ORIGINAL PAPER

Multiple signals in the palmate newt: ornaments help when courting

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Abstract There is increasing evidence that female mate choice is often based on the assessment of multiple male traits, involving both morphology and behavior. We investigated female mate choice for multiple male traits in the palmate newt, *Lissotriton helveticus*, including male tail filament length, hind foot web size, crest development, body size, ventral coloration, and courtship display activity. Observations of courtship display in the field revealed that females spent more time in front of males with longer tail filaments. Laboratory experiments revealed a more detailed relationship between filament length and courtship display. We found that females took more sperm masses from males with both longer filaments and greater display activity. Experimental shortening of the tail filament length substantially decreased the number of male sperm masses transferred. However, when we experimentally reversed relative filament length between two males in mating trials, male mating success was explained by courtship activity and not by filament length. Our results show that female palmate newts value multiple traits during mate choice, including both morphological ornaments and reproductive behaviors in males. Our results further suggest that, when filament length is below a certain threshold, females may value the information content of courtship activity over that of filament length.

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Electronic supplementary material The online version of this article (doi:10.1007/s00265-012-1355-y) contains supplementary material, which is available to authorized users.

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Introduction

In many species, males express complex courtship displays involving elaborate ornaments combined with conspicuous behaviors. The evolution and maintenance of such morphological and behavioral traits are commonly explained by their signaling role in intersexual communication (Møller and Pomiankowski 1993; Candolin 2003; Bro-Jørgensen 2010). Multiple signals that potentially could be used for mate choice were empirically identified before the first theoretical explanations had been proposed. Indeed, theoretical models of sexual selection generally predict that females should evolve a preference for a single signal because of the costs of assessing multiple signals (Schluter and Price 1993; Johnstone 1996; Fawcett and Johnstone 2003; but see van Doorn and Weissing 2004). However, the idea that females do use multiple signals to choose their mate is supported by an increasing number of empirical studies, leading to a growing interest in the evolution and maintenance of such signals (Candolin 2003; Hebets and Papaj 2005; Bro-Jørgensen 2010).

Several hypotheses have been put forward to explain why and how females use numerous signals: multiple messages (different signals convey information on different properties or temporal aspects of male qualities), redundant signal hypothesis (different signals convey the same information), unreliable signal hypothesis (signals not linked to quality), or receiver psychology (several signals are better detected than a single one, or a signal amplifies another signal;

Guilford and Dawkins 1991; Møller and Pomiankowski 1993; Rowe 1999; Hebets and Papaj 2005). Disentangling these hypotheses mainly requires unraveling the information content of multiple signals (Candolin 2003). To date, less work has been undertaken to examine the relative use of each signal, their prioritization, and how signals are processed by choosy females (Witte and Ryan 1998; Künzler and Bakker 2001; Candolin 2003; Scheuber et al. 2004).

Recently, it was proposed that a fluctuating environment is likely to drive the evolution of multiple signals because few signals may be sufficiently flexible to remain reliable when the environment changes (Chaine and Lyon 2008; Bro-Jørgensen 2010; Cornwallis and Uller 2010). Morphological and behavioral signals commonly differ in their temporal flexibility. Some morphological signals, such as an elongated tail or elaborate plumage, are relatively fixed (static sensu Hill et al. 1999) since they cannot be extensively modified on a short time scale by the signaler. Morphological signals are generally produced once per year before or at the beginning of the breeding season and remain unmodified until the next year (Hill et al. 1999; Loyau et al. 2005a, b; Baird et al. 2007; van Dongen and Mulder 2008). Morphological signals are thus expected to better reflect genetic quality or long-term viability (Kokko et al. 1999; Scheuber et al. 2004). However, if the bearer's quality or the environmental conditions change between production and assessment, morphological signals may become unrepresentative of the signaler's quality (Bro-Jørgensen 2010). On the contrary, behavioral signals such as vigorous displays or song rates can be finely tuned by the signaler depending on the signaler's current quality, motivation, or environmental conditions. Behavioral signals are typically more labile (dynamic; sensu Hill et al. 1999) than morphological ones and thus are expected to accurately reflect signaler condition at the time of assessment (Hill et al. 1999; Loyau et al. 2005a; Baird et al. 2007; van Dongen and Mulder 2008).

Given that individual quality and environmental conditions may vary on a short time scale, it has been predicted that females should use a mixture of static morphological and dynamic behavioral signals to adaptively choose their mates (Bro-Jørgensen 2010). Females are further expected to differ in the degree to which they pay attention to these two types of signals during mate choice. This potential difference can only be assessed when both signals are contradictory, e.g., when a male exhibits an ornament of high quality while expressing poor courtship behaviors or vice versa. However, it is still disputed how females deal with such contradictory information (Hill et al. 1999; Loyau et al. 2005a; Baird et al. 2007; van Dongen and Mulder 2008). One can predict that females should value the information content of behavioral signals over morphological signals because behavioral signals provide more accurate information on current male condition (Hill et al. 1999; Loyau et al.

2005a; Baird et al. 2007; Bro-Jørgensen 2010). This is particularly the case when females prefer mating with healthy individuals not only to obtain indirect benefits, in terms of good genes for their offspring or more viable sperm, but also to avoid contracting infectious diseases, since a recent decline in male condition could indicate a challenged immune system (Sheldon 1993; Loyau et al. 2005a; Chargé et al. 2010). On the contrary, it has been suggested that the expression of dynamic (behavioral) signals is sensitive to very short term, motivational influences, social circumstances, or stochastic environmental factors, and thus these signals may not be as valuable to conspecifics as fixed signals (Green et al. 2000; van Dongen and Mulder 2008).

We wished to test this set of predictions in the palmate newt, *Lissotriton helveticus*, an amphibian with strong sexual dimorphism (Griffiths and Mylotte 1988). Males are smaller and thinner than females. Males express a yellow-to-dark-orange ventral coloration whose role remains unclear. During the reproductive period, males exhibit both morphological and behavioral traits that may be assessed by females when choosing a mate. Morphological male traits that appear to have a sexual function are the hind foot web, a caudal filament, and a low dorsal caudal crest. In the crested newt *Triturus cristatus* and the smooth newt *Lissotriton vulgaris*, females prefer males with high crests (Hedlund 1990a; Green 1991; Gabor and Halliday 1997; Malmgren and Enghag 2008). In the palmate newt, Haerty et al. (2007) investigated female visual preference for filament length and body size and found that females spent more time close to males with longer filaments and smaller bodies. In addition to morphological traits, male *L. helveticus* express a conspicuous courtship behavior called "fan display", which mainly consists of displaying the tail and the filament to the female in a rapid vibrating fan movement (Halliday 1975a). This display behavior is very variable between individuals (Halliday 1975a, b; 1976). If the female is receptive to the displaying male, she touches his tail with her snout, which elicits spermatophore deposition by the male. The female can then pick up the sperm mass in her cloaca.

We used an approach combining: (1) a correlative study in the field; (2) a correlative study in the laboratory; and (3) a manipulative study of a morphological signal in the laboratory. We first examined the impact of tail filament length on the maximum time spent by the female watching a male fan display in the field. We then examined whether female palmate newts use multiple morphological and behavioral traits to choose their mates, focusing particularly on the role played by both filament length and courtship activity, while controlling statistically for potential additional morphological signals (including crest development, hind foot web, body size, and coloration). Finally, we manipulated filament length of pairs of males so that the male with the shorter filament became the male with the relatively longer filament, while courtship

activity was not manipulated. We predicted that, if females only value filament length, female preference should be reversed.

Materials and methods

Correlative study in the field

Our study was designed to investigate whether females spent more time watching courtship displays by males with longer tail filaments. In amphibians, female preference has rarely been observed in the wild, and such observations are likely to provide valuable insights into the evolutionary processes involved in sexual selection and are complementary to laboratory experiments.

Our behavioral observations of newts were conducted in two small ponds near the Refuge des Etangs de Bassiès in the Pyrenean Mountains (Ariège, France), at 1,655 m above sea level, between 30 June and 09 July 2010, during the peak of the reproductive season. These study ponds are ideal for behavioral observations because they are shallow with crystal clear water, were only a few meters apart, and had little vegetation and few rocks. The behavioral observations were performed for 5 days between 07:00 am and 07:00 pm. The average male courtship fan display lasted 237.5 ± 25.5 s and was not significantly different between observation days (Kruskal–Wallis tests $\chi^2=1.95$, $df=4$, $P=0.75$), between morning and afternoon (Wilcoxon rank sum test $W=150$, $P=0.39$), and between the two ponds ($W=121$, $P=0.62$).

Reproductive behaviors of newts were well described by Halliday (1975a). The male first approaches the female. He then smells the female cloaca, follows her when she moves away, and orients himself in front of the female. After this orientation phase, courtship activity begins, defined by "fan" movements of the male tail and filament in front of the female. The female either stays still, seemingly watching male fan display or moves away. When the female moves, the male stops the fan movement. A new sequence of orientation and fan display can then occur.

Sperm mass transfer is rarely observed in field conditions (Hedlund 1990b). We used the time spent by females watching courtship fan display as a proxy for female preference (Wagner 1998). Behavioral observations were conducted by one observer (JC) who walked around the two ponds (at 1-m distance) until seeing a male in either the orientation or displaying phase. During behavioral observations, we recorded the time between the start of the male display and the end, due to departure of the female or the male surfacing to breathe. Taking a breath is a critical moment in courtship by male newts, as they are likely to lose visual contact with the female to whom they were displaying (JC, personal observation). Therefore, males increase the time between breathing episodes when the females are receptive (Halliday and Sweatman 1976; Halliday 1977). We obtained several displaying periods for each male. We used length of the longest bout of the male display to a female for statistical analysis. Males were caught to measure snout–vent length (SVL, from the snout tip to the posterior end of the cloaca) and filament length with a caliper (accuracy, 0.01 mm). We recorded individually distinctive features such as shape, black spots, or coloration by drawing the male on paper. These drawings and the two measures (SVL and filament length) allowed us to ensure that a given male was not included twice in our data set. Each male was then immediately released at the site of capture. A total of 33 males (24 and 9 per pond) were observed.

Laboratory experiments

Newts caught in the wild were maintained in captivity at the Station d'Ecologie Expérimentale du CNRS à Moulis (Ariège, France) at 18 °C (±0.5 °C) and a natural light/dark cycle of 10:14 h (ZooMed ReptiSun 2.0 fluorescent bulb). Unisex groups of ten individuals were placed in opaque tanks ($52\times33.5\times29.5$ cm) with de-chlorinated water and some plants collected in their native environment. The water depth was about 5 cm, corresponding to about 9 l of water. Each tank was provided with a shelter (a perforated clay brick with 17 holes). The top of each brick was just above the water level to allow newts to get out of the water. Newts were fed ad libitum with chironomid larvae, daphnia, tubifex, and earthworms. Time between capture and release back into the wild was less than a month.

Behavioral experiments were designed to offer two males to a single female and were conducted in the animal housing facility between 0800 and 1500 hours local time. Experimental tanks were divided into three compartments by two removable transparent perforated Plexiglas separations, defining a central compartment ($20\times33.5\times29.5$ cm) and two external compartments ($16\times33.5\times29.5$ cm). We followed a protocol used by Haerty et al. (2007) with the palmate newt, to be able to compare our results with the previous results (data not shown). The experiment comprised four steps. In step 1, a focal female was placed in the central compartment where she could move freely for 5 min, allowing the female to explore her new environment. In step 2, the experimentalist placed the female in the center of her compartment in a circular opaque PVC tube for 5 min, and a male was placed in each external compartment. The tube was then removed so that the two males and the female could move freely in their compartments and explore their new environment for 15 min (step 3). Finally, in step 4, separations were removed and the three individuals could interact freely for 40 min. At the end of the experiment, we checked for the presence of sperm mass in the tank and we examined the female's cloaca for sperm mass transfer. Each individual was used only once in the experiment and the experimental tank was thoroughly

cleaned with tap water between trials. Four trials were performed simultaneously in four experimental tanks. Each trial was recorded using a video camera (Panasonic HDC-TM60, full HD, 1080p) placed above the experimental tank, totaling 65.3 h of video observation.

Morphological traits were measured immediately after the experiment. We recorded body mass (accuracy, 0.01 g), SVL (snout–vent length), and filament length as previously described using an electronic caliper. The crest is small in this species, so we used tail size as an index of crest development (Green 1991). Tail area and the left and right hind foot web areas were measured by taking a photo on a millimeter paper background (grid 1 mm²) with a digital camera (Nikon D5000). Photo analysis was conducted with ImageJ 1.43 (http://rsb.info.nih.gov/ij; developed by Wayne Rasband; Abramoff et al. 2004). With a subsample of 60 males, we tested the reliability of two measurements with ImageJ. Filament length, hind foot web area, and crest development were all highly repeatable (respectively, $r^2 = 0.99$, $r^2 = 0.98$, and $r^2 = 0.96$). Ventral coloration was measured using a spectrometer (USB2000, Ocean Optics Inc., Dunedin, FL, USA) with a Xenon light source (PX-2) and a R400-7-UV/VIS fiber optic cable and probe at a 45° angle to the skin surface. Five spectrometer measures were taken at the middle of the belly. We used Endler's segment classification method on percent reflectance between 300 and 700 nm to obtain indexes of hue, chroma, and brightness (Endler 1990). We tested the repeatability of these indexes with Kendall's coefficient of concordance (Loyau et al. 2007) with a subsample of 100 individuals. Hue, chroma, and brightness were highly repeatable (respectively, $W = 0.93$, $\chi^2 = 361$, $P < 0.001$; $W = 0.95$, $\chi^2 = 471$, $P < 0.001$; and $W = 0.73$, $\chi^2 = 361$, $P < 0.001$).

The videos were analyzed with The Observer 7.0 software (Noldus Information Technology). For each male, we recorded the time spent displaying the fan (courtship activity) and the number of sperm masses transferred to the female.

Experiment 1: female preference of filament length

This experiment was designed to test whether palmate newt females prefer males with longer tail filaments. In total, 76 males and 38 females were used to perform 38 replicate trials. Each trial involved three individuals: one female, one male with a long filament (M⁺), and one male with a shorter filament (M⁻). The pair of competitor males was chosen so that males significantly differed in filament length (paired t test, $t = 14.78$, $df = 37$, $P < 0.001$) and this difference remained stable over trials (Table 1). SVL, body mass, hue, chroma, brightness, and tail area (index of crest development) did not significantly differ between M⁺ and M⁻ males (paired t test, all $P > 0.05$). However, hind foot web area and filament length were correlated ($r = 0.44$, $df = 74$, $P < 0.001$), and M⁺ males had more developed hind foot web areas than M⁻ males (paired t test, $t = 2.8$, $df = 37$, $P = 0.008$).

Experiment 2: manipulation of filament length

In this experiment, we manipulated filament length to test whether a decrease in filament length decreased the number of male sperm masses transferred. Manipulation of a trait (phenotypic engineering) allowed us to avoid potentially confounding factors (Sinervo and Basolo 1996). Filament length could not be elongated because of technical difficulties. Many other studies have used only sexual trait reduction in their experimental manipulation for similar reasons (e.g., Bischoff et al. 1985; Petrie and Halliday 1994; Tomkins and Simmons 1998; Pryke and Andersson 2005). In total, 120 males and 60 females were used in experiment 2 to perform 60 replicate trials. One week before the start of each experimental trial, male filament length was measured to rank the males according to their filament length and to choose the pairs of male competitors as in experiment 1. However, unlike experiment 1, male filaments were immediately cut after measurement, so that the male with the longer filament in the pair of males became the male with the shorter filament (Table 1). Both males in each trial had their filament length shortened to control for a possible effect of the cut itself (Table 1). Filament length was not measured immediately after cutting to avoid further stress to the individuals. The individuals were left un-manipulated until trial. Filament length was measured a second time just after the trial, and that measurement was used for statistical analyses. As in experiment 1, females had the choice between a male with a

Table 1 Measures of filament length (millimeters) between experiments (means±SE)

	Exp 1	Exp 2 (BS) Before shortening	Exp 2 (AS) After shortening	P value	Equalities
M⁺ males	6.39±0.10	6.25±0.12	2.02±0.07	0.001	Exp 1=exp 2 (BS)
M⁻ males	4.40±0.19	4.46±0.09	3.75±0.09	0.001	Exp 1=exp 2 (BS)
|M⁺−M⁻|	1.99±0.14	1.79±0.09	1.74±0.09	0.31	All

"\|M⁺ −M⁻\|" represents the difference in filament length between the males with longer filaments (M⁺ male) and the males with smaller filaments (M⁻ male) for a given experiment. P values correspond to the results of the linear model that explains filament length by experiment. Equalities show that filament length was not significantly different between experiments (Tukey's tests)

longer filament (M$^+$ male) and a male with a shorter filament (M$^-$ male). The SVL, body mass, hue, chroma, brightness, and tail area did not differ between M$^+$ and M$^-$ males (paired t test, all $P>0.05$). Again, hind foot web area and filament length (before cut) were positively correlated ($r=$ 0.44, $df=118$, $P<0.001$), as a consequence M$^-$ males had more developed hind foot web areas than M$^+$ males (paired t test, $t=3.9$, $df=59$, $P<0.001$).

We performed a study involving five newts to ensure that shortening the male filament would not result in distress. We used the same design describe previously (see Fig. S1). We did not observe any behavioral differences for a male before, immediately after, or 1 week after shortening the filament (Fig. S1). Moreover all males used in experiment 2 behaved normally after filament shortening.

Statistical analysis

In the field experiment, the effect of male SVL and filament length on the time a female spent watching male display (log-transformed) was assessed with a model II regression using an ordinary least squares method.

For the laboratory experiments, we calculated indexes of hind foot web and crest developments as the residuals of a regression of the square root of hind foot web area or tail area on the SVL (Baker 1992). Because morphological traits were strongly correlated, we performed a principal component analysis (PCA) on male traits (SVL, body mass, hue, chroma, and brightness) using the *dimdesc* function of the FactoMineR package in R (R Development Core Team 2010). We did not include filament length, crest development, and hind foot web size in the PCA to fully explore their explanatory potential with model selection. Including or not, filament length, crest development, and hind foot web size in the PCA did not change the overall conclusions of the study. The PCA resulted in principal components of body size (PC$_{body\ size}$, loading on SVL, and body mass), of coloration (PC$_{coloration}$, loading on hue, and chroma), and of brightness (PC$_{brightness}$, loading on brightness; Table S1).

The effects of sexual traits on sperm mass transfer were assessed using generalized linear mixed models with the *lmer* function of the *lme4* package in R (R Development Core Team 2010). Morphological traits (filament length, hind foot web size, crest development, PC$_{body\ size}$, PC$_{coloration}$, and PC$_{brightness}$) and the behavioral trait (courtship activity) were set as fixed factors, and tank, day-of-trial, and trial identity were included as random factors. To investigate the effects of male traits on the number of sperm mass transferred to the females, we used models with a Poisson distribution of error terms and the log link function. We analyzed data and made biological inferences using the information theoretic approach that identifies the best set of models according to several competing hypotheses via information criteria (Burnham and Anderson 2002; Garamszegi et al. 2009; Burnham et al. 2011; Grueber et al. 2011). We evaluated a set of candidate models transferred to combinations of all morphological and behavioral variables and all interaction terms using Akaike's Information Criterion corrected for small sample size (AICc; Burnham and Anderson 2002).We used the *stdz.model* and *top.models* functions of the *arm* and *MuMIn* packages in R to calculate AICc, AICc weight, and other parameters needed for the analysis (Grueber et al. 2011). We present only models that were well supported by the data (models with a ΔAICc <2, Burnham and Anderson 2002). Because several equally likely models were identified and no model had $w >0.90$, we model-averaged parameter estimates ("θ") and the associated variances from the 95 % confidence interval (CI) set of candidate models using the *model.avg* function of the *MuMIn* package in R (Grueber et al. 2011). We considered the effect of one variable as having an important predictive value only when the 95 % CI did not include zero (Garamszegi et al. 2009; D'Alba et al. 2010). We calculated the coefficient of determination (percentage of deviance explained) of the best models as follows: (deviance of the null model − deviance of the model)/deviance of the null model. We additionally used chi-square tests in experiment 1 and experiment 2 to compare the global sperm mass transfer of males with longer and shorter filaments with and without cuts.

Results

Correlative study in the field

In our correlative study in the field, male size and filament length were uncorrelated ($N=33$, $r=0.16$, $P=0.36$). The time spent by a female watching a male display was explained by male filament length ($N=33$, $r=0.42$, $P=$ 0.02, Fig. 1) and not by male size ($N=33$ $r=0.04$, $P=0.81$).

Laboratory experiments

During experiment 1, 89.5 % (34/38) of females accepted at least one sperm mass (60.5 % of females accepted a sperm mass from a M$^+$ male, 18.4 % from a M$^-$ male, and 10.5 % from both males). Among males, 71.1 % of M$^+$ and 28.9 % of M$^-$ males successfully transferred a sperm mass to a female. The latency between the beginning of the experiment and the first courtship display did not differ significantly between M$^+$ and M$^-$ males (Wilcoxon signed rank test, $V=392$, $N=76$, $P=0.98$).

Four models were retained to explain the number of sperm masses transferred in experiment 1, among which the best had a coefficient of determination of 33.3 % (Table S2). Filament
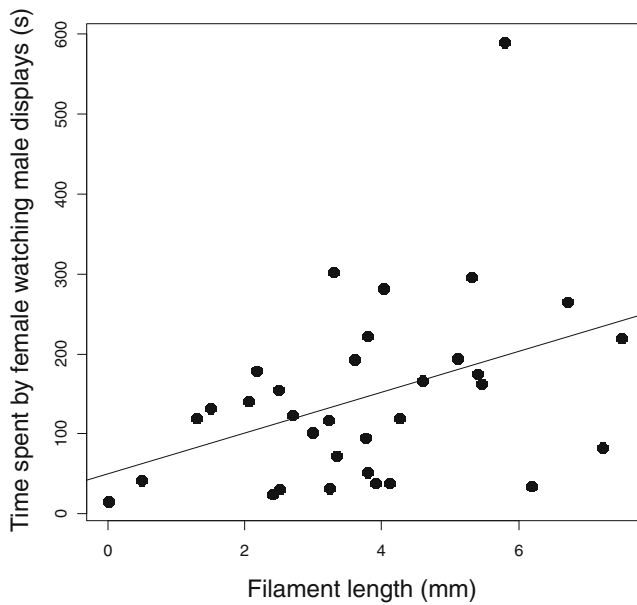
Fig. 1 Relationship between the time spent by a female watching male display and male filament length ($N=33$, y$=25.5x+50.15$, $r=0.42$, $P=0.02$). The variable "Time spent watching" represents the length of the longest bout of the male display to a female

length, hind foot web size, $PC_{body\ size}$, and courtship activity (but not $PC_{brightness}$ and crest development) were included in at least one model (Table S2). The number of sperm masses transferred to a given female was best explained by both male filament length and courtship activity, with females taking more sperm masses from males having longer filaments and higher courtship activity (Table S2, Table 2, Fig. 2).

In experiment 2, the reduction of filament length decreased the number of sperm masses transferred. During the entire experiment, only 53.3 % (32/60) of

females accepted at least one sperm mass, and this number was significantly smaller than in experiment 1 ($\chi^2=12.2$, $df=1$, $P=0.001$). The number of sperm masses transferred of M^- males (after filament shortening) was significantly smaller compared to M^+ males in experiment 1 (Wilcoxon rank sum test, $W=1{,}666.5$, $N=98$, $P<0.001$, Fig. 3), while the number of sperm masses transferred of M^+ males (after filament shortening) was similar compared to M^- males in experiment 1 (Wilcoxon rank sum test, $W=1{,}163$, $N=98$, $P=0.831$, Fig. 3).

In experiment 2, the latency between the beginning of the experiment and the first courtship did not differ between M^+ and M^- males (Wilcoxon signed rank test, $W=832$, $N=120$, $P=0.544$). Five models were selected by AICc and all contained courtship activity (Table S2). The remaining variables included in at least one model were hind foot web size, filament length, and $PC_{coloration}$ (but not $PC_{brightness}$ and crest development; Table S2). The top ranked model explaining male mating success had a coefficient of determination equal to 26 %. Model-average parameter estimates showed that the number of sperm masses transferred increased with courtship activity (Table 2, Fig. 4). However, contrary to experiment 1, filament length had very little influence on the number of sperm masses transferred (Table 2).

Discussion

Our approach combining behavioral observations in the field and in the laboratory showed that female palmate newts value both a morphological and a behavioral trait

Table 2 Effects of morphological and behavioral sexual traits on the number of sperm mass transfers for experiment 1 (exp1) and experiment 2 (exp2)

	Parameter	Estimate	Unconditional SE	Confidence interval	Relative importance
exp1 (no. of sperm mass transfers)	Intercept	−0.52	0.18	(−0.87, −0.17)	
	Hind foot web	0.42	0.25	(−0.08, 0.93)	0.55
	$PC_{body\ size}$	−0.36	0.26	(−0.88, 0.16)	0.44
	Filament length	**1.46**	**0.44**	**(0.61, 2.32)**	**1**
	Courtship activity	**1.06**	**0.35**	**(0.38, 1.74)**	**1**
exp2 (no. of sperm mass transfers)	Intercept	−1.53	0.25	(−2.02, −1.04)	
	$PC_{coloration}$	−0.33	0.32	(−0.96, 0.31)	0.32
	Filament length	−0.13	0.33	(−0.78, 0.51)	0.12
	Hind foot web	−0.53	0.34	(−1.2, 0.14)	0.61
	Courtship activity	**2.48**	**0.47**	**(1.55, 3.41)**	**1**

In exp 1 female had the choice between two males (respectively with long and short filament length). In exp 2, we reversed the relative length of filament before trial. The bold values indicate the variables selected after model averaging (i.e., for which the 95 % confidence interval did not include zero). Fixed effects present in the full models: filament length, hind foot web, crest development, $PC_{body\ size}$, $PC_{coloration}$, $PC_{brightness}$, and courtship activity. For details on the PCA see Table S4
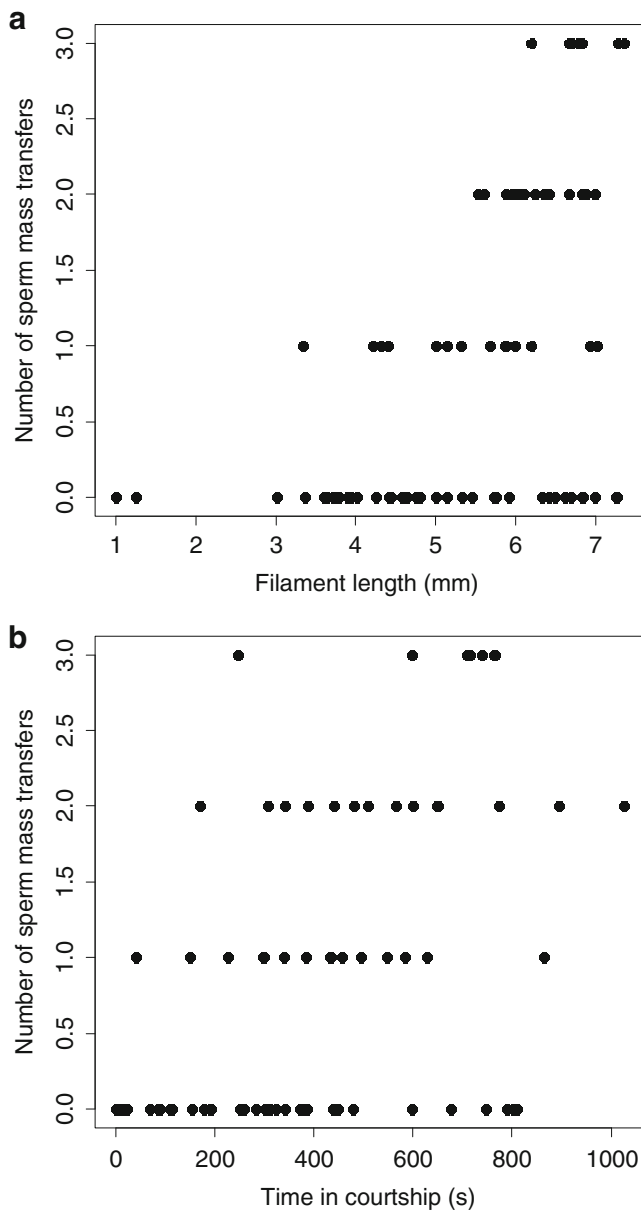
Fig. 2 Relationship between the number of sperm masses transferred and **a** filament length and **b** the time spent in courtship during experiment 1. The females had the choice between a male with a long filament and a male with a short filament length. Filament length was not manipulated

when choosing a mate: the length of the tail filament and courtship display activity. It further suggests that, when filament length is experimentally reduced, females may value the information content of courtship activity over filament length.

Female mate choice for males with long filaments

Direct observations in the field revealed that females spent more time watching males that had longer filaments. Our result is congruent with previous work suggesting that

filament elongation may be the consequence of female preference in this species (Haerty et al. 2007). In a second step, we experimentally tested female preference for filament length in the laboratory. In this experiment, males with longer filaments transferred more sperm masses to females than males with shorter filaments, as predicted by our observations in the wild. However, we could not reject the hypothesis that female preference was directed to an unknown factor correlated with filament length (e.g., hind foot web size). We therefore experimentally reduced filament length, while other traits were not manipulated. This reduction decreased the number of male sperm masses transferred compared to what was observed in the previous experiment. Altogether, our three experiments provided strong evidence that female palmate newts value the development of a morphological ornament, the filament length, to choose their mates. Several not mutually exclusive hypotheses can explain why female palmate newts evolved a preference for males with long filaments. First, such a preference could result from a sensory bias, so that a higher length of filaments catch a female's attention better (Basolo 1990; Ryan and Rand 1990), as filaments strongly resemble the worms that represent a major food resource for (female) newts. Filament length could also be a reliable indicator of male condition, so that only males in good condition can produce and maintain long filaments, as previously reported for interactions between filament length and environmental stress (Secondi et al. 2007, 2009).

In our study, we observed a pre-copulatory female preference for males with longer filament length. However, female newts can accept many sperm masses from the same male and from different males, likely to allow sperm competition and cryptic post-copulatory female choice (Jones et al. 2002; Garner and Schmidt 2003; Jehle et al. 2007). Females of the alpine newt *Mesotriton alpestris* and smooth newt *L. vulgaris* show cryptic mate choice, by favoring sperm of particular males over others after accepting sperm masses (Garner and Schmidt 2003; Jehle et al. 2007). Further work is therefore needed to evaluate true fitness consequences of the preference we observed in terms of number of offspring produced.

Female mate choice for males with high courtship activity

Our results failed to support a direct role of coloration and/or body size on female preference, although there is increasing evidence of the use of coloration for mate choice in amphibians (Rosenthal et al. 2004; Vasquez and Pfennig 2007; Maan and Cumming 2009; Gomez et al. 2009; Doucet and Mennill 2010; Sztatecsny et al. 2010), including newts (Davis and Grayson 2008). Male filament length

Fig. 3 Number of sperm masses transferred during experiments 1 and 2 (without or with filament shortening). M^+ male with longer filament length, M^- male with shorter filament length, *exp 1* without filament shortening, and *exp 2* after filament shortening. The M^- males from this experiment had longer filaments before the cut and the M^+ had smaller filaments before the cut (see Table 1)
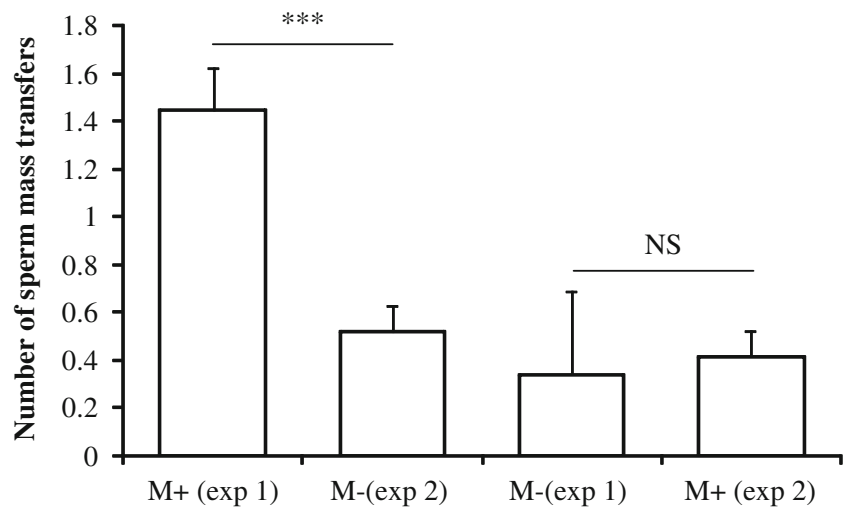
was, however, not sufficient to explain all of the variation in male sperm masses transfer in our study, and courtship activity also strongly predicted male sperm mass transfer. This result indicates that a male with a small filament can acquire access to reproduction if this male has a high court-ship activity and that a male with a long filament but with a very low courtship activity has a low likelihood to breed. The importance of courtship to explain reproductive success raises two conclusions. First, males increased their likeli-hood to breed by increasing their courtship activity, and second, females selected males with the higher courtship activity. Newt courtship activity is well described (Halliday 1975a; 1976), yet its potential to contribute to female mate choice has received surprisingly little attention (but see Vinnedge and Verrell 1998). Females of many species prefer males exhibiting vigorous courtship displays, and measure-ments of courtship energy expenditure show that this activ-ity may be costly (Vehrencamp et al. 1989; Kotiaho et al. 1998; Usherwood 2008; Hasselquist and Bensch 2008; Byers et al. 2010; but see Dearborn et al. 2005). Courtship can thus reveal many aspects of male quality, such as the ability to cope with an immune challenge (Loyau et al. 2005a), body condition (Holzer et al. 2003), ejaculate qual-ity (Matthews et al. 1997; Weir and Grant 2010), or hetero-zygosity (Drayton et al. 2010).

Female mate choice for multiple traits

Our study demonstrates that female palmate newts value multiple traits during mate choice. Why males produce and females assess several traits, and not a single one despite the associated costs, remains an open issue in evolutionary ecology (Møller and Pomiankowski 1993; Candolin 2003; Bro-Jørgensen 2010). During courtship display, males present and agitate their filament in front of females, a behavior likely improving the perception and facilitating the assessment of the

filament length (Rowe 1999). Amplifiers may entail costs by increasing conspicuousness to predators, thereby potentially revealing their ability to escape predators ("amplifying handi-caps" Hasson 1990; 1997; Berglund 2000; Castellano and Cermelli 2010). In line with this idea, male palmate newts can suffer from a high rate of filament predation in habitats with high density of odonates and other insect larvae (JC, personal observation). Because ornamentation acts in conjunction with visual display, it is not clear whether the exhibition of an ornament during courtship should be consid-ered as several signals or a multi-component signal, and whether a visual display could be viewed as an amplifier of the ornament (Hasson 1989; 1990; 1991; Taylor et al. 2000) or whether the filament length may amplify the expression of the behavior (Hebets and Uetz 2000).

Morphological ornamental traits, such as the filament, and behavioral traits (e.g., the courtship) vary in their flex-ibility and may also vary in their reliability, depending on the stability of the ecological and/or social environment (Hill et al. 1999; Loyau et al. 2005a, b; Baird et al. 2007; van Dongen and Mulder 2008; Bro-Jørgensen 2010). For example in field crickets *Gryllus campestris*, females based their mate choice according to carrier frequency (link to harp size development) and chirp rate, which provide reliable information about past and present condition (Scheuber et al. 2003, 2004). Assessing both filament length and courtship activity, female palmate newts could therefore gather information about male past and present conditions to adaptively mate with high-quality males (multiple message hypothesis, Møller and Pomiankowski 1993). However, to date, experimental evidence about the relative flexibility of filament length and courtship activity is lacking in the palmate newt. In newts, morphological sexual traits could indicate male body condition (Green 1991). More-over, the link between behavioral sexual traits and current male quality remains unresolved in newts (but see Halliday and
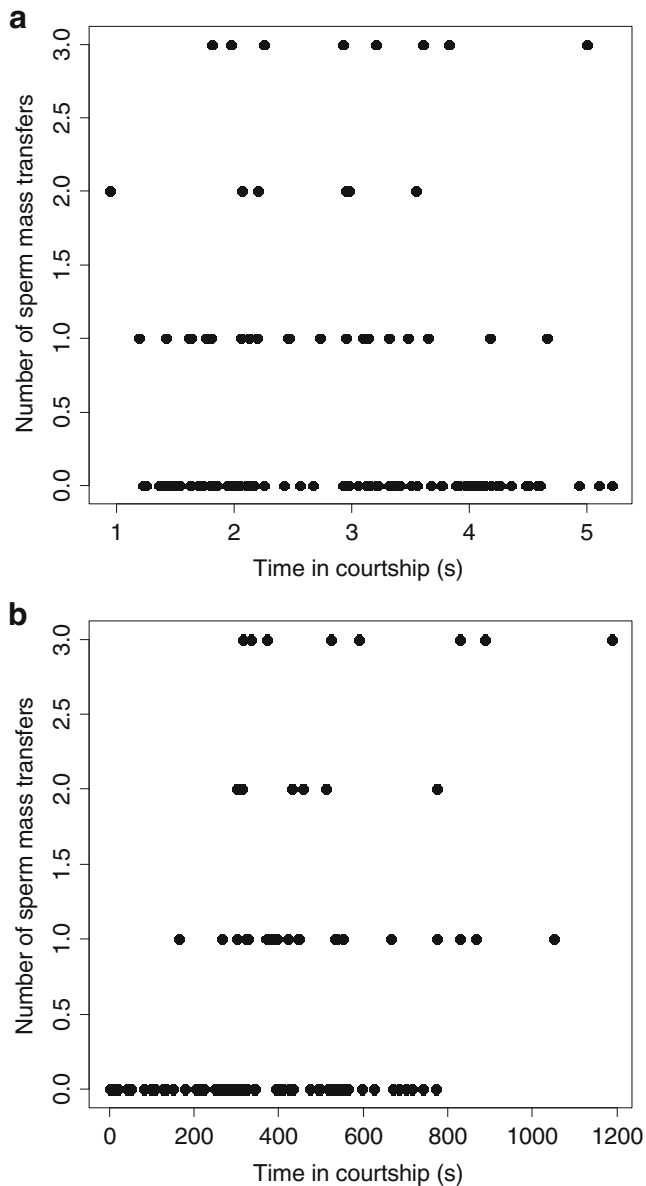
Fig. 4 Relationship between the number of sperm masses transferred and **a** filament length and **b** the time spent in courtship during experiment 2. The females had the choice between a male with a longer filament length and a male with a shorter filament length. Filament length was shortened before trial, so that, in the pair of males, the male with the longer filament length became the male with the shorter filament

Houston 1978). Additional work with newts and other species is needed to clarify if morphological and behavioral sexual traits could give information about past and current male condition.

Female prioritization of behavioral traits over morphological ones?

While evidence is accumulating that females often use multiple traits to choose their mate, relatively few studies have

explored how these traits are processed and integrated. Choosers may assess multiple traits altogether to get a global idea of the overall quality of potential mates, or choosers may value one piece of information over another, depending on the degree to which the information conveyed is error-prone or particularly useful (Hebets and Papaj 2005). Because morphological and behavioral traits strongly differ in their temporal sensitivity to individual condition, females may weigh morphological and behavioral traits differently in the mate choice process. Our results show that when filaments were experimentally reduced and their relative length was reversed, male mating success was explained by courtship activity and not by filament length, suggesting that, in that case, females base their mate choice on courtship activity.

In this experiment, the difference in filament length (2 mm) between the two males was comparable to that in experiments without filament length manipulation. According to the Weber–Fechner law, this difference should be easier to assess for smaller filament length than for longer filaments (Shettleworth 1998). Recently Akre et al. (2011) elegantly demonstrated the relevance of this law during the mate choice process. The authors showed that, for the same discrepancy in ornament size, females were better able to detect the difference in two small ornaments than in two large ornaments. Thus, there is little likelihood that, in our experiment, female newts were not able to detect the difference in filament length after filament manipulation. Instead, our manipulation may have reduced filament length below a certain stimulation threshold, so that females may not have been stimulated by either of the two males. Another interpretation is that females may have ignored an ornament (the filament) as a cue for mate choice because females detected a lack of concordance in the development of two ornaments (the filament length and the hind foot web size) that are normally correlated. Indeed, females may commonly use several signals to avoid mistakes (Johnstone 1996). In a recent experiment in the tree frog *Hyla arborea*, females were provided with the choice between two conflicting sexual traits, attractive call and unattractive vocal sac coloration or unattractive call and attractive vocal sac coloration. This trial resulted in random female mating with respect to the acoustic and visual traits manipulated (Richardson et al. 2010). Finally, our results are consistent with the hypothesis that, when the morphological signals are expressed below a threshold, females may value the information content of behavioral traits over morphological ones because behavioral traits provide more accurate and reliable information on current male condition (Hill et al. 1999; Loyau et al. 2005a; Baird et al. 2007; Bro-Jørgensen 2010).

To conclude, we found that female palmate newts use multiple signals to choose their mates. Hence, in that species,

females value both a morphological trait (the filament) and a behavioral trait (courtship activity). Future investigation should establish whether these two traits that may differ in their temporal flexibility provide different kinds of information to the female.

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