Olfactory and visual species recognition in newts and their role in hybridization

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Summary

Mating patterns between hybridizing taxa are often conditional to the mechanisms underlying species recognition. During mate choice, individuals often assess information displayed by potential mates on several sensory channels. The reliance on more than one modality is particularly expected whenever transmission conditions are variable or signals subject to wear. Determining the sensory bases of species recognition is, thus, crucial to assess the effect of the signalling environment on the hybridization process between species where mate choice occurs. We addressed this issue in two newt species, *Lissotriton helveticus* and *L. vulgaris*, that hybridize and breed in aquatic habitats disturbed by various natural processes. We measured visual and olfactory preferences in males and females. Visual and olfactory recognition was detected in *L. helveticus* males and *L. vulgaris* females. In contrast, we observed limited olfactory recognition in *L. helveticus* females and no evidence of recognition at all in *L. vulgaris* males. In addition, one single variable, body size, strongly influenced female preference. Ecological factors modulating visual signalling conditions and the body size ratio in males are, thus, likely to influence the probability of heterospecific mating. This study highlights the need to consider more largely environmental factors affecting communication in the hybridization process.

Keywords: sensory ecology, multimodal communication, vision, olfaction, *Lissotriton*.

Introduction

Species recognition is a major component of reproductive isolation in animals where mate choice occurs. This pre-zygotic mechanism determines the frequency of mixed pairs or mating attempts with heterospecifics, and hybrid frequency when hybrids are viable (Butlin, 1987; Liou & Price, 1994; Seehausen et al., 1997). Thus, the transfer of information about species identity is crucial for individual decisions that may have consequences at the population level. Species recognition is essentially a sensory process based on the discrimination of signals and cues presented by conspecific and heterospecific individuals. Inputs from different sensory channels can then be used to reduce the probability of heterospecific mating (Baker & Baker Miller, 1990), a strategy often used in the assessment of potential partners (Hebets & Uetz, 1999; Partan & Marler, 1999; McLennan, 2003; Taylor et al., 2007). However, the level of signal degradation, which vary with the sensory channel and habitat type, is expected to affect the signalling efficiency and ultimately to influence the probability of mismating.

In this regard, freshwater species may experience strong constraint on communication processes because of spatial and temporal variations in the properties of the transmission medium. Visual signals depend on the spectrum of available light that is a combination of the light spectrum transmitted from the surrounding terrestrial habitat, and the spectrum resulting from optical water characteristics like colour and turbidity (Endler, 1990, 1991). Similarly, transmission and reception of olfactory signals depend on chemical water characteristics like pH or humic acid content (Hubbard et al., 2002; Fisher et al., 2006; Heuschele & Candolin, 2007). Visual and olfactory parameters can change over time (Candolin et al., 2007) within a habitat but they can also vary in space (Endler & Théry, 1996; Secondi et al., 2007). Thus, environmental conditions may strongly affect the assessment of cues from one or several sensory modalities in aquatic habitats. Which sensory channels are used and how the communication process is affected by the variation of transmission properties may largely determine mating patterns and eventually whether hybridisation will occur in the presence of heterospecifics (Seehausen et al., 1997).

We investigated the sensory bases of species recognition between the Palmate newt *Lissotriton helveticus* and the Smooth newt *L. vulgaris*, two species known to hybridize across a broad sympatric zone where syntopy is

common (Nöllert & Nöllert, 2003). Hybrid viability has been demonstrated by experimental crossings (Spurway & Callan, 1960) and field observations (Griffiths et al., 1987; Schlüppman et al., 1999). An earlier study found a low frequency of hybrids (0.7%; Arntzen et al., 1998). More recent analyses detected 1.7% hybrids and introgression in 73% of syntopic sites (Johanet, unpublished data). Yet, this figure is not likely to reflect the actual costs associated with interspecific reproductive interactions. It does not consider the frequency of heterospecific matings or the energy and time resources wasted in failed attempts. Earlier experiments suggested that visual and olfactory species recognition was suprisingly weak given the geographical extent of sympatry and the frequency of syntopic sites (Roberts, 1990). The stochasticity of signalling conditions may contribute to the ongoing hybridization. Investigating the contribution of different sensory channels to species recognition may shed light on the hybridization process.

Although, the use of vision and olfaction in mate selection is acknowledged in Amphibians, their contribution to species recognition has not received much interest (but see Cogalniceanu, 1994; Michalak et al., 1997). Olfactory communication is used during mating in Urodeles (Malacarne & Giacoma, 1986; Kikuyama et al., 2002), and in the genus *Lissotriton* in particular (Cogalniceanu, 1994; Secondi et al., 2009) where sex pheromones have been identified in *L. vulgaris* (Osikowski et al., 2008). Visual communication is used too (Halliday, 1977; Himstedt, 1979; Haerty et al., 2007). Males of the two species produce an elaborate courtship display that differ in a few details, like the frequency of fanning, i.e., a rapid wave movement of the tail tip (Halliday, 1977; Roberts, 1990). Males can also be identified by a suite of conspicuous secondary sexual traits that differ markedly between species (Halliday, 1975; Griffiths, 1996). *L. helveticus* males display a caudal filament, a smooth caudal crest and hind foot webs whereas *L. vulgaris* males express a denticulate dorsal and caudal crest, narrow skin extensions on hind foot toes and no caudal filament. Head, ventral and tail colour patterns are different, *L. vulgaris* being overall more colourful and contrasting (Griffiths, 1996). Females are much more similar than males but they can be identified by several characters like a short caudal filament in *L. helveticus*, a pointed tail tip and a marbled throat in *L. vulgaris*. Female newts have an exclusive control over fertilization (Halliday, 1990) so that their ability to recognize conspecific partners will determine the hybridization rate. However, males are expected to show species recognition too because courtship

is time and energy consuming (movements, spermatophore production), and make males more conspicuous to predators. Sperm production is also limiting during the breeding season (Verrell et al., 1986; Takahashi & Parris, 2009). These elements suggest that heterospecific mating attempts are costly for males too.

We measured visual and olfactory species recognition abilities of each sex in order to address several questions. (i) Does one sex perform better than the other at species recognition? Given the morphological similarity of females, we predicted males to rely mainly on olfaction. In contrast, the large differentiation of epigamic traits in males suggests that females may rely more on visual communication. (ii) Is one crossing type more likely to occur than the other because both partners exhibit poorer recognition at one or both channels? If so, relative sex and species ratio could be a predictor of the hybridization rate. (iii) What type of environmental disturbance is likely to reduce species recognition efficiency? Testing both visual and olfactory channels provides information about the consequences of environmental disruption of one channel on the hybridization risk. (iv) Is there interference between species recognition and intraspecific mate assessment? Some authors have proposed that the same traits are used in both situations (Ryan & Rand, 1993). A reduced difference in such traits between conspecific and heterospecific mates is expected to increase the probability of mispairing (Pfennig, 1998). Both males and females often show a preference for larger mates in vertebrates. This trait reflects fecundity in female amphibians (Verrell, 1986a,b) and survival, competitive ability, or dominance in males (Friedl & Klump, 1997; Mathis & Britzke, 1999). The effect of body size on female preference is more controversial in Urodeles than in Anurans (Halliday & Verrell, 1986). However, in a previous experiment, we showed that *L. helveticus* females preferred smaller males (Haerty et al., 2007). We, therefore, tested the effects of body size on the strength of male and female preference for conspecifics.

Methods

Sampling and housing conditions

We captured 64 *L. helveticus* and *L. vulgaris*, 16 of each sex and species, on 29 March 2007 for female preference tests and 72 breeding *L. helveticus*

and *L. vulgaris*, 18 of each sex and each species, on 3 April 2008 for male preference tests. Tests were carried out between 3 and 12 April 2007 and 7 and 21 April in 2008. All individuals originated from the same syntopic site, a long pond surrounded by meadows and periodically connected to the Loire River during flood events, located in western France near the village of Liré (Maine-et-Loire, 1°09′57″W, 47°20′32″N). We chose to test the sensory basis of species recognition in sympatry because reproductive barriers are often strengthened in areas where species co-occur (Brown & Wilson, 1956; Butlin, 1987).

In 2007, males and females were kept indoor in pairs of the same sex and the same species. They were housed in plastic tanks (length 51.5 × width 34 × height 32.5 cm) at 18°C, filled with 4 l aged tap water. We added two semi-circular sections of PVC tube that provided a shelter for each individual and water plants (*Elodea* sp.). Standard fluorescent tubes provided light that we dimmed by partially covering the tubes with plastic sheets. Individuals were fed daily with chironomid larvae. At the end of the experiments all newts were released in their pond. In 2008, all individuals were housed singly in indoor aquaria at a room temperature of 15°C. Females were housed in plastic tanks (length 51.5 × width 34 × height 32.5 cm), filled with 8 l aged tap water whereas males were housed in aquaria (length 33 × width 17.5 × height 25 cm) filled with 3 l aged tap water. Individuals have been caught and kept in captivity according to permits delivered by Préfécture de Maine-et-Loire and Loire-Atlantique.

Species assignment and morphological interspecific difference in syntopic sites

All individuals selected for this experiment exhibited characteristics of the species they were assigned to. Males displayed typical secondary sexual traits (crest shape and position and hind foot webbing, presence/absence of caudal filament, colour pattern) and females presented the usual criteria (tail and head shape, colour pattern). Females are much more similar than males and may, therefore, be more difficult to identify. We, therefore, additionally recorded body length, tail height and cloaca width in all females. These three traits differ between species (Johanet et al., 2009). Methodological details for measurements are given elsewhere (Secondi et al., 2007).

Preference test of visual and chemical species recognition

We tested sexual preference for conspecific visual and chemical stimuli in two series of tests. Male and female preferences were measured in two experiments using the same protocol as described below. We used a linear olfactometer with a neutral zone on one end and two preference zones on the other end (Figure 1). The whole apparatus was 15 cm high. Water depth was 4 cm. A pump kept water flowing at 0.27 l/min. In all experiments, we used aged tap water. Subjects could move freely between the neutral and both preference zones. Stimuli were paired randomly by selecting one *L. helveticus* and one *L. vulgaris* individual. Each stimulus individual was used in one pair only.

   We habituated subjects to experimental conditions the day before the first test by placing them in the apparatus for at least 30 min without any individual stimulus. During a test, subjects were placed on the distal end of the neutral zone where they were kept for 5 min in a mesh cage that allowed them to perceive visual and olfactory cues from both stimuli. After that period, the cage was lifted. For the next 15 min, we recorded the time spent in and the latency to enter each preference zone. At the end of that period, the subject was brought back into the cage. The whole apparatus was drained, rinsed, and wiped to remove chemical cues. This operation is unnecessary for visual tests but we carried it out for comparison purpose between the two test series. Stimuli position was swapped between the two periods. Once the olfactometer was refilled with water, we started the second period following the same procedure. We checked sexual behaviours during visual tests because they could bias response but none were observed. The same pair of
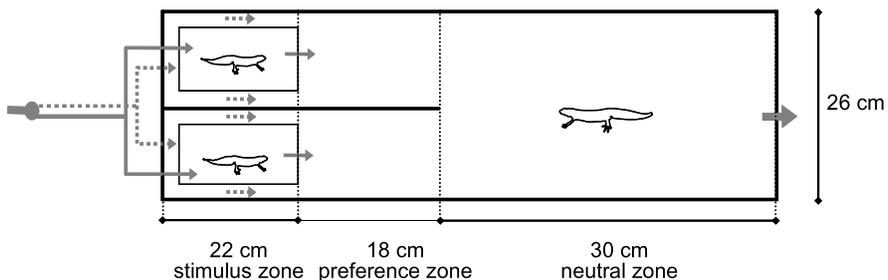

Figure 1. Olfactometer used for species recognition tests. Grey lines indicate water circulation for visual (dotted lines) or chemical tests (solid lines). Observation window was located on the right hand side of the apparatus.

stimuli was used for a given subject in the visual test and the chemical test. In addition, each stimulus pair was tested with one subject of each species. Mean size difference in body length between stimuli (*vulgaris–helveticus*) was 2.01 ± 2.83 mm (min $= -4.04$ mm; max $= 6.61$ mm) for male pairs and 1.18 ± 0.77 mm for female pairs (min $= -0.77$ mm, max $= 3.50$ mm). Because of the overall size difference between the two species, the distribution of size contrasts between stimulus pairs was not centred on zero.

For visual tests, each stimulus individual was kept in a transparent plastic box (18 length $\times$ 12 width $\times$ 7.5 cm height) in one of the stimulus zones so that subjects had no access to chemical information. Shutters ensured that subjects had visual contact with only one stimulus once entered in a preference zone. Within a preference zone, water flowed underneath and along the sides of the box (Figure 1). No water exchange between preference zones was possible. Water level in the box was the same as in the rest of the apparatus. We used a 25 W light tube spanning the range of daylight spectrum (98 CRI, 6700 K, Repti Glo 2.0). Because light can disturb newt motor activity, we shaded the neutral and preference zones by covering them with a black plate. Finally, the apparatus was located in a chamber with black walls so that no spatial cues could alter the subject's response. An observation window on the 'neutral zone' end of the apparatus was used to record subject's behaviour.

For olfactory tests, we used the same apparatus and the same box model as for visual tests. However, a different set of boxes was used. Here, we allowed water to circulate through the boxes so that chemical cues emitted by the stimulus males were conveyed to the preference and neutral zones (Figure 1). We covered box fronts with black shutters so that subjects had no access to visual cues. All other conditions for visual and chemical tests were similar between the two test series. Each subject was tested in each experiment on two different days. The presentation order of experiments (visual or chemical stimuli) was alternated between subjects.

Statistical analyses

We used Discriminant Function Analysis to test the robustness of species assignment for females using the three morphological variables body length, tail height, and cloaca width.

For recognition experiments, we carried out two distinct analyses in order to investigate the following questions. (i) Can we detect visual and olfac-

tory species recognition in males and females? (ii) What factors affect the efficiency of species recognition? Both questions could not be tested using a single statistical procedure. Thus, we first tested preference for conspecific olfactory and visual stimuli using Wilcoxon signed-rank tests as the objective was to determine whether subjects preferentially associated with one stimulus type. Non parametric tests were selected because some duration and latency variables significantly departed from normality in the male and female experiments. Then, we tested the effect of species identity and body length on visual and chemical preference for conspecific stimulus using linear mixed models. We considered the score difference in mean time spent and the difference in mean latency to enter each preference zone between the conspecific and heterospecific stimulus as response variables. Differences in responses will hereafter be referred to as contrasts. Contrary to raw variables, contrasts were normally distributed. We considered the difference in body length between the two stimulus males as a covariate. We did not consider other morphological traits as covariates in our models as their biological meaning was less clear. Furthermore, body length is usually positively correlated with most morphological traits. For a given subject, the same pair of stimuli was used for the chemical and the visual test. Each stimulus pair has also been tested with one subject of each species. We considered stimulus pair as a random factor in our analyses. For this reason, we decided upon testing visual and chemical preference in two different procedures in order to avoid the crossing of random effects. We also tested sexes separately because predictions about the relationships between size and preference differed for males and females. For model selection, we used a backwards selection procedure by removing the effect with the highest p-value, keeping all effects with significant p-values (<0.05), and testing whether the reduced model provided a better fit to end up with the minimum adequate model. Analyses have been carried out using Statistica 7.1 (Statsoft) and R (R Development Core Team, 2007).

Results

Species assignment of experimental individuals

We tested the robustness of species assignment for females using a discriminant function analysis based on the three morphological traits. We ob-

served a highly significant difference between female types used as subjects in 2007 (Lambda Wilk: 0.463, $F_{\text{approx } 3,28} = 10.784$, $p < 0.0001$). The classification matrix confirmed the separation between the species as all individuals but two *L. helveticus* and three *L. vulgaris* were correctly assigned to the right species (84.37% correct assignments). Species discrimination was more efficient in stimulus females tested in 2008 (Lambda Wilk: 0.253, $F_{\text{approx } 3,32} = 31.543$, $p < 0.0001$) as two *L. vulgaris* females were classified as *L. helveticus* (94.44% correct assignments). All individuals assigned to the other species by discriminant analysis exhibited traits typical of the species which they were visually assigned to (body colour, pattern, and shape), and that we did not consider in the discriminant analysis. We are, thus, confident that we tested non hybrid, or only slightly introgressed individuals.

Chemical and visual species recognition

Visual tests

We observed a significant female preference for conspecific over heterospecific visual stimuli in both newt species (Figure 2). Females spent significantly more time close to the conspecific male (*helveticus*: 13/16 females, $Z = 2.844$, $p = 0.004$; *vulgaris*: 15/16 females, $Z = 3.361$, $p = 0.001$). They also entered earlier in its compartment (*helveticus*: 12/16 females, $Z = 2.379$, $p = 0.017$; *vulgaris*: 13/16 females, $Z = 3.206$, $p < 0.001$).

Results on male preference were more complex (Figure 2). *L. helveticus* males spent more time close to conspecific females (15/18 males, $Z = 2.154$, $p = 0.031$) but they did not approach significantly faster (13/18 males, $Z = 1.681$, $p = 0.093$). *L. vulgaris* males did not express preference for conspecific females (time spent: 11/18 males, $Z = 0.806$, $p = 0.420$; 10/18 males, latency: $Z = 0.152$, $p = 0.879$).

From the full model, including species, body length contrast and the interaction as explanatory variables, we carried out a backwards model selection procedure on the two response variables, i.e., contrasts for time spent and latency. For females, the full model was selected for latency. We detected significant effects of body size contrast ($p = 0.005$) and the interaction between body size contrast and species ($p = 0.0009$) (Table 1). This result is
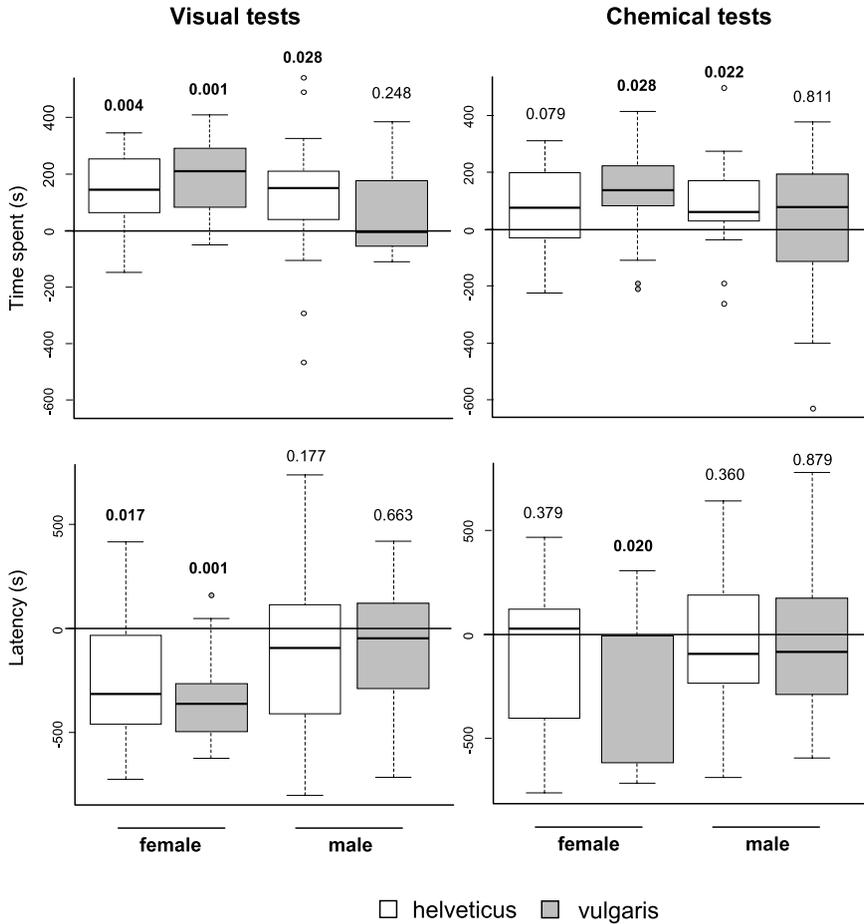
Figure 2. Difference in time spent in, and latency to enter each preference zone (conspecific-heterospecific) for males and females *Lissotriton helveticus* and *L. vulgaris*. Box plots represent medians and interquartile ranges. *P*-values for Wilcoxon sign-ranked tests are given above each box. Numbers in bold indicate significant differences at $p = 0.05$. Sixteen subject females and 18 subject males of each species have been used in visual and olfactory tests.

explained by the opposite response of *L. helveticus* and *L. vulgaris* to body size contrast (Figure 3). The preference for conspecific individuals faded as the normal size ratio got smaller and preference was even reversed when the size ratio was inverted. The procedure retained no minimal model for time spent but the overall female response pattern was the same as for latency (Figure 3). For males, no minimal model was selected (Figure 4).

Table 1. Results of model selections for visual preference tests in males and females.

	Coefficient	SE	df	*t*	*p*
Female preference					
Time spent		No minimal model selected			
Latency					
Intercept	24.303	1.822	28	13.341	0.000
Species (TV)	−2.910	2.576	28	−1.130	0.268
dSVL	1.644	0.536	28	3.066	0.005*
Species (TV) × dSVL	−2.819	0.758	28	−3.718	0.001*
Male preference					
Time spent		No minimal model selected			
Latency		No minimal model selected			

Only the minimal model is presented. dSVL is the difference in body length between the conspecific and the heterospecific stimulus. *Significant effect at $\alpha = 0.05$.

Chemical tests

L. vulgaris females showed a significant preference for conspecific males, both in time spent and latency (*time spent*: 13/16 females, $Z = 2.198$, $p = 0.028$; *latency*: 12/16 females, $Z = 2.327$, $p = 0.020$) whereas males did not show any preference for conspecific females (time spent: 8/18 males, $Z = 0.327$, $p = 0.744$; latency: 10/18 males, $Z = 0.152$, $p = 0.879$) (Figure 2).

In contrast, *L. helveticus* females did not significantly prefer conspecific males (*time spent*: 10/16 females, $Z = 1.758$, $p = 0.079$; *latency*: 7/16 females, $Z = 0.879$, $p = 0.379$) but males spent more time close to conspecific females (time spent: 15/18 females, $Z = 2.199$, $p = 0.028$). Latency was not shorter though (latency: 12/18 females, $Z = 0.893$, $p = 0.372$).

Discussion

Sex and species asymmetry in species recognition

The efficiency of species recognition strikingly differed between sexes in *L. vulgaris*. Females showed strong visual and chemical species recognition. In contrast, males expressed no visual or olfactory preferences. They seemed
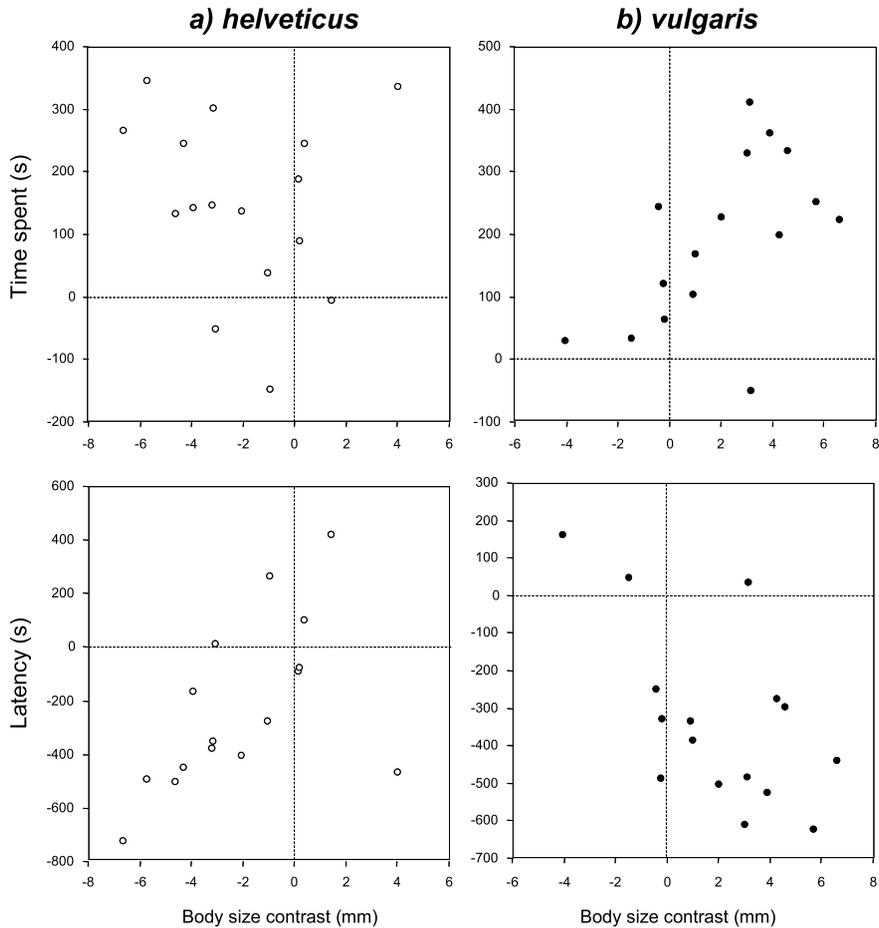
Figure 3. Relationship between contrasts in stimuli size and contrasts in female response (time spent in and latency to enter each preference zone) observed during the visual experiment. Contrasts were computed as the difference conspecific-heterospecific so that a preference for the conspecific stimulus is indicated by a positive value for time spent and a negative value for latency.

unable to perform better than chance, at least when only cues from one single sensory channel were available. A different pattern was observed in *L. helveticus*. Females expressed species recognition in visual tests but not in olfactory tests. Even if the difference in time spent close to each male was close to significance, olfactory recognition seemed weaker than visual recognition. In contrast, males did show visual and olfactory species recognition.
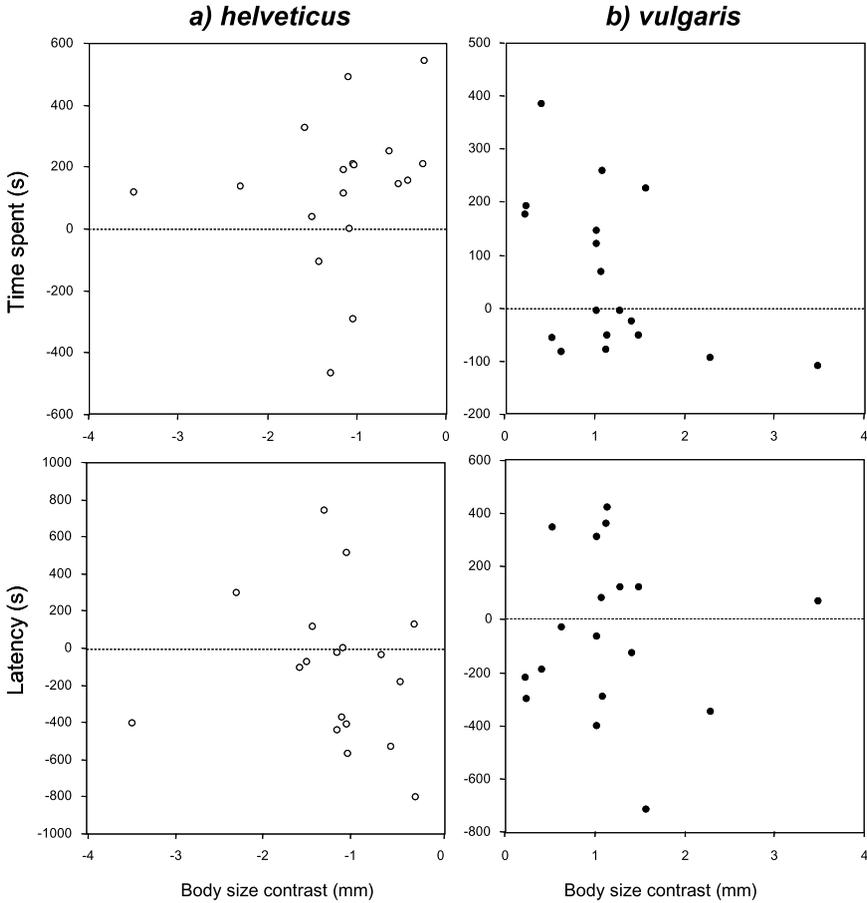
Figure 4. Relationship between contrasts in stimuli size and contrasts in male response (time spent in and latency to enter each preference zone) observed during the visual experiment. Contrasts were computed as the difference conspecific-heterospecific so that a preference for the conspecific stimulus is indicated by a positive value for time spent and a negative value for latency.

They spent more time close to the conspecific female in both tests. However, latency was not significantly shorter which suggests that recognition requires close examination of females, or that males usually assess all females in their vicinity. Such an explanation is not unlikely in species where male mating preference exists (Verrell, 1986a). Overall, our results supported predictions about the type of information used for females but not for males.

Species recognition is typically a mechanism where inputs from different sensory systems can be important. Resorting to different communication channels increases the probability of correct discrimination of conspecific and heterospecific individuals when transmission conditions are variable or signals are subject to wear. In *L. vulgaris* females and *L. helveticus* males, efficient species recognition occurred even when information from a single channel was available at a time. Visual and olfactory cues alone provided enough information for a subject to express a preference for the conspecific individual. Thus, species recognition can occur even if signal transmission is disrupted in one channel. This is not true for the two other groups. *L. helveticus* females did not compensate for the loss of visual information and male *L. vulgaris* failed to achieve species recognition using one channel only. Such a result does not imply that errors systematically occur in nature. The combination of information from different channels may be necessary instead. In those two groups, visual and olfactory signals are, thus, not redundant. They probably carry each a part of the information required to achieve correct identification.

Authors have frequently observed that one species, or one sex, performs better than the other at species recognition (Wirtz, 1999; Randler, 2002). Species or sex asymmetry in recognition ability generates skewed distributions of genotypes and phenotypes (if one crossing is more frequent than the other) and contributes to hybridization and introgression dynamics. Tests confirmed that encounters between males and females of the two species should generally result in the avoidance of heterospecific partners, which is consistent with the low hybridization rate observed in nature (Arntzen et al., 1998). However, we expect this general outcome to be modulated by local conditions.

Factors modulating the efficiency of species recognition

L. helveticus males and *L. vulgaris* females achieved both visual and olfactory discrimination. On the contrary, *L. helveticus* females showed visual recognition only and *vulgaris* males no evidence of discrimination at all. Thus, the crossing between *L. helveticus* females and *L. vulgaris* males is more likely than the reciprocal crossing (Figure 5). Its frequency is expected to depend on the probability of encounter of partners, i.e., on the sex ratio

female *helveticus*

	visual	chemical
male *vulgaris* visual	N/Y	-
chemical	-	N/N

female v*ulgaris*

	visual	chemical
male *helveticus* visual	Y/Y	-
chemical	-	Y/Y

Figure 5. Matrix showing the occurrence of species recognition in males and females *Lissotriton helveticus* and *L. vulgaris* for visual and chemical stimuli. In each cell, Y indicates a significant conspecific preference for one or both variables measured in males (left character) and females (right character). N indicates that conspecific preference was not detected on none of the two variables (latency and time spent). The table shows the asymmetry in efficiency of species recognition between crossing types.

and the relative abundance of each species. We explain below how environmental conditions could contribute to enhance such an asymmetric mating pattern.

In addition, one crossing type may be more frequent if individuals of one species prefer a trait expressed by the other species or if overlaps in distributions of traits under sexual selection occur (Parsons et al., 1993). Then, the species with the smaller trait is more likely to seek mating with the species expressing the larger trait (Pfennig, 1998). Difference in body size between the conspecific and the heterospecific female did not affect male preference. In contrast, tests revealed the large effect of body length contrast on the efficiency of visual species recognition in females. In both species, the degree of conspecific preference decreased as the difference in size between the conspecific and heterospecific males decreased. Such a drop in recognition efficiency was unexpected because males of the two species differ by the presence and shape of several conspicuous traits (hind foot web, caudal filament, caudal crest shape, colour) that all could be assessed. Thus, body size seems to have a major effect on female preference in both species.

Females actually expressed divergent preferences for body size. *L. helveti-cus* females preferred smaller males and *L. vulgaris* females preferred larger males. Strictly speaking, our experiment is not a test of female preference for male size as we did not present selected pairs or series of conspecific males to each female but random pairs of males of different species instead. Nevertheless, in both species the strength of female preference decreased with male body length contrast and shifted towards the heterospecific male when the relative size order was reversed, i.e., the male of the normally smaller species was the larger stimulus male. Female preference in *L. helveticus* is striking as preference for smaller males is more an exception than a rule in sexual selection studies (but see Morrison et al., 2001; De Block & Stock, 2006; Poschadel et al., 2007). Results are consistent with other experimental data on *L. helveticus* though (Haerty et al., 2007). Viewed in the context of hybridization, divergence in mating preferences for body size makes sense as this pattern is consistent with reproductive character displacement. Our data cannot demonstrate this hypothesis but the result is intriguing. Testing preference of females from allopatric sites may shed light on the evolutionary process underlying the divergence in mating preferences.

Environmental variation and hybridization risk

Like any communication process, the efficiency of species recognition is bound to depend on the quality of signal transmission. Signal transmission occurs under less optimal visual and chemical conditions in breeding sites than in the laboratory. Habitat irradiance and water absorbance spectra vary with time, shading, aquatic vegetation, water colour and turbidity. The perception of colour patterns and, thus, visual species recognition, is, therefore, expected to be modulated by habitat visual characteristics. For chemical signals, transmission can be altered by compounds common in natural environments (Hubbard et al., 2002; Fisher et al., 2006; Heuschele et al., 2007). *L. helveticus* females achieved efficient visual recognition but poorly discriminated chemical cues of conspecific and heterospecific males, which suggest that they rely mainly on vision for species recognition. Visual conditions are often degraded during the breeding period because of runoffs, aquifer surges or floods that all increase water turbidity. Poor visual conditions may not affect the risk of hybridization between *L. vulgaris* females and *L. helveticus* males but they may increase it in the reciprocal crossing in which males and

females perform poorly at olfactory recognition. Thus, the quality of transmission conditions for visual signals may be a key factor in the hybridization process, probably more important than transmission conditions for chemical recognition. Increased hybridization rate resulting from sudden changes in visual signalling conditions has been observed in cichlid fishes (Seehausen, 1997). In newt, hybridization is unlikely to result from recent changes in human activities. The influence of transmission conditions on reproductive interactions between species may be a general phenomenon. It is probably more common in aquatic species because natural disturbance regimes often alter their visual environment.

Ecological factors can affect body size in amphibians (Morrison & Hero, 2003). This trait is involved in the mate selection process (Verrell, 1986a,b; Haerty et al., 2007). In our study area, interspecific difference in male body size varied substantially (Johanet et al., 2009). We, therefore, expect hybridization rate to depend on the local size distribution of males of the two species. Interestingly, terrestrial habitat may have an indirect effect on hybridization risk as body size increases with the proportion of wooded area around the pond in *L. helveticus* (Johanet et al., 2009). We do not know whether this variable affects *L. vulgaris* males but landscape composition may influence body size ratio in syntopic ponds.

The present study suggests that environmental factors could be major predictors of hybridization rate. Surprisingly, how environment affect the expression or perception of traits assessed for species recognition are questions that received relatively little attention. Understanding the relationships between proximal factors at the behavioural level and ultimate factors at the genome level is still a necessary step to get further insight into the speciation process. The integration of multimodal signalling theory in the study of hybridization and hybrid zone dynamics is to this regard a promising approach that may contribute to refine predictions about the relationships between habitat, signal degradation and hybridization rate.

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