions that this asymmetry can adopt: fluctuating asymmetry (FA), directional asymmetry (DA) and anti-symmetry (AS) (Palmer & Strobeck, 1986). The three types of symmetries differ in distribution and mean of right minus left side (Palmer & Strobeck, 1986): FA is normally distributed with a mean around zero. In this case the majority of the individuals are approximately symmetrical with a few individuals lateralised in one or the other direction, for example wing length in birds (Møller, 1994). DA is normally distributed around a mean higher or lower than zero indicating that the majority of population has a tendency to one side over the other, this has for example been observed in the location of wing spots in butterflies (Brunton *et al.*, 1999). AS shows a bimodal distribution around zero where about half of the population are biased towards the right side and the other half are biased towards the left side, for example claw size in fiddler crabs (Graham *et al.*, 1998).

Fluctuating asymmetry has been suggested to be the result of developmental instability (DI) which is an individual's inability to maintain developmental homeostasis in the face of environmental and genetic stress (Møller & Pomiankowski, 1993). As both sides of an organism share the same genes and experience the same environmental conditions DI has been hypothesized to manifest itself in small deviations in otherwise bilaterally symmetrical traits: i.e. FA (Van Valen, 1962). An important topic for discussion has been whether FA is in fact correlated with DI (and thereby fitness) or not (Polak, 2003). FA has indeed been found to be positively correlated with a number of different stressors such as food deficiency, various parasites and diseases, inbreeding etc. indicating that FA could be used as a measure of the individual's overall fitness (Møller & Pomiankowski, 1993, and references therein). But the relationship between FA and fitness is still being debated and the results from different studies are far from consistent (i.e. Knierim *et al.*, 2007; Van Dongen, 2006). The underlying reasons for the diverging results have among other things been attributed to measurement error, small sample sizes or use of the wrong statistical tools etc. (see Lens *et al.*, 2002, for a review). There is also a lack of complete

understanding of the underlying reasons for DI, for example what environmental variables affect DI or exactly how it works at the cellular level (Polak, 2003).

FA and sexual selection

It is in the interest of the female to, in her choice of a mate, use a cue which is an accurate indicator of male quality. A good signal would be one that reveals the individual's ability to cope with environmental and genetic stress (Møller & Pomiankowski, 1993). Because individuals can not control their FA it has been suggested that this could be used in sexual selection as an honest signal of male genetic quality: high quality males have higher developmental stability and can cope with different kinds of stress better and therefore show low FA (Møller & Pomiankowski, 1993). Because FA is larger in sexually selected traits (e.g. 5-10 times larger in feather ornaments in birds) than in other characters (1-2%) (Møller & Höglund, 1991) it has been suggested that FA could be a detectable indicator of male quality (Møller & Pomiankowski, 1993). For example, the ability in birds to detect FA has been estimated to lie between 1.25-1.8% asymmetry (Swaddle, 1999). Another important issue is that if FA is to be targeted by females as an honest signal in mate choice it must have a significant heritable variation to be beneficial to the females (Polak, 2003). But the heritability of FA has repeatedly been shown to be low (around 0.03) whereas heritability in DI has been estimated to be considerably larger and could therefore provide the female with offspring with lower DI and hence genetic quality (Polak, 2003). This brings us back to the discussion of whether there is in fact a correlation between DI and FA or not.

Regardless of whether FA is an honest signal of male quality or not, the male could potentially deceive the female by biasing his behaviour towards his better side and thus complicate her assessment of him. A possible relationship between FA and behavioural lateralisation has been discussed (Bisazza *et al.*, 1998), but has not been observed until very recently. Gross *et al.* (2007) were the first to show that male guppies bias their behaviour towards their more colourful side during courtship. Female guppies prefer more colourful males (Gross *et al.*, 2007) and may hence be deceived to think that a male showing more of his best side is more colourful than he actually is. How the male knows

which side is his better side may have several possible explanations. One possibility is that the male knows from prior experience with a female which is his most attractive side. If showing one side more than the other was successful with one female it might work with this one as well. However, no previous experience would be required if the male can instantly adapt to signals given, deliberately or not, by the female suggesting what kind of display or side she prefers. In the case of the guppies mentioned above Gross *et al.* (2007) found that the male display bias was mediated by cues given by the female. Another example is male satin bowerbirds that were found to adjust their display intensity according to cues given by the female (Patricelli *et al.*, 2006). Supposing that the male knows what the female prefers yet another possibility is that he could simply look at himself and compare both sides to see which side is his best. Another possible explanation could be that FA is the result of a genetically related handedness which is reinforced by handedness in the use of the trait. This can for example be seen in human tennis players where handedness is inherited and the size of the preferred arm increases because of the increased muscle training associated with the more frequent use of that arm.

Study species

When studying FA and behavioural biases in response to FA the swordtail characin (*Corynopoma riisei*), subfamily Glandulocaudine, is a very suitable study species: it has pronounced bilateral secondary sexual characters and easily observed courtship behaviours (see below). *C. riisei* is a small (30-50 mm standard length) freshwater fish found in Venezuela and Trinidad. The sexual dimorphism between the sexes is pronounced with the male having enlarged anal and dorsal fins and the lower lobe of the caudal fin is clearly elongated (Nelson, 1964). The most extreme secondary sexual character in the male is a thin paddle-like structure extending from the operculum which is enlarged at the end into a flag (Nelson, 1964).

In nature reproduction and spawning coincides with the rainy seasons (Alkins-Koo, 2000). Nelson (1964) on the other hand suggested that reproduction should be most favourable during the dry season when the low water volume increases the concentration

of fish and thus the chances of finding a mate. Under laboratory conditions courting and spawning can be seen at all times of the year (Nelson, 1964). Mate preference is poorly investigated in *C. riisei*. Nelson (1964) noted that males, when given a choice of females to court, showed a mate preference but would often switch from one female to another unpredictably. What this preference was based on remains unclear. Courting in this species is time consuming and may extend over several days (Niclas Kolm, pers. comm.). During courtship the male extends the paddle in front of the female (Kutaygil, 1958). The female can be seen to bite at the flag when it is extended and Kutaygil (1958) therefore suggested that the female might think it is food (see also Arnqvist & Rowe, 2005). The male may thus use the flag to lure the female closer to him at which point insemination may take place. Fertilization is internal in *C. riisei* where the male transfers aggregations of spermatozoa to the female. As the males lack external genitalia (Nelson, 1964) exactly how and when this transfer takes place is still unclear. Kutaygil (1958) showed that when the anal fin was removed no insemination occurred and therefore proposed that the anal fin is in somehow involved in this transfer. There are a number of hooks on the anal fin that perhaps serve to hold the female in place while the transfer is made (Kutaygil, 1958). Another fact that speaks for the use of the anal fin in insemination is how the male during courtship bends the fin towards and around the female seemingly trying to hold or pull her closer (Kutaygil, 1958). The male's paddle displays may thus serve to position the female so that the male can take hold of her using the hooks on the anal fin, pull her closer and/or hold her in position to be able to inseminate her.

Following insemination the female can store the sperm for up to 10 months before she, when the conditions are favourable, lays the eggs (Kutaygil, 1958; Nelson, 1964). *C. riisei* shows no parental care so the survival of the young depends on the prevailing conditions. During the wet season there is much room due to increasing water volumes, low concentration of predators and a lot of food items and nutrients are washed into the water by the rain (Nelson, 1964). These are perfect conditions for the growing fry and the female's ability to store sperm and control the timing of the egg laying enables her to provide her young with the best possible conditions.

Hypotheses and predictions

This study describes the morphological variation in paddle length, flag length and flag area in *C. riisei*. Since the paddle structure is a sexually selected character FA was predicted to be quite large, normally distributed around zero and negatively correlated with average trait size. The females' response to males of varying size and FA was investigated. In this case the females were predicted to show a preference for more symmetrical males as well as a males with a larger total trait size as this in addition to symmetry may serve as indicators of genetic quality. Furthermore, the males' behavioural response in relation to their own FA was investigated. It was hypothesized that males with high FA would bias their behaviour towards their most attractive side, presumably their bigger side. Males with low FA were expected to show little or no behavioural bias. If the male shows a behavioural bias, the underlying mechanism could either be that the male is trying to deceive the female, a result of a mediated preference by the female for either of the male's sides or a result of handedness (i.e. laterality). To disentangle the possible explanations to any behavioural bias the males were manipulated to alter the relative relationship between the paddles. The outcome of the manipulation was hypothesized to be a corresponding change in the male's behavioural bias following the male's assumed perception of himself or alternatively according to consistent cues given by the female indicating a specific preference. In the event that a behavioural bias was due to handedness no change in behavioural bias was expected.

Materials and methods

All experiments were conducted from September 2007 to February 2008 at the Department of Animal Ecology, Evolutionary Biology Centre, Uppsala University. The experimental fish were purchased from an aquarium fish wholesaler. They were kept in groups of 50 specimens each in 100 l tanks and were fed daily with dry flake food *ad libitum*. To ensure that all fish used in the experiments were virginal before the experiment they were isolated into smaller tanks of 50 l at first sign of sexual maturity. All tanks were kept on a 12 hour light-dark cycle through fluorescent lighting with 1 hour's dusk and dawn periods. The water was composed of 50% de-ionised water and 50% tap-

water. The temperature was held at 23-25 C° and pH ranging from 6-7.5. The tanks had motor-driven filters and were cleaned from food residuals, snails and algae once a week. In addition approximately 20% of the water was changed weekly. At each cleaning and water change the water quality was maintained through use of water conditioner and pH was kept at a stable level using a pH-reducing agent.

Morphological measurements

In order to investigate the morphological variation within the laboratory population 72 males were anaesthetized with benzocaine and photographed on both sides on September 24-26 2007. The photos were analyzed using the public domain image processing and analysis program Image J 1.38x (http://rsb.info.nih.gov/ij/). The fish's standard length (snout to caudal fin split), lower caudal lobe (dark caudal glandular spot to caudal fin tip),
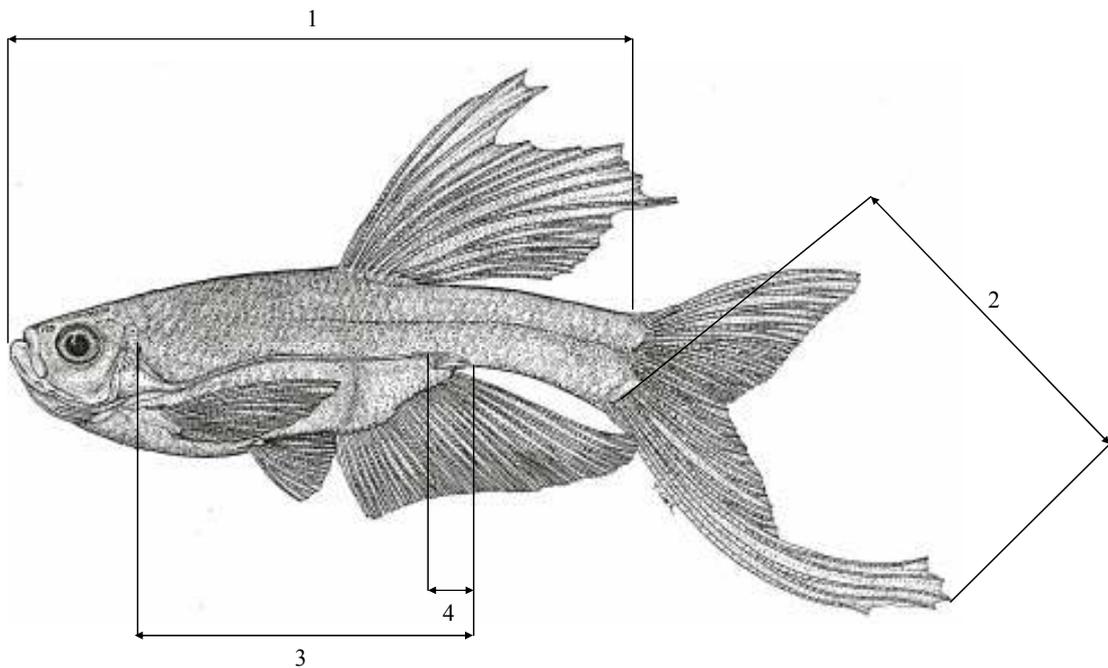
Figure 1. Illustration of measurements taken on *Corynopoma riisei*. 1. Standard length. 2. Lower caudal fin length. 3. Paddle length. 4. Flag length. (From Weitzman & Fink, 1985, p 421)

total paddle (operculum to flag tip), flag (base to tip) and flag area (figure 1) on both sides were measured. To reduce measurement error standard lengths and lower caudal fin lengths were measured on both sides and an average was used. Paddle length, flag length and flag area where measured four times and an average for each side was used.

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Behavioural investigation 1

To investigate if males showed a behavioural bias in response to their own FA in paddle length, flag length or flag area, a behavioural investigation was made in which a male and a female were observed over a period of 5 days (see specifics below). The female's response to the male's FA and behaviour was also investigated (see below). The experiment was conducted 13 October – 13 November 2007. 20 females from the laboratory population were chosen and ordered randomly. They were anaesthetized and standard length was measured to the nearest 0.5 mm. 20 males were chosen randomly to take part in the experiment.

The females were fed frozen Artemia (brineshrimp) *ad libitum* daily starting three days before the observations started and the water level was reduced to approximately 60% to simulate dry season. One day later the male was introduced into the female's tank in a 13x17x12 cm fish net breeder with nylon netting through which the fish could see each other and interact in all ways except physically. This allowed 48 hours for the male and female to acclimate to each other. Starting from the day the male was introduced to the female's tank he too was fed frozen Artemia *ad libitum* daily until the end of the experiment. The first day of observation the fish were fed dry flake food 1.5 hours prior to the start of the experiment. At the start of the experiment the male was gently released into the tank with the female using a landing net and the fish net breeder was removed. The behaviours of the male and female were then observed for a two hour period between 10 and 12 am and thereafter for seven 10 minute periods every half hour until 15.30. Thereafter the fish were observed for a 10 minute period morning and afternoon for four days. In total the fish were observed 270 minutes.

To measure female response to the presence and behaviour of a male four behaviours were observed: flight, chase, aggressive bite and paddle bite. Flight was recorded as total time the female spent swimming rapidly against the wall at a 90° angle or swimming along the wall with the head pressed against it. Aggressive bites and paddle bites were counted and were defined as bites that were directed at and made contact with the male's body or either of the male's paddles respectively. Chase was defined as an extended

attack where the female continued to follow the male even after he responded by swimming away from her. During the first two hours of the experiment, time until first aggressive bite and first chase by the female were noted. Aggressive bites, chase and flight were interpreted as expressions of aversion by the female whereas paddle bites were used as an indicator of interest shown by the female. To measure the male's response to his own morphology the number of displays were counted separately for both paddles.

Manipulation

To see how the males responded to a change in paddle length the relative paddle size was manipulated. The 20 experimental males were anaesthetized on December 5 2007 and the flags were cut on each side using a scalpel. On the paddle that had been longest before the experiment started the flag was cut leaving approximately 1 mm of the flag. On the other paddle, which was shorter before the experiment, the flag was cut only a little, approximately 0.5 mm. This resulted in that 15 males switched which paddle was longest from one side to the other. The remaining 5 males had an initial difference between the paddles that was too large for the manipulation of the flags to have an effect. Although the paddles had not changed regarding which was longer in 5 cases the relative relationship in length was altered (i.e. the difference between the paddles was smaller) which could still potentially alter the male's behaviour. Because females naturally bite at the males' paddles and sometimes even bite them off completely (pers. obs.) I have reason to believe that the manipulation did not affect males detrimentally and so would not cause a behavioural change, but see below for further discussion.

Behavioural investigation 2

The second experiment was conducted 18 January – 1 February 2008. 18 of the manipulated males were used in this experiment; two had died of natural causes. The males were used in the same order as in the first experiment. 18 new females were chosen and ordered randomly. They were anaesthetized and standard length was measured to the nearest 0.5 mm. This time the experimental setup was slightly altered: the female was introduced immediately into the male's tank without any prior acclimating period. This

was done because the females showed a lot of aggression towards the males when they were introduced into the females' tanks thereby possibly constraining the males from courting. The water level was not changed prior to the experiment. I do not think the slight differences between the experiments introduced any biases in the data since I was only interested in any behavioural change within the individual males. The female was gently released into the male's tank in the morning using a landing net and the behaviour of the male was observed for a two hour period. Thereafter they were observed for 30 consecutive minutes in the afternoon the same day and 30 minutes the following day. The number of displays with each paddle was recorded.

Statistical analyses

All statistical analyses were performed in STATISTICA 7 (StatSoft Inc., Tulsa, OK, USA). Significance levels were set at $\alpha = 0.05$ and two-tailed analyses were used.

Results

Morphological measurement

The male laboratory population (N=72) had an average standard length of 36.34 mm (range =30.63-41.83 mm, S. D.=2.44 mm) and an average lower caudal fin length of 20.45 mm (range=11.72-24.95 mm, S. D.=3.03 mm). Average paddle length, flag length

Table 1. Descriptive statistics of average, average absolute FA (|left-right side|), range, standard deviation (S. D.) and normality distributions (Shapiro-Wilk) in paddle length, flag length and flag area (**p<0.01, ***p<0.001).

	Paddle length (mm)	Flag length (mm)	Flag area (mm^2)
Average	18.92	3.01	3.28
Range	9.14-22.32	1.42-4.00	0.77-6.01
S. D.	2.66	0.55	1.11
Average |FA|	1.07	0.40	0.62
Range	0.04-6.82	0.002-1.60	0.02-3.00
S. D.	1.31	0.36	0.66
Distribution mean	-0.08	-0.20	-0.25
Normality test			
W	0.88	0.95	0.91
p	***	**	***

and flag area were calculated as well as average absolute FA (right – left side) (table 1). The average magnitude of FA observed was 5.7%, 13.3% and 18.9% for paddle length, flag length and flag area respectively. The observed FA was not normally distributed for any of the traits (table 1) but had means close to zero (figure 2). No statistically significant correlations were found between FA and any of the paddle-related measures (mean paddle length: N=72, r=-0.19, p=0.12; mean flag length: N=72, r=-0.07, p=0.56; mean flag area: N=72, r=0.18, p=0.13). Male body size was positively correlated with all the secondary sexual characters: lower caudal fin length (N=72, r=0.67, p<0.001), mean paddle length (N=72, r=0.60, p<0.001) mean flag length (N=72, r=0.61, p<0.001) and mean flag area (N=72, r=0.61, p<0.001).
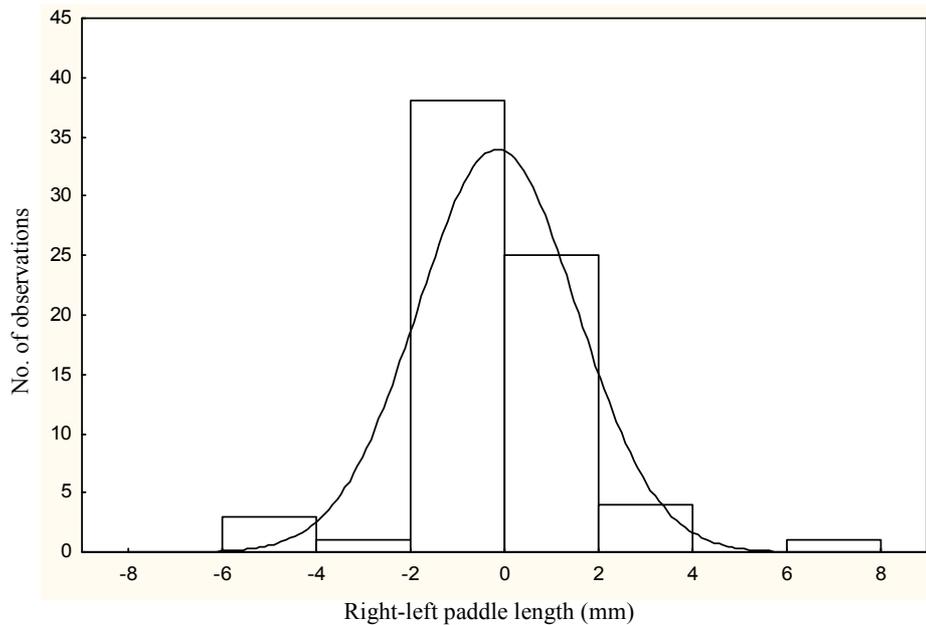
Figure 2. Distribution of right – left paddle length with the expected normal distribution curve.

Behavioural investigation 1

Neither total paddle length, total flag length nor total flag area were found to be correlated with any of the aggressive behaviours shown by the female (total number of aggressive bites, time until first aggressive bite, time until first chase and time spent fleeing) or male courting activity (total number of displays with both paddles over the entire experimental period) (table 2). Total time spent fleeing was positively correlated with FA in paddle length and flag area (table 2) indicating that the female will try to flee

Table 2. Correlation matrix with correlations between |FA|, total (right + left side) paddle length, flag length, flag area and total number of aggressive bites (N=20), time until first aggressive bite (N=17), time until first chase (N=20), total time spent fleeing (N=20) and courting activity (total number of displays with both paddles) (N=20).

		Total paddle length	Total flag length	Total flag area	|FA| paddle length	|FA| flag length	|FA| flag area
Aggressive bites	r	0.09	0.06	0.16	0.01	-0.02	0.08
	p	0.70	0.79	0.51	0.95	0.94	0.73
1st aggressive bite	r	0.28	0.18	0.32	0.26	0.26	0.48
	p	0.28	0.50	0.22	0.32	0.31	0.05
1st chase	r	0.15	0.14	0.30	-0.03	0.24	0.15
	p	0.52	0.56	0.20	0.89	0.31	0.54
Flight	r	-0.02	-0.02	-0.01	0.79	0.15	0.45
	p	0.95	0.93	0.97	0.000	0.53	0.046
Courting activity	r	-0.22	-0.20	-0.13	-0.11	0.14	0.09
	p	0.36	0.41	0.58	0.65	0.55	0.72

away more from an asymmetric male than a symmetric male. But two females were found to be spending much more time fleeing than the others (>50 minutes) (average=12.2 min, median=6 min), if these two individuals are excluded from the analysis the correlation disappears (paddle length: N=18, r=0.12, p=0.64; flag area: N=18, r=0.24, p=0.34). No relationship between absolute FA and female aggressive behaviour or male courting activity was found (table 2). Further, no relationship was found between male courting activity and time of first aggressive bite (N=17, r=0.23, p=0.37), total number of aggressive bites (N=20, r=-0.15, p=0.52) or flight (N=20, r=-0.02, p=0.93). But time until first chase was positively correlated with male courting activity (N=20, r=0.61, p=0.004) suggesting that the less aggressive the female is the more the male courts her (or vice versa). Neither the female's nor the male's size affected the aggressive behaviours shown by the female (table 3) or male courting activity (table 3). Not enough paddle bites were observed to support statistical analyses (19 bites by 7 females).

Because measurement error is a big source of error in analyses of FA, I chose to use paddle length in all analyses of male display in response to FA as it is the biggest character and thus measurement error is relatively small. Strong correlations were found

between average paddle length and the other paddle-related measurements (flag length: N=20, r=0.90, p<0.001; flag area: N=20, r=0.81, p<0.001) which supports the use of paddle length as representative for the other measures. Similar relationships were seen in FA indicating that FA in paddle length is also representative for FA in flag length and flag area.

Table 3. Correlation matrix with correlations between female and male standard lengths (SL) and total number of aggressive bites (N=20), time of first aggressive bite (N=17), time of first chase (N=20), total time spent fleeing (N=20) and courting activity (total number of displays of both paddles) (N=20).

|		SL female	SL male
Aggressive bites	r	-0.27	0.25
	p	0.26	0.30
1st aggressive bite	r	-0.18	0.06
	p	0.49	0.81
1st chase	r	-0.25	0.14
	p	0.29	0.55
Flight	r	-0.20	0.35
	p	0.39	0.13
Courting activity	r	0.21	-0.22
	p	0.38	0.35

The males (N=17, 3 males displayed their paddles less than 5 times in total and were therefore excluded from the analysis) were found to display more with the longer paddle (figure 3) also when controlling for allometry (table 4).

Table 4. Results from multiple regression with displays using the right paddle relative to total number of displays as the dependent variable. Independent variables are right-left paddle length and average paddle length. (Multiple R^2=0.39, $F_{(2, 14)}$=4.38, p=0.03).

Independent variables	β	S. E.	$t_{(14)}$	p
Right-left paddle length	0.51	0.21	2.44	0.03
Average paddle length	0.36	0.21	1.70	0.11

No change in overall courting intensity (number of extensions per 10 minutes) could be seen over time in the first experiment (one-way ANOVA: $F_{5, 114}$=0.21, p=0.96). Nor

could any change in relative use of the longer paddle over the other be found over time (one-way ANOVA: $F_{4, 95}=0.33$, p=0.86).
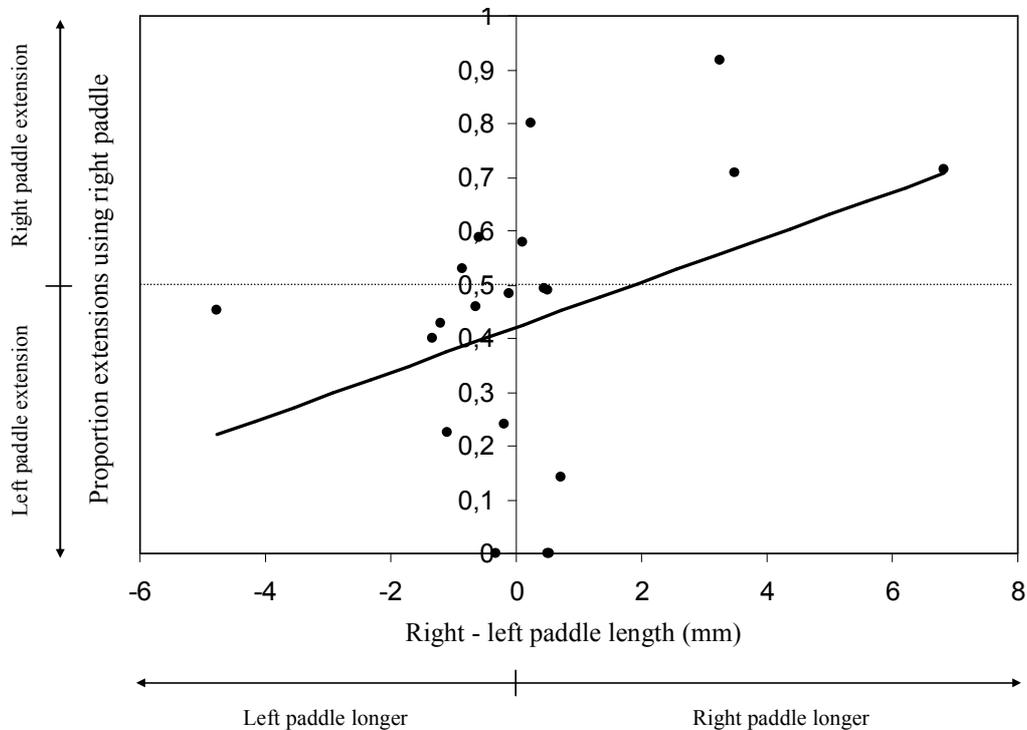

Figure 3. Male bias in number of right displays compared to total number of displays corresponds to the morphological variation in right – left paddle length indicating a preference for using the longer paddle. N=17, y=0.04x+0.42, r=0.51, p=0.038.

Behavioural investigation 2

In total 14 males extended their paddles in both the first and second experiment, two males displayed their paddles less than five times and were therefore excluded from the analyses. The males showed a clear behavioural response to the manipulation and lowered the display activity using the paddle which was longest before the first experiment in favour of the other following manipulation (figure 4).

To see if the males had responded negatively to the manipulation, a comparison between the relative use of the shorter paddle in the first experiment and the use of the manipulated paddle in the second experiment was done. No significant difference in use of the manipulated paddle as compared to the use of the shorter paddle was found (paired t-test:

N=12, t=-0.33, p=0.75). This indicates that the manipulation had not affected the males detrimentally.
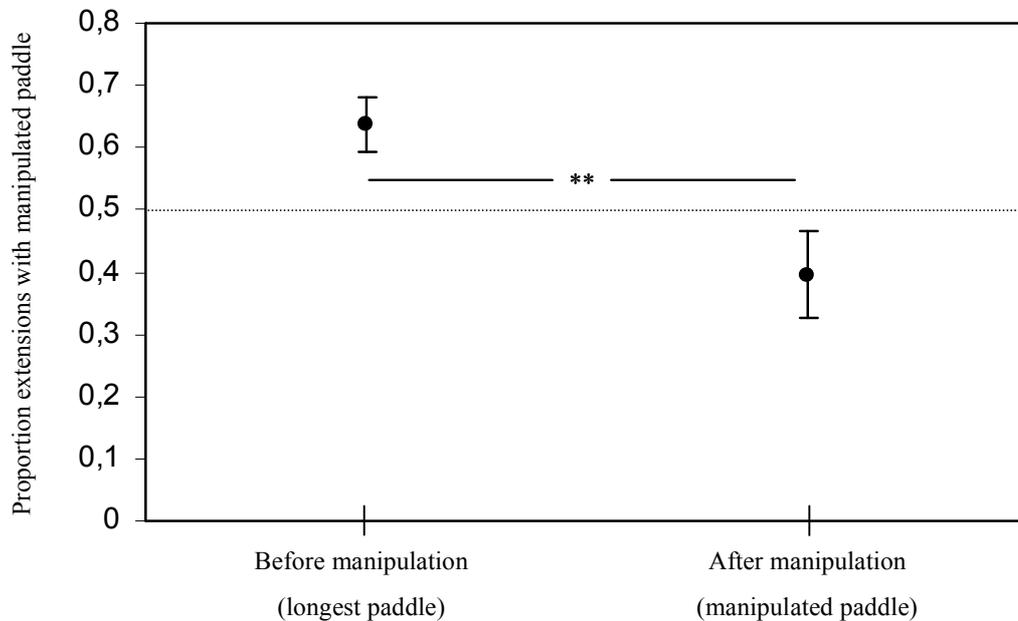
Figure 4. Proportion of displays with the manipulated paddle before and after manipulation (mean ± S. E.). Paired t-test: N=12, t=4.06, p=0.002. (**p<0.01).

Discussion

The majority of studies on lateralisation have focused on lateralisation at the level of the population rather than on the individual level (Gross *et al.*, 2007). It is important however to investigate laterality at the level of the individual as population lateralisation has been proposed to originate from individual lateralisation (Vallortigara, 2006) and a greater understanding of the origins will enhance our knowledge of lateralisation in general. In this study I show, in concordance to the findings of Gross *et al.*, (2007), that individuals respond behaviourally to their own morphology. In addition this study shows that male *C. riisei* not only display their longer paddle when courting a female but also alter this behavioural bias following manipulation of the paddles. It is quite remarkable that a simple animal like a fish has such an advanced perception of their own morphology but my results suggest that male *C. riisei* are indeed aware of which is their "best side" and display their paddle ornament accordingly.

Morphology

The observed magnitude of FA in this laboratory population was found to be rather high which agrees well with the idea that FA in sexually selected trait is larger than in other traits (Møller & Pomiankowski, 1993). No significant relationship between paddle FA and mean paddle size was found but a negative tendency could be seen. If paddle size is an honest signal of male quality a negative relationship between paddle FA and average paddle size is expected (Møller & Pomiankowski, 1993). But paddle size does not have to be exclusively related to male quality, for example if this is a case of sensory exploitation, which has been suggested (Kutaygil, 1958), big paddles will still be selected for by the female but size is not necessarily correlated with fitness. In this case individuals that grow larger traits will be subjected to greater stress during development and the FA will instead be positively correlated to mean trait size (Møller & Pomiankowski, 1993). Paddle size in *C. riisei* could be an example of a signal which works both as a quality indicator and is the result of sensory exploitation. In either case it is likely to be costly for the male to produce these paddles that are only useful to him during courtship so it would probably require a strong, viable male to produce large paddles. This could explain why no significant negative correlations between FA and any or the paddle-related traits were found: trait size is selected for in both cases but a relationship to fitness is required only if the signal is an indicator of quality. That the correlation shows a negative tendency could indicate that both average paddle size and FA are signals of male quality and may be used by the female as such.

Female preference

The fact that individuals influence each other's relative paddle sizes by biting at each other's paddles speaks against the hypothesis that females can use total trait size, mean trait size and/or individual FA as honest signals of male fitness. However, a male with large, untouched paddles could signal fitness by being able to resist biting from others and therefore be attractive to females. Although unlikely, the opposite could also be true if small, bitten paddles indicate that the male is attractive among other females. But since males also bite at each others' paddles a small paddle could mean that the male is being beaten by other males which would not seem like a positive thing from the female's

perspective. Since so few paddle bites from females were observed little can be said about female preference. Females were not observed to be more aggressive towards males of a particular kind of morphology. On the contrary, a positive tendency between FA in flag area and time of first aggressive bite could be seen indicating that females show aggression sooner with more symmetric males, although this finding was not supported by a decrease in total number of aggressive bites or any of the other aggressive behaviours with increasing asymmetry.

The observation that females tended to flee more from asymmetric than symmetric males needs to be interpreted with great care as the behaviour of two single females seemed to draw the correlation in this direction. But if it is indeed true that females are reluctant to spend time with asymmetric males it could be because the female is not willing to pay the costs associated with mate choice. For females mate choice can be costly in many different ways: it requires energy to look for males and assess their quality, time is lost when she could have been foraging, the proximity of a male may increase risk of acquiring diseases as well as increase predation risk for the female (see Arnqvist & Nilsson, 2000, and references therein). For example, Dill *et al.* (1999) showed that female guppies were less receptive to male courtship in the face of predation. In the case of *C. riisei*, the females are usually larger than the males and may therefore be more exposed to predators. Although considering that it is generally the female who is dominant in *C. riisei* (Niclas Kolm, pers. comm. & pers. obs.) one would expect the female to chase the male away from her rather than trying to flee away from him herself.

The only relationship found between any of the females' aggressive behaviours and the males' behaviour was a positive correlation between time of first chase and male courting activity suggesting that males court less aggressive females more. This agrees well with the fact that females are dominant over males and thus will not accept courting unless she wants to. Alternatively this result could indicate that females are less aggressive towards males that court more intensively. This could relate to the trade-off mentioned above: A male who is willing to risk his paddles being bitten as well as predation in order to get a mating opportunity could be more attractive to females. But again this was not supported

by similar relationships between male courting activity and any of the other aggressive behaviours shown by the female.

Male behaviour

The males showed a bias towards the longer paddle in the first experiment and clearly responded to the manipulation by changing their display bias from one side to the other. This suggests males are aware of which their best side is and that the bias in display rates towards the longer paddle was not simply caused by an innate handedness effect where the preferential use of one paddle could have affected its appearance like that in a tennis player where the arm used to hold the racket tends to be much stronger. Nor is it a classical example of laterality defined as a bias in behaviour resulting from the organisation of different behaviours to different parts of the brain (Vallortigara, 2006). If this would have bee the case the behavioural bias would not have changed following manipulation. Instead the lateralised behaviour seems to be a result of variation in morphology.

The mechanism behind the observed male preference to display the longer paddle in the first experiment could not be attributed to experience as all males and females were naïve prior to the experiment. But the males could still be showing one side more than the other in an immediate response to cues given by the female. Unless the male adapts instantly to the female's cues one could expect an increase in display bias over time as the male learns which side the female prefers. The fact that no such increase was observed contradicts this hypothesis. Moreover, during courtship in *C. riisei* the male is seen to be swimming rapidly from behind the female then quickly swimming closely underneath her coming up on one side or the other of the female and displaying the paddle when he is in front of her. Meanwhile, the female seems to be swimming calmly around the tank and the male follows. This leads me to believe that it would be hard for the female to affect which paddle the male displays but rather it seems like the male decides which side of the female he is on and hence which paddle he displays in front of her.

An alternative explanation to the observed change in display bias could be that the more manipulated paddles were affected in such a way that the males are reluctant to use them as much as before. In the event that the manipulation of the paddles had a detrimental effect on the paddle which was most cut this would affect the behavioural bias in the same way as a deliberate change in behaviour by the male confounding the interpretation of the results. Two months had elapsed since the manipulation so it could be reasonable to think that any negative effects of the manipulation should have worn off. The fact that the males used the manipulated paddle relative to the total number of paddle displays in the second experiment as much as they used the shorter paddle in the first experiment speaks against any negative effects from the manipulation that could confound the results. In the first experiment one female was even seen to bite one paddle off completely and therefore I argue that the manipulation was not only within the natural range of asymmetry that can be seen in nature but also a repeatedly occurring natural event.

The adaptive benefits of this display bias could be a case of males trying to trick the females into believing that they are of better quality than they really are. If paddle size and/or FA is an honest indicator of good genes its honesty can be complicated in this species. If a male of low genetic quality is able to gain mating advantages in spite of having a trait that signals low quality they can obstruct the assessment by the females' by displaying their bigger side more. This could also be a case of sensory exploitation were the signal is not correlated to good genes but one that serves to trigger, in the case of *C. riisei,* a feeding response in the female (Kutaygil, 1958). The selective use of the bigger paddle over the other could then invoke a more intensive feeding response in the female.

The paddles of males are bitten not only by females but also by other males (pers. obs.) so the size and shape of the paddles change continuously. It would therefore seem to be in the interest of the male to know what he looks like at any given time. As he extends the paddles at a right angle from his body he could potentially assess the relative size of his paddles by looking at and comparing both sides. As large size of the paddle seems to be an important signal in courtship in this species the females' biting of the paddles creates

and interesting trade-off situation for the male: When a female is allowed to bite at the paddle the male may get to mate with that female, but at the same time his chances of mating with another female later are reduced as the size of his paddles decreases. Therefore one might expect the male to try to reduce the number of displays so that he can attract the female's attention but without providing her with too many chances of biting the paddle.

It is worth noting that when observing the males' displays it seems to be more strenuous for the males to extend a large paddle than a small one. When displaying the larger paddle it meets more resistance in the water which could be more costly for the male in terms of energy expenditure. None the less the males not only chose to display the longer and bigger paddle more in the first experiment but changed this display bias following manipulation of the paddles.

Implications and future studies

My findings have important implications for our understanding of the evolution of sexual signalling. If males are able to assess which side is more attractive to females and adjust their displays accordingly only allowing females to see the better, more attractive side, this could potentially drive the evolution towards asymmetry with one side being big and the other very small. Males would not have to waste energy developing two paddles but could instead allocate more energy to one side and then only show that side to the female in courtship. In this case females would have to start paying more costs in their mate choice to assess males more thoroughly to reveal deceivers. One could also imagine that having one side bigger than the other could be costly to the male for example if this imbalance causes limits to or obstructs normal locomotion. This would oppose the evolution of one very large paddle and one very small.

In the case of the guppies (Gross *et al.*, 2007) the male's display bias was seen to disappear when they courted a dead, unresponsive, female, suggesting that cues given by the female are important if not crucial for the male to show a bias. I have reason to believe that in *C. riisei* it would be hard for the female to influence which paddle the

male displays (see above). One way to investigate this is to perform an experiment in which the male and female are separated by a one way mirror so that the male can see the female and court her but she can not see him. In this way cues given by the female can be ruled out should the male still show a behavioural bias.

The fact that the males bias their displays towards their better side makes it difficult for the females to assess them accurately and complicates the idea of honest signalling. If symmetry is associated with good genetic quality and females use symmetry as a signal of male viability in mate choice males could deceive females to think he is of better quality than he actually is by only allowing the female to see one side of him. Again females would have to start paying higher costs in mate choice to avoid deceit. Further studies to investigate female preference should be undertaken to see if females actually prefer symmetric males over asymmetric ones but also to investigate the female's perception of the male both regarding FA and morphology in general as well as behaviour. Also male fitness should be investigated in this system to see if symmetric males are of better genetic quality or not.

Acknowledgements

References

Ades, C. & Ramires, E. N. 2002. Asymmetry of leg use during prey handling in the spider *Scytodes globulata* (Scytodidae). *J. Insect Behav.* **15**, 563-570.

Alkins-Koo, M. 2000. Reproductive timing of fishes in a tropical intermittent stream. *Environ. Biol. Fishes* **57**, 49-66.

Andersson, M. 1994. Sexual selection. 1st ed. Priceton Univ. Press, Princeton, NJ.

Andrew, R. J., Tommasi, L. & Ford, N. 2000. Motor control by vision and the evolution of cerebral lateralization. *Brain Lang.* **73**, 220-235.

Arnqvist, G. & Nilsson, T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Behav.* **60**, 145-164.

Arnqvist, G. & Rowe, L. 2005. Sexual conflict. 1st ed. Priceton Univ. Press, Princeton, NJ.

Basolo, A. L. 1990. Female preference predates the evolution of the sword in swordtail fish. *Science* **250**, 808-810.

Bisazza, A., De Santi, A. & Vallortigara, G. 1999. Laterality and cooperation: mosquitofish move closer to a predator when the companion is on the left side. *Anim. Behav.* **57**, 1145-1149.

Bisazza, A., Rogers, L. J. & Vallortigara, G. 1998. The origins or cerebral asymmetry: a review of evidence of behavioural and brain lateralization in fishes, reptiles and amphibians. *Neurosci. Biobehav. Rev.* **22**, 411-426.

Brunton, C. F. A., Atkinson, R. J., Ager, M. L. & Majerus, M. E. N. 1999. An observation of directional symmetry in wing spots of two Arctic butterflies (*Colias*, Pieridae). *Ann. Zool. Fennici* **36**, 121-123.

Byrne, R. A., Kuba, M. J. & Meisel, D. V. 2004. Lateralized eye use in *Octopus vulgaris* shows antisymmetrical distribution. *Anim. Behav.* **68**, 1107-1114.

Candolin, U. 2003. The use of multiple cues in mate choice. *Biol. Rev.* **78**, 575-595.

Carranza, J. 1994. Female attraction by males versus sites in territorial rutting red deer. *Anim. Behav.* **50**, 445-453.

Dawkins, M. S. & Guilford, T. 1991. The corruption of honest signaling. *Anim. Behav.* **41**, 865-873.

Dill, L. M., Hendrick, A. V. & Fraser, A. 1999. Male mating strategies under predation
risk: do females call the shots? *Behav. Ecol.* **10**, 452-461.

Gonzales Voyer, A., Fitzpatrick, J. & Kolm, N. In review. Sexual selection governs
parental care in cichlid fishes. *Evolution.*

Graham, J. H., Emlen, J. M., Freeman, C. D., Leamy, L. J. & Kieser, J. A. 1998.
Directional asymmetry and the measurement of developmental instability. *Biol. J.
Linn. Soc.* **64**, 1-16.

Gross, M. R., Suk, H. Y. & Robertson, C. T. 2007. Courtship and genetic quality:
asymmetric males show their best side. *Proc. R. Soc. B.* **274**, 2115-2122.

Hopkins, W. D., Hook, M., Braccini, S. & Schapiro, S. J. 2003. Population-level right
handedness for a coordinated bimanual task in chimpanzees: replication and extension
in a second colony of apes. *Int. J. Primatology* **24**, 677-689.

Jennions, M. D., Møller, A. P. & Petrie, M. 2001. Sexually selected traits and adult
Survival: A meta-analysis. *Quarterly Rev. Biol.* **76**, 3-36.

Knierim, U., Van Dongen, S., Forkman, B. Tuyttens, F. A. M. Špinka, M. Campo, J.L. &
Weissengruber, G. E. 2007. Fluctuating asymmetry as an animal welfare indicator – a
review of methodology and validity. *Phys. Behav.* **92**, 398-421.

Kokko, H., Brooks, R., Jennions, M. D. & Morley, J. 2003. The evolution of mating
biases. *Proc. R. Soc. B.* **270**, 653-664.

Kokko, H. & Jennions, M. D. 2003. It takes two to tango. *Trends Ecol. Evol.* **18**, 103-
104.

Kokko, H. & Jennions, M. D. In press. *J. Evol. Biol.*

Krebs, J. R. & Davis, N. D. 1993. An Introduction to Behavioural Ecology. 3rd ed.
Blackwell Science, Oxford, UK.

Krebs, J. R. & Davis, N. D. 1997. Behavioural Ecology: An Evolutionary Approach. 4th
ed. Blackwell Science, Oxford, UK.

Kutaygil, N. 1958. Insemination, sexual differentiation, and secondary sex characters in
Stevardia albipinnis Gill. *Istanbul Univ. Fen Fakultesi Mecmuasi (B)* **XXIV**, 93-128.

Lande, R. 1981. Models of speciation by sexual selection on polygenetic traits. *Proc.
Natl. Acad. Sci.* **78**, 3721-3725.

Lens, L., van Dongen, S., Kark, S. & Matthysen, E. 2002. Fluctuating asymmetry as an

indicator of fitness: can we bridge the gap between studies? *Biol. Rev.* **77**, 27-38.

Lippolis, G., Bisazza, A., Rogers, L. J. & Vallotrigara, G. 2002. Lateralisation of predator avoidance in three species of toads. *Laterality* **7**, 163-183.

Møller, A. P. 1994. Sexual selection in the barn swallow (*Hirundo rustica*). IV. Patterns of fluctuating asymmetry and selection against symmetry. *Evolution* **48**, 651-670.

Møller, A. P. & Höglund, J. 1991. Patterns of fluctuating asymmetry in avian feather ornaments: implications for models of sexual selection. *J. Evol. Biol.* **6**, 481-491.

Møller, A. P. & Pomiankowski, A. 1993. Fluctuating asymmetry and sexual selection. *Genetica* **89**, 267-279.

Nelson, K. 1964. Behavior and morphology in the glandulocaudine fishes (Ostariophysi, Characidae). *University of California Publications in Zoology* **75**, 59-152.

Palmer, A. R. & Strobeck, C. 1986. Fluctuating asymmetry – measurement, analysis, patterns. *An. Rev. Ecol. Syst.* **17**, 391-421.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**, 525-568.

Patricelli, G. L., Coleman, S. W. & Borgia, G. 2006. Male satin bowerbirds, *Ptilonorhynchus violaceus*, adjust their display intensity in response to female startling: an experiment with robotic females. *Anim. Behav.* **71**, 49-59.

Polak, M. 2003. Developmental instability: causes and consequences. Oxford Univ. Press, Oxford, UK.

Pomiankowski, A., Iwasa, Y. & Nee, S. 1991. The evolution of costly mate preferences. I. Fisher and biased mutation. *Evolution* **45**, 1422-1430.

Queller, D. C. 1997. Why do females care more than males? *Proc. R. Soc. B.* **264**, 1555-1557.

Ryan, M. J. & Keddyhector, A. 1991. Directional patterns of female choice and the role of sensory biases. *Am. Nat.* **139**, 4-35.

Swaddle, J. P. 1999. Limits to length asymmetry detection is starlings: implications for biological signalling. *Proc. R. Soc. B.* **266**, 1299-1303.

Trivers, R. L. 1972. Mother-offspring conflict. *Am. Zool.* **12**, 648-648.

Vallortigara, G. 2006. The evolutionary psychology of left and right: costs and benefits of lateralization. *Dev. Psychobiol.* **48**, 418-427.

Vallortigara, G. & Rogers, L. R. 2005. Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. *Behav. Brain Sci.* **28**, 575-633.

Van Dongen, S. 2006. Fluctuating asymmetry and developmental instability in evolutionary biology: past present and future. *J. Evol. Biol.* **19**, 1727-1743.

Vanpé, C., Gaillard, J-M., Kjellander, P., Mysterud, A., Magnien, P., Delorme, D., Van Laere, G., Klein, F., Liberg, O. & Hewison, M. A. J. 2007. Antler size provides an honest signal of male quality in roe deer. *Am. Nat.* **169**, 481-493.

Van Valen, L. 1962. A study of fluctuating asymmetry. *Evolution* **16**, 125-142.

Versace, E., Morgante, M., Pulina, G. & Vallortigara, G. 2007. Behavioural lateralization in sheep (*Ovis aries*). *Behav. Brain Res.* **148**, 72-80.

Weitzman, S. H. & Fink, S. V. 1985. Xenurobryconin phylogeny and putative pheromone pump in glandulocaudine fishes (Teleostei: Characidea). Smithsonian contribution to zoology. 421.

Zahavi, A. 1975. Mate selection – a selection for a handicap. *J. Theor. Biol.* **53**, 205-214.