Female preference for male dorsal crests in great crested newts (*Triturus cristatus*)

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Studies of mate choice in great crested newts have established a difficulty in separating the visual constituent of the male’s dorsal crest from its importance to cutaneous respiration and the conveying of pheromones during courtship. We used image manipulation to test if size differences in the dorsal crest alone can be evaluated visually by female newts, controlling for other differences between males. Females responded well to the experimental design and did not remain in front of simulated male ‘models’ independently of differences in dorsal crest height. Instead, they spent more than twice as much time in front of the manipulated high-crested male, than in front of the non-manipulated low-crested male, which was significant also after controlling for zone area. However, the design failed to determine if females remained true to their first choice, probably due to a combination of low sample size and male ‘models’ remaining unnaturally indifferent to female interest.

**KEY WORDS:** Salamandridae, sexual selection, mate choice, ornamentation.

**INTRODUCTION**

The great crested newt, *Triturus cristatus*, is a pond-breeding caudate amphibian (family Salamandridae) currently in focus as a concern for conservation across Europe (see, e.g., THIESMEIER & KUPFER 2000, LANGTON et al. 2001, ARNTZEN 2003, EDGAR & BIRD 2006). This newt belongs to a group of species particularly well studied as models in reproductive biology (reviewed by HALLIDAY 1990, HALLIDAY & ARANO 1991, HALLIDAY & TEJEDO 1995), and has a set of characteristics with interesting implications for evolutionary ecology.

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(reviewed in Malmgren 2001). Particularly, males aggregate in arenas to display to females during courtship, participating in mating settings similar to true leks (Hedlund & Robertson 1988, Arntzen & Sparreboom 1989, MacGregor et al. 1990, Zuiderwijk 1990, Höglund & Alatalo 1995). The potential significance of male crests to female mate choice in these newts was already adduced by Darwin (1874: 359-360) to illustrate the principle of sexual selection. However, in spite of attempts (e.g., Malacarne & Cortazza 1983, Hedlund 1990a), it has proven difficult to separate the visual constituent of the crest from its potential importance to cutaneous respiration and the conveying of pheromones during courtship (e.g., Malacarne & Vellano 1987, Cogălniceanu 1994, Griffiths 1996, Yamamoto et al. 2000, Secondi et al. 2005). The combination of nocturnal habits and an overall complexity of behavioural repertoires have further complicated a resolution to the problem.

In this paper we report results from an experiment conducted to test the hypothesis that the dorsal crest alone can act as a visual attractant for mate choice to female great crested newts, using digital image manipulation and flat screen projection. We thus controlled for the effect of male variation in morphology and behaviour to focus on the visual constituent of the crest, eliminating the potential contribution by other functions of the crest and variation in male condition and performance.

METHODS

Unmated adult female great crested newts (n = 21) were collected at a drift fence with pitfall traps during a spring migration episode in April 2003, when they moved from terrestrial hibernation quarters to a breeding pond in south-central Sweden (Lillsjön, 16 km W Örebro: 59°15’N 14°58’E). They were transported to a laboratory housing facility, where they were kept individually in small aquaria (340 × 190 × 210 mm) with aerated water and ventilated lids. Housing conditions were held at 17 °C with softened ambient lighting. All individuals were allowed 2 days of acclimatisation to captive conditions before being used in experiments, and were fed chopped earthworms at regular intervals. All in all, females were kept captive for 7 days before being released at the site of capture.

An experimental arena was constructed using a specially designed aquarium (length of sides 575 × 135 (2) × 335 (2, for projection) × 150 mm, height 230 mm), two flat computer screens (15” TFT LCD) and a Plexiglas cylinder (height 300 mm, inner diameter 145 mm). After construction and careful washing, the arena was conditioned with 0.1 litres of water from each female’s housing aquaria and then filled up with aerated water. We assumed that this would convince the females that other females were present, making them more secure with the artificial situation. It also eliminated the need for rigorous cleansing of the arena between trials.

We produced two male ‘models’ that only differed in respect of their dorsal crests, to be used for mate choice simulations. A photograph of a male great crested newt in early breeding dress was used as a source image for ‘both’ males in the test. The image was cleaned from background information and minor adjustments in contrast and sharpness was performed using standard digital image software. Male body size was scaled to the mean and median size of females used in the experiment. Females averaged 79.8 mm ± 0.6 SE (ranging from 73.6 to 86.3 mm, median 80.2) in snout-vent
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length (SVL, measured from the tip of the snout to the posterior end of the cloacal lips), and we therefore scaled the male to 80.0 mm SVL. A male of this size is generally found in the upper quartile of the size range in populations of great crested newts where details on size are available (J.C. Malmgren unpubl. data). We cloned the original male image (Fig. 1A) and enhanced the crest by digital image manipulation (cut, copy, paste and minor cloning), arbitrarily adding 137.5% in height and 91.3% in surface area, to produce a competing male with a dorsal crest typical to males in breeding condition during the peak of breeding activity (Fig. 1B). The images were finalised by being placed against a semi-natural soft and murky background colour gradient, facing each other and centred low in each screen area. A subtle synchronised movement loop was assigned to both males by slightly tilting their noses down by 1° and back again; to better catch the attention of females. The images were transformed into two avi movie files; one with the larger-crested male to the right and the lower-crested male to the left, and a mirrored vice versa. The virtual ‘models’ were displayed on two flat LCD monitors that were pressed against the outside of the aquarium. Simulations were run in full screen mode, covering the two screens at a resolution of 2048 × 768 (each at 1024 × 768), on a PC system using a graphics card with multi-screen functionality. Thus, two slightly different copies of a single male were simultaneously projected in synchronised animation sequence in each of two adjacent display surfaces in the aquarium (Fig. 2). The aim was to present females with the illusion that two males, identical in all respects

Fig. 1. — Images of great crested newt male ‘models’ used in behavioural experiments on the dorsal crest as a visual attractant for female choice. (A) Original image source and model for a male with low crest, and (B) manipulated image in which the dorsal crest has been extended upwards, adding 137.5% in height and 91.3% in surface area, acting as a model for a male with a high crest. The two males were assumed to compete for female attention, and were identical in all respects but their dorsal crest.
but their dorsal crests, competed for her attention at the bottom of a pond, simulating a semi-natural scenario.

Because great crested newts are predominantly nocturnal, the experiment was conducted in a darkened room. The walls of the aquarium, except for those surfaces facing the monitors, were covered with black cloth to prevent females being distracted by outside disturbances. The arena was invisibly divided into three bottom zones (excluding the surface layer); one neutral, in which we treated females as resting or retreating away from males, and two triangular zones on each side of the centre in the aquarium — immediately in front of the screens. A trial was started by placing the Plexiglas cylinder in the neutral zone, placing a female inside and allowing her to acclimatise for 5 min. During this time she had a clear view of the entire experimental arena and the two displaying males. The cylinder was thereafter removed and behavioural recording commenced by recording the time females spent in each zone during a total trial time of 15 min. We paid particular interest to the time portions females used in front of the male models, and subsequently used these as an indicator of female interest for each male. All trials were taped on video for documentation purposes. Females were immediately returned to their housing quarters after taking part in a trial, allowing them to rest and feed for 3 days before being subjected to a second trial in which the position of the males were reversed.

RESULTS

Individual data \( (n = 21) \) were pooled for both trials to calculate the mean total time use in relation to the a priori zones, correcting also for differences in zone area. Females clearly used more time residing in refuge in the neutral zone than in front of male images (Fig. 3). Most likely, this outcome was mainly due to females either resting or retreating as a first response to the experimental situation. However, as they settled in the artificial environment

![Fig. 2. — Schematic illustration showing the experimental arena and the trial situation from the female’s view. Two male models, identical in all respects but for their dorsal crests, were displayed on two screens in the arena. A synchronised movement loop was assigned to the males to evoke the interest of females in trials. The time budget and relative position of females were recorded to keep track of behaviour as an indicator of female preference.](image)
they soon began to explore the experimental arena. The time portion used by females in front of male images averaged almost a third (32%) of the total time in trials. Four females did not spend any time in front of male images and these were excluded from the data set, leaving as a conservative approach $N = 17$ for subsequent tests. A closer analysis of the mean total time females were spending in front of either male image during repeated trials revealed that they used more than twice as much time in the area closest to the male with the higher crest, mean 476 sec ± 99 SE, than in front of the male with the lower crest, mean 232 sec ± 65 SE. The difference was significant (one-tailed paired $t$-test: $t_{16} = 1.95, P = 0.034$), and consistent also when the data were corrected for differences in zone area (Fig. 3, $P < 0.05$). We used one-tailed tests since our main hypothesis of female preference for greater crests predicted a directed difference. To test if individuals behaved consistently, we calculated the difference in time spent in front of either male for each individual and both trials. These differences were then used to derive a mean difference for each individual (positive if more time was spent in front of the male

![Graph showing mean time per area unit (sec/cm²) spent by female great crested newts in each categorical zone.](image)

Fig. 3. — Mean total time (sec) per area unit (cm²) spent by female great crested newts in each categorical zone at the bottom of the arena during experimental trials (data pooled for two trials per individual). The negligible fraction of time spent in the surface layer is excluded here, as is three individuals that failed to reside in front of males in both trials. Error bars show standard errors around the mean. The neutral zone was a place of refuge away from the display zones, where the images of two courting males were projected. The two males differed only with respect to their dorsal crest, one having a high crest produced through image manipulation and the other having a low crest.
with the higher crest), summed and averaged for all individuals. The derived grand mean, 125.6 sec ± 62.4 SE, was significantly different from zero (one-tailed \( t \)-test: \( t_{16} = 2.01, P = 0.031 \)), and we rejected the null hypothesis of no preference in favour of female preference for remaining longer in front of the male with the higher crest.

To differentiate between female preferences we tested if females (1) remained in front of the male images independently, and (2) if they remained true to their first choice in repeated trials. In both cases we assumed as the null hypothesis that females behaved independently of male dorsal crest differences. Of the 21 females used in the experiment, only 12 returned to repeat their activities in front of the male images in their second trial. This limitation of sample size weakened the opportunities for a full repeatability analysis of female preference. However, the evenly distributed data (presented in Table 1) allowed for at least a limited test. Based on these results, females on average chose to remain in front of the high-crested male in 68% ± 17 (CI, confidence interval) of the trials, and the null hypothesis of female preference independent of male dorsal crests could be rejected (\( P < 0.05 \)). This indicates that a female from the population choosing to remain in front of the male with the higher crest, relative to the low-crested male, would choose the former in 51-81% of all repeated trials. Although 50% of the females repeated their preference for the high-crested male, and only about 8% of the females remained true to the low-crested male (Table 1), results from repeated trials could not demonstrate with statistical confidence that females remained true to their first preference (Chi-square test: \( \chi^2_3 = 6.00, P = 0.112 \)). This indicates that although female newts can discriminate between males with subtle dorsal crest differences, the limited sample size available in this experiment did not allow for a full resolution of the issue of choice repeatability.

### Table 1.

Female preference data for male images from repeated trials. The columns list possible response categories in relation to repeated trials and male dorsal crests. The number of females (\( n = 12 \)) responding according to each category is listed as the observed result. The number expected under the null hypothesis of no repeatability of preference is also given.

<table>
<thead>
<tr>
<th>First trial: Low crest</th>
<th>Second trial: Low crest</th>
<th>First trial: High crest</th>
<th>Second trial: High crest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Expected</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Although females in this experiment initially responded to the trial conditions by seeking refuge in the neutral zone, they spent a significant proportion of time in front of the male projections. This demonstrates that the experi-
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Experimental design was successful in invoking the attention of females towards a set of virtual male ‘models’. Further, the results clearly indicate that females did not remain in front of the males at random or independently of differences in dorsal crest height. Instead, females spent significantly more time in front of the manipulated high-crested male, than in front of the low-crested male, which was consistent also after controlling for zone area.

That females spent the majority of their trials in the neutral zone, away from the males, may have been due to retreating behaviour because of the artificial experimental conditions. Alternatively, the male models did not respond adequately to female attention. In natural situations, male and female feedback interplays in initiating different courtship behavioural sequences towards spermatophore delivery (Arntzen & Sparreboom 1989). Such behavioural feedback components were entirely lacking in this experiment, due to the simplistic design. Females may therefore simply have lost interest, since the male dummies were stubbornly showing-off without initiating courtship sequences in response to female approach. Great crested newts are also notorious among researchers trying to study mating behaviour, since they quickly lose sexual motivation under captive conditions (T.R. Halliday pers. communication). This could contribute to explain why so few females returned to remain near the males in trial repeats.

From a general perspective, sexual selection theory predicts that mate choice results from the combination of individual preferences for certain phenotypes of the opposite sex and the extent to which individuals sample the available phenotypes before mating (e.g., Kokko et al. 2006). The evolution of sexual ornaments and displays in males are thought to coevolve with female preference because the expression of larger sexual traits is costly and may be condition-dependent (Zuk 1991, Cotton et al. 2004). Assuming that there is a trade-off between differences in genetic constitution among males and the propensity to utilize resources in the environment, condition-dependence is mirrored in the expression of sexual traits and display vigour. Hence, these features may provide females with honest information about both a male’s genetic quality and condition. Males in good condition gain the benefits of high reproductive success, whereas males in poor condition pay the costs of fewer mating opportunities.

Furthermore, female mate preferences — like male ornaments — can also be condition-dependent, because high quality females show the strongest mate preference (Cotton et al. 2006) and are assumed to lose more by making a bad choice. Hence, sexual selection with condition-dependence can be a strong evolutionary driving force, capable of accelerating both population adaptation and speciation processes, especially when environmental conditions are fluctuating, and to reinforce both the expression of sexual traits and displays, as well as female preference (Lorch et al. 2003, but see Cotton et al. 2004, Kokko et al. 2006).

The hypothesis of condition-dependent sexual selection has not been tested in the great crested newt (Cotton et al. 2004, but see Green 1991). However, this is certainly plausible. In fact, the great crested newt may be a species where signals of male condition and quality have particular merit, due to the genetical constraint posed by the development arrest syndrome present in
the species (reviewed in Malmgren 2001). Male traits that are both condition-dependent and easy to assess for females may therefore provide useful information, and the dorsal crest is a likely candidate for being a key feature for mate choice — as is emphasized by our results. For example, Hedlund (1990a, 1990b) demonstrated that both male body size and dorsal crest height were important for female mate choice in semi-natural experiments. In her study, females chose to mate with large males early in spring (when dorsal crests are not yet fully developed among males), shifting later to prefer males with large dorsal crests, and there was a positive correlation between male body size and crest height. This demonstrates that crest development is a dynamic feature, and that females may be guided by different traits for evaluating honest signals of male quality and condition, even over the same season. Further, since female newts mate for as long as they lay eggs, which can extend over several months, it is likely that female reproductive success is limited by the availability of sperm from high-quality males. Females therefore have reason to be selective, whatever the season, although that selectivity may take different directions (e.g., Gabór & Halliday 1997, Osikowski & Rafinski 2001).

Large body size and the development of a large crest are likely to indicate vigour as well as age and experience, traits that can be interpreted as signals of prime qualities (Hedlund 1990a, Baker 1992, Griffiths 1996, Malmgren & Thollesson 1999, Baker & Halliday 2000). Interestingly, Baker & Halliday (2000) demonstrated that the morphology of dorsal crests in male great crested newts differ with age. Older males have higher and more irregular crests and are generally larger than younger males. The combination of a more densely shaped crest and a larger overall body size and surface area is likely to enhance the appearance of a male and present to females stronger visual stimuli. Further, Dolmen (1982) demonstrated that older and thereby larger males develop more testis lobules, which can aid in providing more sperm to females and the ability to mate more often with increasing age. Increased crest height is also the result of increased testosterone levels, which is positively correlated with male mating success (Malacarne & Cortassa 1983). It would appear likely that many of these features are costly, particularly under Scandinavian conditions, where more than six months each year are spent in terrestrial hibernation. Hence, the dorsal crest appears useful for indicating several condition-dependent states in male great crested newts, that need further attention (see Kokko et al. 2006). In contrast to other attempts to study mate choice by female great crested newts, our results clearly demonstrate that visual information conveyed by the dorsal crest may be sufficient for females to differentiate between males. Hence, it provides important clues to the underlying patterns determining mate choice under more natural conditions.

From this experiment we conclude that it is possible to use computer models and screen projections to manipulate and study behavioural responses in great crested newts. It appears that female newts respond well to this experimental method, which is non-destructive, and we encourage follow-up experiments. Future tests may benefit from a refinement of the design, for example by using 3D-modelling of movements and common courtship behaviour in response to female approach, to allow for more resolved responses. Further, the results clearly suggest that differences in dorsal crests among males can
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act as a first visual attractant to female mate choice, regardless of other functions of the crest and variation in male performance. Hence, the results offer a resolution to an issue of lengthy debate in salamander science.

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