Information-content of morphological and behavioural sexual traits in the Palmate newt (*Lissotriton helveticus*)

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ABSTRACT

The question of why females evaluate more than one sexual trait to choose their mates has received increasing attention in recent years. Here, we investigated the information-content of both morphological and behavioural sexual traits that have been identified as predictors of male reproductive success in the palmate newt, *Lissotriton helveticus*. We examined the co-variation of multiple traits with one aspect of male quality, the male body condition, using both a correlative study and an experimental diet restriction. We found that the development of the three morphological sexual traits (filament length, hind-foot-web size, and crest size) was positively inter-correlated, and was correlated to body condition. In contrast, courtship activity, an important indicator for male reproductive success, was uncorrelated to male body condition. Our results suggest that females likely obtain redundant information on male condition when evaluating filament length, hind-foot-web size and crest size during mate choice. Contrary to our expectations, display activity was not a reliable indicator of male condition, leaving the information-content of this trait unraveled. Our results further suggest that complex, multiple traits may evolve because redundant message, unreliable signals and, possibly, multiple messages can coexist.

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1. Introduction

In many species, females assess several sexual traits during mate choice, such as coloration, morphological structures, song/calls and behavioural displays (Candolin, 2003; Hebets and Papaj, 2005; Bro-Jørgensen, 2010). Usually, females prefer mating with the males that are able to express the most extravagant and conspicuous sexual traits. This preference has been explained by the cost of expression and maintenance of the sexual traits, so that only males of good quality and/or in good condition can afford these costs. The development of a sexual trait, then, reliably signals male quality to the choosing females (Zahavi, 1975). For example, females could base their choice on male coloration because coloration is a good indicator of male body condition (e.g. Griggio et al., 2011) or

male immune abilities to cope with parasites and pathogens (e.g. Hamilton and Zuk, 1982; Faivre et al., 2003; Alonso-Alvarez et al., 2004). Females could also prefer mating with attractive males to gather genes for attractiveness for their male offspring. As long as the trait is heritable and female preference for this trait is also heritable (Fisher, 1930), an exaggerated male trait can evolve even though it is unreliable (i.e. does not indicate quality other than attractiveness).

If females can assess male attractiveness or quality using only one single trait, why do females commonly use multiple traits during mate choice? This question has received growing consideration over the past two decades and continues to be an active field of research (Candolin, 2003; Hebets and Papaj, 2005; Bro-Jørgensen, 2010). To date, several sets of theories have been developed to explain the evolution and maintenance of multiple sexual traits and have been explored with mathematical models (reviewed by Candolin, 2003). Firstly, according to the efficacy-based hypotheses, multiple traits can be selected by their efficacy to produce, transmit or process the signal across environments (Rowe, 1999;

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Hebets and Papaj, 2005). For example, multiple traits may have evolved because they enhance the probability a signal is perceived, making it easier to assess male attractiveness (amplifiers; Harper, 2006) because they influence the receiver's ability to detect or discriminate sexual partners (receiver psychology, Rowe, 1999) or because they allow signal transmission across multiple sensory environments (Hebets and Papaj, 2005).

Although the efficacy-based hypotheses are extremely useful to understand the evolution of multiple signals, they do not address the potential information extracted by the receiver that could be an essential aspect of signal evolution. This issue is addressed by the information content-based hypotheses (Møller and Pomiankowski, 1993; Hebets and Papaj, 2005). In this framework, quality is understood as being different to attractiveness. Three main content-based theories have been proposed: the unreliable signal, the multiple message and the redundant signal hypotheses (Møller and Pomiankowski, 1993). The unreliable signal hypothesis states that male traits do not reflect current male quality/condition. Such traits could evolve because of a pre-existing sensory bias unrelated to female mate choice. Another explanation could be that traits could have ceased to be connected to quality/condition because of the costs of secondary sexual traits and the costs of assessing multiple traits, which could favor a single, most-revealing trait, rendering additional traits unreliable (Møller and Pomiankowski, 1993). Hence, the expression of only one trait may co-vary with an aspect of male quality. The multiple message hypothesis suggests that evolution of multiple traits can also be based on multiple reliable sexual traits, with each trait being related to a different aspect of male quality. The redundant signal hypothesis advocates that each reliable sexual trait can be linked to the same aspect of male quality with a certain error (Møller and Pomiankowski, 1993). Therefore, redundant traits should be inter-correlated, should co-vary with a given measure of individual quality, and mate choice should be more accurate when several traits are used compared to only one trait (Møller and Pomiankowski, 1993; Partan and Marler, 1999; Partan and Marler, 2005). However, an empirical study suggested that these three hypotheses are not necessarily mutually exclusive (Doucet and Montgomerie, 2003). Indeed, these authors showed that, in the Satin bowerbird, *Ptilonorhynchus violaceus*, both the multiple message and the redundant signal hypotheses contribute to explain the evolution of multiple sexual traits. Although Doucet and Montgomerie (2003) predicted that future studies will reveal additional examples of such a "mosaic" of multiple signals, to our knowledge, no further example has been identified so far.

Several approaches have been proposed to experimentally challenge the unreliable signal, the multiple message, and the redundant signal hypotheses (Partan and Marler, 1999, 2005; Candolin, 2003; Hebets and Papaj, 2005). One powerful approach consists of examining the intensity of the receiver's response to each trait separately versus altogether (Partan and Marler, 1999; Partan and Marler, 2005). Similar responses are interpreted as redundant traits, whereas different responses are interpreted as non-redundant traits. However, it is not always possible to manipulate each trait separately with non-invasive methods, and this approach does not explore the potential information-content of the signal. Another approach focuses on the relationships between the traits and their information-content, with similar information-content interpreted as redundant traits (Candolin, 2003; Hebets and Papaj, 2005). Interestingly, these two approaches could lead to opposite conclusions about trait redundancy. Two traits could provide similar responses (i.e. redundant) while reflecting different pieces of information (i.e. non-redundant).

Here, we investigated the evolution of multiple traits using the content-based approach. Our approach entails examining whether the expression of each trait co-varies with male quality (other than attractiveness) to explore the information-content of the trait, testing whether multiple traits co-vary altogether and, if so, positively or negatively. We applied our framework to the palmate newt, *Lissotriton helveticus*, for which we have recently reported that multiple sexual traits of male adult newts influence female mate choice (Cornuau et al., 2012). These traits include both a morphological trait (a caudal filament at the end of the tail) and complex behavioural sexual traits (Cornuau et al., 2012). However, the information-content of these multiple traits is still unknown. In addition to these traits, adult male newts also develop hind-foot-webs and a low dorsal caudal crest, although their role in sexual communication remains unclear (Halliday, 1975; Cornuau et al., 2014). In the present study, we have tested whether the expression of four male traits (filament, hind-food-webs, crest and courtship display) co-vary and correlate with a widely used index of male quality, the male body condition. We assessed the expression of morphological as well as behavioural sexual traits and their natural correlation with male body condition. We further examined how food restriction impacted both morphological and behavioural sexual traits.

2. Materials and methods

2.1. Palmate newts

Male and female adult palmate newts hibernate during the winter period in deep leaf litter. After hibernation, they migrate over land to their reproductive sites, i.e. ponds and water bodies, and develop a strong sexual dimorphism at the beginning of the aquatic phase. Male sexual traits include hind-foot-webs, a caudal filament at the end of the tail, and a low dorsal caudal crest, which start to grow at the very beginning of the breeding period. This development can last for several weeks and likely implies energetic costs, so that males in better condition or with higher lipid storage may be better able to express these traits. In contrast, the development of these traits may be determined by reproductive hormones, i.e. independent of male condition. In addition to morphological traits, male palmate newts express a conspicuous courtship behavior called "fan display" which consists of displaying the tail and filament to the female in a rapid, vibrating fan movement. 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. Previous studies showed that the time spent in courtship, the length of the filament, the size of the hind-foot-webs and potentially the size of the crest contribute to explain male mating success in this species (Haerty et al., 2007; Cornuau et al., 2012). Manipulation of a trait (filament length) suggests that when filament length is reduced, females may prioritize the information-content of the behavioural trait over morphological ones (Cornuau et al., 2012). At the end of the breeding period, male morphological sexual traits decline and disappear, male and female skin becomes dry and rough, and adult newts leave the water.

2.2. Study subjects

Mature adult newts were collected by dip netting from two large ponds at a very early stage of the breeding season. For the experimental study, we collected 126 males and 64 females in a pond near Caumont (Ariège, France; N43.01182, E1.09142, alt. 438 m, February 07 2011). For the correlative study, we captured 123 males in the Etang de Bouteve (Haute-Garonne, France; N42.90295 E0.77374, alt. 1682 m, April 26 2011) to collect morphological data. Because the population sex ratio was strongly male biased at this time of the breeding season, we were unable to obtain enough

females. Therefore, 1 year later, we caught 24 males and 24 females in another pond (Ariège, France; N43.01182, E1.09142, alt. 438 m, April 10 2012) to obtain both morphological and behavioural data.

In contrast to the experimental study, behaviours were observed after only 1 week in captivity to ensure that the behavioural data collected during the experimental study were not biased by the time spent in the laboratory (nearly 1 month). We obtained consistent patterns between populations and years, suggesting that the patterns we observed were not related to the population, the environment or the year.

We used the same protocol to maintain the newts in the laboratory as reported in Cornuau et al. (2012) and applied all precautions against infectious diseases as suggested earlier (Schmeller et al., 2011). Briefly, unisex groups of ten newts were placed in opaque tanks (52 cm × 33.5 cm × 29.5 cm) with aged tap water, plants collected in their native environment, and a clay brick perforated with holes to ensure shelter. Each tank was kept at 18 °C (±1) under fluorescent tubes (ReptiSun 2.0, ZooMed) to simulate natural light with a 12-h light:12-h dark cycle. To allow individual identification, the newts were marked at the base of one of the four legs with subcutaneous injections of colored visible elastomers (VIE, Northwest Marine Technology, Washington, Shaw Island, WA, USA). The newts involved in the correlative study were fed *ad libitum* with larvae of chironomids, daphnia and tubifex during their entire captivity. The newts were released in their respective ponds at the end of the experiment.

2.3. Study 1: Correlative study

As male morphological traits develop over the breeding season, we measured male morphological traits at the beginning and the end of the experiment (i.e. 32 days). The beginning of the experiment corresponds to the beginning of the breeding period, and the end of the experiment corresponds roughly to the peak of the breeding period, as this period can last up to 2 months in the area where the study was conducted. The development of each morphological trait was calculated as the difference between the two measures (Δtrait = trait$_{end}$ − trait$_{start}$). We calculated the change in trait size rather than an absolute trait size to better capture the development potential of each trait.

We recorded body mass with a digital scale (accuracy: 0.01 g). As a measure of body size we used Snout-Vent-Length (SVL) (Băncilă et al., 2010), measured on a photo taken on a millimeter paper background (grid 1 mm^2) using the ImageJ software (http://rsbweb.nih.gov/ij/, see also Cornuau et al., 2012). Similarly, we used photos for measuring filament length, the size of the hind-foot-web (mean of the left and the right hind-foot), and the tail size as an index of crest development (Green, 1991).

To explore the trade-offs between sexual traits and male condition we calculated a body condition index (BCI) for each individual using the residual of the linear regression of the cube root of body mass on SVL, as recommended for amphibian species (Băncilă et al., 2010). The use of the BCI is probably the most widespread and validated measure of male quality (Cotton et al., 2004; Băncilă et al., 2010), reflecting the lipid content in newts (Denoël et al., 2002) and body condition in other species (Cotton et al., 2004).

We measured behavioural sexual traits for 24 males after 1 week in the laboratory. The courtship activity of the males was measured during a 40-min period during which one male could freely interact with one female coming from the same population. The behaviours were recorded using a video camera (Panasonic HDC-TM60, full HD, 1080p) placed above an opaque experimental tank (52 cm × 33.5 cm × 29.5 cm), filled up with aged tap water and sand spread on the bottom. The videos were subsequently analyzed using the software The Observer v7.0 (Noldus Information Technology). In the Palmate newt, the behavioural sexual traits are

composed of several phases. Here, we measured the time spent in courtship display during 40 min as a proxy of courtship activity, which is highly repeatable and not influenced by female features (Cornuau et al., unpublished), but strongly affects male reproductive success (Cornuau et al., 2012). As a proxy for the metabolism of the males, we counted the number of breathings per newt, allowing us to test the hypothesis that courting increases the metabolic rate in this species.

2.4. Study 2: Experimental study

We proceeded as detailed above for the correlative study, except that after 1 week of acclimatization in the laboratory we randomly assigned the individuals to either a high or a low food treatment. The 63 males under high diet were fed with 100 mg of chironom larvae per individual every 2 days complemented with tubifex and daphnia every week for 28 days, while the 63 males under low diet were not fed. This treatment followed from a previous experiment on the condition-dependence of sexual traits in the Smooth newt, *Lissotriton vulgaris* (Green, 1991). The male phenotypes were randomly assigned to the two different treatments, showing no statistical differences at the beginning of the experiment (*t*-tests, all $P > 0.50$). The morphological traits were measured for all males as described for the correlative study at the beginning and after 28 days. The experiment was prolonged for 1 week for a sub-sample of 64 males (32 males per treatment group) to assess courtship activity. The time spent in courtship display was assessed as for Study 1, and morphological traits were measured immediately after the behavioural trial.

2.5. Statistical analysis

Statistical analyses were performed using R (R development core team 2009, version 2.9.2). Assumptions for the parametric analyses were met for both morphological and behavioural variables. We used generalized linear mixed models (GLMMs) with the *gls* function of the *nlme* package. Tank identity was always included in the models as a random factor, and its effect was never significant.

2.5.1. Data analysis of Study 1

We first assessed the relationships between Δfilament length, Δhind-foot-web size, Δcrest size and ΔBCI. All possible relationships between Δfilament length, Δhind-foot-web size and Δcrest size were tested with one morphological sexual trait as a dependent variable, and another morphological trait and SVL included as fixed factors. ΔSVL was included in these models, as the morphological sexual traits can be linked to the body size (Cotton et al., 2004). We then examined whether the development of each morphological trait could be explained by ΔBCI. We built a separate model for each trait and included ΔBCI as a fixed factor in the models. Finally, we assessed the relationships between courtship activity and the predictor variables (BCI, number of breathing events, filament length, hind-foot-web size and crest size measured at the time of the trial). We built a separate model for each predictor variable, with courtship activity as the dependent variable and the predictor as a fixed factor.

2.5.2. Data analysis of Study 2

We first tested whether ΔBCI, Δfilament length, Δhind-foot-web size and Δcrest size were affected by the experimental treatment. We built a separate model for each dependent variable, with the treatment as a fixed factor. We then examined how the relationships between pairs of Δmorphological traits evolved during the experimental treatment. We built models in which the experimental treatment (high/low diet), one Δmorphological trait,

Table 1
Relationships between the developments of the filament length, hind-foot-web size and crest size during the correlative study ($N = 123$, Δtrait = trait$_{end of experiment}$ − trait$_{start of experiment}$, df = degree of freedom, SVL = Snout-Vent-Length).

Response	Predictor	df	$F_{1,120}$	P
ΔFilament	ΔHind-foot-web	1	15.001	<0.001
	ΔSVL	1	27.095	<0.001
	ΔCrest	1	73.695	<0.001
	ΔSVL	1	2.882	0.092
ΔHind-foot-web	ΔFilament	1	21.122	<0.001
	ΔSVL	1	0.349	0.555
	ΔCrest	1	19.779	<0.001
	ΔSVL	1	0.165	0.685
ΔCrest	ΔFilament	1	76.594	<0.001
	ΔSVL	1	7.515	0.007
	ΔHind-foot-web	1	24.308	<0.001
	ΔSVL	1	17.62	<0.001

Table 2
Relationships between the developments of the filament length, hind-foot-web size and crest size during the experimental study ($N = 126$, Δtrait = trait$_{end of experiment}$ − trait$_{start of experiment}$, df = degree of freedom, SVL = Snout-Vent-Length).

Response	Predictor	df	$F_{1,121}$	P
ΔFilament	ΔHind-foot-web	1	86.17	<0.001
	Experimental treatment	1	0.82	0.367
	ΔSVL	1	0.02	0.888
	ΔHind-foot-web: Experimental treatment	1	0.096	0.757
	ΔCrest	1	82.093	<0.001
	Experimental treatment	1	0.166	0.684
	ΔSVL	1	0.001	0.98
	ΔCrest: Experimental treatment	1	0.958	0.33
ΔHind-foot-web	ΔFilament	1	138.209	<0.001
	Experimental treatment	1	68.303	<0.001
	ΔSVL	1	4.511	0.038
	ΔFilament: Experimental treatment	1	2.637	0.107
	ΔCrest	1	196.946	<0.001
	Experimental treatment	1	26.54	<0.001
	ΔSVL	1	1.012	0.316
	ΔCrest: Experimental treatment	1	4.156	0.044
ΔCrest	ΔFilament	1	113.214	<0.001
	Experimental treatment	1	36.278	<0.001
	ΔSVL	1	7.808	0.006
	ΔFilament: Experimental treatment	1	1.544	0.216
	ΔHind-foot-web	1	175.205	<0.001
	Experimental treatment	1	3.917	0.05
	ΔSVL	1	4.313	0.04
	ΔHind-foot-web: Experimental treatment	1	5.444	0.021

the interaction between the Δmorphological trait and the experimental treatment, and ΔSVL were included as fixed factors. Finally, we explored the impact of ΔBCI on Δmorphological traits with models including ΔBCI, the experimental treatment, the interaction between ΔBCI and experimental treatment, and ΔSVL as fixed factors.

We examined the impacts of the treatment, number of breathings, BCI, filament length, hind-foot-web size and crest size on courtship activity with GLMMs. We built a separate model for each predictor, including the predictor and the treatment as fixed factors.

3. Results

3.1. Study 1: Correlative study

The development of filament length, hind-foot-web size and crest size was significantly and positively linked during the experiment (Table 1). The development of filament length, hind-foot-web size and crest size was also positively explained by the development of BCI during the experiment (Δfilament length: $F_{1,121} = 6.362$, $P = 0.013$; Δhind-foot-web size: $F_{1,121} = 22.018$, $P < 0.001$; Δcrest size: $F_{1,121} = 34.822$, $P < 0.001$).

We observed an important variation in the courtship activity of males (mean = 814s, sd = 415, min = 0s and max = 1736s) and the higher the courtship activity, the higher was the number of breathings ($F_{1,22} = 4.662$, $P = 0.042$). Our data further indicates that courtship activity was not explained by male body condition at the time of the trial ($F_{1,22} = 0.138$, $P = 0.713$) and was not linked to any of the three morphological sexual traits at the time of the trial (filament length: $F_{1,22} = 1.024$, $P = 0.323$; hind-foot-web size: $F_{1,22} = 0.032$, $P = 0.859$; crest size: $F_{1,22} = 1.628$, $P = 0.215$).

3.2. Study 2: Experimental study

The BCI was significantly affected by the diet treatment, with a constant BCI in the high diet treatment group and a decrease of BCI in the low diet treatment group ($F_{1,124} = 176.752$, $P < 0.001$, Fig. 1a). The diet treatments also impacted the development of the three morphological sexual traits, with filament length, hind-food-web and crest size increasing significantly more in the high diet treatment group as compared to the low diet treatment group (Δfilament length: $F_{1,124} = 20.758$, $P < 0.001$; Δhind-foot-web size: $F_{1,124} = 96.038$, $P < 0.001$; Δcrest size: $F_{1,124} = 62.916$, $P < 0.001$; Fig. 1). Similarly to our correlative study, the development of filament length, hind-foot-web size and crest size were significantly and positively linked (Table 2, Fig. 2). The development of the three morphological sexual traits was also

significantly linked to the ΔBCI (Table 3). This relationship was independent of the diet treatment, except for crest size where the relationship was stronger in the low diet group than in the high diet group (low diet: $F_{1,62} = 4.665$, $P = 0.035$; high diet: $F_{1,62} = 8.379$, $P = 0.005$; Table 3).

In our experimental study, we observed a high variation in the courtship activity of males (mean = 681s, sd = 337, min = 0s and max = 1451s), as well. The diet treatment did not impact the courtship activity ($F_{1,62} = 0.025$, $P = 0.874$), and our data shows a clear relationship between the courtship activity and the number of breathings (breathings: $F_{1,60} = 8.882$, $P = 0.004$). The courtship activity was unrelated to the BCI and independent of the diet treatment (BCI: $F_{1,60} = 1.046$, $P = 0.311$; treatment: $F_{1,60} = 0.058$, $P = 0.810$). Further, the morphological sexual traits did not explain the courtship activity (Δfilament length: $F_{1,60} = 2.824$, $P = 0.098$; Δhind-foot-web size: $F_{1,60} = 0.947$, $P = 0.334$; Δcrest size: $F_{1,60} = 0.263$, $P = 0.610$) in both diet treatments (Δfilament length: $F_{1,60} = 0.633$, $P = 0.429$; Δhind-foot-web size: $F_{1,60} = 2.185$, $P = 0.145$; Δcrest size: $F_{1,60} = 0.092$, $P = 0.763$).

4. Discussion

Mate choice is often based on several morphological and behavioural sexual traits (Candolin, 2003; Hebets and Papaj, 2005; Bro-Jørgensen, 2010), as also found for the palmate newt (Cornuau
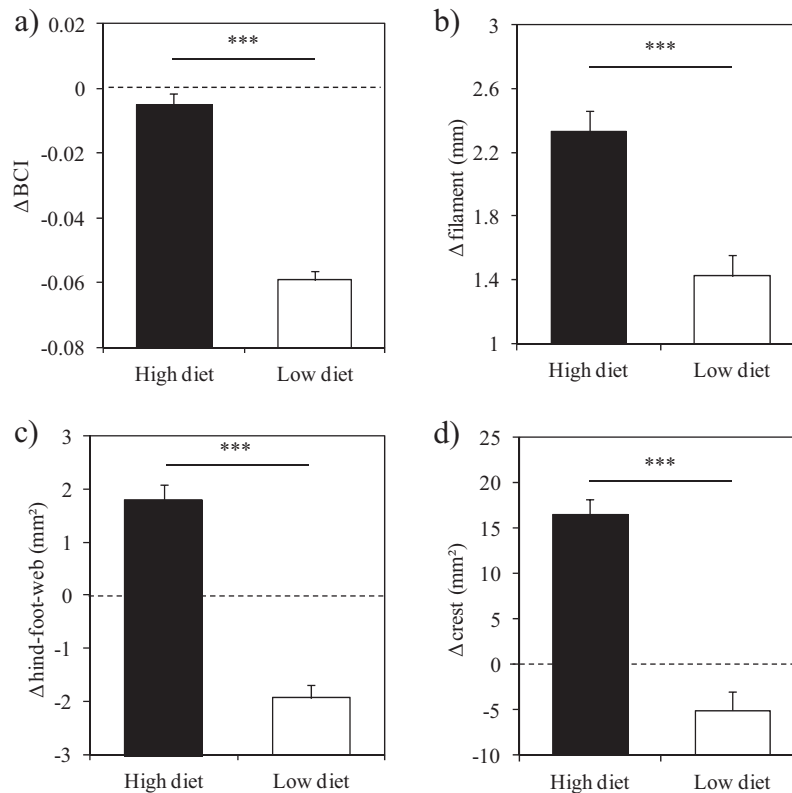
Fig. 1. Effect of the diet treatment on the development of (a) BCI (body condition index), (b) filament length, (c) hind-foot-web size, and (d) crest size ($N = 63$ per treatment). We show the mean and the standard error of the mean. Differences between treatments were all significantly different (all $P < 0.001$). (Δtrait = trait$_{end of experiment}$ − trait$_{start of experiment}$).

et al., 2012). Here, we investigated the information-content of multiple sexual traits in the palmate newt with both a correlative and an experimental study that provided congruent results. We found a correlation between the three morphological sexual traits measured here and a positive correlation with male condition, suggesting that filament length, hind-foot-web size and crest size have the potential to reliably signal male condition. The diet restriction efficiently altered male condition and the expressions of

Table 3
Relationships between the developments of the three morphological sexual traits (filament length, hind-foot-web size, crest size) and BCI (body condition index) during the experimental study ($N = 126$, Δtrait = trait$_{end of experiment}$ − trait$_{start of experiment}$, df = degree of freedom, SVL = Snout-Vent-Length).

Response	Predictor	df	$F_{1,121}$	P
ΔFilament	ΔBCI	1	23.919	<0.001
	Experimental treatment	1	3.042	0.084
	ΔSVL	1	7.19	0.008
	ΔBCI: Experimental treatment	1	0.161	0.689
ΔHind-foot-web	ΔBCI	1	106.193	<0.001
	Experimental treatment	1	22.459	<0.001
	ΔSVL	1	21.455	<0.001
	ΔBCI: Experimental treatment	1	0.942	0.334
ΔCrest	ΔBCI	1	96.658	<0.001
	Experimental treatment	1	8.693	0.004
	ΔSVL	1	35.466	<0.001
	ΔBCI: Experimental treatment	1	4.032	0.047

the morphological traits, confirming a correlative link. Therefore, the three morphological traits may provide redundant messages to the female. However, they did not correlate with courtship activity. Courtship activity was also unrelated to male condition and remained unaffected by the diet restriction. Hence, courtship activity may either be an unreliable signal, or may signal an unknown aspect of male quality.

A first assumption of the redundant message hypothesis is that the traits conveying information should co-vary. The development of filament length, crest area and hind-foot-web area was positively correlated in our studies. Our experimental treatment clearly showed that these positive relationships were true in different environments (high and low food availability). It further shows that the production of one trait does not endorse an energy or physiological constraint for the production and maintenance of the other traits.

A second assumption of the redundant message hypothesis is that all traits should co-vary with one aspect of the sender's condition (Hebets and Papaj, 2005). In agreement with this assumption, all three morphological sexual traits were good predictors of male condition. Hence, the information in these traits appears to be redundant, supporting the redundant signal hypothesis (Partan and Marler, 2005; Hebets and Papaj, 2005) and adding evidence to the few other experimental studies supporting redundant signals (birds: Birkhead et al., 1998; Jawor et al., 2004; Roulin et al., 2011; frogs: Vásquez and Pfennig, 2007; lizard: Martǐn and López, 2010, and spiders: Gibson and Uetz, 2008). Morphological sexual traits usually grow at the start of the breeding season in newts, when males arrive at the breeding site. The information contained in these morphological traits may relate to the food intake at the beginning of the breeding season, allowing the female to obtain reliable information on overall male quality, increasing the
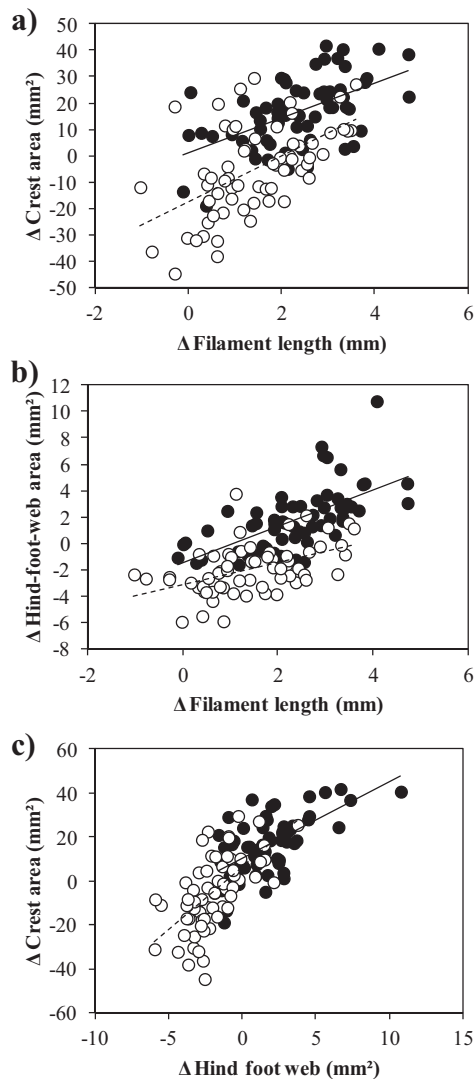
Fig. 2. Relationships between the development of the three morphological sexual traits (filament length, hind-foot-web area and crest area) in high (filled dots) and low diet treatment (open dots; $N = 63$ for each treatment). (Δ trait $= $ trait$_{end\ of\ experiment}$ $-$ trait$_{start\ of\ experiment}$).

probability of choosing a high quality male (Møller and Pomiankowski, 1993).

A third assumption of the redundant message hypothesis is that the probability of choosing a high quality male is increased when females assess two or more male traits when choosing a mate (Møller and Pomiankowski, 1993, see also Partan and Marler, 1999). This assumption is generally difficult to test. With our study organisms, a separate manipulation of different traits was impossible without harming the individuals. However, an earlier experiment (Cornuau et al., 2012) strongly suggests that this third assumption of the redundant message hypothesis might also be fulfilled in the palmate newt. In that former study, we explored female mate choice by manipulating filament length. When the relative filament length between two males was experimentally reversed (the higher became the smaller), the choice of the females was not completely reversed, suggesting that two or more traits may be evaluated by female newts to make the best mate choice. However, further experiments with independent manipulation of multiple sexual traits are needed to confirm this hypothesis (see Poole and Murphy, 2007 for an example on the barking tree frog *Hyla gratiosa*).

Males with higher courtship activity have higher reproductive success; but despite an apparent basal metabolic cost of courtship revealed by the number of breath events, courtship activity was unaffected by body condition as well as by food availability. It was also unaffected by an experimental injection of LPS, a potent activator of the immune system (Cornuau et al., 2014). One explanation could be that courtship activity is an unreliable signal, i.e. a Fisherian trait unrelated to aspects of male condition, whose expression is merely determined by reproductive hormones. In line with this idea, nitrate, an endocrine, nervous and immune disruptor, impacted the probability to court, with exposed males being more likely to court compared to unexposed males (Secondi et al., 2013). Courtship display could also contain information on aspects of male quality that were not assessed in this study, such as parasitic load or health status (Lailvaux and Kasumovic, 2011), immune capacity (Loyau et al., 2005; but see Cornuau et al., 2014), sperm quality (Chargé et al., 2010), locomotor ability (Byers et al., 2010), or heterozygosity (Drayton et al., 2010).

In a previous study on the palmate newt, we found that male mating success was mainly explained by display activity and, to a lesser extent, by morphological sexual traits (Cornuau et al., 2012). Why females base their choice primarily on the apparently unreliable trait rather than on the reliable ones may appear intriguing. However, male display activity may contain important information (as yet not unraveled by our study) which the females prioritize over the information on male body condition. Another possible explanation is that the reliable traits may not be reliable in every environment or when the environment has changed, with the risk that the environment the sire has experienced differs from the one his offspring will experience, lowering indirect benefits of female mate choice (Bro-Jørgensen, 2010). In contrast, if the sons inherit their father's display activity and the daughters inherit their mother's preference for this trait (Fisher, 1930), the adaptive significance of the female preference for such a behavioural trait remain high in any environment experienced by the offspring.

In our study the three morphological sexual traits were inter-correlated and contained information related to male body condition. In contrast, the three sexual traits were not correlated to courtship activity, an important behavioural trait allowing access to reproduction (Cornuau et al., 2012). Our study showed that females can evaluate the same aspect of male quality using different morphological sexual traits (redundant message hypothesis), and other independent male characteristics using behavioural sexual traits (multiple message and/or unreliable signal hypothesis). Hence, our study supports the notion that the three content-based hypotheses are not mutually exclusive (Doucet and Montgomerie, 2003). For future studies, it would be of great interest to investigate the multiple signal theory using efficacy-based components (Hebets and Papaj, 2005).

Ethical standards

The experiments comply with the current laws of the country in which they were performed.

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References

Alonso-Alvarez, C., Bertrand, S., Devevey, G., Gaillard, M., Prost, J., Faivre, B., Sorci, G., 2004. An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals an antioxidant activity. Am. Nat. 164, 651–659.

Băncilă, R.I., Hartel, T., Plaiasu, R., Smets, J., Cogalniceanu, D., 2010. Comparing three body condition indices in amphibians: a case study of yellow-bellied toad *Bombina variegate*. Amphib. Reptil. 31, 558–562.

Birkhead, T.R., Fletcher, F., Pellatt, E.J., 1998. Sexual selection in the zebra finch *Taeniopygia guttata*: condition, sex traits and immune capacity. Behav. Ecol. Sociobiol. 44, 179–191.

Bro-Jørgensen, J., 2010. Dynamics of multiple signaling systems: animal communication in a world in flux. Trends Ecol. Evol. 25, 292–300.

Byers, J., Hebets, E., Podos, J., 2010. Female mate choice based upon male motor performance. Anim. Behav. 79, 771–778.

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

Chargé, R., Saint Jalme, M., Lacroix, F., Cadet, A., Sorci, G., 2010. Male health status, signalled by courtship display, reveals ejaculate quality and hatching success in a leking species. J. Anim. Ecol. 79, 843–850.

Cornuau, J.H., Rat, M., Schmeller, D.S., Loyau, A., 2012. Multiple signals in the palmate newt: ornaments help when courting. Behav. Ecol. Sociobiol. 66, 1045–1055.

Cornuau, J.H., Schmeller, D.S., Pigeault, R., Loyau, A., 2014. Resistance of morphological and behavioral sexual traits of the palmate newt (*Lissotriton helveticus*) to bacterial lipopolysaccharide treatment. Amphib. Reptil. 35, 63–71.

Cotton, S., Fowler, K., Pomiankowski, A., 2004. Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? Proc. R. Soc. Lond. B 271, 771–783.

Denoël, M., Hervant, F., Schabetsberger, R., Joly, P., 2002. Short- and long-term advantages of an alternative ontogenetic pathway. Biol. J. Linn. Soc. 77, 105–112.

Doucet, S.M., Montgomerie, R., 2003. Multiple sexual ornaments in satin bowerbirds: ultraviolet plumage and bowers signal different aspects of male quality. Behav. Ecol. 14, 503–509.

Drayton, J.M., Milner, R.N.C., Hunt, J., Jennions, M.D., 2010. Inbreeding and advertisement calling in the cricket *Telegryllus commodus*: laboratory and field experiments. Evolution 64, 3069–3083.

Faivre, B., Grégoire, A., Préault, M., Cézilly, F., Sorci, G., 2003. Immune activation rapidly mirrored in a secondary sexual trait. Science 300, 103.

Fisher, R.A., 1930. The Genetical Theory of Natural Selection. Clarendon Press, Oxford.

Fisher, M.C., Schmidt, B.R., Henle, K., Schmeller, D.S., Bosch, J., Aanensen, D.M., Garner, T.J.W., 2012. RACE: Risk Assessment of Chytridiomycosis to European Amphibian Biodiversity Froglog 101, 45–47.

Gibson, J.S., Uetz, G.W., 2008. Seismic communication and mate choice in wolf spiders: components of male seismic signals and mating success. Anim. Behav. 75, 1253–1262.

Green, A.J., 1991. Large male crests, an honest indicator of condition, are preferred by female smooth newts, *Triturus vulgaris* (Salamandridae) at the spermatophore transfer stage. Anim. Behav. 41, 367–369.

Griggio, M., Valera, F., Cassas-Crivillé, A., Hoi, H., Barbosa, A., 2011. White tail markings are an indicator of condition and affect mate preference in rock sparrows. Behav. Ecol. Sociobiol. 65, 655–664.

Haerty, W., Gentilhomme, E., Secondi, J., 2007. Female preference for a male sexual trait uncorrelated with male body size in the palmate newt (*Triturus helveticus*). Behaviour 144, 797–814.

Halliday, T.R., 1975. On the biological significance of certain morphological characters in males of the Smooth newts *Triturus vulgaris* and the Palmate newt *Triturus helveticus* (Urodela: Salamandridae). Zool. J. Linnean Soc. 56, 291–300.

Hamilton, W.D., Zuk, M., 1982. Heritable true fitness and bright birds: a role for parasites? Science 218 (4570), 384–387.

Harper, D.G.C., 2006. Maynard Smith: amplifying the reasons for signal reliability. J. Theor. Biol. 239, 203–209.

Hebets, E.A., Papaj, D.R., 2005. Complex signal function: developing a framework of testable hypotheses. Behav. Ecol. Sociobiol. 57, 197–214.

Jawor, J.M., Gray, N., Beall, S.M., Breitwisch, R., 2004. Multiple ornaments correlate with aspects of condition and behaviour in female northern cardinals, *Cardinalis cardinalis*. Anim. Behav. 67, 875–882.

Lailvaux, S.P., Kasumovic, M.M., 2011. Defining individual quality over lifetimes and selective contexts. Proc. R. Soc. Lond. B 278, 321–328.

Loyau, A., Saint Jalme, M., Cagniant, C., Sorci, G., 2005. Multiple sexual advertisements honestly reflect health status in peacocks (*Pavo cristatus*). Behav. Ecol. Sociobiol. 58, 552–557.

Martı́n, J., López, P., 2010. Multimodal sexual signals in male ocellated lizards *Lacerta lepida*: vitamin E in scent and green coloration may signal male condition in different sensory channels. Naturwissenschaften 97, 545–553.

Møller, A.P., Pomiankowski, A., 1993. Why have birds got multiple sexual ornaments? Behav. Ecol. Sociobiol. 32, 167–176.

Partan, S.R., Marler, P., 1999. Communication goes multimodal. Science 283, 1272–1273.

Partan, S.R., Marler, P., 2005. Issues in the classification of multimodal communication signals. Am. Nat. 166, 231–245.

Poole, K.G., Murphy, C.G., 2007. Preferences of female barking treefrogs, *Hyla gratiosa*, for larger males: univariate and composite tests. Anim. Behav. 73, 513–524.

Roulin, A., Almasi, B., Meichtry-Stier, S., Jenni, L., 2011. Eumelanin- and phenomelanin-based colour advertise resistance to oxidative stress in opposite ways. J. Evol. Biol. 24, 2241–2247.

Rowe, C., 1999. Receiver psychology and the evolution of multicomponent signals. Anim. Behav. 58, 921–931.

Schmeller, D.S., Loyau, A., Dejean, T., Miaud, C., 2011. Using amphibians in lab studies—precautions against the emerging infectious disease Chytridiomycosis. Lab. Anim. 45, 25–30.

Secondi, J., Lepetz, V., Cossard, G., Sourice, S., 2013. Nitrate affects courting and breathing but not escape performance in adult newts. Behav. Ecol. Sociobiol. doi:10 1007/s00265-013-1583-9.

Vásquez, T., Pfennig, K., 2007. Looking on the bright side: females prefer coloration indicative of male size and condition in the sexually dichromatic spadefoot toad, *Scaphiopus couchii*. Behav. Ecol. Sociobiol. 62, 127–135.

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