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**CONDITION-DEPENDANCE ET HONNETETE DU SIGNAL
DANS UN SYSTEME DE SIGNALEMENT SEXUEL MULTIMODAL**



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Signal honesty and condition-dependence in a multimodal sexual signaling system

In a sexual selection context, signals displayed by males are supposed to reflect honestly their condition. Given that condition is a multifactorial concept, it has been proposed that the multiple components often involved in sexual signals reflect different aspects of the emitter's condition (multiple messages hypothesis). My thesis aimed at testing the condition-dependence of sexual signals and the multiple messages hypothesis by manipulating different aspects of male condition; and at investigating ways to circumvent an honest signalling system through alternative reproductive tactics. We conducted these experiments on the European treefrog (*Hyla arborea*), a species in which males display signals with both acoustic and visual components used by females during mate choice. Our results suggest that the within-bout call rate, a dynamic call property, is a reliable indicator of current condition while call dominant frequency, a static call property, seems to be independent from current condition but is linked to past condition. Visual signal components are independent from variations in nutritional status on short term but are linked to male condition in the field and may be indicators of past condition. The adoption of a satellite tactic depends more on past condition than on current condition. As several signal components are related to the emitter's condition and seem to be dependent upon distinct aspects of this condition, our results mainly support the multiple messages hypothesis. However, the weak correlations between signal components and condition aspects found in this study highlight the necessity to consider also the evolutionary dynamics of signalling systems.

Condition-dépendance et honnêteté du signal dans un système de signalement sexuel multimodal

Dans un contexte de sélection sexuelle, les signaux émis par les mâles sont censés refléter honnêtement leur condition. Etant donné que la condition est un concept multifactoriel, il a été proposé que les différentes composantes des signaux sexuels reflètent différents aspects de la condition de l'émetteur (hypothèse des messages multiples). Ma thèse a pour but de tester la condition-dépendance des signaux sexuels et l'hypothèse des messages multiples en manipulant différents aspects de la condition des mâles ; et d'étudier les moyens de contourner un système de signalement honnête par des tactiques de reproduction alternatives. Nous avons conduit ces expériences chez *Hyla arborea*, espèce chez laquelle les mâles émettent des signaux comprenant des composantes acoustiques et visuelles impliquées dans le choix du partenaire sexuel. Nos résultats suggèrent que le taux de chant intra-bout, une propriété dynamique, est un indicateur de la condition actuelle, alors que la fréquence dominante, une propriété statique du chant, semble indépendante de la condition actuelle mais est liée à la condition passée. Les composantes visuelles du signal sont indépendantes des variations du statut nutritionnel à court terme mais pourraient être des indicateurs de la condition passée. L'adoption d'une tactique satellite dépend plus de la condition passée que de l'actuelle. Plusieurs composantes du signal sont liées à différents aspects de la condition du mâle, ce qui supporte l'hypothèse des messages multiples. Cependant, les relations entre les composantes et les aspects de la condition sont faibles et nous poussent à considérer également la dynamique évolutive des systèmes de signalements.

Ecologie comportementale

Keywords : alternative reproductive tactics; anurans; communication; condition-dependence; honesty; multicomponent signals; multiple messages; sexual selection.

Mots-clés : anoures ; communication ; condition-dépendance ; honnêteté ; messages multiples ; sélection sexuelle ; signaux multi-composantes ; tactiques de reproduction alternatives.

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« Si un problème a une solution, ce n'est pas la peine d'être malheureux. Si un problème n'a pas de solution, être malheureux n'y changera rien. »

Proverbe Tibétain.

« Un problème sans solution est un problème mal posé. »

Albert Einstein.

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INTRODUCTION

- Sexual Selection

Sexual selection is a concept initially introduced by Darwin (1859) - and more widely developed in his second authoritative book (1871) - to explain the amazing dimorphisms between males and females found in many species, and particularly the extravagant secondary sexual characters often observed in males. Darwin defined it as a particular form of natural selection favouring the ability of males to compete for the acquisition of mates. This form of selection should favour both ornaments developed to attract and seduce females and weapons used to win contests with other males.

A first explanation of the competition between males to access females was brought by Bateman and his competition experiments with fruit flies (1948). In this study, he found that male reproductive success varied much more widely than that of females and that it was limited by male's ability to attract or seduce females. In addition, male reproductive success increased with the number of copulations experienced by the male whereas female reproductive success did not increase after the first copulation. Bateman thus concluded that males have a greater interest than females at competing for access to several mates and argued that this difference could be explained by the difference in energy investment made by each sex in its gametes. Indeed, given that males generally invest very little energy in the production of a given sex cell in comparison to females, a male's reproductive success is not limited by its ability to produce spermatozoids but by its ability to fertilise eggs with these sex cells. Conversely, a female's reproductive success is not limited by its ability to have her eggs fertilised but by its ability to produce these cells. Males thus engage in a competition over the access to a limiting resource: the female eggs.

This insight has been later extended to a broader concept: the parental investment (Trivers 1972). Indeed, the energetic investment in gametes is not the only investment made by parents into their progeny that can limit the number of produced offspring. Trivers defined parental investment as "any investment made by the parent in an individual offspring that increases the offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring". This definition includes all investments (in terms of energy, other kind of limiting resources, time, risk of predation, etc....) that benefit the young, and helps to understand why females compete over the access to males in species where males provide more care than females to offspring (see also Kokko & Monaghan 2001 and Kokko & Jennions 2008). According to this concept, the sex with the highest parental investment will become a limiting resource for the other sex. Given that individuals of the sex investing less can increase their reproductive success by investing successively in the offspring of several members of the limiting sex, they will engage in contests

among themselves to access members of the other sex (Figure 1). On the other hand, individuals of the limiting sex are not limited by the access to members of the other sex and should become choosy to select mates that can improve their own reproductive success (Figure 1), for instance by providing them (and/or offspring) protection, resources, territory, etc... (direct benefits) or by transmitting genes conferring a greater fitness to offspring (indirect benefits, Kirkpatrick & Ryan 1991).

Contests among individuals of the same sex (intrasexual selection) and seduction of the limiting sex by the competing sex (intersexual selection) imply interactions between individuals of the same sex and between the two sexes (Figure 1). Sexual selection can thus be viewed as a particular case of social selection, a form of selection where an individual's fitness is not solely determined by its own phenotype but also by the phenotypes of the individuals with which it interacts (West-Eberhard 1979; Lyon & Montgomerie 2012). Since interactions between individuals are determinant, communication takes a central place in this kind of selection regimes and can lead to the development of extravagant secondary sexual characters that troubled Darwin.

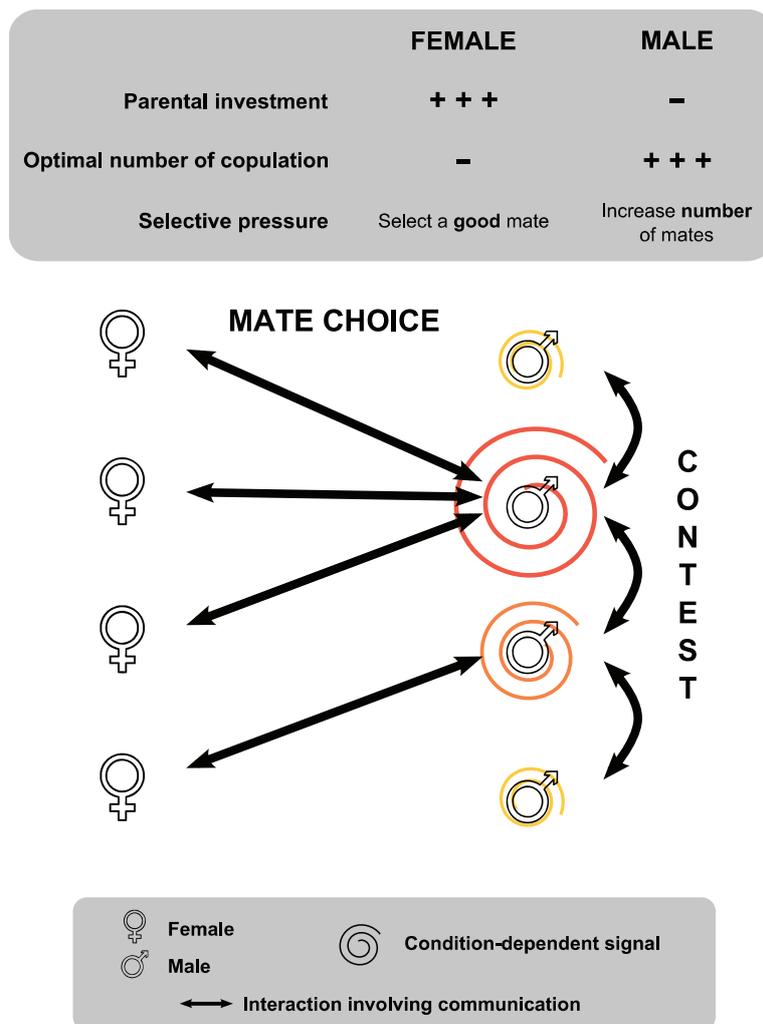


Figure 1. Simplified representation of sexual selection processes.

- Communication, Signal, Honesty

In its broadest biological definition, the term “communication” refers to any interaction between an individual (the emitter) who delivers information and an individual (the receiver) who use this information to make a decision (Shannon & Weaver 1949; Otte 1974; Marler 1977). This information transfer takes place through cues evaluated by the receiver. Signals constitute a particular case of cues provided by the emitter that have evolved precisely to deliver information, while other cues like the size of the emitter for instance, may have evolved for other reasons (Maynard-Smith & Harper 1995). A signal is under two main selective pressures, one on its content (what information is being transmitted), the other on its efficiency (effectiveness with which it is transmitted and received). Indeed, to be effective, a signal has not only to be able to travel from the emitter to the receiver (through a transmission channel, Shannon & Weaver 1949) but also to be detected by the sense organs, discriminated from background noise and from other signals and categorized by the receiver (Guilford & Dawkins 1991).

Dawkins & Krebs (1978) were among the first to define the term “signal” in an evolutionary perspective. They described it as an action or structure which increases the fitness of an individual (the signal emitter) by altering the behaviour of other organisms (the signal receivers). In this seminal publication, the authors particularly emphasised the manipulative purpose of sending a signal, providing examples where the emitter benefits from sending a signal and the receiver suffer from being manipulated through this signal. In such signalling system, the selective pressure exerted by the emitter on the receiver should lead to an improvement of discrimination abilities of the receivers; which in turn should lead to the selection of emitters with the best ability to lure receivers. This kind of situation thus leads to an arms race between emitters and receivers and does not reach an evolutionarily stable equilibrium.

Nevertheless, there are cases where signalling systems can reach an evolutionarily stable equilibrium: when both the emitter and the receiver benefit from their interaction. Communication in this case has been termed “mutualistic communication” (Otte 1974) or “true communication” (Marler 1977). The prerequisite for the receiver also benefits from communication is that the transferred information is reliable (Maynard-Smith & Harper 1995). In this case, the receiver is not manipulated by the emitter but rather obtains true information from it. The signal provided by the emitter is then called an “honest signal”. There are two main types of signals according to the type of information that they convey: “self-reporting signals” provide information about some property of the emitter, “other-reporting signals” convey information about an object or an organism other than the emitter (Maynard-Smith & Harper 1995).

In an evolutionary perspective, it is intuitive to imagine the potential benefits (in terms of inclusive fitness, Hamilton 1964) that individuals can obtain by providing reliable information to

related individuals. However, in most interactions (and even sometimes between related individuals), the emitter and the receiver have conflicting interests. This is for instance the case when two conspecifics are competing for a limited resource, when a female would benefit from choosing a particular mate while a male would benefit from mating with as many partners as possible, or even when a predator would benefit from eating a prey while the prey would benefit from not being eaten. Yet, these situations lead to signalling equilibrium where emitters provide reliable information to receivers (for a review see Searcy & Nowicki 2005). The central issue in explaining the evolution of communication is thus to understand what maintains signal honesty despite the incentive of the emitter to deceive the receiver when their interests conflict.

- The Costly Signalling Theory

For the signals to be honest even under conflict of interest between the emitter and the receiver, the incentive of the emitter to deceive the receiver must be balanced by a countervailing incentive against deceit. Most of research on the evolution of communication has focused on understanding the source and form of this countervailing incentive (Lachmann *et al.* 2001). The current dominant paradigm to explain the maintenance of signal honesty is the costly signalling theory. It derives from the handicap principle developed in a sexual selection context by Zahavi (1975) to explain the evolution of extravagant secondary sexual characters observed in males of many species. In its original version, the handicap principle stated that these characters were used by males to signal honestly their quality to females (in order to be chosen as mates) because only high quality males should be able to survive while bearing such handicaps. Hence, males that are still alive with such extravagant characters have successfully passed a “test”, proving their quality. This idea was first criticised by theoreticians, notably because formal models showed that such a situation could not lead to an evolutionarily stable equilibrium since females would suffer from choosing ornamented males because a part of their male progeny would not survive to such an inherited handicap (Maynard-Smith 1976). The handicap principle was thus updated (Zahavi 1977) to refine this too simplistic vision, by introducing the notion of cost of the handicap linked to male’s quality: the higher the cost of the handicap, the higher the male’s quality. This link could theoretically be realised through two ways: conditional handicaps (produced only if the male has both “genes of high viability” and “genes of the handicap”) and revealing handicaps (handicaps with a magnitude directly correlated with the quality of the male). Even if it was still debated (see Maynard Smith 1985 for a review), some formal models began to support the idea that the cost of sending a signal could maintain the reliability of the information transmitted. These models were applicable to several types of interactions with conflict of interest between the emitter and the receiver, such as a contest between two individuals to obtain a resource (Enquist 1985), offspring signalling their level of need

to parents (Godfray 1991), males signalling their quality to females or preys signalling their fleeing abilities to predators (Grafen 1990a,b). By making the assumption that the marginal cost of increasing the signal is greater for low quality individuals than for high quality individuals, *i.e.* that at an equal level of signalling it is costlier for a low quality individual than for a high quality one to increase his signalling level of one arbitrary unit (see Johnstone 1997 for a graphic representation and an adaptation to the level of need of the emitter instead of the level of quality), these models showed that a signalling system with conflict of interest between the emitter and the receiver can reach an evolutionarily stable equilibrium with honest signalling provided that signals emitted are costly. After the publication of the two Grafen's models (1990a,b), Maynard-Smith (1991) provided supplemental formal support to the idea that a signal should be costly to be honest through the Philip Sidney game; which finally convinced the scientific community to accept this idea and made Zahavi's (1975, 1977) and Grafen's (1990a,b) papers among the most cited references in behavioural ecology.

Nevertheless, all these models implicitly assumed a constraint linking the marginal cost and the realised cost of signalling (*i.e.* the cost of producing and/or maintaining the signal at the signalling equilibrium). Yet, this constraint was not necessary to reach an evolutionarily stable signalling equilibrium and several models have since shown that signal honesty can be maintained even with null or even negative realised costs (Hurd 1995; Szamado 1999; Lachmann *et al.* 2001). Hence, the signal needs not be costly at the signalling equilibrium provided that the cost of leaving this equilibrium, *i.e.* to send a dishonest signal, is sufficiently high ("stabilising cost" *sensu* Hurd 1995). In addition, Getty (1998a) showed that the differential cost criterion is neither a necessary nor a sufficient condition to reach signal honesty. Instead, he showed that in a general way, high quality signallers must be more efficient at converting signalling into fitness than low quality ones (Getty 1998b), highlighting the fact that not only differences in costs should be considered but also differences in benefits acquired by each emitter through signalling.

To summarise, although it may seem trivial, the general paradigm to explain signal honesty in the current state of knowledge states that the emitter of a signal should have a better benefits/costs balance when providing a signal transmitting a reliable information (about itself or about the state of the world) than when providing a dishonest signal (Getty 2006; Szamado 2011). Inter-individual differences in benefits/costs balance can theoretically be generated by differences in benefits but research has mainly focused on the costs of leaving the signalling equilibrium (*i.e.*, stabilising costs). These stabilising costs are not necessarily linked to realised costs of signalling. Nevertheless, in several cases realised costs can probably facilitate the maintenance of stabilising costs, leading to the mental shortcut that signals have to be costly to be honest (Szamado 2011).

Stabilizing costs have been classified by several authors according to their origin or the moment at which the emitter has to support them. Table 1 provides a classification of these costs based on those developed by Searcy & Nowicki (2005) and Fraser (2012). Costs are here divided into two wide categories: intrinsic costs and imposed costs. Imposed costs are those which are dependent upon the reaction of another organism. This organism may be the receiver of the signal, but also organisms outside the signalling system that can intercept the signal (generally termed “eavesdroppers”, McGregor 1993) and act to the detriment of the emitter, like parasites or predators. The receiver can impose stabilising costs on the emitter because signalling can increase the risk of being attacked by the receiver (“retaliation” cost), but also the risk of being injured since signalling may imply to expose some body parts (“vulnerability” cost). In some cases, there may also be costs associated with the “punishment” of dishonest signallers when they are recognised as such (Fraser 2012). Intrinsic costs are those which depend only on the emitter itself and its management of limited resources and time. These intrinsic costs can be subdivided in three categories according to the timing at which they occur relative to the emission of the signal (Searcy & Nowicki 2005). Hence, production costs are costs supported at the time the signal is emitted to the receiver (*e.g.* energy consumed by calling crickets, time taken away from foraging activities by singing in birds). Developmental costs are those supported before the signal is emitted, when the signal develops (*e.g.* resources invested in the development of antlers in deer, pigments invested in the development of bright feathers in birds). Maintenance costs are costs supported after the signal structure has been developed regardless of whether the signal is actually emitted to the receiver or not (*e.g.* colouration of some body parts involving the derivation of pigments from other function, elongated tail in birds).

Some signals have been conventionally considered separately from others because they are assumed to be physically linked to an emitter’s property of interest for the receiver and thus to be unfalsifiable (*i.e.* “index” *sensu* Maynard-Smith & Harper 1995). This is for instance the case of scratching marks made by tigers on trunks that indicate their size to conspecifics, or dominant frequency in many insect or frog sexual calls that are directly dependent upon the size of the vibrating structures and thus often indicate body size of the emitter. However, according to the classification of stabilising costs proposed above, we can merely consider that these signals are kept honest by developmental costs. Indeed, it seems possible for a frog or an insect to develop larger vibrating structures during its ontogeny to appear larger when calling. However, the size of these structures probably results from the optimisation of an allocation trade-off of limited resources towards different body parts or functions; and increasing the size of certain parts would certainly engender a cost for the individual. In the same way, a tiger could probably invest in longer legs to make scratching marks higher on the trunks but it will certainly involve developmental costs and

perhaps also maintenance costs along its life. Thus with such a classification of stabilising costs, there is *a priori* no reason to treat an index differently from another honest signal (Searcy & Nowicki 2005).

Table 1. Classification of stabilising costs (based on those developed in Searcy & Nowicki 2005 and Fraser 2012).

Imposed		Intrinsic	
Receiver-dependent:	- Vulnerability	Developmental:	- Limited resources
	- Retaliation	Production:	- Limited resources
	- Punishment		- Limited time
Receiver-independent:	- Predation	Maintenance:	- Limited resources
	- Parasitism		- Foraging difficulties

- Sexual Selection & Signalling Quality/Condition

The honest signalling of quality/condition appears central in sexual selection processes. Hence, females, by selecting high quality/condition mates, are expected to obtain direct (*e.g.* good parental care, good territory, avoidance of diseases transmission) and/or indirect (*i.e.* good genes transmitted to offspring) benefits (Kirkpatrick & Ryan 1991; Andersson 1994). Moreover, males can use signals emitted by other males to assess their quality/condition and then take decision on the best way to react (typically fight or flight, Andersson 1994).

Despite the apparent centrality of quality/condition in sexual selection processes, these two terms are rarely defined and most of the time they are used indifferently, just as in the previous paragraph (*e.g.* Johnstone 1995). There is currently no *consensus* on the definition of individual quality. It sometimes refers to one or several phenotypic traits related to an estimates of individual fitness, but in many studies it is invoked as an unmeasured trait explaining differences among individuals in their way to manage trade-offs (reviewed in Wilson & Nussey 2009). Individual genetic quality, however, has been precisely defined by Hunt *et al.* (2004) as the sum of the additive effects of the genes of an individual on total fitness. Nevertheless, while this genetic quality may be crucial for female choice in order to obtain indirect benefits, it is probably not the most relevant emitter's property when females are looking for direct benefits and when males are engaged in contests. Hence, most studies in sexual selection consider that the information transmitted through sexual signalling is a phenotypic quality, or condition (Andersson 1994). Individual condition has often been defined as the relative part of energy stores of an individual (Peig & Green 2010). However, it can be

seen as a broader concept, concerning not only energy stores but all kind of limited resources. Indeed, several authors define it as the total pool of resources acquired by an individual and available for allocation to the production and maintenance of fitness-enhancing traits (Rowe & Houle 1996; Hunt *et al.* 2004; Tomkins *et al.* 2004). Individual condition, defined in this way, is thus a phenotypic characteristic of an individual depending both on its genotype and its experienced environment. It includes both aspects of nutritional status (*e.g.* the amount of a particular resource acquired through foraging and assimilated) and health status (*e.g.* the amount of a particular resource that has not to be invested in combating pathogens). In addition, the optimality of resource allocation and the efficiency of resource use can be viewed as factors acting on individual condition, even if they are not *per se* aspects of condition as defined above (Hunt *et al.* 2004). Individual condition is hardly measurable as such, but it can be assessed through indices (*e.g.* Jakob *et al.* 1996; Kotiaho 1999; Green 2001; Peig & Green 2010). It can also be experimentally modified through manipulation of the environment (*e.g.* food availability: Scheuber *et al.* 2003a; exposure to parasites: McGraw & Hill 2000) or manipulation of the genotype (*e.g.* half-sib breeding design: Kotiaho *et al.* 2001; inbreeding: Ketola & Kotiaho 2010; for a review see Cotton *et al.* 2004).

There are several reasons to think that individual condition, as defined above, is the main information provided by males during signal emission in a sexual selection context. First, condition should be directly related to most of the direct benefits that a female can acquire through mate choice. For instance, it should be highly linked to the ability of the male to feed juveniles and/or defend a good territory (*e.g.* Senar *et al.* 2002), to male's health status (and thus his probability to transmit a disease to the female, *e.g.* Faivre *et al.* 2003), or even to the probability that a male eat the progeny to enhance its own future reproductive success (*e.g.* Candolin 2000a). Secondly, condition should be directly related to the ability of an individual to win a male-male contest (through fighting ability for instance, Olsson 1994). At last, condition probably harbours a great genetic variance and is dependent upon environmental factors, allowing the maintenance of indirect genetic benefits for females despite a constant directional selection on it (via the genic capture process and/or genotype \times environment interactions, Rowe & Houle 1996; Greenfield & Rodriguez 2004; Hunt *et al.* 2004; Tomkins *et al.* 2004).

In line with the costly signalling theory, provided that it is less advantageous to invest a certain amount of resources in sexual signals for a male of low condition than for a male of high condition, sexual signals should reliably reflect male condition. Indeed, several theoretical works have shown that in this case, we should expect variability in the signalling level, both between and within individuals, honestly reflecting the variability in male condition. This has been termed the hypothesis of condition-dependence of sexual signals (Andersson 1982, 1986; Kodric-Brown & Brown 1984; Nur & Hasson 1984; Zeh & Zeh 1988). Several empirical studies have since supported this

hypothesis, notably through experimental manipulation of individual condition and estimation of its impact on sexual signals (*e.g.* Frischknecht 1993; Mappes *et al.* 1996; David *et al.* 1998). Some studies even showed that it is costlier to increase the level of signalling for low condition individuals than for high condition ones (Kotiaho 2000); and that when another factor disturbs the link between cost and condition (like the perspective of future reproductive events for high condition individuals) the relationship between the level of signalling and male condition may not be monotonic (Candolin 1999). More recently, it has also been shown that the strength of the relationship between the level of signalling and condition becomes stronger when environmental conditions are worse (Vergara *et al.* 2012) and is positively correlated with the strength of selection through mate choice when comparing several traits in the same species (Delcourt & Rundle 2011).

- Multicomponent Signals

Many signals consist in a combination of several components that are assessed and considered by the receiver when taking a decision. These signals are termed “multicomponent signals” (Candolin 2003). This is for instance the case of the song produced by male field crickets which contains several components assessed by females like the chirp rate or the carrier frequency (Scheuber *et al.* 2003a). Some multicomponent signals have the particularity of being composed of several components transmitted via several sensory modalities. These particular signals are generally termed “multimodal signals” (Candolin 2003). This is for instance the case of the colouration and the smell emitted by ladybugs as a signal of unpalatability towards predators (Marples *et al.* 1994). Several hypotheses have been formulated to explain the evolution and maintenance of multicomponent signals (reviewed in Candolin 2003 and Hebets & Papaj 2005), some of them are based on signal efficiency selection, the others on signal content selection.

Among hypotheses based on signal efficiency, the “receiver psychology hypothesis” states that producing several components together can improve detection, discrimination and/or learning of the signal by the receiver and may thus provide benefits for both the emitter and the receiver (Guilford & Dawkins 1991; Rowe 1999). Another closely related and non-exclusive hypothesis proposes that the efficiency of the signal may be enhanced by adding several components if communication occurs in several sensory environments; it is the “multiple sensory environments hypothesis” (Candolin 2003). Indeed, the different signal components, especially those occurring in different sensory modalities, may be, for instance, used by the receiver at different distances from the emitter because the sensory environment changes as the receiver goes up to the receiver (*e.g.* Backwell & Passmore 1996; Candolin & Reynolds 2001; Lopez & Martin 2001).

The hypotheses based on signal content are of two main types: either multicomponent signals provide more information to receivers than simple signals, or multicomponent signals provide

less information to (or even exploit) receivers. According to the latter category, one of the hypotheses formulated by Møller & Pomiankowski (1993) to explain the existence of multicomponent sexual signals suggested that many signal components do not currently provide information about male condition (the “unreliable signal hypothesis”). In their seminal study, they made a distinction between “Fisher traits” and “handicaps”. According to their definitions, a Fisher trait is unconnected or has ceased to be connected to male condition, while a handicap is a condition-dependent trait. Assuming a more rapid loss of benefits acquired by females through mate choice and a quicker genetic variance depletion in Fisher traits than in handicaps (because of fewer genes involved in the expression of Fisher traits), Møller & Pomiankowski (1993) hypothesized a quicker evolutionary turnover in Fisher traits than in handicaps. This rapid turnover, with sometimes maintenance of some traits because they are relatively cheap to produce, may generate the existence of multicomponent signalling situations. This hypothesis is supported by data collected by Møller & Pomiankowski (1993) in a comparative study of feather ornament in birds since male secondary sexual characters in species with multiple ornaments show far weaker condition-dependence than those in species with only a single ornament. Several theoretical (*e.g.* Pomiankowski & Iwasa 1993; Iwasa & Pomiankowski 1994, 1995) and empirical studies (*e.g.* Ligon & Zwartjes 1995; Omland 1996a, b) have since supported the idea that uninformative Fisher traits can develop and be maintained in multicomponent signalling systems.

Based on the conflict of interest between males and females and on the statement that female preferences may sometimes evolve before, and be exploited by, male sexual traits (Ryan *et al.* 1990; Basolo 1990), Holland & Rice (1998) developed another model where multicomponent signals are used by emitters to exploit receivers. This model of sexually antagonistic coevolution, called the “chase-away model”, could explain the evolution of exaggerated multicomponent signals in the context of sexual selection. Females are here assumed to resist to exploitation by lowering their level of sensitivity to a particular signal component. In response to this resistance, males increase the level of this signal component to still elicit a female response. When the costs associated with this increase become too high to be compensated by benefits of being chosen, males shift to another signal component to exploit another pre-existing female sensory bias. This mechanism could result in several costly signal components that have little value as condition indicators but are maintained as thresholds for maintaining female attention. Actually, the chase-away model does not conceptually differ from benefits-based models for the evolution of female choice. Indeed, in both cases, females benefit from accepting or rejecting a subset of males that differ in the benefits and costs they provide (Rosenthal & Servedio 1999; Getty 1999; Rice & Holland 1999). Female resistance to males that provide weak stimuli can thus be considered as female preference for males with strong stimuli (Kokko *et al.* 2003). Hence, insights provided by this model are close to those brought

by Iwasa & Pomiankowski (1995) and highlight the fact that dynamics of signalling system can allow the evolution of several signal components that are little or non-informative about male's condition.

- Multicomponent Signals & Multicomponent Condition

Among the hypotheses based on the signal content proposed to explain the evolution and maintenance of multicomponent signals, one suggests that multicomponent signals provide more information to receivers than simple signals: the "multiple messages hypothesis". This hypothesis states that the different components of a signal provide different information to the receiver (Møller & Pomiankowski 1993; Candolin 2003; van Doorn & Weissing 2004; Hebets & Papaj 2005). In the context of sexual selection, where signals are expected to reflect honestly the condition of the emitter, this hypothesis seems particularly relevant. Indeed, condition appears to be a multicomponent concept as it is dependent on several nutritional and/or health aspects and may vary over time. In fact, the different components of a signal may rely on different limited resources acquired and kept by the emitter like energy, pheromone precursors, or even molecules involved in both immune system and signals like some pigments. Moreover, depending on the type of costs associated with the reliability of each signal component (development, production or maintenance), they are expected to be plastic at different time scales, allowing the transmission of information about the condition of the emitter at different stages of its life (past condition vs. current condition, Møller & Pomiankowski 1993). Indeed, even if dynamic and static signal components have been suggested to serve respectively to male condition assessment and species recognition (Gerhardt 1991), they can theoretically both convey information about male's condition. Hence, dynamic components of a signal that vary greatly on short term and involve mainly production costs may be indicators of the current condition, while static components, less variable on short term and relying mainly on developmental costs, may be reliable indicators of the past condition.

The multiple messages hypothesis first received little support from theoretical works (*e.g.* Pomiankowski & Iwasa 1993; Schluter & Price 1993; Iwasa & Pomiankowski 1994). Indeed, these models showed that receiver preference for different indicators of a same condition should not be maintained and should lead to the evaluation of condition through a unique (the most reliable) component. However, further models showed that the multiple messages hypothesis can lead to an evolutionarily stable equilibrium with multicomponent signalling provided that emitters are assumed to vary in several distinct aspects of condition rather than varying in a single inclusive condition (Johnstone 1995, 1996; van Door & Weissing 2004). Note that, in such models, even if the cost of a particular signal component depends only on one particular aspect of the condition (*e.g.* the availability of a particular resource), its expression is likely to be influenced by other aspects of the

condition. Hence, there is no need for one-to-one relationship between signal components and aspects of the emitter condition at signalling equilibrium (Johnstone 1995).

Since these theoretical works, several empirical studies have supported the multiple messages hypothesis (reviewed in Candolin 2003). For instance, McGraw & Hill (2000) showed in the American goldfinch that carotenoid-based plumage and bill coloration are sensitive to infection by intestinal coccidians, while the melanin-based black cap is not but instead is dependent on social status. In the satin bowerbird, a correlative study suggested that bower quality (a component of the signal sent by males to females) was linked the ectoparasite load and body size of the male, whereas ultraviolet plumage coloration predicted the intensity of infection from blood parasites, feather growth rate, and also body size (Doucet & Montgomerie 2003). By experimentally manipulating current and past condition of male field crickets, Scheuber et al. (2003a,b) showed that the chirp rate (a dynamic call component) reliably indicated short-term condition changes while the carrier frequency (a static call component) was an honest indicator of past condition because constrained by the development of vibrating structure during ontogeny. Similarly, Gibson & Uetz (2012) recently showed in a wolf spider that dynamic components of the signal sent by males to females were dependent upon current condition while static ones were influenced by past condition.

In line with the multiple messages hypothesis, it has been suggested that the use of multiple components in a signal may prevent emitters to deceive receivers because sending a dishonest signal would imply to cheat on several aspects of condition simultaneously (Candolin 2003). Nevertheless, there are other ways to circumvent the honesty of a signalling system: Alternative Reproductive Tactics.

- Deceit & Alternative Reproductive Tactics

As developed above, signal honesty means that the information transmitted from the emitter to the receiver is reliable. Based on the classification of the types of communication made by Otte (1974), Searcy & Nowicki (2005) defined a signal as honest if some characteristic of this signal (including perhaps, its presence/absence) is consistently correlated with some attribute of the emitter or its environment and if the receiver benefits from having information about this attribute. Conversely, according to their definition, deception occurs if a receiver registers something Y from an emitter; if the receiver responds in a way that benefits the emitter and is appropriate if Y means X; and if it is not true that X is the case. This definition of deceit implies that the emitter benefits from sending a dishonest signal and that the receiver suffers from trusting the false information conveyed by the signal. It has been theoretically shown that deceit may occur in honest signalling systems provided that the proportion of "cheaters" (or the proportion of deceit made by individuals) is sufficiently low to maintain on average the benefits of the receiver to consider all signals as honest.

Hence signals need only be honest “on average” for a signalling system to be stable (Johnstone & Grafen 1993; Kokko 1997), and a distinction can be made between the honesty of a signal and the honesty of a signalling system (Castellano 2009).

Deceit as defined by Seracy & Nowicki (2005) implies signal emission. The use of multiple components in a signal may prevent emitters to deceive receivers because sending a dishonest signal would imply to cheat on several aspect of condition simultaneously (Candolin 2003). However, there are cases where some individuals can circumvent the honesty of a signalling system, even those involving multicomponent signals, by exploiting signals emitted by conspecifics without signalling themselves. In a sexual selection context, this is often the case of males using a parasitic reproductive tactic (Taborsky *et al.* 2008). Indeed, there are many examples of sexual signalling systems where some individuals honestly signal their condition while some others do not emit signal at all and try to obtain copulation (or even only egg fertilisation) by exploiting the investment made by signalling males. For instance, in the Pacific salmon, some males called “jacks” do not develop secondary sexual characters and try to steal fertilisation of some eggs when other males are copulating with females (Gross 1985). Similarly, in the bluegill sunfish, some males look like females to gain access to spawning and fertilise a part of the eggs (Gross & Charnov 1980). In ruffs, while some males are displaying signals to court females, some others, called “satellites”, stay in territories of signalling males and try to copulate with some females attracted toward it (van Rhijn 1973). This kind of satellite behaviour is also widely observed in mammals (Wolff 2008) and in chorusing anurans (Zamudio & Chan 2008).

The use of a parasitic reproductive tactic is often determined by male condition; low condition males using more often this kind of tactic than high condition ones (Gross 1996; Taborsky *et al.* 2008). In some cases however, the condition-dependence is reverted. For instance, in the Pacific salmon, high condition males (condition measured through growth rate) mature earlier and use a parasitic reproductive tactic while other males mature later and use a reproductive tactic implying the development of secondary sexual characters (Gross 1991).

- Biological model

The European treefrog (*Hyla arborea*) is a lekking anuran in which males gather in choruses during the reproductive season to attract females. In this species there are strong inter-individual differences in male reproductive success, due to female choice and/or male-male competition (Jaquier *et al.* 2009). Females are known to select their mates on the basis of a multimodal sexual signal involving several acoustic (call, Figure 2) and visual (vocal sac colouration, Figure 3) components (Richardson *et al.* 2010b; Gomez *et al.* 2010). Males *H. arborea* produce calls in series named bouts containing on average 25 calls and lasting four seconds on average (Figure 2ab). Each

call has a dominant frequency – frequency with the highest energy – ranging from 2000 to 3000 Hz (Figure 2c). Among call components used in mate choice, there are both static and dynamic call properties (Castellano *et al.* 2002), potentially linked to male condition at different time scales. Indeed, on one hand, call dominant frequency in this species is negatively correlated with male body size (Marquez & Tejedo 1990; Richardson *et al.* 2010b) and may thus be an indicator of male’s past condition. On the other hand, call production is highly energy demanding in this species (Grafe & Thein 2001) and several dynamic call properties like call amplitude, call duration or within-bout call rate may be honest indicators of current condition. In addition, vocal sac colouration in *H. arborea* relies mainly on carotenoid pigments (Richardson *et al.* 2009). These pigments are known to be a limited resource since animals cannot synthesise them *de novo* and because they are involved in crucial functions in the immune and antioxidant systems (Lozano 1994; Olson & Owens 1998). Hence visual signal components in *H. arborea* may be honest indicators of male ability to obtain and manage this resource. At last, in this species males are known to sometimes use a satellite parasitic reproductive tactic, which consists in waiting silently near a displaying male and intercepting females attracted toward it (Berec & Bajgar 2011).

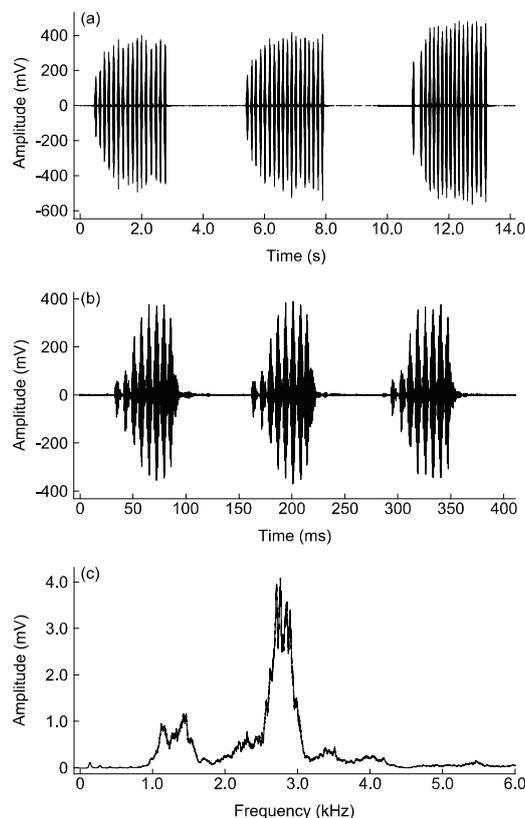


Figure 2. Temporal and spectral characteristics of *Hyla arborea*'s advertisement call. (a) oscillogram of 3 call bouts (b) oscillogram of 3 calls (c) power spectrum of one call.



Figure 3. A male *H. arborea* displaying both advertisement calls and vocal sac colouration.

H. arborea is thus a model that allows testing (1) condition-dependence of several components of a multimodal sexual signal; (2) whether the different components are dependent upon different aspects of male condition (multiple messages hypothesis); (3) whether the use of a parasitic reproductive tactic is condition-dependent and upon which aspects of condition it depends.

To investigate these 3 axes, we first studied variability in the metabolic cost of call production according to call properties and male condition (article [1]). We then manipulated male condition through starvation / feeding experiments to investigate condition-dependence of the different call components (article [2]). We investigated the reliability of a static call component (dominant frequency) in reflecting past condition (articles [3]), and a methodology to properly assess this call property (article [4]). We also manipulated several aspects of male condition through starvation / feeding / carotenoid supplementation experiments to investigate condition-dependence of visual signal components (article [5]). At last, we investigated condition-dependence of the use of a parasitic reproductive tactic by comparing behaviours of males with different past condition and by manipulating their current condition (article [6]).

The tested hypotheses are:

H1.1: Some attractive call properties involve a higher production cost.

H1.2: For a given level of signalling, high condition males support a lower production cost than low condition ones.

- H2.1: An energetic constraint can generate trade-offs between several dynamic signal components and/or between these components and the amount of signal produced.
- H2.2: According to their condition, males have different ways to manage these trade-offs.
- H3.1: The within-individual variability of call dominant frequency is higher than previously believed.
- H3.2: This variability can impact the selective strength driving female choice on this signal component.
- H4.1: The assessment of variability should be better when using several records discontinuous in time over the night than by using samples made of consecutive calls.
- H5.1: Colouration components selected by females should be correlated to male condition in the field.
- H5.2: Changes in condition should be rapidly mirrored in colouration components.
- H6.1: The probability to adopt a parasitic tactic depends on past condition which has influenced traits conditioned by ontogeny like body size or size of a particular body part (inherent disadvantage hypothesis).
- H6.2: The probability to adopt a parasitic tactic depends on current condition which limits the energy availability for signal production (energetic constraint hypothesis).

ARTICLES

- [1] Voituron, Y., Brepson, L., Richardson, C., Joly, P. & Lengagne, T. 2012. Energetics of calling in the male treefrog *Hyla arborea*: when being large means being sexy at low cost. *Behaviour*, 149, 775-793.
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Article 1: **Energetics of calling in the male treefrog *Hyla arborea*: when being large means being sexy at low cost**

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Behaviour, 149 (2012), 775-793.

Issue:

Condition-dependence of a signal in the context of sexual selection implies stabilising costs supported by the emitters if sending a dishonest signal. In chorusing anurans, these costs seem to be essentially production costs maintained through an elevated metabolic realised cost supported by males when calling. Stabilising costs may occur if (1) increasing the attractiveness of the signal implies increasing production costs; and (2) for a given level of signalling, low condition males support a higher production cost than high condition ones. However, inter-individual variability in this cost has rarely been investigated.

Hypotheses:

H1.1: Some attractive call properties involve a higher production cost.

H1.2: For a given level of signalling, high condition males support a lower production cost than low condition ones.

Methods:

Call production and oxygen consumption (estimation of the production cost) of males differing in condition were simultaneously measured.

Results:

- Oxygen consumption was mainly explained by the quantity of call produced by males.
- For a given quantity of call produced, increasing the within-bout call rate (WCR, *i.e.* increasing the attractiveness for females) led to an increase in oxygen consumption.
- For a given level of signalling (*i.e.* a given number of call produced and a given WCR), large males showed a lower mass-specific oxygen consumption than small ones.
- Large males emit calls with a lower dominant frequency, more attractive for females.

Conclusion:

Increasing the attractiveness of the signal, notably through modification of dynamic call properties, implies increasing production costs. For a given level of signalling, high condition males seem to bear a lower production cost than low condition ones. Moreover, large males produce more attractive calls independently of their current investment in call production since they produce calls with a lower dominant frequency (a static call property). These results together suggest that both static and dynamic call components may be reliable indicators of male's condition and that the condition-dependence of dynamic call components may be maintained through a stabilising cost due to a higher production cost for low condition males than for high condition ones, all else being equal.



Energetics of calling in the male treefrog *Hyla arborea*: when being large means being sexy at low cost

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Abstract

Female mate choice is often thought to be based on signals that honestly reflect male quality as a potential mate. However, while particularly costly acoustic signals have often been studied, the existence of differences in energetic costs among males has rarely been considered. These differences may be decisive in many 'income breeding' species in which males face a trade-off between calling in order to attract females and foraging to renew their energetic reserves. We thus examined calling energetic costs in an income breeder, *Hyla arborea* (L.), known to produce costly energetic calls, in order to explore the sources of inter-individual variability. After an arginine vasotocin injection, we determined the relationships between energetic costs (via oxygen consumption), call characteristics and male morphology. We found a strong variation in male calling energetic costs. For the same call rate, some males consumed oxygen at a rate four times greater than others. This difference was mainly explained by male size, bigger males consuming less oxygen per gram of tissues than smaller ones during calling. In addition, an acoustic parameter, within bout call rate, also influenced the energetic cost of emitted signals. These findings highlight the importance of calling energetic cost in income breeding species which have strong consequences on sexual selection processes. Indeed, energetic constraints are key parameters to understand the inter-individual variation in call characteristics. Males with a reduced cost may increase their chorus tenure and/or the attractiveness of their calls. Regarding female mate choice, choosing a big male may involve indirect benefits linked both to male size and to calling cost.

Keywords

sexual selection, acoustic communication, calling cost, body size, oxygen consumption, anurans, honest signal, arginine vasotocin.

1. Introduction

Sexual selection is a form of selection which favours individuals, male or female, that are more adept at exploiting their partner's capacities to produce numerous, viable and fertile offspring (Andersson, 1994). In many species, females invest more than males in reproduction, and also often select their sexual partner (Emlen & Oring, 1977; Andersson, 1994). Many studies have investigated the phenotypic male traits females assess when choosing their mate (Richardson et al., 2008, 2010a,b; Richardson & Lengagne, 2010). These traits are expected to honestly reflect male quality. Zahavi (1975) suggested that signals will be honest when they are costly to produce or to maintain and when this cost is related to the physical condition of the emitter. Signals can also be honest when their production is mechanically related to characteristics of the sender (Maynard-Smith & Harper, 1995).

In anurans and orthopterans, acoustic signals play a crucial role in reproduction (Gerhardt & Hubert, 2002). Many experimental studies have shown female preference for energetically costly call parameters, such as high call rates, or for call parameters which are related to certain morphological characteristics (e.g., preference for low frequencies which reflect large body size, reviewed in Gerhardt, 1994). Field studies have also found a strong correlation between male mating success and the number of nights spent calling throughout the breeding season (known as chorus tenure, Gerhardt, 1987; Sullivan & Hinshaw, 1992; Meuch & Grafe, 2009). Males are, therefore, expected to maximize their chorus tenure. Surprisingly however, the majority of field studies conducted to date on anurans have found that males spend relatively few of the nights of the breeding season actively calling (e.g., Jacobson, 1985; Murphy, 1994a; Friedl & Klump, 2005; Grafe & Meuche, 2005).

Why then do males not lengthen their calling activity in order to increase their mating success? The energetic constraint hypothesis (Murphy, 1994b) suggests that the energetic cost of calling is such that callers are unable to obtain sufficient energy to call for more than a small portion of the breeding season. Laboratory analyses have indeed found that calling imposes an extremely high metabolic cost in anurans. In most species studied to date this cost (assessed through O_2 consumption) by far exceeds that of forced locomotory exercises (reviewed in Wells, 2007 and see Stoddard & Salazar, 2011). This constraint is particularly high in 'income' breeding species (*sensu* Jonsson, 1997) that face a time trade-off between producing sexual

signals and foraging. In his study of *Bufo rangeri*, Cherry (1993) indeed observed that males lose weight when they call whereas they gain weight during their movements outside the breeding site, which suggests that they leave it in order to forage for food. A few studies suggest that the weight lost due to calling varies amongst individuals. Tejedo (1992) found that big *Bufo calamita* males lost weight slower than small males during the time spent at the breeding site and could, therefore, remain there for a greater number of nights. The physiological basis of this relationship may be similar to the allometric scaling of the metabolic cost of transport to $(\text{body mass})^{-0.32}$ (Alexander, 2005). Such allometric relationship suggests a higher efficiency of large animals' muscles involved in locomotion (Casey & Ellington, 1989) and we suggest that this process could also be involved in calling energetic.

Call rate is an important predictor of the cost of acoustic signals, with a linear correlation between O_2 consumption and call rate (Taigen & Wells, 1985; Wells & Taigen, 1986, 1989; Grafe, 1996; Wells et al., 1996). However, Grafe & Thein (2001) underline that “there is an additional unappreciated variation resulting from differences in the morphology and/or physiology of males such that calling at the same rate can cause a threefold differences in O_2 consumption”. Surprisingly, very few studies address the sources of this inter-individual variability (McLister, 2001) but based on the potential higher muscle efficiency of large individuals (Alexander, 2005), we can hypothesize that large males will exhibit lower energetic cost of calling compared to small ones.

Hyla arborea is a European hyloid known to produce costly energetic calls (Grafe & Thein, 2001). During the seven weeks of the reproductive season, males alternate periods of calling and foraging (Grafe & Meuche, 2005; Meuche & Grafe, 2009). Friedl & Klump (2005) found that the *H. arborea* males they followed in the field were only present in the chorus for a few nights (mean \pm SD 12.53 ± 6.61). In the present study which focuses on the energetic cost of acoustic signals, we investigated (i) whether large males are more effective than small ones during calling and (ii) whether some call characteristics are correlated with these differences in energy expenditure.

2. Materials and methods

2.1. Study area, source populations and animals

Hyla arborea is a small treefrog (mean weight \pm SD 5.3 ± 1.5 g and snout–vent length 42.2 ± 2.7 mm; Richardson et al., 2008) found throughout central

and eastern Europe. During its prolonged breeding season, from mid-April to the end of May in these latitudes, the males congregate around ponds from which they call at night during several hours in favourable meteorological conditions (no wind, minimal temperature: 4.5°C; Friedl & Klump, 2002). In this study conducted in 2008, males came from three French populations located around Lyon, one called 'Planches' (P), near Crémieu (5°21'7"E, 45°44'20"N), and the two others from the Gresivaudan valley, 'Cheylas' (C) and 'Laissaud' (L) (5°59'48"E, 45°22'53"N and 6°3'51.02"E, 45°28'18.02"N, respectively).

2.2. Arginine vasotocin treatment

Each male was weighed to the nearest 0.01 g and then injected with a dose of arginine vasotocin (AVT, Sigma; $2 \mu\text{g}\cdot(\text{g wet weight})^{-1}$). AVT is a disinhibiting hormone (Marler et al., 1995) which generates calling behaviour in males regardless of their quality, size, motivation and stress. Indeed, calls from males as small as 3.1 g and as large as 9.3 g were obtained, thus constituting a representative sample of both high and low quality males. To avoid a potential bias induced by the hormone, two preliminary studies have been conducted to assess the AVT effect on signal structure and on oxygen consumption adjusted for the calling activity. First, the effect of AVT injection has been determined by comparing calling activity of 18 males in two situations: with AVT ($2 \mu\text{g}\cdot(\text{g of AVT wet weight})^{-1}$) versus 'with no injection' (control period). Then, the effect of AVT by itself has been evaluated by comparing calling activity of 12 males in two situations: with AVT ($2 \mu\text{g}\cdot(\text{g of AVT wet weight})^{-1}$) versus 'with saline injection' (equivalent volume of 0.9% saline). In all cases, the order of the treatment was determined at random and analyses were based on recording from 10 to 20 min. In the second preliminary study, the calling activity and oxygen consumption of 19 males have been recorded (using the methodology described below) without AVT injection to test if the mass effect on oxygen consumption is affected by the hormone (by comparing these data with those obtained with AVT injected males).

2.3. Oxygen consumption

In order to obtain relevant mass values of the frogs, individuals were kept for 24 h before the experiments, ensuring the purge of the gastrointestinal tract (Brepson, unpublished data). In addition, the mass of the individuals were only used if the frogs urinated just before being weighted.

Energy expenditure was measured by indirect calorimetry using an open-circuit system. After capture, the frogs were placed in a 750 ml metabolic chamber in the dark. The chamber was placed in a thermostatic box ($22 \pm 1^\circ\text{C}$) and connected to a high pressure air bottle (Air Liquide, SA) filled with air (Respal-CT), which pushed air through the chamber since a regulator-flowmeter (Minibloc, Air Liquide) at a rate of $150 \text{ ml}\cdot\text{min}^{-1}$ ($\pm 5\%$). A subsample of the exhaust air from the chamber was drawn continuously through columns of Drierite to trap moisture. Oxygen content was then measured by a Servomex O_2 Analyser (Servomex). The gas analyser constantly recorded the fractional oxygen concentration in the modified air and sends the values every 10 s to a computer to track the time-course of oxygen consumption. A delay of 60 s between the time of a breath and a deflection in oxygen concentrations measured by the analyzer occurred and has been taken into account.

Excel software (Windows) was used to convert changes in fractional oxygen concentration to values of \dot{V}_{O_2} in ml O_2 per gram of fresh tissue per hour using a simplified version of the method of Depocas & Hart (1957), taking into account only oxygen. All gas measurements were corrected to standard temperature and pressure dry (STPD). Frogs were allowed to settle in the chamber for at least 1 h before the start of a trial to reach a thermal equilibrium with the chamber air and were simultaneously stimulated with a chorus playback.

2.4. Call recording

Simultaneously to oxygen consumption measurements, the calling activity of males was recorded using a Sony ECM-T6 directional microphone (Sony), placed outside the respirometric chamber, connected to a Tascam US 144 soundcard (Teac) and a notebook computer (recording software: Cool Edit Pro, sampling frequency 16 kHz). Each male was recorded for a minimal period of 30 min.

We stimulated male calling by broadcasting chorus noise recorded at the study site, via an amplified loudspeaker (KH pas-100). We chose a recording in which no male was louder than the others in order to avoid any risk of the tested male responding to the calls of a particular competitor (for more details, see Richardson et al., 2008).

Simultaneous measurements of \dot{V}_{O_2} and calling activity were collected from 35 frogs (11 from Planches, 15 from Laissau and 9 from Cheylas)

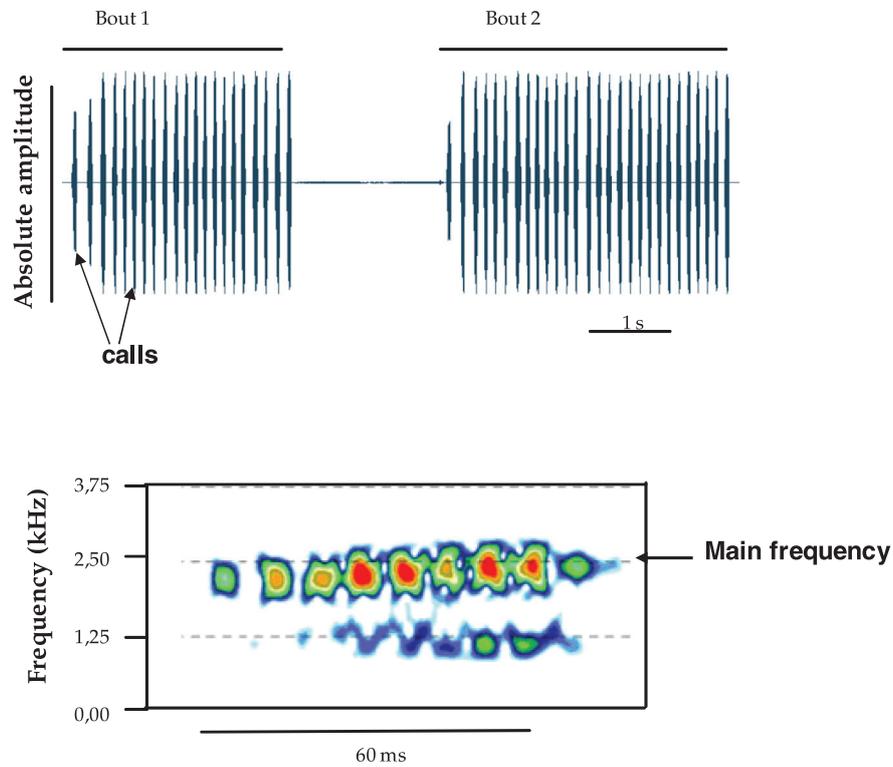


Figure 1. Organisation of mating calls of *Hyla arborea*. (a) Waveform demonstrating the organisation of calls into bouts. (b) Spectrogram of a single call. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/1568539x/149/7>.

between 2200 h and 0200 h. Concomitantly, different calling properties were measured over the corresponding periods of \dot{V}_{O_2} after allowing for the 60-s time lag. In this species, mating calls are produced in series, known as bouts (Figure 1), which usually contain between 10 and 30 calls. From the entire recording, we extracted the main acoustic characteristics using Avisoft Saslab (Avisoft BioAcoustics): call duration (ms), dominant frequency (the frequency with the highest energy, in Hz), within-bout call rate (WCR, in $\text{calls}\cdot\text{s}^{-1}$) and the call rate (in $\text{calls}\cdot\text{h}^{-1}$). Due to the potential displacement of the animal into the respirometric chamber, the call amplitude was not correctly assessed and, thus, not included into statistical analyses.

All the animals were released at the breeding site on the night following the metabolism and calling recording.

2.5. Statistical analysis

Statistical analyses were conducted using R™ v2.7.2 (2010) software. First, the effect of AVT treatment on call properties was assessed using paired *t* tests. In addition, regarding the analyses focused on metabolism, the potential population effect was considered as a random effect in mixed-effects linear models fitted by maximum likelihood (package ‘nlme’, Pinheiro et al., 2009). Since the variability due to population effects was negligible and the estimated parameters were rigorously the same with or without random effect, we used linear regression for the following models. The first model explains oxygen consumption (in ml O₂·g⁻¹·h⁻¹) by call rate (in calls·h⁻¹). The following models are looking for factors inducing variability around the first model. The chosen models have been selected by the Akaike Information Criterion (AIC). Factors tested were: call duration, within-bout call rate (WCR) and mass. All second-order interactions between factors have been tested and removed if not significant. For all models, variance homogeneity, residuals normality and residuals independence have been checked. Each factor effect has been drawn using the package ‘effects’ (Fox, 2003) which represent the relationship between one factor and the response variable (with its 95% confidence envelope) averaging the other factor effects.

3. Results

During the recordings (50.9 ± 16.9 min; mean ± SD) tested males (mean mass ± SD: 5.47 ± 1.54 g, range: 3.13–9.31 g) emitted on average 3023 ± 2669 calls (mean ± SD).

3.1. Effect of AVT on signal structure and O₂ consumption

As shown in Table 1, AVT injection induced a significant difference on only one parameter that is WCR by lowering it. However, the interaction between AVT effect on WCR and mass was not significant (regression between WCR changes due to treatment and mass, $R^2 = 0.056$, $p = 0.276$). Nevertheless, when considering the effect of AVT on mass-specific oxygen consumption, the interaction between log(mass) and AVT injection was significant and positive (estimate ± SE = 3.58 ± 0.94, $t = 3.811$, $p < 0.001$). This result indicates that large males showed a stronger increase in their oxygen consumption than small ones with AVT injection, generating a bias in results observed with AVT injection. However, since we expected a negative

Table 1.

Effect of arginine vasotocin (AVT) injection ($2 \mu\text{g}\cdot(\text{g wet weight})^{-1}$) on different call properties in *Hyla arborea*.

Call property	Type of comparison	N	Mean of the difference	95% CI boundaries		df	t	p
				Bottom	Top			
Call duration	AVT vs. no injection	18	-2.333	-5.273	0.606	17	-1.675	0.112
Call duration	AVT vs. saline injection	12	-1.833	-6.477	2.811	11	-0.869	0.404
Dominant frequency	AVT vs. no injection	18	17.250	-43.386	77.886	17	0.600	0.556
Dominant frequency	AVT vs. saline injection	12	-17.792	-91.987	56.403	11	-0.528	0.608
WCR	AVT vs. no injection	18	-0.372	-0.574	-0.171	17	-3.902	0.001**
WCR	AVT vs. saline injection	12	-0.578	-0.998	-0.158	11	-3.027	0.012*
Call rate	AVT vs. no injection	18	432.674	-1403.032	2268.379	17	0.497	0.625
Call rate	AVT vs. saline injection	12	1642.959	-1628.510	4914.428	11	1.105	0.293

WCR, within-bout call rate.

* Significant difference, $p < 0.05$.

** Significant difference, $p < 0.01$.

correlation between mass and mass-specific oxygen consumption, the bias generated by AVT injection was conservative and did not affect the validity of our results.

3.2. Effect of calling and mass on O_2 consumption

Figure 2 shows a positive linear relation between mean O_2 consumption and call rate during the recording. Call rate explained approximately 70% of the variance in O_2 consumption. It is important to notice that our purpose was not to obtain absolute O_2 consumption measurements but to investigate how males mass affect O_2 consumption per gram.

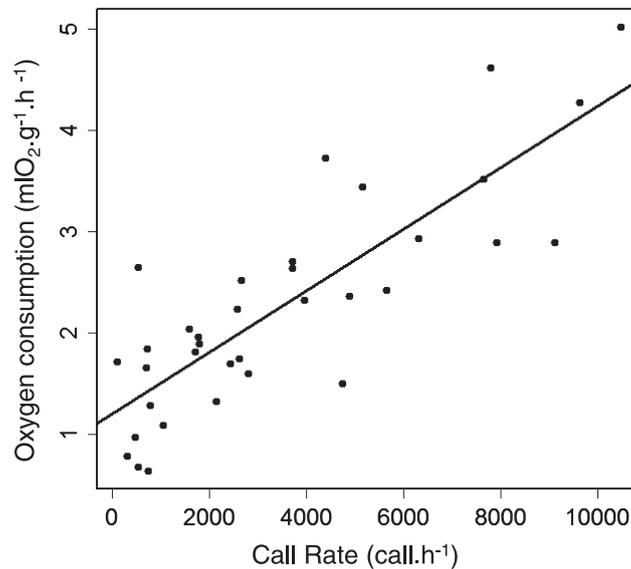


Figure 2. Effect of call rate, on oxygen consumption of *Hyla arborea* during the entire calling recording in 35 males. (Linear model equation: $Y = 1.199 + 0.0003043X$; $R^2 = 0.695$; $p = 5.03 \times 10^{-10}$).

In order to test the mass effect, two analyses have been conducted: first, we used a linear model taking into account the call rate + call duration + WCR + mass to explain oxygen consumption in $\text{ml}\cdot\text{h}^{-1}$. With such analyses, the data showed a positive effect of all parameters (call rate, WCR and mass) on oxygen consumption (see Figure 3A–C). In addition, to test the relative cost of one gram of fresh tissue taking into account the call rate + call duration + WCR + mass, we used another linear model to explain variability in term of mass-specific oxygen consumption estimated in $\text{ml}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ (see Figure 3D–F). In both models, the call duration effect has been tested and removed bringing no information into the model (see AIC values, Table 2). Since models including call duration or not had close parameter estimations for the three other factors, model averaging was not necessary.

Residual variance showed that for the same call rate, some males consumed less energy than others (Figure 2). For instance, for a given call rate some males needed twice as much oxygen as predicted by the model while others consumed only half the predicted amount.

The results of the chosen linear models (Table 3) showed that oxygen consumption was influenced by male body mass, call rate and by mean in-

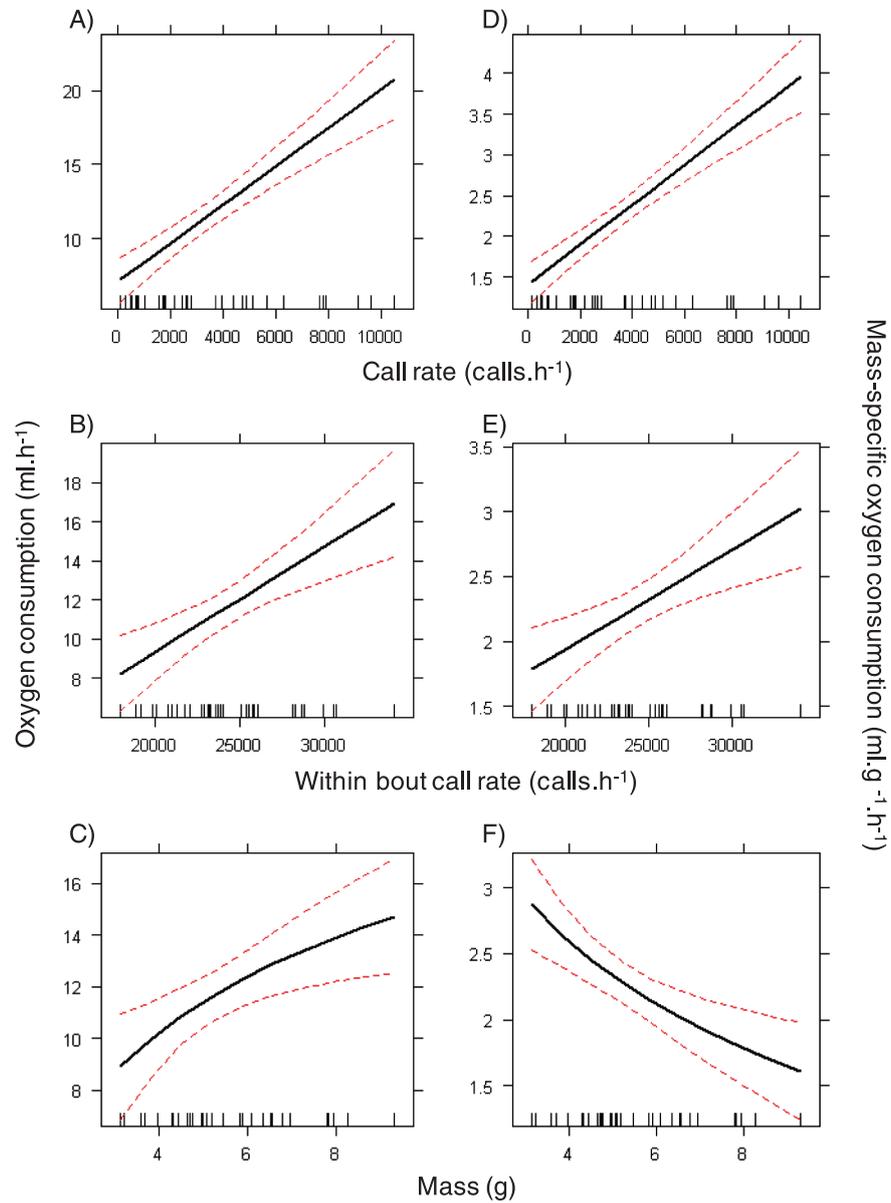


Figure 3. Effect sizes of call rate, within bout call rate and mass on oxygen consumption and mass-specific oxygen consumption drawn from the two linear models. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/1568539x/149/7>.

Table 2.

Akaike information criterion (AIC) testing the relative impact of the call rate, call duration, within-bout call rate (WCR) and mass on oxygen consumption of *Hyla arborea* during calling and mass-specific oxygen consumption of *Hyla arborea* during calling.

Model	AIC
Oxygen consumption (in ml·h ⁻¹) of <i>Hyla arborea</i> during calling	
$\dot{V}_{O_2} \sim \text{call rate} + \text{WCR} + \log(\text{mass})$	174.2273
$\dot{V}_{O_2} \sim \text{call rate} + \text{WCR} + \text{call duration} + \log(\text{mass})$	176.0289
$\dot{V}_{O_2} \sim \text{call rate} + \text{WCR}$	181.7973
$\dot{V}_{O_2} \sim \text{call rate} + \text{WCR} + \text{call duration}$	183.6029
$\dot{V}_{O_2} \sim \text{call rate}$	192.6814
Mass-specific oxygen consumption (in ml·g ⁻¹ ·h ⁻¹) of <i>Hyla arborea</i> during calling	
Mass-specific $\dot{V}_{O_2} \sim \text{call rate} + \text{WCR} + \log(\text{mass})$	48.98291
Mass-specific $\dot{V}_{O_2} \sim \text{call rate} + \text{WCR} + \text{call duration} + \log(\text{mass})$	50.40303
Mass-specific $\dot{V}_{O_2} \sim \text{call rate} + \text{WCR}$	61.87324
Mass-specific $\dot{V}_{O_2} \sim \text{call rate} + \text{WCR} + \text{call duration}$	63.5695
Mass-specific $\dot{V}_{O_2} \sim \text{call rate}$	67.80628

dividual within bout call rate (WCR). For a given number of calls emitted, a heavy male consumed less oxygen per gram than a lighter male (Table 3 and Figure 3F). Taking the lowest and the highest masses among the tested individuals (3.13 and 9.31 g, respectively), the mass-specific oxygen consumption ranged from 2.86 to 1.60 ml O₂·g⁻¹·h⁻¹. In the same way, regardless

Table 3.

Effect of call rate, mass and withinbout call rate (WCR) on oxygen consumption of *Hyla arborea* during calling and mass-specific oxygen consumption of *Hyla arborea* during calling.

	Estimates	Std. error	numDF	denDF	F	p
Oxygen consumption (in ml·h ⁻¹) of <i>Hyla arborea</i> during calling						
Intercept	-14.793687	4.159994	1	29	661.1230	<0.0001
Call rate	0.001299	0.000172	1	29	85.0529	<0.0001
log(mass)	5.332611	0.707949	1	29	10.3703	0.0032
WCR	0.000536	0.000128	1	29	17.5115	0.0002
Mass-specific oxygen consumption (in ml·g ⁻¹ ·h ⁻¹) of <i>Hyla arborea</i> during calling						
Intercept	1.480038	0.695105	1	29	96.1922	<0.0001
Call rate	0.000243	0.000029	1	29	135.6601	<0.0001
log(mass)	-1.157065	0.285386	1	29	5.7816	0.0004
WCR	0.000076	0.000021	1	29	12.7248	0.0013

of the number of calls emitted and individual body size, an individual calling with a higher WCR consumed more O₂ than a male calling at a slower rate. It is noteworthy that body mass and WCR are not correlated ($N = 35$; $R^2 = 0.0028$; $p = 0.761$), thus influencing the oxygen consumption independent of each other.

4. Discussion

When a male is calling, we can hypothesize that almost all of the metabolic rate would be determined by the muscles' mean force (F , in N), shortening velocity per duty cycle (v , in ms⁻¹) and number of duty cycles (n , dimensionless but assumed for 1 s), leading to the following equation: $P_{\text{metabolism}} = nFv$. In terms of behaviour, mean muscle force would relate most directly to the average energy for each call and the metabolism is, thus, strongly dependent upon this average energy per call and call rate. The present study is in agreement with such assumptions since call rate explained 70% of the variance in O₂ consumption (see Figure 2). Number studies in anurans already showed positive correlation between mass-specific oxygen consumption and call rate (Prestwich et al., 1989; Wells & Taigen, 1989; Grafe, 1996; Wells et al., 1996; Grafe & Thein, 2001; McLister, 2001). In addition, for a given call rate and mass, oxygen consumption increases with within-bout call rate. Calling faster, thus, constitutes a more costly form of courtship for a male.

Similarly to the observations of Grafe & Thein (2001), we also found large inter-individual variations in oxygen consumption in *H. arborea*, with up to a fourfold difference between males for a given call rate. To date only one study (McLister, 2001) has explored the sources of inter-individual variability in O₂ consumption in a hylid (*Hyla versicolor*) but taking into account the metabolism in mJ. Nevertheless, because O₂ consumption was not corrected by individual mass, the results of the McLister's study underline the fact that a big individual needs more O₂ to fuel his greater mass of tissue than a small individual. Using similar analyses, our data (see Figure 3C) confirm those published by McLister (2001). However, examining inter-individual variability in mass-specific O₂ consumption allowed us to highlight that the heavier a male is, the less oxygen per gram it consumes while calling (for a given call rate and within-bout call rate). The heaviest male from our tested individuals consumed 44% less oxygen per g body mass than the lightest.

Such conclusion could suffer from a confounding effect due to call amplitude. This hypothesis can likely be dismissed since published data on the same species at the intra-specific level (Marquez et al., 2005), and studies on 14 other hylid species (Gerhardt, 1975) and 17 African frog species (Passmore, 1981) with inter-specific comparison showed no relationship between size and intensity of calls. Such relation has been found in only one Bufo species (Gerhardt, 1975). Such discovery, thus, demonstrates a scaling of the energy cost of calling in the same way to the scaling of energy cost of locomotion (Alexander, 2005).

4.1. Why do bigger males consume less energy per g body mass in call production?

As we stated before, males produce calls by using what are known as their trunk muscles that undoubtedly consume the majority of O₂ during calling. However, the mass effect as new explanatory factor approach cannot be investigated through the framework described before, since this variability does not appear in the equation explaining metabolic rate with muscle mean force, velocity per duty cycle and number of duty cycles. We can, thus, hypothesize the occurrence of differences in physiological traits between large and small males to explain the observed inter-individual variations and particularly potential differences in muscles functioning. Several inter-specific comparisons of Neotropical hylids and leptodactylids have found a strong positive correlation between male calling rates and trunk muscle lipid content, capillary density, sarcoplasmic reticulum and mitochondria content (Wells, 2007). Such proximal causes of higher calling capacities have also been studied at an intra-specific level (Zimmitti, 1999) demonstrating that males with high calling rates constantly exhibit higher citrate synthase (CS) activity. We can, thus, hypothesize similar physiological differences between big and small males in *H. arborea*. But this still does not explain the inter-individual variation in call production cost. Part of the explanation may involve inter-individual differences regarding mitochondrial content and functioning (Steyermark et al., 2005), especially regarding the number of ATP molecules produced from ADP for each oxygen atom consumed during substrate oxidation and oxidative phosphorylation, which is known as the mitochondrial ATP/O ratio. This parameter, often neglected, is very important in the relationships between acquiring nutrients and ensuring a suitable supply of oxygen to oxidize them, and producing ATP in quantities sufficient for growth, survival and reproduction. Up until now, the huge

majority of studies argued from the oxygen consumed that is not constantly correlated to ATP synthesis but recent studies in ectotherms demonstrated implication of ATP/O into the growth and senescence processes (Robert & Bronikowsky, 2010; Salin et al., 2012). Thus, assessing the ATP/O ratio in large and small individuals could demonstrate that large individuals perform an ATP production similar to that of small individuals but with lower oxygen consumption, reflecting variation in metabolic efficiency (Brand, 2005).

4.2. Male size, energetic calling cost and calling activity

Acoustic signals are very costly to produce in *H. arborea* (Grafe & Thein, 2001; this study), and in this species, males are considered to be income breeders with a prolonged breeding season. Thus, males face a trade-off between calling — which is an energy-consuming activity — and foraging to renew their energetic reserves. In our study, we have shown for the first time in anurans that big males spend less mass-specific energy than smaller males at producing calls (i.e., they consume less oxygen per gram for the same acoustic output).

A lower calling cost can have various consequences for male calling behaviour. With a given amount of energy, males could (i) emit calls that are both costlier energetically and more attractive to females, (ii) spend more consecutive nights calling on the breeding site before leaving to replenish their energetic reserves, (iii) call for a longer time each night. Concerning the first hypothesis, our study found no correlation between male body mass and within-bout call rate or bout length ($N = 35$; $R^2 = 0.0026$; $p = 0.770$). We unfortunately have no data regarding call amplitude, which is also known to determine the energetic cost of call production. Regarding chorus tenure, the second hypothesis seems more attractive than the third: in prolonged breeding anurans, male mating success has often been explained by the number of nights males spent calling at the breeding site (e.g., Gerhardt, 1987; Dyson et al., 1992; Sullivan & Hinshaw, 1992; Murphy, 1994a; Bertram et al., 1996; Friedl & Klump, 2005) and not by the time spent within the chorus during the night (Murphy, 1999). Moreover, Friedl & Klump (2005) and Castellano et al. (2009) found chorus tenure to be positively correlated with male body mass in *H. arborea* and *H. intermedia*, respectively. It now appears necessary to monitor male vocal activity precisely over several successive nights in order to put these various hypotheses to the test.

4.3. Why choose big males?

In our study, we showed that bigger males consume less oxygen per gram during calling than smaller males. These large males are often more attractive to females in many species (Andersson, 1994). What direct and indirect benefits could females obtain by choosing big males?

In non-lekking species, the bigger males have been shown to give indirect benefits to females such as access to the best oviposition sites (e.g., Wells, 1977; Howard, 1978) or a higher fertilisation rate of the eggs (Robertson, 1990). *H. arborea* is a lekking species (Friedl & Klump, 2005) and, thus, males are not expected to defend resources for females. Furthermore, as far as we know, no data showed any difference due to male size in egg fertilisation in *H. arborea*. Once paired, like in other anurans, the female controls the movement of the pair (Lengagne & Joly, 2010) and, thus, chooses the oviposition site. It, therefore, seems that bigger males cannot afford any direct benefits to female in *H. arborea*.

In many anuran species, females can also benefit from mate choice indirectly because attractive males bequeath superior genes to their offspring. In *H. arborea*, as in many other anuran species, there is a negative correlation between call frequency and male size: the bigger a male, the lower its call frequency and females exhibit a strong preference for low frequency calls (Márquez & Tejedo, 1990; Richardson et al., 2010a). By pairing with a larger male, females may gain several indirect benefits. First, we showed that large males consume less energy per gram of fresh tissue in call production than smaller ones. While a particular form of mitochondrial functioning might not be heritable by pairing with a large male (mtDNA is generally passed along the maternal line), other characteristics such as higher muscle quality could be heritable to offspring. Secondly, bigger males may also be viewed as males with better foraging abilities. Indeed, an indirect benefit of female mate preference concerning foraging ability has been shown in guppies (*Poecilia reticulata*) with a probable genetic contribution to the foraging ability from fathers to their offspring (Karino et al., 2005). Lastly, large males may also be viewed as males that, with the same amount of food, exhibit a better assimilation of nutrients and a better growth (Secor & Diamond, 1998). Determining how male calling costs affect both male signal production and female mate choice, thus, promises to significantly advance our understanding of the mechanisms involved in sexual selection.

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Article 2: **Condition-dependent ways to manage acoustic signals under energetic constraint in a treefrog**

Brepson, L., Voituron, Y. & Lengagne, T.
Behavioral Ecology, in press.

Issue:

Dynamic components of sexual signals are expected to be condition-dependent on short term. When investigating condition-dependence of multicomponent signals with several dynamic components we should consider potential allocation trade-offs among the different dynamic components but also trade-offs between these components and the amount of signal produced. Moreover, we should also consider the possibility that, according to their condition, males use different strategies to face these trade-offs. However, studies have rarely considered these trade-offs and even less inter-individual differences in the way to manage them; particularly in chorusing anurans where experimental manipulations of current condition led to very conflicting results.

Hypotheses:

H2.1: An energetic constraint can generate trade-offs between several dynamic signal components and/or between these components and the amount of signal produced.

H2.2: According to their condition, males have different ways to manage these trade-offs.

Methods:

We investigated condition-dependence of call components and potential trade-offs between them in 36 males by manipulating their energetic constraint through starvation/feeding experiments while recording their overall call production during 10 consecutive nights.

Results:

- Even when considering simultaneously several call components and the amount of call produced, the energetic constraint does not appear to impact call production.
- When focusing on individual responses, it appears that males under energetic constraint cannot maintain both the amount of call produced and within-bout call rate (WCR).
- While large and high condition males mainly decrease WCR along the experiment, small and low condition males mainly decrease the amount of call produced per night.
- Males who maintained the amount of call produced per night the most are those who lost the most condition.
- When re-fed, starved males only increased the amount of call produced per night.

Conclusion:

These results suggest that both the amount of call produced per night and WCR are short-term condition-dependent. The amount of call produced per night seems to be costlier to maintain than WCR but may confer a greater advantage in mating success. This may lead to different allocation strategies among males of different conditions, masking condition-dependence of signal components when the trade-offs and the different ways to manage these trade-offs are not considered.

Condition-dependent ways to manage acoustic signals under energetic constraint in a treefrog

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Abstract: Because resources needed to produce sexual displays are limited, courting males are expected to face trade-offs between investing in display properties selected by females and investing in the amount of display. This should be particularly the case in species with energetically expensive displays and high intrasexual competition through endurance rivalry like chorusing anurans. Studies that manipulated the energetic constraint in calling anurans have led to conflicting results, potentially due to non-simultaneous assessment of call properties selected by females and the calling activity (*i.e.*, the total number of calls emitted by a male), and/or to the use of different strategies to face display trade-offs by males. We investigated display trade-offs in 36 males *Hyla arborea* (L.) by manipulating their energetic constraint through starvation/feeding experiments while recording their overall call production during 10 consecutive nights. Even when considering simultaneously calling activity and call properties, the energetic constraint does not appear to impact call production. However, when focusing on individual responses, it appears that males cannot maintain both calling activity and within-bout call rate. While big and high condition males mainly decrease this call property along the experiment, small and low condition males mainly decrease calling activity. Moreover, males who maintained calling activity the most are those who lost the most condition and, when re-fed, starved males only increased calling activity. These results suggest that calling activity is costlier to maintain than call properties but may confer a greater advantage in mating success, leading to different allocation strategies among males of different conditions.

Keywords: advertisement calls, body condition, call rate, chorus attendance, endurance rivalry, mate choice, sexual selection, signal honesty, trade-off, treefrog.

Introduction

Mechanisms of sexual selection like mate choice are known to promote costly displays in males (Zahavi 1975, 1977; Andersson 1994). These displays are supposed to reflect individual condition: only high condition males being able to produce such costly displays. Hence, male mating success generally increases as the investment in displays increases (Andersson 1994). Beyond the signal produced, male attendance at the mating site has also been found to be a major determinant of male mating success in many species (*e.g.*, in birds: Hill 1991; Fiske *et al.* 1994; in mammals: Higham *et al.* 2005; Lidgard *et al.* 2005; in anurans: Wells 1977; Bertram *et al.* 1996; in insects: Banks and Thompson 1985; Dronev 1992). Sexual selection is here supposed to act through endurance rivalry, promoting the ability to remain reproductively active at the mating site (Andersson 1994) and thus promoting traits that increase the ability to obtain energy and / or spend it efficiently (Murphy 1998). Males are thus expected to face trade-offs between increasing investment in display properties selected by females and increasing the amount of display.

In anurans, laboratory studies have often demonstrated female preference for costly call properties in both simple choice situation (*e.g.*, Sullivan and Hinshaw 1992; Gerhardt *et al.* 1996) and more complex conditions (*i.e.*, more than two males and/or chorus noise, *e.g.*, Schwartz *et al.* 2001; Richardson and Lengagne 2010). However, the huge majority of field studies shows that chorus attendance is the major determinant of mating success, whatever the mating system associated with this chorus (review in Wells 2007). Yet, chorus attendance often appears to be very short with respect to the duration of the breeding season (*e.g.*, Sullivan 1982; Murphy 1994a; Grafe and Meuche 2005). Since calling is clearly an expensive activity in many anurans, involving high metabolic rates (review in Wells 2001), the most likely hypothesis to explain such abbreviated chorus attendance is the energetic constraint hypothesis (Mac Nally 1981; Tejedo 1992; Murphy 1994b). Different studies support this hypothesis showing a decrease of the energetic reserves over the reproductive season (*e.g.*, Mac Nally 1981; Tejedo 1992; Cherry 1993) and even during a single night of calling (*e.g.*, Bevier 1997). Moreover, chorus attendance is often positively linked to initial body condition (Murphy 1994b; Judge and Brooks 2001; Castellano *et al.* 2009) and/or negatively linked to the rate of condition loss (Tejedo 1992; Murphy 1994b; Judge and Brooks 2001), suggesting that chorus attendance is limited by the amount of energy reserves and/or the ability to spend this energy efficiently.

Nevertheless, studies that tried to manipulate experimentally the energetic constraint by starvation or feeding treatments during the reproductive season have achieved conflicting results. Indeed, experimentally feeding can result in a longer chorus attendance (Murphy 1994b; Gordon 2004) or not (Green 1990; Judge and Brooks 2001; Meuche and Grafe 2009), a shorter time before

returning to the chorus (Murphy 1994b; Meuche and Grafe 2009) or not (Green 1990) and an increase in calling rate (Grafe 1996; Marler and Ryan 1996; Meuche and Grafe 2009) or not (Green 1990). These results may be puzzling because these studies did not consider the potential trade-offs between investing in call properties selected by females (hereafter merely referred as 'call properties') and investing in calling activity (*i.e.* the total number of calls emitted by a male). Actually, none of them quantified calling activity, they rather estimated it through the time spent in the chorus without recording calls (Murphy 1994b ; Judge and Brooks 2001), through regular scans (Marler and Ryan 1996; Gordon 2004) or by recording calls over a short sample (Green 1990; Grafe 1996; Meuche and Grafe 2009). In addition, the effect of manipulated food availability on call properties selected by females has only been investigated by Green (1990) who found that call complexity, was not enhanced by experimental feeding in *Physalaemus pustulosus*. Since the cost of calling involves both call properties and calling activity, it appears crucial to take into account potential display trade-offs between them with accurate simultaneous assessment of these two aspects of call production. Moreover, as hypothesized by Castellano (2009), males of different condition might use different strategies, to face the display trade-offs imposed by the energetic constraint. Indeed, his theoretical work showed that, under a strong selection imposed by female choice, males of low condition should invest proportionally more on call properties selected by females and less on calling activity than high-condition males. This inter-individual variability in response to the constraint may mask most of the effects when those are investigated at the group level. Hence, to understand better the impact of an energetic constraint upon such display trade-offs, an experimental approach where the effect of this constraint can be measured both on call properties and calling activity of each individual is compulsory.

In the present study, we experimentally investigated the impact of the energetic constraint on call production in the European treefrog (*Hyla arborea*). Males of this lek-mating hylid are known to exhibit an expansive calling behavior (Grafe and Thein 2001), with costliest displays preferentially selected by females (Richardson *et al.* 2010; Voituron *et al.* 2012). Moreover, although chorus attendance seems to be the major determinant of male mating success (Friedl and Klump 2005; Jaquier *et al.* 2010), it is much shorter than the breeding season (Grafe and Meuche 2005). Given that males provided with supplemental food in the field return sooner to the chorus than control males (Meuche and Grafe 2009), this abbreviated chorus attendance is likely to be due to an energetic constraint on calling activity.

In this study we therefore manipulated the energetic constraint of 36 males *H. arborea* through feeding/starvation experiments and recorded their overall call production over 10 consecutive nights to test the hypotheses (1) that an energetic constraint can generate display trade-offs between call properties and calling activity; (2) that, under energetic constraint, males of low

condition invest proportionally more on call properties selected by females and less on calling activity than high-condition males.

Material and methods

Capture and housing conditions

Individuals were obtained from a population located near Lyon, France, on the Crémieu Plateau (5°21'7"E, 45°44'20"N). During the breeding season, 36 males were captured individually by hand when calling at the pond (three capture sessions along the breeding season, 12 males captured by session and respectively assigned to three independent experimental sessions along the breeding season). They were then transported by car in individual dark boxes to the housing room of the laboratory. Males were then immediately placed in individual terrariums (25x17x15cm) with a water-filled basin and a tree branch. Males were subsequently fed *ad libitum* with domestic crickets (*Acheta domesticus*) during three days for acclimation before the recording experiment. During acclimation and throughout the experiment, males were exposed to natural photoperiod and chorus noise was broadcast each night from 08:00pm to 02:00am, mimicking the field conditions during intense nights of chorusing (Friedl and Klump 2002). During the experiment, temperature of the room was kept at $23.1 \pm 1.5^\circ\text{C}$ (mean \pm sd, range: 20.4 - 25.5°C). This temperature was measured both at the beginning (08:00pm) and at the end (02:00am) of the night from exactly the same place each night with a thermocouple. Temperatures from the beginning and the end of the night were always the same to the nearest 0.2°C; hence in subsequent analyses we used the mean temperature as a unique temperature for each night. Note that the temperature was not correlated with the day of the experiment (linear regression of temperature on day, including sessions as a random factor, estimate \pm SE: 0.116 ± 0.068 , $P = 0.101$). After acclimation, males were randomly assigned to two experimental groups: one fed *ad libitum*, the other deprived of food for 10 days. Just after the end of the 10th night recording, starved males were re-fed *ad libitum* in order to investigate the re-feeding effect on call production during the 11th night recording. All males were recorded individually during the 11 consecutive nights of the experiment from 08:00pm to 02:00am to measure both nightly calling activity (*i.e.* the total number of calls emitted by a male during a night) and call properties selected by females. Males were weighted and measured (femur-tibia length) at the end of the acclimation (initial mass) and on day 10, before re-feeding (final mass). A scaled mass index (size-independent condition indicator) was computed according to the methodology proposed by Peig and Green (2010) both at the end of the acclimation (initial SMI) and on day 10, before re-feeding (final SMI). Males from the two groups did not differ in initial SMI (t-test: $t = -0.267$, $DF = 33.95$, $P = 0.791$). After

the experiment, all males were fed *ad libitum* for two days and released at the capture site the following night.

Recording and call analysis

The 12 males belonging to the same experimental session (6 fed and 6 starved in each session) were recorded simultaneously. Each terrarium was placed in an individual semi-anechoic chamber the day of the recording at 08:00pm. Terraria had plastic mesh sides and lid instead of glass sides in order to avoid sound reflectance. Calls were recorded with a Sony ECM-T6[®] microphone placed in each chamber and plugged in a Roland R-44[®] 4-channels recorder or a Tascam US 144[®] soundcard connected to a notebook computer. Each semi-anechoic chamber was open on the front side to avoid stressing males. Males were stimulated by broadcasting a repeated 2-minutes chorus noise, via an amplified loudspeaker (KH pas-100[®]) connected to a CD player. This stimulating record was an artificial chorus noise, constituted of calls from several isolated males *H. arborea*, in which no male was louder than any other to avoid the risk of the tested male responding to the calls of a particular competitor (Richardson *et al.* 2008). Although recording chambers were open on their front side, they were not placed face to face. As a consequence, sounds perceived by a focal male from the other adjacent males were very weak compared to the volume of the stimulating record that was broadcast.

Males *H. arborea* produce advertisement calls in series named bouts containing on average 25 calls and lasting four seconds on average (Figure 1ab). Each call has a dominant frequency – frequency with the highest energy – ranging from 2000 to 3000 Hz (Figure 1c). Males can produce about 15 000 calls during a calling night (Brepson *et al.* submitted). Nightly calling activity and call properties were measured on the entire night recording, using automatic parameter measurement on spectrogram thanks to Avisoft Saslab[®] software with a Fast Fourier Transform of 256 points. Measured call properties are properties known to be used in mate choice by females (Richardson *et al.* 2010): dominant frequency (in Hz), call duration (in milliseconds) and within-bout call rate (WCR; *i.e.*, the number of calls per bouts divided by bout duration, in calls per seconds, this property was named ‘call rate’ in Richardson *et al.* 2010). For dominant frequency and call duration, nightly individual means were computed on all calls emitted over the night. For WCR the nightly individual mean was computed on all call bouts emitted over the night. In order not to disturb call production, males were free to move in their terrarium throughout the recording, making amplitude measure non-standardized. Hence this call property, although known to impact female choice (Richardson *et al.* 2010), was not used in this study.

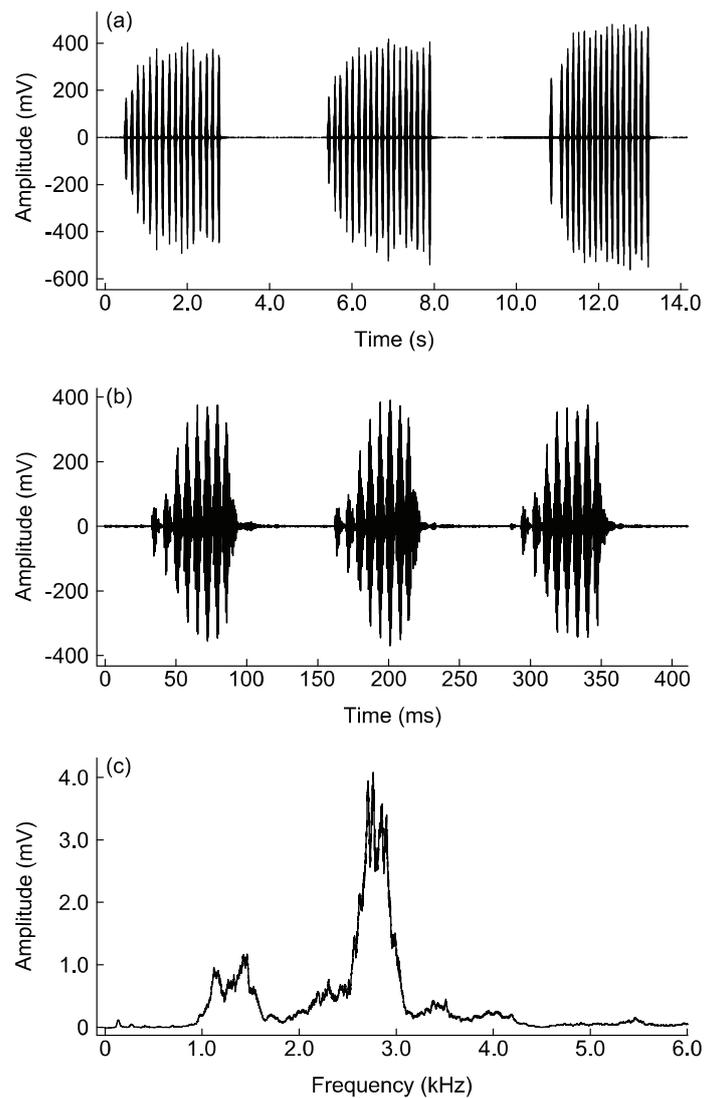


Figure 1. Temporal and spectral characteristics of *Hyla arborea*'s advertisement call. (a) oscillogram of 3 call bouts (b) oscillogram of 3 calls (c) power spectrum of one call.

Statistical analysis

The effects of the energetic constraint on call properties were explored using linear mixed models suited to repeated observations on the same individuals (Bolker *et al.* 2009). All models included temperature, number of days spent housed (thereafter called 'day'), starvation (0 for fed males, 1 for starved males) and the interaction day \times starvation as fixed effects and individual as random effect acting on both the intercept and each fixed effect except the temperature. Indeed, all tested models including a random effect acting on the effect of temperature were less parsimonious than models which did not include it (AIC increase, range: 4 - 10 points), suggesting that the temperature acts in the same way for all individuals. We also tested for a session random effect but all tested models with this supplemental effect were less parsimonious than models which did not

include it (AIC increase, range: 11 - 20 points), hence it was not kept. These effects were investigated both on the nightly calling activity and the three call properties. Coefficients and standard errors (SE) were computed using a restricted maximum likelihood approach. Factor significance was tested using Wald z tests (Bolker *et al.* 2009).

Computed random effects (*i.e.*, individual effects) around the interaction day \times starvation in the starved group are supposed to reflect the strength of the energetic constraint acting on each individual for each call property (or nightly calling activity). Hence, individual trade-offs in call production under energetic constraint have been investigated by correlating these random effects from a call property to another and from call properties to nightly calling activity among starved males. Then, individual traits linked to the different responses to the energetic constraint have been investigated by correlating these random effects with individual condition indicators. These correlations have been explored through linear models.

The effect of re-feeding was explored by comparing values of call properties (or nightly calling activity) predicted at day 11 by models to observed values on day 11 (re-feeding day). Predicted values have been calculated from models computed on 10 days by taking into account both fixed and random effects. The differences between predicted and observed values have been tested with paired t-tests for both starved and fed males.

All statistics were performed using R 2.11.1 (R Development Core Team 2010) with the 'lme4' package for linear mixed models (Bates *et al.* 2011). For all linear models, residual normality, independence and variance homogeneity have been checked.

Results

Starvation and energetic constraint

Along the whole experiment, males emitted a total of 4 565 456 calls. Each male has emitted on average $126\,818 \pm 67\,522$ calls (mean \pm sd, range: 15 393 – 277 210 calls) over the 11 consecutive nights. During one calling night, regardless of their treatment, males emitted on average $11\,529 \pm 8219$ calls (mean \pm sd, range: 0 – 47 627 calls). The two groups differed significantly from one another in their mass loss (Figure 2). The fed group mass loss did not significantly differ from 0 (paired t-test: $t = 1.715$, $DF = 17$, $P = 0.104$) whereas starved males have lost on average 15.3% of their initial body mass in 10 days (paired t-test: $t = 5.754$, $DF = 17$, $P < 0.001$). Hence, only starved males appeared to be under energetic constraint.

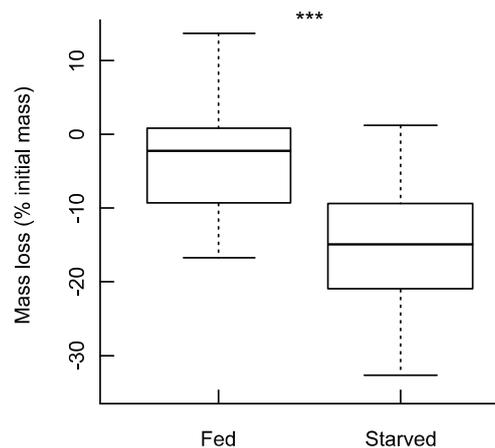


Figure 2. Difference between final and initial mass for fed and starved males expressed in % of initial mass (t-test: $t = 4.577$, $DF = 33.898$, $***: P < 0.001$). Boxplots represent the median, the first and the third quartiles and the range.

Mean effect of the energetic constraint

Temperature appeared to act both on the nightly calling activity and all measured call properties. Indeed, when temperature increased, the number of calls emitted per night increased significantly (Table 1). Hence, a male emitted on average 1013 calls more when temperature rose 1°C. In addition, temperature had different effects on the different call properties. While dominant frequency and WCR increased significantly with increasing temperature, call duration decreased significantly (Table 1).

For both starved and fed males, the number of days spent housed had a negative impact on the nightly calling activity (Table 1, Figure 3). On average, males emitted 576 calls less by day spent housed. Likewise, the number of days spent housed negatively WCR in both groups, but did not affect dominant frequency and call duration (Table 1, Figure 3).

The 'starvation' effect represents the change in intercept between fed and starved males, *i.e.* the estimated difference between the two groups at day 0. Given that males from both groups had exactly the same treatment until day 1, we did not expect any difference at day 0, hence no difference in intercept. The effect of the energetic constraint was thus expected to be revealed by differences in slopes, *i.e.* by an effect of the interaction day \times starvation. Considering the whole data set, neither the nightly calling activity nor the measured call properties were significantly affected by the energetic constraint (Table 1, Figure 3).

Table 1. Fixed effects (estimates \pm se) acting on nightly calling activity and call properties. Significance levels are given by Wald z tests

	Intercept	Temperature ($^{\circ}$ C)	Day	Starvation	Day \times starvation
Nightly calling activity	-8460.70 \pm 6633.40	1012.70 \pm 279.90***	-576.50 \pm 221.10**	375.40 \pm 2365.70	-123.60 \pm 269.90
Dominant frequency (Hz)	2331.87 \pm 109.72	10.12 \pm 4.71*	0.96 \pm 3.33	-26.65 \pm 46.14	3.75 \pm 4.04
Call duration (ms)	87.12 \pm 2.73	-1.58 \pm 0.12***	0.03 \pm 0.09	-0.28 \pm 0.80	0.12 \pm 0.11
WCR (call/s)	0.47 \pm 0.58	0.28 \pm 0.03***	-0.04 \pm 0.02*	0.08 \pm 0.18	0.02 \pm 0.03

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

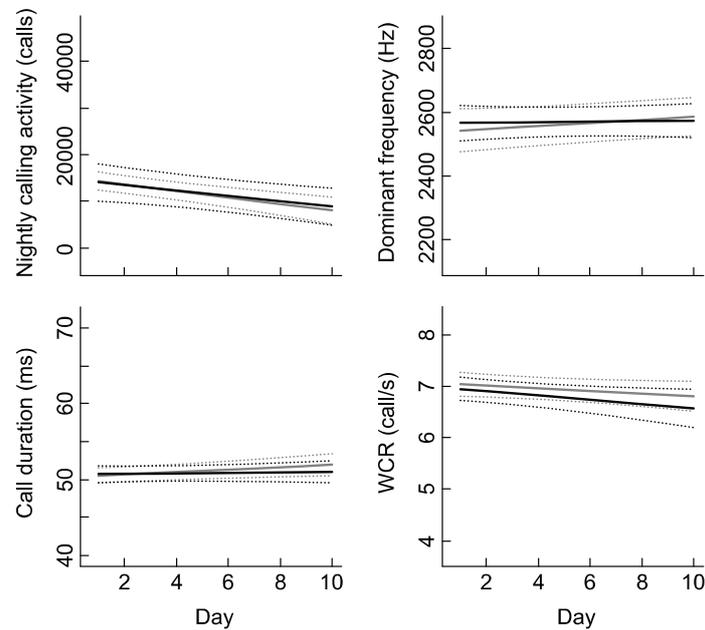


Figure 3. Predicted mean values (and 95% confidence envelopes) of nightly calling activity and call properties depending on the day and the treatment (fed in black, starved in grey). Values are corrected for temperature (given for the mean temperature: 23.05 $^{\circ}$ C). Y axes are scaled between the minimal and the maximal values for each call property (or nightly calling activity).

Energetic constraint and individual trade-offs

Individual display trade-offs under energetic constraint have been investigated by correlating random effect around the interaction day \times starvation (*i.e.*, individual effect of the energetic constraint) between call properties and between call properties and nightly calling activity among starved males. A trade-off between maintaining the nightly calling activity and maintaining WCR was revealed by such analysis (Table 2). Thereby, a male that maintained 0.02 calls per second and per

day of starvation further than the average of the other males, in return, emitted on average 272 calls less per day of starvation (Figure 4). In addition, by correlating random effect around the interaction day × starvation with individual condition indicators among starved males, our results revealed that males who maintained their WCR the most during the course of the experiment were lighter and in poorer condition both at the beginning and the end of the experiment (Table 3, Figure 5). In contrast, even if correlations were not significant due to an outlier that increased his nightly calling activity greatly during the experiment, the results suggest that big and high condition males tended to maintain more their nightly calling activity than small ones (Table 3, Figure 5, without the outlier the correlation was significant both for initial mass and initial SMI, respectively $R = -0.56$, $P = 0.020$, outlier Cook's distance = 0.30 and $R = -0.52$, $P = 0.033$, outlier Cook's distance = 0.33). Hence it appeared that males could not maintain both nightly calling activity and WCR under energetic constraint; small and poor condition males impacting especially their nightly calling activity whereas big and high condition ones impacting WCR. In our data set, there was a male with an extreme initial mass rarely found in this population (10.39g). This point influenced parameter computation significantly in the correlation between WCR and initial mass (Cook's distance = 1.31). However, when removing this point there was still a strong negative correlation between these two variables ($R = -0.56$, $P = 0.020$). Concerning correlation between number of calls per night and initial mass, the point with a high initial mass had little influence on parameter computation (Cook's distance = 0.10) but without this point the correlation became not significant ($R = 0.32$, $P = 0.227$).

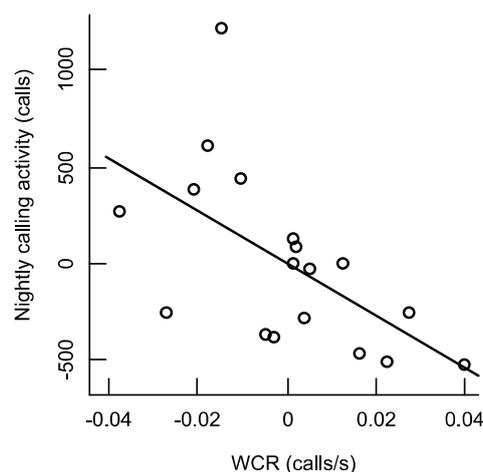


Figure 4. Trade-off between maintaining nightly calling activity and WCR experienced by starved males: negative correlation between individual effects of the energetic constraint on number of calls per night and WCR.

Table 2. Correlation (Pearson's coefficients) between random effects around the interaction day × starvation computed on the 18 starved males.

	Nightly calling activity	Dominant frequency	Call duration
Dominant frequency	-0.34		
Call duration	0.05	0.41	
WCR	-0.59 *	0.24	-0.06

*p<0.05

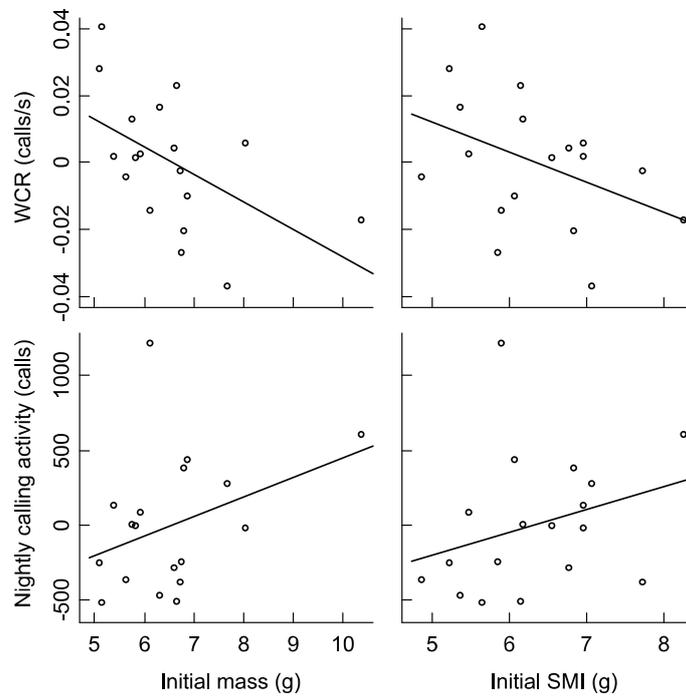


Figure 5. Links between individual condition indicators and individual effects of the energetic constraint on WCR and nightly calling activity.

By correlating random effect around the interaction day × starvation with individual condition indicators among starved males, results also showed that males who maintained the most their nightly calling activity during the course of the experiment were those who lost the most body condition (Table 3).

Table 3. Correlations (Pearson's coefficients) between random effects around the interaction day × starvation and individual condition indicators computed on the 18 starved males. 'Dif mass' is the difference final mass - initial mass; 'Dif mass (%)' corresponds to dif mass/initial mass; 'dif SMI' is the difference final SMI - initial SMI.

	Initial mass	Final mass	Dif mass	Dif mass (%)	Initial SMI	Final SMI	Dif SMI
Nightly calling activity	0.36	0.05	-0.52 *	-0.57 *	0.30	0.10	-0.54 *
Dominant frequency	-0.43	-0.44	0.26	0.13	-0.10	-0.05	0.08
Call duration	-0.23	-0.06	0.31	0.33	-0.06	0.19	0.29
WCR	-0.52 *	-0.47	0.37	0.34	-0.41	-0.19	0.36

*p<0.05

When considering reciprocally random effect around the interaction day × feeding on the fed group, there was no negative correlation between the nightly calling activity and the measured call properties (Table 4); suggesting that fed males did not face trade-off as starved ones did.

Table 4. Correlation (Pearson's coefficients) between random effects around the interaction day × feeding computed on the 18 fed males.

	Nightly calling activity	Dominant frequency	Call duration
Dominant frequency	0.11		
Call duration	0.46	-0.12	
WCR	0.24	0.13	0.00

Re-feeding effect

To test this effect both on fed and starved males, call properties and nightly calling activity predicted at day 11 by models were compared to observed values on day 11 (re-feeding day). For starved males, the nightly calling activity was significantly higher than the predicted value. This was not the case for fed males (Figure 6). However, the average calling activity observed on the night after re-feeding was not significantly higher in re-fed male than in control males (t-test: $t = -0.497$, $DF = 33.98$, $P = 0.622$). Moreover, when focusing on inter-individual variability, the results showed that males that exhibited the highest increases in nightly calling activity after re-feeding were those who lost more mass during the experiment (positive correlation between mass loss in percent of initial mass and difference observed-predicted nightly calling activity, $R = 0.52$, $P = 0.027$). Call properties were not affected by re-feeding (Figure 6).

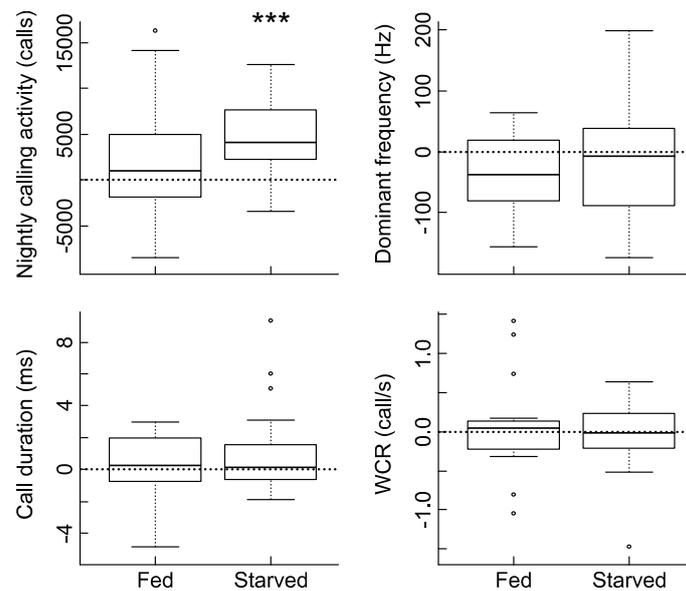


Figure 6. Differences between observed and predicted individual call properties and nightly calling activity at day 11 (re-feeding day) for fed and starved males. The difference in nightly calling activity differ significantly from 0 in the starved group (paired t-test: $t = 4.426$, $DF = 17$, $***: P < 0.001$). Boxplots represent the median, the first and the third quartiles and the range (by convention, points beyond $1.5 \times$ the interquartile distance from the median are represented by isolated spots).

Discussion

Factors affecting call production

As previously demonstrated in anurans (*e.g.*, Zweifel 1968; Gerhardt 1978), temperature appears to be a crucial covariate of both the nightly calling activity and call properties. For call properties, our results showed that temperature is positively correlated with WCR and dominant frequency and negatively correlated with call duration. That is in agreement with previous data in *H. arborea* (Schneider 1974; Castellano *et al.* 2002; Friedl and Klump 2002). However, regarding the nightly calling activity, the present data show for the first time a strong positive impact of temperature on the number of calls emitted during the night, propping up the idea that considering the temperature effect on call production is essential before investigating any impact of another factor.

The number of days spent housed has a negative impact on both the nightly calling activity and WCR despite an *ad libitum* energetic input in the control group. Several non-exclusive hypotheses might explain this result. First, laboratory conditions may be less motivating than field

conditions (solar light, smell of the pond, female presence, etc...). Second, even with a constant energetic input, structures involved in call production (trunk and laryngeal muscles, vocal cords or vocal sac) grow tired as Mitchell *et al.* (2008) previously showed in the oyster toadfish, *Opsanus tau*. At last, male hormonal state may change during the course of the chorus attendance independently from the energetic constraint and impact call production (Leary *et al.* 2008).

Energetic constraint and trade-offs

When considering both the nightly calling activity and call properties in fed and starved groups, the energetic constraint (day × starvation effect) does not appear, at first glance, to impact call production over 10 consecutive nights. Indeed, on average starved males maintain their nightly calling activity as well as fed males. However, a great inter-individual variability appears in regard to the nightly calling activity, and males who maintained the most their number of calls per night during the course of the experiment are those who lost the most condition. This clearly demonstrates a cost in maintaining a high nightly calling activity during a starvation period. Hence, under energetic constraint, these males are expected to decrease call attractiveness by altering some call properties to maintain a high nightly calling activity. Actually, when focusing on individual responses to the energetic constraint, it appears that males who maintained their nightly calling activity were not able to maintain in the same time WCR, whereas when considering individual responses in the fed group there is no such correlation between the nightly calling activity and call properties. Hence, our results suggest that the energetic constraint can generate a trade-off between investing in nightly calling activity and investing on call properties, notably WCR, supporting our first hypothesis.

Moreover, in the starved group, the biggest and highest condition males mainly maintained their nightly calling activity to the detriment of WCR while small and low condition males mainly decreased their nightly calling activity and maintained their WCR. Hence, depending on their initial mass and condition, all males do not respond in the same way to this display trade-off imposed by the energetic constraint. Indeed, as suggested by Castellano's theoretical work (2009), males of low condition invest proportionally more on call properties selected by females and less on calling activity than high-condition males, supporting our second hypothesis.

By lowering WCR, big males *H. arborea* may increase their endurance, since it has been proved that, for the same number of calls emitted, increasing WCR involves an increase of oxygen consumption in this species (Voituron *et al.* 2012). However, decreasing WCR also means being less attractive for females (Richardson *et al.* 2010). It is difficult to assess the impact of the energetic constraint on mating success since (i) each male seems to face the trade-off differently on a continuum between impacting only the nightly calling activity and impacting only WCR, and (ii) little is known about the relative part of female choice on call properties and endurance rivalry through

calling activity in mating success in the field. Nevertheless, it is probably more costly to maintain the nightly calling activity rather than WCR since big males have lost more mass than small ones have (positive correlation between initial mass and mass loss in percent of initial mass; $R = 0.61$; $P = 0.007$) while they are expected to call at lower cost all things being equal (Voituron *et al.* 2012). We can thus hypothesize that this supplemental cost confers a greater mating success; otherwise all males would be expected to decrease their nightly calling activity instead of WCR.

Trade-offs between different call properties have already been investigated in anurans (*e.g.*, Friedl and Klump 2002), birds (*e.g.*, Patricelli and Krakauer 2010) and insects (*e.g.*, Bertram and Warren 2005). However, our study provides three new aspects. First, trade-offs are here investigated between several call properties and the measured nightly calling activity instead of a display rate, as it was usually the case in previous studies. Second, the energetic constraint is usually invoked as an hypothesis to explain observed trade-offs. Here we tested the effect of a manipulated energetic constraint and showed that the observed trade-off is effectively a consequence of this constraint. At last, we found that depending on their mass and condition, all males do not respond in the same way to a display trade-off.

Re-feeding effect

Call properties are not affected by re-feeding the starved males. However, the nightly calling activity is significantly higher than predicted by models for starved males. Hence, re-feeding after a long period of starvation increases the number of calls emitted during the night of about 4500, which represents about 40% of a classical nightly calling activity. Such result shows the promptness of the response to a new energetic input. Although our study is the first one to assess precisely the nightly calling activity, this result is consistent with those of several previous studies which found that experimentally feeding increases call rate, supposed to reflect the nightly calling activity (in *Hyperolius marmoratus* Grafe 1996; in *Physalaemus pustulosus* Marler and Ryan 1996; in *H. arborea* Meuche and Grafe 2009).

Mean predicted values of nightly calling activity at day 11 are very close for the two groups. Individuals from the starved group appear to diverge from this prediction after re-feeding while individuals from the fed group did not. However, the average number of calls observed on the night after re-feeding is not significantly higher in re-fed male than in males that were fed along the experiment. This is probably due to a high inter-individual variability in the nightly calling activity in both groups that masks the re-feeding effect and further highlights the necessity of an individual approach. Moreover, when focusing on inter-individual variability, our results show that males that exhibit the highest increases of nightly calling activity after re-feeding are those who lost more mass during the experiment. This result is consistent with our previous hypothesis that maintaining the

nightly calling activity is costlier and may confer a greater advantage in mating success than maintaining WCR.

Comparison with other experiments and field observations

Our results suggest that the effect of an energetic constraint on call production may be hard to detect for two reasons. First, males seem to face a trade-offs between investing in calling activity and investing in call properties selected by females under energetic constraint, implying simultaneous measures of these two main aspects of call production. Secondly, males seem to have different ways to cope with this kind of trade-offs according to their condition, implying statistical consideration of this inter-individual variability to reveal an effect of the energetic constraint. These two critical points probably explain in part why some studies have found no effect of supplemental feeding on call production (Green 1990; Judge and Brooks 2001), or have found conflicting results across species (*e.g.* Gordon 2004; Meuche and Grafe 2009) and even within the same species (Green 1990; Marler and Ryan 1996).

Starved males in our study have lost on average 15.3% of their initial body mass due to the energetic constraint during the 10 first days of the experiment. This is an important loss compared to data obtained on other anuran species during chorus attendance (*e.g.*, Murphy 1994b) or entire breeding season (*e.g.*, Tejedo 1992). The relatively large mass loss observed in our study is probably due to the fact that males were fed *ad libitum* during three days, then totally food deprived during the experiment whereas males in the aforementioned studies had access to their natural food supply (expected to be relatively abundant during foraging bouts and relatively rare, but not null, during chorus attendance). *H. arborea* is known to rely mainly on carbohydrate to fuel calling during one single night (Grafe and Thein 2001). However, these reserves are quickly depleted during the night especially in species with a high calling rate (Bevier 1997). Hence, prolonged breeding species are expected either to replenish their carbohydrates stores by regular foraging or to rely on lipid stores (review in Wells 2007). In our experiment, starved males probably depleted the majority of their carbohydrates reserves on the first few nights and then fuelled calling thanks to their lipid stores. The great majority of body mass loss is thus probably due to lipid loss.

In a population of *H. arborea*, Friedl and Klump (2005) observed a chorus attendance of about 12 nights whereas in another population Grafe and Meuche (2005) found a chorus attendance of about 7.5 nights. Note that these differences are potentially due to the size of the population, which seems quite small in Friedl and Klump's study and may result in a low calling activity and thus a low rate of energy depletion (see also Friedl and Klump 2002). In our experiment, despite the fact that a large chorus ambience was simulated, all males kept calling until the last day, emitting on average 9402 ± 6180 calls (mean \pm sd; range: 568 – 22 208) after 10 days of starvation. Hence, they

were still able to draw on their reserves to fuel calling. This may be due to the fact that males have been fed *ad libitum* during three days before the experiment. Indeed, Murphy (1994b) found that one day of *ad libitum* feeding provided males *Hyla gratiosa* with enough energy to fuel 6-7 nights of calling. Three days of *ad libitum* feeding may thus be sufficient to fuel 10 nights of calling in *H. arborea*. However, one might question if such food abundance often happened in the field.

To conclude, the present study emphasized for the first time, through an experimental design, that an energetic constraint can generate a trade-off between investing in display properties selected by females and investing in the amount of display. It also suggests that males can use different allocation strategies to cope with this trade-off according to their condition. Our results thus highlight the necessity of taking into account individual differences in response to such constraint since individual variability can mask some effects when investigated at the group level. To get a more comprehensive view of the consequences of such trade-offs on sexual selection processes, future studies should focus on other behaviors that can modulate individual energetic constraint such as foraging abilities or alternative reproductive tactics as well as on consequences of call adjustment performed by males on their mating success.

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Article 3:

Within-individual variability in call dominant frequency and female choice in anurans

Brepson, L., Gomez, D., Voituron, Y. & Lengagne, T.
Submitted to *Behavioral Ecology*.

Issue:

Call dominant frequency in anurans, a call property often involved in mate choice, is considered as a static signal component with little within-individual variability. However, this consideration is based on small samples of consecutive calls and the few studies based on several records of the same individual have always shown heightened within-individual variability. This suggests that a part of variability is not correctly assessed when using small samples. We thus need (1) to assess the within-individual variability of this signal component on a larger time scale; (2) to estimate the impact of this variability on the selective strength driving female choice on this signal component.

Hypotheses:

H3.1: The within-individual variability of call dominant frequency is higher than previously believed.

H3.2: This variability can impact the selective strength driving female choice on this signal component.

Methods:

We recorded the whole call production of 36 males *H. arborea* during one night and measured the dominant frequency of each call produced by each individual. We then simulated female choices (thanks to a purpose-built program) using simple decision rules to assess the proportion “good choice” performed by females in different sampling and variability conditions.

Results:

- When considered on a night scale, the within-individual variability of call dominant frequency is higher than previously believed in this species.
- Great within-individual variability in call dominant frequency can decrease the proportion of good choice up to 30% in the worst case.
- By increasing the number of calls sampled by a female from 10 to 10 000 calls, the proportion of good choice is enhanced up to 10% in the best case.

Conclusion:

The within-individual variability of call dominant frequency is higher than previously believed and can disturb the information transmitted to females in this species. Considering the weak advantage supplied by increasing the number of calls sampled, females should use simultaneously several signal components in short samples instead of increasing disproportionately the sample size. Hence, our study provides indirect evidence supporting the hypothesis that considering several components of the signal improves mate condition assessment and may explain the lack of support for female choice on isolated signal components in field studies.

Within-individual variability in call dominant frequency and female choice in anurans

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Abstract: In a sexual selection context, static signal components are expected to be stable on short term and to convey information about invariable aspects of the emitter's condition. Call dominant frequency in anurans is widely considered as an indicator of male size, little variable within a single individual on short term. Here we show that, if considered at the scale of the entire night, this call property is more variable than previously thought in *Hyla arborea*. This variability was mainly due to sudden discontinuities in dominant frequency over the night. A small part of variability was also due to a warm-up effect leading to a greater variability in the first call bouts emitted during the night. However our analysis did not reveal any exhaustion effect at the end of the night. It appeared unlikely that this short-term variability in dominant frequency can provide any information to signal receivers in this species. To quantify the impact of this variability on the selective strength driving female choice, we simulated female mate choice decisions based on simple rules. A great within-individual variability in call dominant frequency can decrease the proportion of good choice up to 30% in the worst case while by increasing the number of calls sampled by a female from 10 to 10 000 calls, the proportion of good choice is enhanced up to 10% in the best case. Considering the weak advantage supplied by increasing the number of calls sampled, females should use simultaneously several signal components in short samples instead of increasing disproportionately the sample size. Hence, our study provides indirect evidence supporting the hypothesis that considering several components of the signal improves mate condition assessment and may explain the lack of support for female choice on isolated signal components in field studies.

Keywords: sexual selection, mate sampling, within-individual variability, multicomponent signal.

Introduction

In sexual selection processes, individual decisions in mate choice (inter-sexual selection) and male-male competition (intra-sexual selection) are usually conditioned by signals emitted by conspecifics (reviewed in Andersson 1994). Theory posits that, in a sexual selection context, receivers should only pay attention to signals that convey honest information about emitter's condition. Some signals (or signal components) are dynamic and are expected to rapidly mirror changes in the current condition of the emitter. In contrast, static signals (or signal components) are expected to be stable on short term and to convey information about invariable aspects of the emitter's condition, like its past condition (Møller & Pomiankowski 1993; Scheuber et al. 2003a,b; Gibson & Uetz 2012).

Call dominant frequency (DF) – frequency with the highest energy – is one of the static signal components most studied to date, particularly in anurans. It is usually considered as a signal component conveying information about the size of the emitter since many empirical studies have shown a negative correlation between DF and individual mass, both at interspecific (e.g. in 116 Australian species, Hoskin et al. 2009) and intraspecific level (reviewed in Gerhardt 1994; Wells 2007). From a mechanistic point of view, DF depends on the frequency at which vocal cords vibrate; and thus their mass, since heavier vocal cords vibrate at lower frequency (Martin 1971). Vocal cords mass being generally positively linked with individual mass, heavier males call with a lower DF (review in Wells 2007).

DF is often subject to sexual selection in anuran species. In some species, females prefer frequencies close to the population mean (stabilizing selection, e.g. Gerhardt et al. 1987; Castellano & Giacoma 1998; Murphy & Gerhardt 2000). In other species, females prefer low frequency values (directional selection, e.g. Ryan et al. 1992; Wollerman 1998; Richardson et al. 2010). Besides mate choice, DF is used in male-male competition to determine the level of aggressiveness (e.g. Davies & Halliday 1978; Robertson 1986; but see Bee 2002) and which strategy – caller or sneaker – to adopt (e.g. Arak 1988; Humfeld 2008; Brepson et al. 2012a).

To date, almost all studies investigating the within-individual variability in DF have shown a low coefficient of variation (CV, Table 1). Most of the time, call analyses were performed on few calls, but when samples were taken from non-consecutive records of the same male, they yielded higher CV values than when taken from consecutive calls (Table 1). This suggests that a part of the variability in DF is not captured by analysing only a single small call sample. Yet, this variability may impact the reliability of the signal transmitted and therefore disturb the selective strength driving female choice on this call property. It thus appears relevant to investigate within-individual variability in DF over a larger scale: the entire night.

Table 1. Between and within-male variations in dominant frequency in several anurans.

Species	Mean DF (Hz)	Mean between-		Mean within-male CV (%)	Range of within-male CV (%)	Sample	Reference
		male CV (%)	male CV (%)				
<i>Agalychnis moreletti</i>	1233	7.90	8.3 ^a	2.7 - 14.7 ^a	2.7 - 14.7 ^a	from 4 to 68 cc	Briggs 2010
<i>Allobates femoralis</i>	3426 [†]	3.20	2.86 ± 1.77 ^c	0.23 - 6.02 ^c	0.23 - 6.02 ^c	3 x 5 cc	Gasser <i>et al.</i> 2009
<i>Bufo americanus</i>	1795 *	5.79 *	1.3 ^{md,a}	3.2 ^{md,b}	0 - 12.6 ^a	from 2 to 8 cc	Howard & Young 1998
<i>Bufo viridis</i>	~ 1.4k [‡]	6.11 *	1.96 ± 1.38 ^{*a}	3.3 ± 2.6 ^c	0.5 - 12.7 ^b	3 pulses/call from 1 bout	Castellano & Giacoma 1998
<i>Hyla arborea</i>	2139	5.14	0.91 ± 0.39 ^a	2.96 ± 2.27 ^c	0 - 1.92 ^a	from 1 to 26 bouts ¹	Friedl & Klump 2002
<i>Hyla arborea</i>	2121	8.20 **	2.7 ± 1.6 ^a		0 - 9.63 ^c	27 distributed calls ²	Castellano <i>et al.</i> 2002
<i>Hyla ebraccata</i>	3256	4.70 **	1.1 ^a			from 2 to 3 calls	Wollerman 1998
<i>Hyla intermedia</i>	2520	7.54 **	2.2 ^a		1.1 - 4.4 ^a	from 42 to 641 cc (3-14 bouts)	Rosso <i>et al.</i> 2006
<i>Hyla versicolor</i>	2150	4.00	0.8 ^a	3.1 ^c	0 - 4.2 ^a	5 calls from 1 bout	Gerhardt 1991
<i>Hyla versicolor</i>	~ 2k	4.98 *		significant within-male repeatability		from 3 to 5 calls	Sullivan & Hinshaw 1992
<i>Hyla versicolor</i>	2232 *	6.30 *		significant within-male repeatability		5 calls	Gerhardt <i>et al.</i> 1996
<i>Odorrana tormota</i>	6530 ^{††}	13.10	13.1 ± 2.8 ^c	8.2 - 16.1 ^c	8.2 - 16.1 ^c	from 16 to 90 calls	Feng <i>et al.</i> 2009
<i>Physalaemus enesefae</i>	898	10.02 **	1.92 ^a	0 - 15.2 ^a	0 - 15.2 ^a	from 5 to 10 cc (1 bout)	Tarano 2001
<i>Rana catesbeiana</i>	219	7.00	1.5 ^a	0.0 - 3.0 ^a	0.0 - 3.0 ^a	from 19 to 20 cc	Bee & Gerhardt 2001
<i>Rana clamitans</i>	393	5.16	2.04 ^a	0 - 6.4 ^a	0 - 6.4 ^a	from 8 to 10 cc	Bee <i>et al.</i> 2001

DF = dominant frequency ; cc = consecutive calls

* weighted average on several values given for different years or different populations

** recalculated from given sd and mean DF

[†] call mid-frequency instead of DF

[‡] fundamental frequency instead of DF

^{††} mean fundamental frequency on frequency modulated long calls

^a within the same record

^b between different records

^c between different records from different nights

^{md} median instead of mean

¹ about 20calls/bout ; DF determined by averaging spectral properties over a complete bout

² 3 calls at the start, 3 at the middle and 3 at the end of the bout, on 3 bouts from the start, the middle, and the end of the record

Here, we investigated within-individual variability in DF over an entire night in the European treefrog (*Hyla arborea*). This well-studied lek-mating hylid species shows a negative correlation between mass and DF (Marquez & Tejedo 1990; Castellano et al. 2002; Friedl & Klump 2002; Richardson et al. 2010). DF is considered as a static call property in this species, as suggested by its low within-individual variability (Table 1). By recording the overall nightly call production of 36 males, we set out to estimate the within-individual variability of DF on the scale of the entire night and to explore temporal patterns of variation of this call property (warm-up effect at the beginning of the night, exhaustion effect at the end of the night and/or other variations during the night). Then, by simulating female choices thanks to a purpose-built program, we aimed at quantifying the impact of this variability on the selective strength driving female choice in the wild.

Material and methods

Capture and housing conditions

Individuals were obtained from a population located near Lyon, France, on the Crémieu Plateau (5°21'7"E, 45°44'20"N). During the breeding season, 36 males were captured individually by hand when calling at the pond (three capture sessions along the breeding season, 12 males captured by session). They were then transported by car in individual dark boxes to the housing room of the laboratory. Males were then immediately placed in individual terrariums (25x17x15cm) with a water-filled basin and a tree branch. Males were fed *ad libitum* with domestic crickets (*Acheta domesticus*) during 3 to 4 days before the experiment for acclimation. Males were exposed to natural photoperiod and chorus noise was broadcast each night from 08:00pm to 02:00am. Temperature was kept at $22 \pm 1^\circ\text{C}$. After acclimation, males were recorded individually during a whole night. At the end of the record, males were weighted and measured (femur-tibia length) and, for each individual, a scaled mass index (SMI, size-independent condition indicator) was computed according to the methodology proposed by Peig & Green (2010). All males were released to the pond the night after the recording.

Recording

Twelve males were recorded simultaneously during one night. Each terrarium was placed in an individual semi-anechoic chamber the day of the recording at 08:00pm. Terraria had plastic mesh sides and lid instead of glass sides in order to avoid sound reflectance. Calls were recorded with a Sony ECM-T6® microphone placed in each chamber and plugged in a Roland R-44® 4-channels recorder or a Tascam US 144® soundcard connected to a notebook computer.

Each semi-anechoic chamber was open on the front side to avoid stressing males. Males were stimulated by broadcasting a repeated 2-minutes chorus noise, via an amplified loudspeaker (KH pas-100®) connected to a CD player and placed in front of the chambers. This stimulating record was a record in which no male was louder than the others in order to avoid any risk of the tested male responding to the calls of a particular competitor (Richardson et al. 2008). Although recording chambers were open on their front side, they were not placed face to face. As a consequence, sounds perceived by a focal male from the other adjacent males were very weak compared to the volume of the stimulating records that were broadcasted. Hence, we considered that males were physically and acoustically isolated from each other during our experiment.

Measure of dominant frequency variability

We extracted DF for each call of the entire night records, using Avisoft Saslab® software. DF was here defined as the frequency with the highest energy, in a frequency range between 1.8 and 3 kHz (Figure 1). To calculate it, mean power spectra were automatically computed for each call with a Fast Fourier Transform of 256 points. For each male, mean, standard deviation (SD) and CV of DF were calculated on the entire night. We also measured call amplitude, call duration and intervals between calls within a call bout to analyse their relationships to DF. These parameters were computed for each call from the entire records using Avisoft Saslab® software.

We hypothesised that variability in DF would be greater within call bouts at the beginning and/or at the end of the night, due respectively to a warm-up or an exhaustion of the structures involved in call production (i.e. trunk muscle, laryngeal apparatus and/or vocal sac). To test the warm-up hypothesis, we computed CV of DF for each call bout then we compared intra-bout variability of the 12 first call bouts to the mean intra-bout variability computed on the subsequent call bouts (except those involved in the exhaustion effect). Likewise, to test the exhaustion effect we compared intra-bout variability of the 12 last call bouts to the mean intra-bout variability computed on the previous call bouts (except those involved in the warm-up effect).

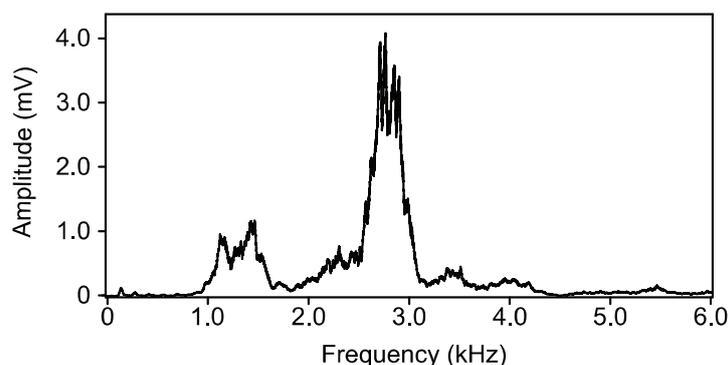


Figure 1. Power spectrum of one *Hyla arborea*'s advertisement call.

Female choice simulations

With the 36 recorded males, we artificially built all possible 630 male pairs. We then simulated female mate choice between two potential mates using SONIO, a purpose-built program (<http://sites.google.com/site/sonioprogram/>). We considered that females would detect a difference in DF above a fixed threshold, in which case they would always prefer the lowest detected DF. In hylid species for which data are available in the literature, female discrimination threshold for DF ranges from 8.6% (*Hyla ebraccata*, Wollerman 1998) to 22.6% (*Hyla intermedia*, Rosso et al. 2006). In *H. arborea*, for which mean DF is approximately 2500Hz, these thresholds correspond to 200Hz and 550Hz respectively. In our data, no male pair differed by more than 550Hz. We therefore considered 3 threshold values: 100, 200 and 400Hz.

For the simulations, the female was allowed to sample male calls using different sample sizes (10, 20, 50, 100, 200, 500, 1000, 2000, 5000 and 10 000 consecutive calls), the same size being used for both males in a pair. For each male, a call sample of the specified size was randomly drawn from the entire night record and the mean value of the DF was computed. A difference in DF between the two males of a pair generated (i) a random choice if below the discrimination threshold and (ii) a systematic choice for the lowest DF if above the threshold. We compared the obtained choice to the reference choice based on the sampling of the entire night records for both males. We considered it a good choice if it targeted the same male as the reference choice. If males of the pair did not differ for more than the threshold on the entire night, the choice was considered random by default with 50% of good choice. Each case (pair, discrimination threshold and window size) was simulated 100 times (1 890 000 simulations in total), which provided a percentage of good choice in each situation.

Here, we used a two-choice sequential sampling in absence of background noise and inter-female variability in sensory ability or decision rules. These conditions are a simplification of reality as females can assess more than two males simultaneously (Murphy & Gerhardt 2002; Richardson & Lengagne 2010), background noise limits the efficiency of communication (Richardson & Lengagne 2010), and females differ in many aspects related to mate choice like hormonal state (Lynch et al. 2006) or body condition (Jennions et al. 1995). Yet, considering a simple evolutionary scenario is a crucial first step to assess the selective force acting on female choice.

Statistical analysis

To test whether the first or the last few call bouts were more variable than the other ones (warm-up and exhaustion effects), we used paired t tests to compare intra-bout variability of the first (or last) call bouts to the mean intra-bout variability computed on the subsequent (or previous) call bouts for the 36 males. Correlations between DF and other call properties over the night were investigated using linear mixed models including individual as a random factor acting both on the

intercept and the slope. Coefficients and standard errors (SE) were computed using a restricted maximum likelihood approach. Factor significance was tested using Wald z tests (Bolker et al. 2009). Correlations between the CV computed on the entire night and individual characteristics were investigated using linear models.

We analysed the probability of choosing the male with lowest DF using generalised linear mixed models with binomial distribution and males as random factors. As fixed effects, we tested the discrimination threshold, the number of calls sampled, the difference in DF between males, the variability in DF and all second order interactions between these factors. To characterize the variability in DF shown by the pair, we computed the sum of the CVs of the two males. We used a maximum likelihood approach with a Laplace approximation and selected the best model by minimizing the Akaike's Information Criterion (Bolker et al. 2009). All statistical analyses were performed using R[®] software v.2.14.1 (R Development Core Team 2011) and the packages 'nlme' (Pinheiro et al. 2009) for linear mixed models and 'lme4' (Bates et al. 2011) for generalized linear mixed models.

Results

Patterns of dominant frequency variability

Males emitted on average $14\,546 \pm 8644$ calls (mean \pm SD; range: 561 – 31 641 calls) grouped in 672 ± 383 call bouts (mean \pm SD; range: 32 – 1317 bouts) during 2.96 ± 1.07 consecutive hours (mean \pm SD; range: 0.22 – 4.23 h). Mean DF was on average 2524 ± 141 Hz (mean \pm SD; range: 2250 – 2803 Hz) and was negatively correlated with body mass ($r = -0.45$, $F_{1,34} = 8.70$, $N = 36$, $P = 0.0057$). Within-individual CVs computed on DF measures from the entire night were $4.57 \pm 2.00\%$ (mean \pm SD; range: 1.94 – 10.29%). This variability is greater than previously thought in this species (Table 1).

Nightly within-individual variability in DF occurred at large temporal scale with some sudden discontinuities (Figure 2). These discontinuities could lead to great changes in DF during the course of the night. Indeed, if we compared the two minutes with the lowest pitch and the two minutes with the highest pitch, the difference was in average 228 ± 103 Hz (mean \pm SD) and could reach 470 Hz in case of pronounced discontinuities. These discontinuities did neither occur at the same time nor in the same direction (higher or lower frequency) for all males.

Individual CV of DF decreased exponentially with the number of calls emitted during the night (Figure 3, $r = -0.54$, $F_{1,34} = 13.82$, $N = 36$, $P = 0.0007$), suggesting a potential trade-off between increasing DF variability and increasing call quantity. In addition, at individual scale, DF was related to

call properties like calling rate or amplitude, but not to call duration (Table 2). Nevertheless, there was no evidence of condition-dependent ability to vary DF. Indeed, there was no relationship between this CV and caller morphology, namely mass ($r = 0.07$, $F_{1,34} = 1.70$, $N = 36$, $P = 0.200$), length ($r = 0.04$, $F_{1,34} = 1.03$, $N = 36$, $P = 0.305$) and SMI ($r = 0.00$, $F_{1,34} = 0.006$, $N = 36$, $P = 0.941$). Since temporal patterns of variation greatly varied from one male to another, we found no relationship between DF and the time elapsed since the first call (Table 2).

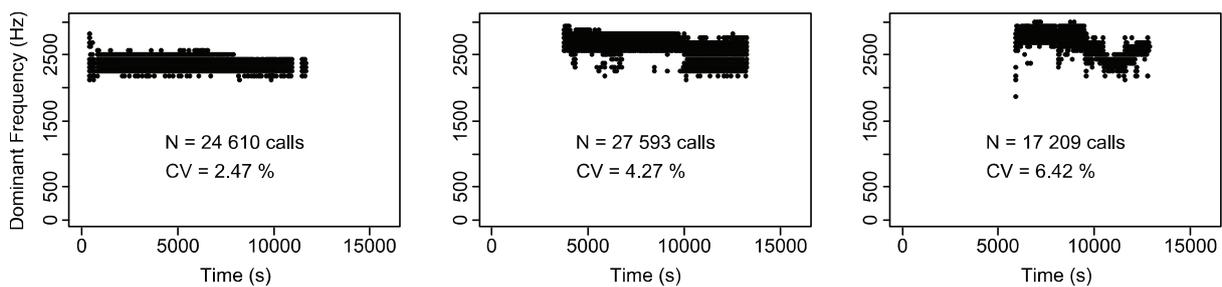


Figure 2. Call dominant frequency (DF) during the course of the night (each circle represents one call) for 3 contrasted males: male on the left had a relatively constant DF whereas male on the right had a highly variable DF at night scale. Male in the centre was an intermediate case reflecting most of the males we recorded. CV values are those calculated on the entire night.

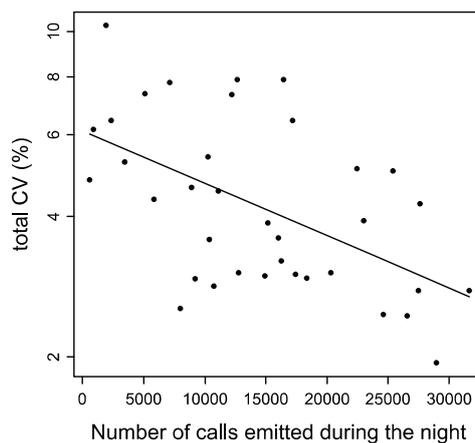


Figure 3. Decrease of the total CV with the number of calls emitted by the male during the night. Each point represents a male ($r = -0.54$, $F_{1,34} = 13.82$, $N = 36$, $P = 0.0007$).

Table 2. Intra-individual correlations between call dominant frequency (DF) and other call properties. Individual is here considered as a random factor acting both on the intercept and the slope. DF is considered in Hz, call duration in seconds and call amplitude in dB. Inter-call delay (inversely proportional to within-bout call rate) is the time between the call and the previous one (only within a calling bout), in seconds. Time represents the time at which each call is emitted since the start of the record, in hours.

Model	Intercept		Call properties	
	fixed effect	random effect	fixed effect	random effect
	estimate \pm se	sd	estimate \pm se	sd
DF~call duration	2511.7 \pm 35.9	215.4	179.68 \pm 550.41	3286.13
DF~amplitude	2175.2 \pm 117.8	706.3	4.78 \pm 1.59**	9.51
DF~inter-call delay	2566.6 \pm 23.4	140.2	-335.94 \pm 41.98***	250.82
DF~time	2608.3 \pm 45.3	271.9	-65.72 \pm 34.82	208.37

** : $p < 0.01$; *** : $p < 0.001$

In addition, we observed that the first four call bouts were significantly more variable in DF than the subsequent ones (paired t test on the 36 males, $P < 0.001$ for the 3 first bouts, $P < 0.01$ for the 4th bout, $P \geq 0.05$ for the following bouts) whereas the last call bouts were not significantly more variable than the previous ones (Figure 4). Hence, we observed a warm-up effect but no exhaustion effect on DF variability.

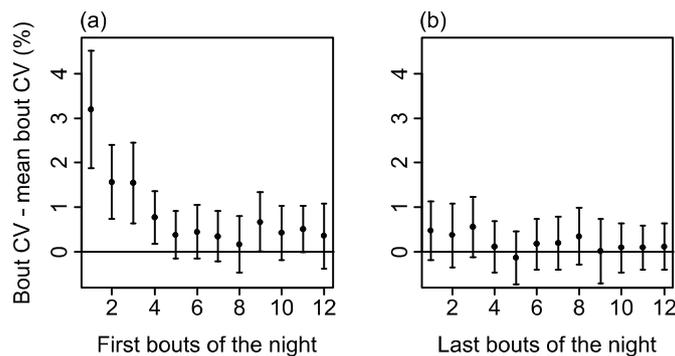


Figure 4. a) Warm-up effect shown through the mean difference (and CI95) between intra-bout variability in dominant frequency (DF) measured on the focus call bout and mean intra-bout variability computed on the subsequent call bouts. The first four call bouts are significantly more variable in DF than the others. b) Exhaustion effects, the last call bouts are not significantly more variable in DF than the previous ones.

Female choice simulations

The model that best explains the proportion of good choice is presented in Table 3. The discrimination threshold appears to be the major determinant of the overall proportion of good choice; a lower threshold leads to a higher proportion of overall good choice (Table 3, Figure 5). For a given threshold, the proportion of good choice is highly affected by the difference between mean DF of the two males; a greater difference leads to a higher proportion of good choice (Table 3, Figure 5). If variability around mean DF increases, the proportion of good choice decreases. The interaction between the difference and the variability in DF is negative; hence the negative impact of the variability on the proportion of good choice is enhanced if the difference increases. Then, a large variability of about 20% in DF (sum of the two CVs) can decrease the proportion of good choice up to 30% in the worst case (Figure 5c). On the other hand, if the size of the sample assessed by the female increases, the proportion of good choice increases. The interaction between the difference and the sample size is positive; hence the positive impact of the sample size on the proportion of good choice is enhanced if the difference increases. By increasing the number of calls sampled by the female from 10 to 10 000 calls, the proportion of good choice is enhanced by up to 10% in the best case (Figure 5d). Although the threshold interacts with the 3 other factors, these predicted effects of sample size and DF variability are almost the same for the 3 tested discrimination thresholds (Figure 5).

Table 3. Estimated parameters (estimate \pm se) of the fixed effects predicting the proportion of good choice. Difference is expressed in Hz, Variability is expressed in % and Sample is expressed in number of calls. Discrimination threshold is considered as a factor with 3 levels, the reference level is 100Hz.

	Estimates	Wald z test
(Intercept)	-5.99E+00 \pm 3.41E-01	***
Sample	-5.21E-04 \pm 6.02E-05	***
Difference	4.87E-02 \pm 2.31E-03	***
Variability	2.45E-01 \pm 3.17E-02	***
Threshold 200	-5.87E+00 \pm 3.87E-01	***
Threshold 400	-2.44E+01 \pm 2.02E+00	***
Difference x Variability	-2.23E-03 \pm 1.99E-04	***
Difference x Sample	5.35E-06 \pm 4.98E-07	***
Sample x Threshold 200	-5.08E-04 \pm 5.65E-05	***
Sample x Threshold 400	-1.61E-03 \pm 1.60E-04	***
Difference x Threshold 200	5.14E-03 \pm 1.32E-03	***
Difference x Threshold 400	2.25E-02 \pm 4.47E-03	***
Variability x Threshold 200	1.76E-01 \pm 3.03E-02	***
Variability x Threshold 400	6.73E-01 \pm 7.28E-02	***

***p<0.001.

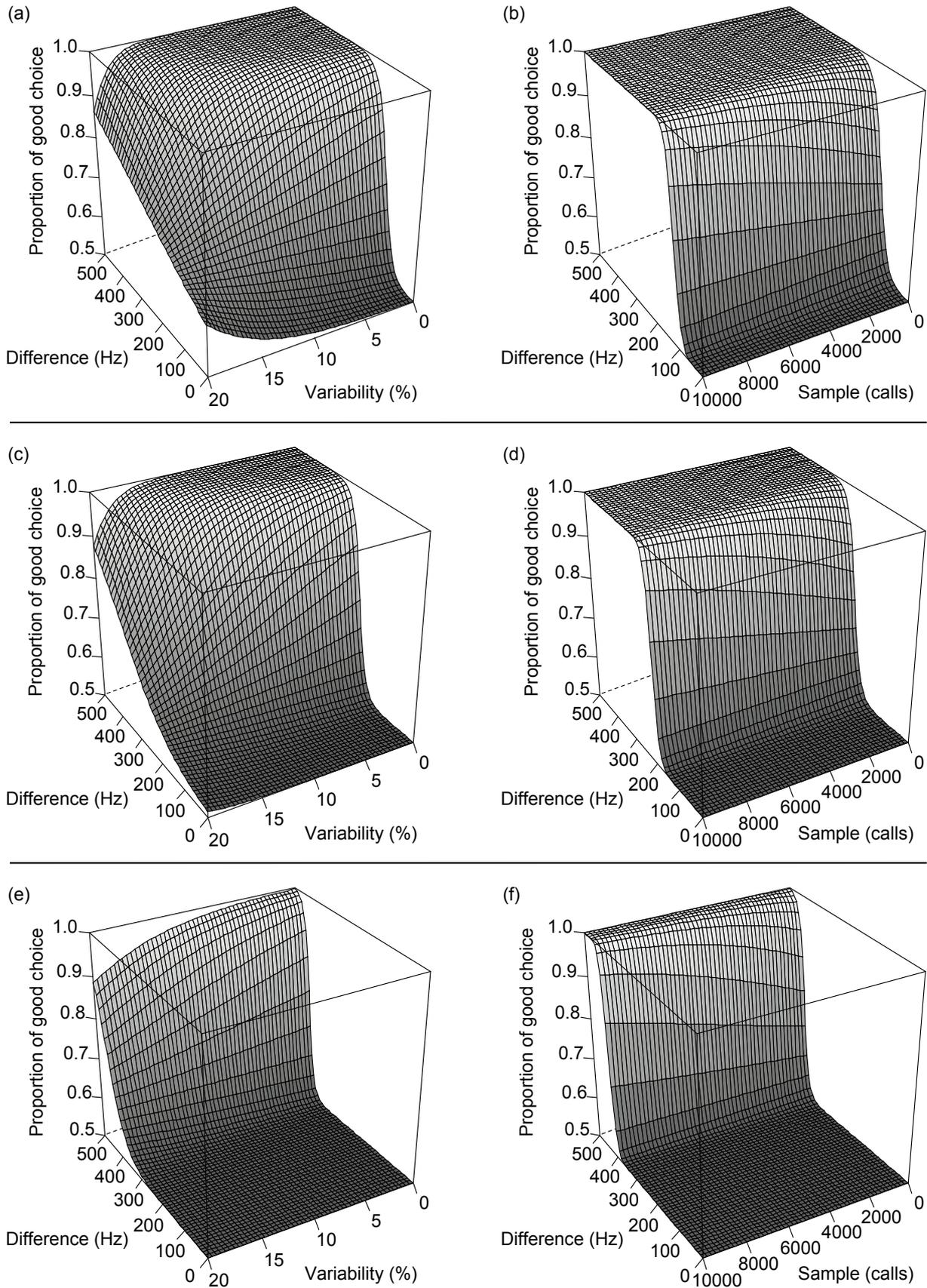


Figure 5. Predicted values of the proportion of good choice (a, c & e) depending on the difference between mean dominant frequencies (DF) of the two males (“Difference”) and the variability around these mean DF (“Variability”); (b, d & f) depending on the difference between mean DF of the two males (“Difference”) and the number of calls sampled by the female (“Sample”). Values are shown for the 3 tested discrimination thresholds: 100Hz (a,b), 200Hz (c,d) and 400Hz (e,f). A 0.5 proportion of good choice means a random choice.

Discussion

Patterns of dominant frequency variability

Our results show that, when considered over the entire night, DF is more variable than previously believed (Table 1). We observed a warm-up effect but no exhaustion effect on DF variability. However, the warm-up effect concerns 4 call bouts (about 0.6% of the bouts emitted) and accounted on average for 1.7% of the total CV. The major part of the frequency variability that we emphasized in our study is thus due to the sudden discontinuities observed over the night.

It is known that a change in vocal cord tension, in arytenoid cartilage shape or position relative to the vocal cords can impact DF (review in Gerhardt 1994) and in some species it has been evidenced that males can modulate their DF on short term according to the social context (e.g. *Leptodactylus albilabris*, Lopez et al. 1988; *Acris crepitans*, Wagner 1989; *Rana clamitans*, Bee and Perrill 1996; *Rana virgatipes*, Given 1999; *Rana catesbiana*, Bee and Bowling 2002). It is thus possible that this call property has some dynamic aspect also providing information to receivers. In anurans, there is a mismatch between the small size of radiating structures (mainly the vocal sac) and the principal wavelengths of the call. Thereby, lowering DF supposes lowering efficiency in converting metabolic energy into acoustic energy during call production (Ryan 1985; Prestwich et al. 1989). All other properties being equal, it should therefore be costlier for a male to produce lower frequency calls (supported by Wagner 1989). Given that females in *H. arborea* and some other species (review in Gerhardt and Huber 2002) prefer males with a lower than average DF, males could increase their probability of being chosen by decreasing their DF. This strategy would inescapably increase the cost of signalling, particularly for males in poor current condition, potentially making DF a reliable indicator of current condition. It seems to be the case in the Blanchard's cricket frog, where changes in DF appear to function as reliable signals of size-independent fighting ability (Wagner 1992). Nevertheless, while hylids are among the most studied frogs for their advertisement calls, most of species known to modulate their DF do not belong to this taxon (see above). Moreover, males *H. arborea* do not seem to be able to modulate their DF when confronted to a noise masking their calls (Lengagne 2008).

Within-individual variability in DF observed in this study can be due to active modulation but also to passive imprecisions during sound production processes. Our data show that increasing call amplitude or within-bout call rate results in an increase in DF, potentially suggesting a trade-off between lowering DF and increasing attractiveness of other call properties since high within-bout call rate and amplitude are selected by females (Richardson et al. 2010). However, this result can also be explained by physical constraints linking these call properties and does not rule out the hypothesis of passive imprecisions during sound production. Moreover, our data also show a negative correlation

between total number of calls emitted during the night and total CV, potentially suggesting a trade-off between varying DF and increasing call quantity. Nevertheless, this can also be explained in case of passive imprecisions, since males with more precise vocal apparatus may be able to call more than other males. In addition, we failed to show any relationship between total CV and body size or condition index revealing differential abilities to shift DF relative to male condition. Moreover, food manipulation experiments conducted in *H. arborea* suggest that DF is not condition-dependent on short term and not involved in an energy allocation trade-off during signal production (Brepson et al. 2012b). Therefore, even if biomechanical processes involved in sound production need to be more accurately studied, our knowledge on this species suggests that within-individual variability in DF observed in this study is more likely to be due to passive imprecisions during sound production processes than active shifts transmitting information to receivers.

Female choice simulations

Female choice and male decisions during contests are generally based on a few minutes listening (e.g. for female choice: Murphy & Gerhardt 2002; Schwartz et al. 2004; for male contests: Robertson 1986; for sneaker strategy: Arak 1988; Humfeld 2008). If it is also the case in *H. arborea*, the sudden discontinuities observed in the present study may lead to an imprecise estimation of male condition through DF. Indeed, if we compared the two minutes with the lowest pitch and the two minutes with the highest pitch, frequency difference is on average 228 Hz (and up to 470 Hz), which represents a difference of about 9% (and up to 19%) of the mean DF observed in this population. This difference is thus on average below the female discrimination threshold for DF generally observed in hylids (20%, Oldham & Gerhardt 1975; 20%, Gerhardt 1987; 15%, Gerhardt 1991; 22%, Rosso et al. 2006) but exceeds the threshold of 8.6% observed in *Hyla ebraccata* (Wollerman 1998). This variability may therefore potentially impact female choice, at least in the case of males with highly variable DF.

Mate choice simulation conducted in this study show that the within-individual variability in DF can strongly affect the proportion of good choice made by females according to this call property, from a quasi-full good choice (90%) with a stable DF for both males, to a quasi-random choice (60%) with a highly variable one. However, to properly measure the influence of DF variability on the selective strength driving female choice on this call property we also have to consider the costs and benefits associated with being selective for a female.

In *H. arborea*, DF is negatively correlated to male body size and females are known to select males with the lowest DF (Richardson et al. 2010). Hence, females appear to select larger males by indirectly assessing size through DF. Given that in this species males do not defend resources, females are not supposed to obtain direct benefits when choosing large males. However, they might

get indirect benefits, in terms of good genes transmitted to offspring. For instance, larger males can be more efficient in foraging, in assimilating resources or in displaying calls (Voituron et al. 2012). Yet, heritability of these individual characteristics has never been investigated in this species. Hence, the benefits of being selective cannot be quantified in the current state of knowledge.

Concerning the costs of being selective (reviewed in Reynolds & Gross 1990), they are assumed to increase as the time allowed to assess male condition increases. In our results, when increasing the number of calls sampled by the female from 10 to 10 000 calls, the proportion of good choice is only enhanced up to 10% in the best case. It thus appears that increasing the time allowed to sample potential mates is not an appropriate strategy for female to overcome the difficulty of choosing due to variability in DF.

Which alternative for females? *In vivo*, DF is not the only cue assessed by females to choose their mates. For instance, in *H. arborea*, it has been experimentally proved that females choose their mate not only on other call characteristics (e.g. amplitude and within-bout call rate, Richardson & Lengagne 2010) but also on visual signal components (vocal sac coloration, Gomez et al. 2010). Considering the weak advantage supplied by increasing the number of calls sampled, females probably use several components of the signal simultaneously in short sample instead of increasing disproportionately the sample size. Our study is thus in agreement with the two aforementioned studies and provides indirect evidence supporting the hypothesis that considering several components of the signal improves mate condition assessment by females (Candolin 2003; Gomez et al. 2011) and may partly explain the lack of support for female choice on isolated signal components in field studies (for instance in *H. arborea* see Friedl & Klump 2005).

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Article 4:

Do we correctly assess the (un)variability of call dominant frequency in anurans? Towards a new methodology

Brepson, L., Gomez, D., Josserand, R., Voituren, Y. & Lengagne, T.

Submitted to *Amphibia-Reptilia*.

Issue:

Call dominant frequency in anurans, a call property often involved in mate choice, is considered as a static signal component with little within-individual variability. However, this consideration is based on small samples of consecutive calls. When measured on the whole nightly call production this call property is more variable than previously believed. This suggests that a part of within-individual variability is not correctly assessed when using small samples made of consecutive calls. We thus need to develop a sampling methodology allowing a good assessment of this variability while keeping a reasonable sampling effort.

Hypothesis:

H4.1: The assessment of variability should be better by using several records discontinuous in time over the night than by using samples made of consecutive calls.

Methods:

We recorded the whole call production of 36 males during one night and measured the dominant frequency of each call produced by each individual. We then used a purpose-built program to draw sub-samples from this data base and estimate the ability of different sampling methods at capturing the whole variability observed on the night scale.

Results:

- The assessment of within-individual variability is better when using 2 or 3 short and well-distributed records (beginning, middle and end of the calling period) than by using samples made of consecutive calls.
- This result still holds when the number of consecutive calls considered is high.

Conclusion:

We advocate the use of several short and well-distributed samples (*e.g.* beginning, middle and end of the calling period) rather than long samples made of consecutive calls to correctly assess the within-individual nightly variability in call dominant frequency in anurans.

Do we correctly assess the (un)variability of call dominant frequency in anurans? Towards a new methodology

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Abstract: Call dominant frequency is considered as a size indicator in anurans and therefore takes a central place in sexual selection processes. Recent insights suggest that the within-individual variability of this call property has potentially been underestimated because of sampling methods resting on few consecutive calls. In this study, we use the overall call production of 36 males *Hyla arborea* during one night, to test the efficiency of different sampling methods at capturing nightly within-individual variability in call dominant frequency. Our results suggest that the assessment of this variability is better by using 2 or 3 short and well-distributed records than by using samples made of consecutive calls. Hence, in *H. arborea*, 3 well-distributed 2-minutes records (beginning, middle and end of the calling period) are sufficient to capture on average all the nightly variability, whereas a sample of 10 000 consecutive calls captures only 86% of it.

Keywords: anurans; call dominant frequency; call spectral properties; European treefrog; frequency shift; sampling method; sexual selection.

Introduction

In many anurans species, call dominant frequency takes a central place in sexual selection processes (Gerhardt 1994). Indeed, many empirical studies have shown a negative correlation between this call property and the mass of the calling male, both at interspecific (*e.g.* in 116 Australian species, Hoskin *et al.* 2009) and intraspecific level (reviewed in Wells 2007). This call property thus seems to act as a reliable indicator of calling male's size.

Given that male's size is unlikely to change on short-term (*e.g.* during one night), call dominant frequency is expected to be little variable within a single individual. Accordingly, almost all studies investigating the within-individual variability in dominant frequency have shown a low coefficient of variation (*e.g.* Gerhardt 1991; Bee *et al.* 2001; Tarano 2001). However, most of the time, call analyses were performed on few calls. When samples were taken from non-consecutive records of the same male, they systematically yielded higher CV values than when taken from consecutive calls (*e.g.* Gerhardt 1991; Castellano & Giacoma 1998, Friedl & Klump 2002), suggesting that a part of the variability in dominant frequency is not captured by analysing only a single small call sample.

By analysing the overall nightly call production of 36 males *Hyla arborea*, Brepson *et al.* (submitted) showed that this variability occurred through sudden discontinuities that led to great changes in dominant frequency during the course of the night and may impact female choice. Here, based on the same data set, we test the efficiency of different sampling methods at capturing this variability with a reasonable sampling effort to offer a convenient tool for future studies on anuran calls.

Material and methods

Test of different sampling methods to assess frequency variability

For capture, housing and recording conditions please refer to Brepson *et al.* (submitted). We developed the program SONIO® (<http://sites.google.com/site/sonioprogram/>) to test different sampling methods on values of dominant frequency obtained for each call emitted by each male during a night. Sampling methods were defined by two parameters: the number of subsamples to randomly draw from the entire night record and the subsample "window" size (in consecutive calls or in seconds). Once the method was defined, the program randomly drew the subsamples and computed the mean value of dominant frequency, its estimated SD and estimated CV value. This sampling was repeated 1,000 times and the program computed a CV ratio (estimated CV value / total

CV computed over the entire night) representing the fraction of the total variability captured. The sampling methods mimicked different possible decisions of an experimenter deciding *a priori* to adapt or not the record duration to male calling activity.

First, we defined windows by their number of consecutive calls (Method A). We explored 10 different window sizes (10, 20, 50, 100, 200, 500, 1000, 2000, 5000 and 10 000 consecutive calls). Whenever the window size exceeded the total number of calls emitted by a male, we took a CV ratio of 100% as the sample captured the overall variability.

Second, we defined windows by their duration in seconds (Method B). We explored 3 cases in which respectively 1, 2 or 3 temporal windows were randomly chosen from the entire night. We constrained the program to choose windows discontinuous in time. When 2 windows (3 windows) were chosen, the period of the night where the studied male called was divided in 3 equal parts (5 parts) and the 2 windows (3 windows) were chosen in the 1st and 3rd parts (1st, 3rd, and 5th parts). For each of the 3 case, we explored 5 window sizes (1, 2, 5, 10 and 20 minutes). For cases with several temporal windows, the CV value was computed over all calls of all windows taken together. Since windows were drawn regardless of the number of calls, some windows were without call. We thus computed the number of effective windows (with calls), which ranged from 0 to the number of drawn windows. We took this parameter into account in the analysis.

Statistical analysis

We explored the variations of CV ratio using linear mixed models suited to repeated observations on the same individuals (Bolker *et al.* 2009). All models included individual as random effect (on both the intercept and the slope). We tested window size as fixed effect. Since the relationship between window size and CV ratio appeared to be log-linear, we computed the natural logarithm of window size in the models. Coefficients and standard errors (SE) were computed using a restricted maximum likelihood approach. Factor significance was tested using Wald z tests (Bolker *et al.* 2009). All statistics were performed using R 2.14.1 (R Development Core Team 2011) with the package 'nlme' for linear mixed models (Pinheiro *et al.* 2009). For all models, we checked for variance homogeneity, residual normality and independence.

Results

According to Brepson *et al.* (submitted) results, males emitted on average $14\,546 \pm 8644$ calls (mean \pm SD; range: 561 – 31 641 calls). Mean dominant frequency was on average 2524 ± 141 Hz

(mean \pm SD; range: 2250 – 2803 Hz). Within-individual CVs computed on dominant frequency measures from the entire night were $4.57 \pm 2.00\%$ (mean \pm SD; range: 1.94 – 10.29%).

When using consecutive calls (method A), the CV ratio increased with the logarithm of window size, resulting in a better estimation of the total CV (table 1, figure 1a). A sampling of 10 000 consecutive calls captured on average up to 86% of the nightly variability in dominant frequency. Note that this percentage is influenced by the fact that 11 of the 36 males have emitted less than 10 000 calls during the night; hence such a sample captured 100% of the variability for these males.

Using discontinuous time windows (method B) yielded a better estimate of nightly variability than using a continuous window (table 1, figure 1b). A total of 3 distinct 10-minutes time windows captured 94% of the nightly variability. Increasing window size to 20 minutes captured an additional 3% of the nightly variability. By contrast, decreasing the number of windows resulted in an underestimation of dominant frequency variability. For instance, 2 distinct 10-minutes windows captured 83% of the total variability while one 10-minutes window only captured 52% of the total variability (table 1, figure 1b). In fact, if we refine this result by considering the number of effective windows, two 10-minutes windows captured 95% of the total variability (table 1, figure 1c,d) whereas three 10-minutes windows captured all the variability whatever the window size (table 1, figure 1c). Finally, the effect of window size is due to the probability of getting a window without call being negatively correlated to window size. For instance, the probability of getting 3 effective windows is greater for a window size of 20 minutes compared to a window size of 2 minutes (figure 1e, f).

Table 1. Effect of window size on the CV ratio (estimated CV / total CV) using the different sampling methods. Individual is here considered as a random factor acting both on the intercept and the slope. For method A, windows size is considered in consecutive calls whereas for methods B, it is considered in seconds.

	windows (eff.)	Intercept		Log(window size)	
		fixed effect estimate \pm se	random effect sd	fixed effect estimate \pm se	random effect sd
Method A	1 (1)	$0.492 \pm 0.034^{***}$	0.207	$0.040 \pm 0.005^{***}$	0.029
Method B	1 (0 or 1)	0.013 ± 0.056	0.337	$0.079 \pm 0.009^{***}$	0.054
	2 (from 0 to 2)	$0.336 \pm 0.083^{***}$	0.499	$0.078 \pm 0.012^{***}$	0.069
	2 (2)	$0.832 \pm 0.053^{***}$	0.311	$0.019 \pm 0.009^*$	0.052
	2 (1)	$0.471 \pm 0.057^{***}$	0.327	$0.051 \pm 0.011^{***}$	0.061
	3 (from 0 to 3)	$0.639 \pm 0.053^{***}$	0.315	$0.047 \pm 0.008^{***}$	0.047
	3 (3)	$1.048 \pm 0.060^{***}$	0.347	-0.004 ± 0.008	0.047
	3 (2)	$0.829 \pm 0.044^{***}$	0.258	$0.018 \pm 0.006^{**}$	0.034
	3 (1)	$0.546 \pm 0.081^{***}$	0.351	0.025 ± 0.016	0.061

eff. = effective windows

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$

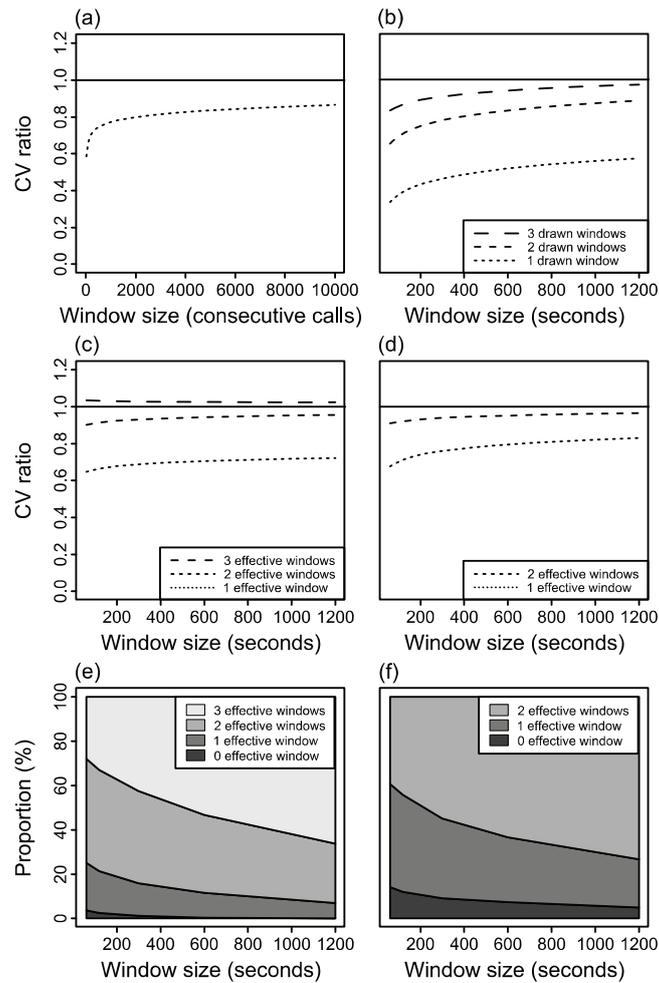


Figure 1. Mean effect of window size on the CV ratio (estimated CV / total CV); a) using method A (sampling consecutive calls), b) using method B (sampling one or several temporal window(s)). Methods B with 3 and 2 drawn windows are respectively refined in c) and d) by taking into account the number of effective windows (drawn windows containing calls). The proportions of effective windows are shown for these two methods respectively in e) and f). The solid lines on a), b), c) and d) represent perfect estimations of the nightly variability.

Discussion

At night scale, total CV found in Brepson *et al.* (submitted) exceeds the values generally observed in the literature for dominant frequency in this species (Castellano *et al.* 2002; Friedl & Klump 2002). To get a better assessment of dominant frequency variability over the night, a first solution can consist in increasing the number of consecutive calls recorded. Yet, the window size required to obtain a good estimate of total CV is huge and unrealistic to implement (even 10 000 consecutive calls capture only 86% of the total CV). Increasing the number of consecutive calls

recorded is thus not the most efficient way to capture within-individual variability in dominant frequency. Therefore, instead of increasing the number of consecutive calls to be analysed, we advocate to use records made at different times during the night.

Depending on recording conditions, two sampling strategies can be adopted. Firstly, when records are made in the field in a large population with many calling males, it should be easy to find calling males and thus to avoid the risk of 'recording' a silent male. The experimenter should decrease window size and maximize the number of samples – two or three – recorded for each male. For instance, in the European treefrog, two 2-minutes effective windows largely separated in time capture 92% of the variability and three 2-minutes effective windows are sufficient to capture all the variability. Alternatively, when records are made on a small number of individuals (small population or small number of males to record), the experimenter should optimize the number of males correctly recorded. The experimenter should thus increase window size to reduce the probability of recording males during a silent period. Since this probability is not negligible, we suggest to draw 3 temporal windows evenly distributed in time and to maximize the duration of these windows in order to optimize the probability of getting at least 2 effective windows for each male. For instance, in our case, by drawing randomly three 10-minutes windows, an experimenter estimate on average 94% of the variability in dominant frequency.

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Article 5: **Fading of a condition-dependent coloured signal irrespective of food and carotenoid availability**

Brepson, L., Bellvert, F., Desprat, J., Josserand, R., Voituron, Y. & Lengagne, T.
(in prep.)

Issue:

Under the hypothesis of condition-dependence of sexual signals, both inter and intra-individual variability in the level of signal expression is expected according to variation in male condition. Carotenoid-based visual signals are known to be more or less rapidly plastic depending on whether costs involved in their expression are mainly developmental or maintenance costs. For instance, this kind of signals should respond particularly rapidly to changes in individual condition when they are located in fleshy tissues and when carotenoids are likely to be remobilised for other functions, like in *Hyla arborea* during the breeding season.

Hypotheses:

H5.1: Colouration components selected by females should be correlated to male condition in the field.

H5.2: Changes in condition should be rapidly mirrored in colouration components.

Methods:

We collected males in the field and manipulated their condition through starvation and carotenoid supplementation to investigate the impact of male condition on the expression of vocal sac colouration.

Results:

- Colouration components selected by females are correlated to male condition in the field.
- Neither food nor carotenoid availability affected colouration components across 12 days of manipulation.
- Vocal sac colouration became less attractive for females in all males regardless of their treatment.

Conclusion:

Components of the vocal sac coloration may be long-term condition indicators rather than short-term ones. The observed fading of vocal sac colouration in all males may be due to condition-independent changes in the hormonal state of males during the breeding season.

Fading of a condition-dependent coloured signal irrespective of food and carotenoid availability

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Abstract: Sexual signals are supposed to be costlier to express, at equal level, for poor-condition males than for high-condition ones. Both inter and intra-individual variability in the level of signal expression is thus expected according to variation in male condition. Carotenoid-based visual signals are known to be more or less rapidly plastic depending on weather costs involved in their expression are mainly developmental or maintenance costs. For instance, this kind of signals should respond particularly rapidly to changes in individual condition when they are located in fleshy tissues and when carotenoids are likely to be remobilised to other functions, like Reactive Oxygen Species (ROS) scavenging. Here, we investigated condition-dependence of *Hyla arborea*'s vocal sac colouration, a sexual signal expected to be particularly plastic across the reproductive season, by manipulating food and carotenoid availability. Although vocal sac colouration was significantly related to condition in the field, neither food nor carotenoid availability affected the signal across 12 days of manipulation. Instead, vocal sac became less colourful for all males regardless of their treatment. This fading may be due to condition-independent changes in the hormonal state of males during the breeding season. We discuss the possibility that vocal sac colouration may be a long-term condition indicator, conveying additional information to females who also base their choice on acoustic cues known to be short-term condition-dependant.

Keywords: carotenoids; condition-dependence; *Hyla arborea*; starvation; supplementation; treefrog; visual signal; vocal sac; skin colouration.

Introduction

Both in the sphere of sexual selection and in other fields related to the evolution of animal communication, the issue of honest signalling in case of interest conflict between the signaller and the receiver has long troubled, and still troubles, behavioural ecologists. The current dominant paradigm to explain the maintenance of signal honesty is the costly signalling theory. It derives from the original version of the handicap principle (Zahavi 1975, 1977) and implies that the honesty of the signal is guaranteed by stabilising costs (i.e. costs of sending a dishonest signal; Grafen 1990; Hurd 1995; Lachmann et al. 2001). In a sexual selection context, males generally compete to get access to females, notably by emitting signals expected to mirror their phenotypic condition (Andersson 1994). According to the costly signalling theory, emitting a signal of a given intensity should be costlier for a low-condition male than for a high condition one. Hence, we should expect both inter and intra-individual variability in the level of signal expression depending male's nutritional status and/or health status; this is the hypothesis of sexual signal condition-dependence (Andersson 1982, 1986; Kodric-Brown & Brown 1984; Nur & Hasson 1984).

According to the recent classification of Searcy & Nowicki (2005), intrinsic costs related to signal emission can be placed in three categories: development, production and maintenance. We therefore expect to observe condition-dependent signals indicating animal condition at different temporal scale depending on the type of cost associated to it. For instance, signals whose main cost is a developmental cost (e.g., size of antlers in deer, Clutton-Brock et al. 1982) are expected to be plastic at a relatively large time scale, while signals whose main cost is a production cost (e.g., call rate in anurans, Wells 2001) are expected to be plastic at a relatively short time scale. Signals involving mainly maintenance costs are intermediate cases, with some short-term plastic signals (e.g., coloration of some body parts in birds, Velando et al. 2006) and other long-term plastic ones (e.g., size of elongated tail in birds, Evans and Thomas 1992). Moreover, in case of multicomponent signalling, the different components of the signal may be plastic at different time scales and may thus inform the receiver on the emitter's condition at different moments of its life (Moller & Pomiankowski 1993). For instance, in the calling song of *Gryllus campestris*, the chirp rate involves mainly production costs and indicates short-term condition changes while the carrier frequency involves mainly developmental costs and is linked to past condition (Scheuber et al. 2003a,b).

Among traits considered as honest condition indicators, are found in many species coloured visual signals involving the use of carotenoid pigments like coloured plumage, beak or vocal sac (reviewed in Fox 1979 and Swensson & Wong 2011). These traits are considered as costly signals for several cumulating reasons (according to Olson & Owens 1998 and von Schantz et al. 1999). First, carotenoids are not synthesisable *de novo* by animals and may be rare and difficult to obtain, at least

in some ecosystems and at some levels of the trophic chain. Second, they play a role in some important mechanisms like immune and antioxidant systems and using them for coloured displays turns them away from these functions. Third, they are potentially toxic for the organism that metabolise them. Depending on the species, these costs can be mainly developmental costs, if carotenoids are permanently invested in the signal (e.g., during the development of feathers in the house finch *Carpodacus mexicanus*, Hill 2000), or maintenance costs, if these carotenoids are deposited in the skin (e.g., in the stickleback *Gasterosteus aculeatus*, Candolin 2000) and can be remobilised to other functions (Lozano 1994). The plasticity of this kind of signal can therefore be very different from one species to another.

During the breeding season, males generally face a trade-off between allowing time to display and allowing time to forage for food (Jonsson 1997). They must therefore often draw on their reserves, especially in species where the parade period between two foraging periods is long. In such cases, fasting males are both deprived of energy, carotenoids and any other antioxidant. Hence, under the hypothesis that carotenoid-based visual signals are condition-dependent, we can expect that their level of expression should decrease along the parade period in species where carotenoids can be remobilised for other functions. This decrease should be particularly obvious in species where the parade itself involves high metabolic rates, potentially generating a large amount of reactive oxygen species (ROS, Speakman 2005) and implying the remobilisation of carotenoids in the antioxidant system (Olson & Owens 1998).

Male European treefrogs, *Hyla arborea*, are known to exhibit a coloured vocal sac, used as a signal by females during mate choice (Gomez et al. 2009, 2010), that involves carotenoid pigments (Richardson et al. 2009). Given that these pigments are deposited in the skin, they may be remobilised for other functions and the signal is expected to be plastic at a short time scale. Moreover, males in this species can call at least for 10 consecutive nights without any food supply (Brepson et al. 2012) while the production of advertisement calls is known to involve high metabolic rates (mean aerobic scope of 18 ± 9 , Grafe & Thein 2001), potentially generating a large amount of ROS. Hence, under the honest signal condition-dependence hypothesis, vocal sac colouration in *H. arborea* should be plastic at a short time scale and, during the parade period, we would expect (1) a correlation between coloration and male condition in the field; (2) a greater decrease in signal expression in starved males than in fed males; (3) a better maintenance, or even an increase, in signal expression in males fed on carotenoid supplemented food than in males fed on carotenoid deprived food.

Material & Methods

Capture and housing conditions

Individuals came from several breeding ponds belonging to a French meta-population located near Lyon, on Crémieu plateau. During the 2011 breeding season, 89 males were captured individually by hand when calling at the pond (three capture sessions along the breeding season, respectively 15, 48 and 26 captured males). They were transported by car in individual dark boxes to the housing room of the laboratory. Males were then immediately placed in individual terrariums (25x17x15cm) with a water-filled basin and a tree branch. Males were subsequently hand-fed with one domestic cricket (*Acheta domesticus*) per day during three days for acclimation before the experiment. During acclimation and throughout the experiment, males were exposed to natural photoperiod under full colour spectrum daylight fluorescent lamps. Chorus noise was broadcast each night from 08:00pm to 02:00am. Temperature was kept at $22 \pm 1^\circ\text{C}$. After acclimation, males were randomly assigned to one of the three treatments: control (hand-fed with one cricket per day), starved (food deprived), supplemented (hand-fed with one cricket per day and carotenoid supplement). Given that, under these conditions, males *H. arborea* are still able to call after 10 days of starvation (Brepson et al. 2012), in this study we decided to apply the treatment for 12 days. Males were weighted and measured (snout-vent length, SVL) at the beginning of the acclimation (field mass), at the end of the acclimation (initial mass) and after the treatment (final mass). To estimate male condition, a size-independent condition index, the Scaled Mass Index (SMI), was computed from mass and length measures. The SMI is a condition index corresponding to the predicted mass of the individual at the mean length of the population based on the log-log standardised major axis regression between mass and length. This condition index is particularly relevant because it considers both length and mass as two indicators of the 'real size' instead of considering traditionally the length as the measure of the 'real size' with the mass carrying almost of the variability of the condition (Peig & Green 2010). After the experiment, all males were re-fed *ad libitum* during three days and then, released to their pond of origin.

Carotenoid supplementation

Anurans are known to be able to assimilate β -carotene (Fox 1979). Moreover, analyses through HPLC coupled to mass spectrometry in a pilot study revealed that the carotenoid pigment present in the greatest concentration in *H. arborea* plasma samples was close to β -carotene (see supplementary material). Hence, in this study we decided to supplement males *H. arborea* with β -carotene (Sigma: 22040). This pigment has been shown, in a nutritional study conducted on mice, to be as bioavailable in its free form as when included in a complex (Blair 2010). Hence, in our

experiment males were supplemented with β -carotene in its free form. Each male of the supplemented group received 5mg of β -carotene powder suspended in 50 μ L of ultra-pure water each day during the treatment period. This suspension was provided directly in the back of the mouth thanks to a micropipette and followed by a cricket during hand-feeding. Males from control and starved groups were given 50 μ L of ultra-pure water each day according to the same protocol, followed by a cricket for the control group but not for the starved one. Analyses through HPLC in a pilot study revealed that house crickets did not contain carotenoids (see supplementary material).

Colouration measurements

Male vocal sac colouration was measured at the beginning of the acclimation (field measure), at the end of the acclimation (initial measure) and after the treatment (final measure), in reflectance spectrometry using a JAZ - Ocean optics spectrometer with a Deuterium-Tungsten Halogen light source and a coaxial optic fibre. For each measure, 3 replicates were made and a mean reflectance spectrum computed using AVICOL (Gomez 2006). Then, mean reflectance spectra were analysed thanks to AVICOL by computing *brightness* (total brightness over the range 300–700 nm) and *chroma* (difference between minimal and maximal reflectance over 300–700 nm divided by the mean reflectance over the same range), two properties known to influence the mate choice in this species (Gomez et al. 2010).

Statistical analyses

Inter-individual correlations between field condition and field colouration were investigated through simple linear models using the two colouration properties (brightness and chroma) as response variables and the three morphological indicators (mass, SVL and SMI) as explicative variables.

The effects of the treatments on male colouration were investigated by comparing the treated groups (starved and supplemented) to the control group using two linear mixed models, the first to investigate the impact on brightness, the second to investigate the impact on chroma. These two models included in fixed effects both the time (before vs. after the experiment), the treatment (control, starved, supplemented; with the control group taken as the reference) and the interaction between these two factors. These models also included the individual as a random effect acting on the intercept (initial measure). Coefficients and standard errors (se) were computed using a restricted maximum likelihood approach. Factor significance was tested using Wald z tests (Bolker et al. 2009). Using exactly the same procedure, we built a third model to investigate the effect of the treatment on male body mass.

All statistics were performed using R 2.14.1 (R Development Core Team 2011) with the ‘nlme’ package for linear mixed models (Pinheiro et al. 2009). For all models, residual normality, independence and variance homogeneity have been checked.

Results

Coloration and condition in the field

Field coloration of the vocal sac is linked to field condition. Indeed, individuals in poorer condition are less colourful, i.e., brighter and less chromatic (Table 1, Figure 1a,b). However, condition explained about only 7% of variance in coloration in both cases. When removing the two points with high values of chroma (see Figure 1b), the correlation is still significant ($r = 0.236$, $F_{1,85} = 5.015$, $p\text{-value} = 0.028$). In contrast, field coloration does not seem to be linked to male size, since there is no correlation between coloration variables and size indicators like mass or SVL (Table 1).

Table 1. Correlations between vocal sac coloration and body condition in the field.

	Brightness			Chroma		
	r (Pearson)	$F_{1,87}$	p-value	r (Pearson)	$F_{1,87}$	p-value
Mass	-0.201	3.658	0.059	0.002	0.000	0.982
SVL	-0.047	0.190	0.664	-0.149	1.983	0.163
SMI	-0.264	6.526	0.012	0.271	6.894	0.010

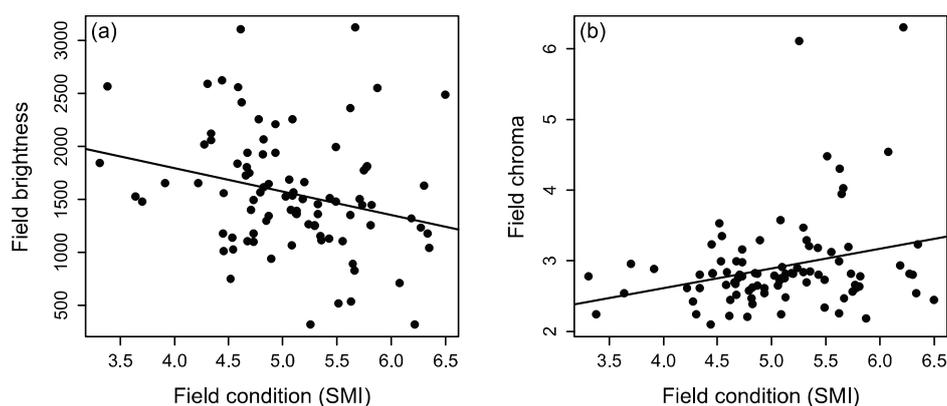


Figure 1. Correlations between vocal sac coloration properties (a. brightness; b. chroma) and male body condition in the field (SMI, expressed in gram). Coloration properties of the vocal sac preferred by females during mate choice (i.e., a lower brightness and a higher chroma) are associated with a better male body condition.

Coloration and food manipulation

Males from the starved group significantly loss mass during the experiment whereas control and supplemented males did not (Table 2, Figure 2a). However, neither the starvation nor the supplementation treatments affected the coloration properties of the vocal sac (Table 3,4, Figure 2b,c). Nevertheless, all males, regardless of their treatment, became brighter and less chromatic during the 12 days of the experiment.

Table 2. Factors affecting the body mass estimated through linear mixed models (N = 89).

	Estimate	se	z-value	p-value
(Intercept)	5.34	0.23		
Starvation	0.15	0.33	0.47	0.64
Supplementation	0.06	0.33	0.17	0.86
Time	0.13	0.11	1.17	0.24
Time:starvation	-0.79	0.15	5.13	< 0.001
Time:supplementation	0.17	0.15	1.10	0.27

Table 3. Factors affecting the brightness estimated through linear mixed models (N = 89).

	Estimate	se	z-value	p-value
(Intercept)	1629	109		
Starvation	39	150	0.26	0.80
Supplementation	-69	154	0.45	0.65
Time	668	139	4.82	< 0.001
Time:starvation	320	216	1.48	0.14
Time:supplementation	124	183	0.67	0.50

Table 4. Factors affecting the chroma estimated through linear mixed models (N = 89).

	Estimate	se	z-value	p-value
(Intercept)	2.71	0.07		
Starvation	-0.01	0.09	0.08	0.93
Supplementation	0.04	0.11	0.42	0.67
Time	-0.30	0.09	3.46	< 0.001
Time:starvation	-0.10	0.11	0.84	0.40
Time:supplementation	-0.12	0.12	0.95	0.34

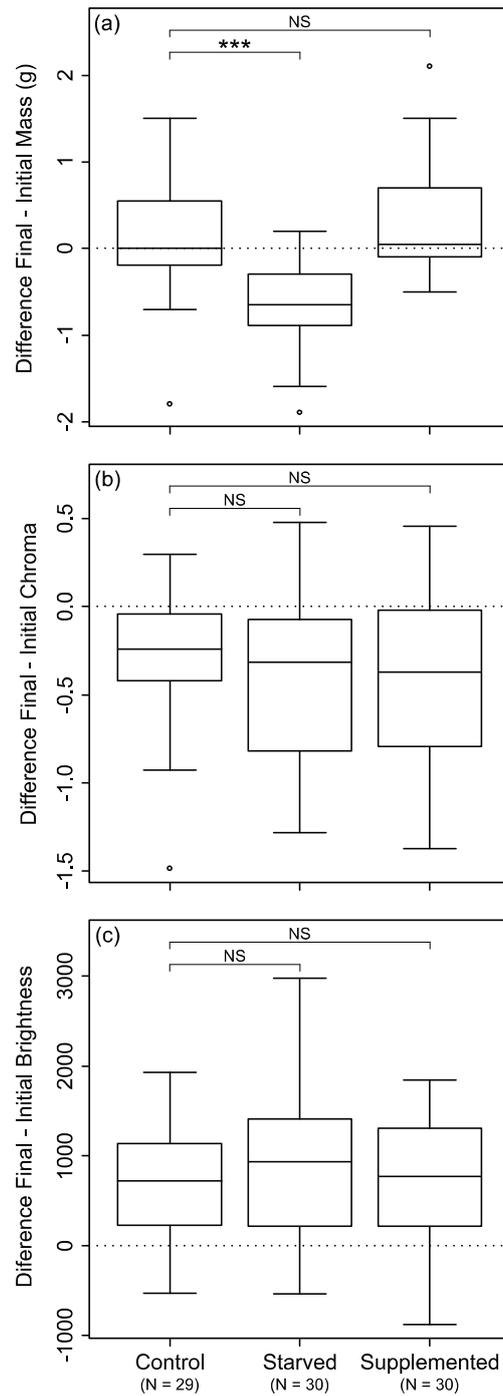


Figure 2. Effect of the different treatments on male body mass (a) and vocal sac coloration properties (b. brightness; c. chroma). Starved males lost on average 0.8g while control and supplemented males did not. All males, regardless of their treatment, became less colourful in a way that made them less attractive to females.

Discussion

A long-term condition-dependent signal?

Coloration properties of the vocal sac preferred by females during mate choice (i.e., a lower brightness and a higher chroma, Gomez et al. 2010) are associated with a better condition of males in the field. Hence this signal appears to be condition-dependent and may reflect what an individual has been able to eat, its parasitic status and/or the way he managed his oxidative stress (Lozano 1994). However, the link between coloration and condition is weak and there are probably several genetic and/or environmental factors that strongly affect coloration properties independently from condition as measured in this study.

To investigate the coloration response to changes in nutritional status during the breeding season, we conducted an experiment where food and carotenoid availabilities were manipulated during 12 days. Males from the starved group have lost on average 0.8 g (about 15 % of their initial mass) during the treatment while males from the control group and the supplemented group did not. Hence, starvation did have a strong negative effect on male condition. However, starved males have not lost more coloration than males from the control group. Moreover, males fed on a diet supplemented in β -carotene did not increase or maintain vocal sac coloration better than males from the control group. These two results suggest that this visual signal does not reflect the short-term nutritional status of the individual.

The fact that vocal sac coloration is linked to condition in the field but does not respond to food and carotenoid availabilities during the breeding season may reflect that this visual signal is a long-term condition indicator. Indeed, there is a growing body of literature demonstrating that some signals, or some signal components, do not reflect current condition but rather past condition (e.g., Uetz et al. 2002; Scheuber et al. 2003a,b; Vergara & Fargallo 2011). Moreover, a recent carotenoid supplementation experiment conducted on the red-eye treefrog (*Agalychnis callidryas*) revealed that carotenoid availability during larval and post-metamorphic development had a strong impact on adult coloration while carotenoid availability on a 3-months period during adulthood did not influence coloration anymore (Ogilvy et al. 2012). Hence, coloured visual signals involving carotenoid pigment in anurans seem to be independent of short-term variations in nutritional status even if they are expected to be plastic at a short time scale because of their location in fleshy tissues (Lozano 1994).

Breeding season fading

In our experiment, all males, regardless of their treatment, became less colourful in a way that made them less attractive to females. This kind of nutritional status-independent variability in

coloured visual signals during the breeding season has already been reported in the red-legged partridge (*Alectoris rufa*, Perez-Rodriguez 2008), but never in anurans. In our experiment, the observed fading may be due to seasonal variation in hormone profiles, especially testosterone levels (Blas et al. 2006; McGraw et al. 2006). Indeed, as it has been shown in other anurans (e.g. Delgado et al. 1989), there seems to be a testosterone peak in the middle of the season in *H. arborea* (R. Jossierand & N. Mondy, unpublished data). Given that our experiment was conducted in the second part of the breeding season (*i.e.*, from 1 to 26 May whereas the breeding season in this meta-population runs from early April to late May), it is possible that males were in a decreasing phase of testosterone levels inducing a decrease in coloration.

Why is it short-term condition-independent?

The fact that vocal sac colouration is condition-independent but still plastic on short-term remains puzzling. Actually, even under the hypothesis of decreasing levels of testosterone during the experiment, we would expect a greater decrease in colouration in starved males than in control males and in control males than in supplemented males because they did not have the same amount of anti-oxidant molecules to neutralise ROS produced by the calling activity. Indeed, even if house crickets did not contain carotenoid pigments, they probably contained other anti-oxidant molecules like vitamins or other kind of pigments that can help in maintaining a colourful signal (McGraw 2005; Bertrand et al. 2006; Catoni et al. 2008; Perez et al. 2008). Two non-mutually exclusive hypotheses can explain these results. First, it is possible that the carotenoids involved in the visual signal constitute a negligible part of carotenoid stores of an individual and that the majority of carotenoids mobilised for ROS scavenging during the breeding season come from the liver or fatty tissues, especially for starved males (Negro et al. 2001). Second, the metabolism of carotenoid pigments or other antioxidant molecules seems to be energetically costly (Hill 2000; McGraw et al. 2005). It is thus possible that, during the reproductive season, the energy allocated to the calling activity is such that males cannot afford to allocate it to the metabolism of such molecules. Then, when facing different food availability conditions during the breeding season, the short-term adjustment variable would be call activity rather than vocal sac colouration.

Multicomponent signals and condition-dependence

One of the hypotheses proposed to explain the emergence and persistence of multicomponent signals is the “multiple messages hypothesis” (Møller & Pomiankowski 1993; Candolin 2003; Hebets & Papaj 2005). This hypothesis states that the different components of a signal convey different kind information. This includes cases where different condition-dependent signal components indicate the condition of the signaller during different periods within its lifetime

(Scheuber et al. 2003a,b; Gibson & Uetz 2012). In *H. arborea*, it has been shown that at least one component of the acoustic signal, the within-bout call rate, is short-term condition-dependent (Brepson et al., 2012). Given the results of the present study and the lack of correlation between acoustic and visual signal components in the field in this species (Gomez et al. 2011), it is probable that some signal components indicate the condition of the signaller during different periods within its lifetime, thus supporting the multiple messages hypothesis. In addition, when females *H. arborea* are presented with conflicting acoustic and visual signal components, they equally preferred attractive calls or intense vocal sac coloration (Richardson et al. 2010), suggesting the possible existence of inter-individual variation in rules for signal component prioritisation and then variation in preference for short-term or long-term condition indicators. Even if this hypothesis remains to be specifically tested in *H. arborea*, Castellano & Rosso (2007) provided evidences of within-female choice consistency according to acoustic signal components in a closely related species (*H. intermedia*) supporting the idea that females may have different rules for component prioritisation. Such potential pattern of non-consensual mate choice between short-term and long-term condition indicators has already been reported in other species (*Junco hyemalis*, Hill et al. 1999; *Poecilia reticulata*, Kodric-Brown & Nicoletto 2001). Future studies should therefore investigate correlates of female signal component prioritisation in order to define if such non-consensual mate choice may be adaptive according to the type of male condition chosen.

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Article 6:

Cheating for sex: Inherent disadvantage or energetic constraint?

Brepson, L., Troïanowski, M., Voituron, Y. & Lengagne, T.
Animal Behaviour, 84 (2012), 1253-1260.

Issue:

In many species, some individuals can circumvent the honesty of a signalling system by exploiting signals emitted by conspecifics without signalling themselves. In a sexual selection context, this is often the case of males using a parasitic reproductive tactic. The use of a parasitic reproductive tactic is often determined by male condition; low condition males using more often this kind of tactic than high condition ones. However, little is known about the different aspects of condition (*e.g.* current condition vs. past condition) and their relative importance in the decision to switch from a classical to a parasitic reproductive tactic.

Hypotheses:

H6.1: The probability to adopt a parasitic tactic depends on past condition which has influenced traits conditioned by ontogeny like body size or size of a particular body part (inherent disadvantage hypothesis).

H6.2: The probability to adopt a parasitic tactic depends on current condition which limits the energy availability for signal production (energetic constraint hypothesis).

Methods:

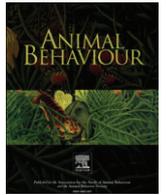
We tested and confronted these two hypotheses in an experimental design by manipulating food availability of males with different degrees of inherent disadvantage in the caller/satellite system of the European treefrog.

Results:

- Small males were more likely to be satellites than large individuals.
- Starved males were not more likely to be satellites than those that were fed.
- All else being equal, males were more likely to act as satellites if confronted with an attractive competitor than if confronted with an unattractive one.
- Males had a greater probability of being satellites at the beginning and end of the night, whereas they were more likely to call during the middle of the night.

Conclusion:

Our results support the inherent disadvantage hypothesis but not the energetic constraint one. Past condition thus seems to have a greater influence of the decision to use a parasitic tactic than current condition. Moreover, our results suggest that, when switching from one tactic to another, males use the same acoustic signal components as those used by females to select mates. At last, the fact that the probability to use a parasitic tactic changes during the course of the night may denote a synchronisation between male tactics and the expected female availability.



Cheating for sex: inherent disadvantage or energetic constraint?

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In many species, some males gain access to females by cheating, i.e. by exploiting the reproductive investment made by other males. There are two main nonmutually exclusive hypotheses to explain the decision of an individual to switch from the classical reproductive tactic to the parasitic one: the inherent disadvantage and energetic constraint hypotheses. For the first time, we tested and compared these two hypotheses in an experimental design by manipulating food availability of males with different degrees of inherent disadvantage in the caller/satellite system of *Hyla arborea*. Small males were more likely to be satellites than large individuals as predicted by the inherent disadvantage hypothesis. However, food deprived males were not more likely to be satellites than those that were fed, suggesting that the energetic constraint is not the main process explaining the use of a parasitic reproductive tactic. Moreover, all else being equal, males were more likely to act as satellites if confronted with an attractive competitor than if confronted with an unattractive one. In addition, males had a greater probability of being satellites at the beginning and end of the night, whereas they were more likely to call during the middle of the night. These results could highlight synchronization between male tactics and expected female availability during the course of the night.

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Extravagant attributes of males in many taxa such as the antlers of deer, the horns of beetles or bright coloration in birds have long intrigued naturalists. They found an initial explanation in the second seminal work of Darwin (1871) through sexual selection. According to Darwin's theory, these secondary sexual characters provide an advantage to males that have them in attracting females and/or fighting other males. Soon after, Bateson & Brindley (1892) were among the first to describe a dimorphism in secondary sexual characters within a single sex (male) in two insect species. However, it was not until the early 1970s, after the accumulation of examples in various taxa, that Gadgil (1972) suggested that this dimorphism within males may be linked to alternative strategies to achieve reproduction and would be under the influence of sexual selection. Nowadays, the concept of alternative reproductive tactics (ARTs) is considered in a more extensive manner and includes alternative tactics in both males and females. ARTs are thus defined as sets of discontinuous traits (behavioural or not) expressed within a sex, that allow individuals to achieve reproduction in two or more

alternative ways (Taborsky et al. 2008). They represent a key concept for understanding the mechanisms of sexual selection and are considered as a model system for studying the evolution of phenotypic variation, providing interactions between different fields of study such as animal behaviour, biology of development, phenotypic plasticity, life histories or game theory (e.g. Gross & Charnov 1980; Arak 1988; Lucas & Howard 1995). One of the most common cases in which ARTs are expected to evolve is when the investment in reproduction made by some individuals is exploitable by competitors of the same sex, generally males (Taborsky et al. 2008). In this case, two reproductive tactics are typically maintained within the same sex: 'bourgeois' (or 'classical tactic') and 'parasitic' (or 'sneaking tactic'). The bourgeois tactic supposes a high investment for access to reproduction, whereas the parasitic tactic allows access to reproduction by exploiting the investment of competitors that use the bourgeois tactic. In the vast majority of cases the adoption of a bourgeois or a parasitic tactic is condition dependent, depending on both intrinsic and extrinsic factors (Gross 1996). One of the current issues in the study of ARTs is to understand how variations in intrinsic and extrinsic factors can influence the probability of adopting one or the other tactic.

Among species exhibiting condition-dependent ARTs, two main types of phenotypic plasticity have been described. First, ARTs can be fixed throughout an individual's lifetime. In this case the

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adoption of one or the other tactic depends on intrinsic and extrinsic factors during the individual's ontogeny, before sexual maturity (e.g. development towards parental or cuckolding males in the bluegill sunfish, *Lepomis macrochirus*: Gross & Charnov 1980; development towards fighter or scrambler males in the dimorphic mite *Sancassania berlessei*: Radwan et al. 2002). Second, individuals can switch from one tactic to another to maximize their reproductive success according to the intrinsic and extrinsic factors at a given time (simultaneous ARTs sensu Taborsky et al. 2008; conditional strategy with plastic alternatives sensu Gross 1996; e.g. switch between solitary and colonial nesters in the longear sunfish, *L. m. megalotis*: Jennings & Philipp 1992; switch between territorial and nonterritorial tactics in the solitary bee *Ptilothrix fructifera*: Oliveira & Schlindwein 2010). In these cases, the adoption of one or the other tactic depends on extrinsic factors potentially interacting with two types of intrinsic factors: short-term factor, that is, nutritional status at a given time limiting the expression of the bourgeois tactic through energetic constraint, and long-term factor, that is, inherent advantage or disadvantage in adopting the bourgeois tactic owing to traits conditioned by ontogeny such as body size or size of a particular body part. While extrinsic factors such as the challenger's competitiveness have been well studied in numerous bourgeois/parasitic tactic systems (e.g. in insects: Brockmann 2008; in crustaceans: Shuster 2008; in anurans: Zamudio & Chan 2008), the distinction between 'energetic constraint' and 'inherent disadvantage' effects on the probability of adopting a parasitic tactic has received less attention. Dissecting these two effects will undoubtedly increase our understanding of the elements involved in the dynamics of switches between tactics in a sexual selection context but also more broadly of short-term phenotypic plasticity in other contexts (e.g. in a foraging context with producer/scrounger tactics: Mathot et al. 2009).

Anurans represent one of the most studied taxa in the field of ARTs. Indeed, in many species two simultaneous ARTs can be used by males to achieve reproduction: calling (bourgeois tactic) or being a satellite male near a calling male (parasitic tactic). In most cases, the adoption of one or the other tactic seems to be condition dependent (reviewed in Zamudio & Chan 2008). While the challenger's competitiveness seems to influence the probability of using one tactic or the other in the vast majority of cases (e.g. Arak 1988; Leary et al. 2006; Humfeld 2008; Castellano et al. 2009), intrinsic factors interacting with it are not fully understood. The energetic costs involved in calling and/or defending a territory in anurans are often high (reviewed in Wells 2001) and render the energetic constraint a relevant hypothesis to explain the switch from a calling to a satellite tactic (Emerson & Hess 2001). However, most studies conducted to date have shown no apparent difference between callers' and satellites' body condition, providing little support for the energetic constraint hypothesis (e.g. Arak 1988; Lance & Wells 1993; Leary et al. 2008a; Castellano et al. 2009; but see Leary et al. 2004; Humfeld 2008). Moreover, studies of steroid hormonal profiles in two *Bufo* species suggest a switch mechanism dependent on corticosterone levels (Leary et al. 2006, 2008a, b) but apparently independent of energy stores, contrary to the predictions of the energetics–hormone–vocalization model proposed by Emerson & Hess (2001). In addition, in most cases satellite males are smaller than calling males (e.g. Wells 1977; Arak 1988; Castellano et al. 2009; but see Lance & Wells 1993). Hence, causes referring to an inherent disadvantage (typically low fighting abilities or unattractive calls) are often invoked to explain the use of a satellite tactic in anurans (Zamudio & Chan 2008). Nevertheless, even if these elements mainly support the inherent disadvantage hypothesis, they do not rule out the energetic constraint hypothesis since small males may have lower energy stores and/or may suffer higher metabolic costs than large males when calling (Voituron

et al. 2012); however, these differences are not considered in body condition indices (Peig & Green 2010). In addition, several theoretical models have taken into consideration a potential energetic constraint that has led to patterns of tactic use dynamics in anurans close to those observed in the field, indirectly supporting this hypothesis (Lucas & Howard 1995; McCauley et al. 2000). Hence, to disentangle the effects of the inherent disadvantage and the energetic constraint on the decision-making process and to compare them quantitatively, we need an experiment in which the energetic constraint is manipulated through feeding/food deprivation experiments rather than inferred through body condition indices.

In this study, we experimentally tested and compared the effects of both inherent disadvantage and energetic constraint in interaction with the challenger's competitiveness on the probability of adopting a satellite tactic in the European treefrog, *Hyla arborea*. Males in this species do not defend resources but are known to exhibit a very expensive calling behaviour to attract females (mean aerobic scope of 18 ± 9 ; Grafe & Thein 2001). Small males seem to bear an inherent reproductive disadvantage because they are less attractive to females (Marquez & Tejado 1990; Richardson et al. 2010). Moreover, small males have been reported occasionally to use a satellite tactic, preferentially parasitizing larger males (Berec & Bajgar 2011). In addition, *H. arborea* is a prolonged breeder and, during the breeding season, males seem to alternate calling periods to attract females and foraging periods to replenish their energy stores (Meuche & Grafe 2009). Hence, our predictions are threefold: males should be more likely to use a satellite tactic (1) if they are small (under the inherent disadvantage hypothesis), (2) if they are deprived of food (under the energetic constraint hypothesis) and (3) if they are confronted with an attractive competitor versus an unattractive one (all else being equal).

METHODS

Capture and Housing Conditions

Hyla arborea were obtained from a population located near Lyon, France, on the Crémieu Plateau (5°21'7"E, 45°44'20"N). Over the course of the breeding season, we captured 100 males individually by hand (in a net) when they arrived at the pond. Under this capture regime, there is no selection of particular males since we avoided the bias of capturing only calling males on the pond. The frogs were then transported by car in individual boxes to our laboratory where they were immediately weighed and measured (snout–vent length, SVL). After weighing, the males were stomach-flushed according to the methodology described by Solé et al. (2005) to ensure that all animals began the treatment with an empty stomach to standardize the effect of this treatment regardless of what the individual ate before. We then placed males in individual terraria (25 × 17 cm and 15 cm high) constructed of plastic mesh (sides and lid) containing a water-filled basin and a tree branch. During the treatment period (described below), the males were exposed to a natural photoperiod, and chorus noise (75 dB) was broadcast each night from 2000 to 0130 hours (mimicking the field conditions during intense nights of chorusing). We used the same chorus noise during the behavioural experiment (see below). To construct the chorus noise track, we recorded 10 males from the same population in the laboratory in individual semianechoic chambers using Sony ECM-T6 microphones and Roland R-44 recorders. Then, we modified the call amplitude of each individual record (using Avisoft Saslab software, Avisoft Bioacoustics, Berlin, Germany) to reach the same level and mixed them carefully to avoid amplitude saturation of the constructed track. We thus used a chorus noise in which no male was louder

than any other to avoid the risk of the tested male responding to the calls of a particular competitor during the behavioural experiment (Richardson et al. 2008).

Treatment

We randomly assigned males to one of two treatments: fed or food deprived. The fed group was fed house crickets, *Acheta domesticus*, and bluebottle flies, *Calliphora vomitoria*, ad libitum for 7 days before beginning the experiment. We ensured that all of the fed males ate by observing them individually after distributing the food. The males of the food-deprived group were maintained during the same time period without any food supply. Their terraria were manipulated in the same manner as those of the males of the fed group. We assessed the impact of the treatment on the body condition by reweighing the males after the treatment and by computing a size-independent condition index, the scaled mass index (SMI), both before and after the treatment. SMI is a condition index corresponding to the predicted mass of the individual at the mean length of the population based on the log–log standardized major axis regression between mass and length. This condition index is particularly relevant because it considers the mutual interdependence of mass and length, with both being inherent indicators of 'true size', rather than considering only length as an indicator of 'true size' (Peig & Green 2010). Owing to a technical issue with the balance used for weighing, only 88 males out of the original 100 were reweighed after the treatment. Whereas the SMI did not differ between the fed and the food-deprived groups before the treatment (t test with Welch's 1947 approximation for degrees of freedom: $t_{97.5} = -0.969$, $P = 0.335$), it was significantly lower in the food-deprived group after the treatment (t test with Welch's approximation for degrees of freedom: $t_{85.7} = 2.352$, $P = 0.021$; mean SMI \pm SE in the fed group = 5.69 ± 0.09 g (range 4.38–6.94 g), in the food-deprived group = 5.39 ± 0.09 g (range 4.18–6.60 g)).

Experimental Design

We conducted tests between 2030 and 0100 hours in a rectangular experimental arena (Fig. 1). This arena was lit by a weak red light allowing behavioural observations. Walls were 35 cm high. The floor of the arena was covered with moist hessian and the inside of the walls were lined with 60 mm-thick anechoic foam wedges (Plastiform) to limit sound reverberation. We used the terrarium of each male as the release box at one end of the arena, allowing us to avoid male handling before the experiment. Two loudspeakers were placed in the arena: the first was placed 1 m above the centre of the arena for broadcasting chorus noise, and the second was placed at the opposite side of the release box for broadcasting competitor display. We defined the satellite zone as the area within 10 cm of the competitor loudspeaker.

Acoustic displays of unattractive and attractive competitors were constructed with calls from individuals of the same

population recorded in the laboratory in a previous study (same recording methodology as that described above for the chorus track) and artificially modified (using Avisoft Saslab software) to fit the first and the third quartiles of the population for three sexually selected call properties: dominant frequency, within-bout call rate and amplitude. For dominant frequency, lower values are preferred by females whereas for amplitude and within-bout call rate, higher values are preferred in this species (Richardson et al. 2010). Hence, the display of the unattractive competitor had a 2750 ± 46 Hz mean dominant frequency (mean \pm SD throughout), an amplitude of 74.5 ± 1.9 dB at the release point and a within-bout call rate of 5.87 ± 0.02 calls/s. The display of the attractive competitor had a 2400 ± 53 Hz mean dominant frequency, an amplitude of 80 ± 1.6 dB at the release point and a within-bout call rate of 7.20 ± 0.03 calls/s. In addition, the displays differed in the number of calls. The attractive competitor produced 172 calls grouped into five call bouts during the 30 s, whereas the unattractive competitor produced 102 calls grouped into three call bouts during the same period. Number of calls per bout were comparable for the two displays (34 ± 13 calls for the attractive competitor, 34 ± 5 calls for the unattractive one), as well as call duration (55 ± 6 ms for the attractive competitor, 55 ± 5 ms for the unattractive one).

Throughout the 20 min of each test, a repeated 2 min chorus noise was broadcast at a mean amplitude of 73.4 dB at the release point. After 9 min, we opened the terrarium by removing the side facing the competitor loudspeaker. After 1 min, the artificial competitor began calling (a 30 s display that was loop repeated) during the last 10 min of the test. Each male was tested in two situations, with an attractive competitor and an unattractive competitor. The first competitor (attractive or unattractive) was randomly chosen, and the two tests of a single male were conducted consecutively.

During the treatment (7 days before the experiment), chorus noise was broadcast each night from 2000 to 0130 hours using a Hitachi CX70 CD player. During the experiment, the chorus noise and competitor display were broadcast using two Monacor SP155X loudspeakers connected to a computer via an IMG Stage Line STA102 stereo amplifier. The sound levels were calibrated using a Voltcraft MT-8820 Sound Level Meter (C-weighted, 'fast' root-mean-square (RMS) setting, tolerance ± 1.5 dB).

Behavioural Observations

In this experiment, males could behave in three ways: being a caller, being a satellite or no response. A male was defined as a caller if he produced at least one call while the competitor was calling, whereas he was defined as a satellite if he stayed silent in the satellite zone around the competitor loudspeaker for at least 20 s while the competitor was calling. In all other cases (staying inactive or moving into or out of the arena), the male was considered as not responding to the test in contrast to males that responded to the test (i.e. callers or satellite males). The criteria used to categorize satellite males in this experiment were selected according to a pilot study conducted with the same experimental design. Males that remained silently close to the loudspeaker for at least 20 s all adopted the typical low posture of satellite males observed in the field in this species and described in other hylids (Forester & Lykens 1986; Castellano et al. 2009; Berc & Bajgar 2011). Almost none of the males that explored without paying attention to the competitor stopped moving, while males that were not active at all stayed in the release box during the entire experiment. No male stayed stationary for 20 s outside the satellite zone either during the pilot study or during the experiment, except for calling. It is therefore very unlikely that males classified as satellite were nonresponding males resting in the satellite zone.

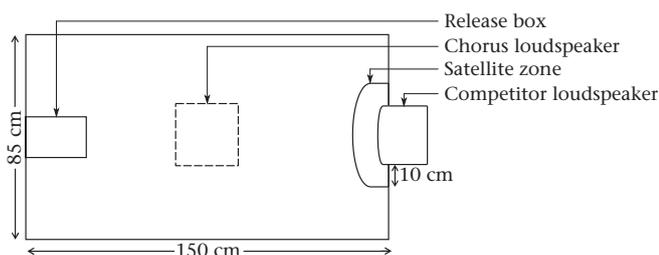


Figure 1. Top view of the experimental arena.

Statistical Analysis

To ensure that our experimental design did not bias the results obtained, we first investigated the factors influencing the probability of responding to the test. Then, among the tests in which a male responded, we investigated the factors influencing the probability of being a satellite rather than a caller. These two points were investigated through generalized linear mixed models with a binomial error and an individual random effect to take into account repeated observations for the same individuals (Bolker et al. 2009). We tested several explanatory factors according to the three predictions: the attractiveness of the competitor (attractive versus unattractive), the body mass of the tested male before treatment (as a size indicator to test the inherent disadvantage hypothesis) and the treatment (fed versus food deprived to test the energetic constraint hypothesis). We tested other key factors: the start time of the test (varying from 2030 to 0030 hours, coded as 8.5 to 12.5), the date of the test (varying from 20 April to 16 May, coded as 1 to 27) and the order of the test (first or second test of each male). The quantitative factors (body mass, start time and date) were standardized by subtracting the mean and dividing by the standard deviation to facilitate the interpretation of each isolated parameter by averaging the others by default. Given that the frog's calling activity is centred on particular hours in the night and a particular season in the year, we also tested the quadratic effects of the start time and date. We tested all of the second-order interactions between the factors too. The parameters were estimated through an 8-point adaptive Gauss–Hermite quadrature that approximates the log-likelihood using the package 'lme4' (Bates et al. 2011). We selected the best models using the Akaike information criterion (AIC) and examined the significance of the effects using Wald *z* tests. All of the statistics were performed using R 2.14.1 (R Development Core Team 2011).

Ethical Note

The population from which the individuals were collected belongs to a metapopulation consisting of more than 20 000 tree-frogs. Hence, our sampling did not significantly affect reproduction of the population. The stomach flushing method consisted of injecting water at 20 °C with a syringe and a flexible hose. Stomach flushing is discussed by many authors as a gentle and commonly used method for assessing diet in reptiles and anurans (e.g. Legler & Sullivan 1979; Harr et al. 2000; Solé et al. 2005). This method is especially well adapted for hylids of this size (Solé et al. 2005). No complications were noted for either fed or food-deprived males (i.e. no mortality, no lungs filled with water, Solé et al. 2005); all males behaved normally on the following days (i.e. resting on the tree branch or on the sides of the terrarium during the day, calling and exploring activities during the night). Moreover, all fed males caught food items by themselves the day after their stomach flushing. The duration of food deprivation was chosen carefully to mimic what happens in the wild. Indeed, in a field study, the average chorus attendance was estimated to be 7.5 days by Grafe & Meuche (2005) in *H. arborea* and so this duration appeared to be relevant to reflect the field situation. After the behavioural experiment, all males were fed ad libitum for 2 days and kept under observation. All of them ate during these 2 days and were observed calling almost every night during the whole experiment. Hence, we are confident that housing conditions and treatment did not permanently affect their health. The night following these 2 days, they were all released at the capture site. This study was conducted in accordance with French laws and with the approval of the Préfecture de l'Isère (decision 2007-03328) and the Direction of Veterinary Services (permit DSV no. 69266347).

RESULTS

Probability of Responding to the Test

Among the 100 tested males, 57 responded to the test (i.e. were caller or satellite) at least once. Among these 57 males, 25 responded to both consecutive tests, 22 responded only if confronted with an attractive competitor, and 10 responded only if confronted with an unattractive competitor, resulting in a sample size of 82 males responding over 200 tests. Two factors significantly affected the probability of responding to the test: the date of the test and the attractiveness of the competitor (Table 1). The effect of the date was not a quadratic effect. Nevertheless, this factor negatively impacted the probability of responding to the test. Hence, we observed that even though males responded to the test in 43% of the cases during the first 5 days of the experiment, they only did so in 22% of the cases during the last 5 days. Moreover, the males responded more frequently if confronted with an attractive competitor (47%) than if confronted with an unattractive one (35%).

Inherent Disadvantage and Energetic Constraint

Among the 200 tests conducted, the males responded 82 times: 47 times by performing a calling tactic, 35 times by adopting a satellite tactic. Consistent with our predictions, males were more likely to act as a satellite if confronted with an attractive competitor than if confronted with an unattractive one (Table 2, Fig. 2). As predicted by the inherent disadvantage hypothesis, the probability of being a satellite was higher for small males than for large males (Table 2, Fig. 2a, b). Moreover, contrary to the prediction of the energetic constraint hypothesis, the food-deprived males were not significantly more likely to be satellites than the fed males (Fig. 2e, f). Indeed, when we added the factor 'treatment' to the selected model, its effect was not significantly different from 0 (estimate \pm SE = 1.33 \pm 1.50; Wald *z* test: $P = 0.373$). When we considered the change in the SMI during the treatment instead of the binary factor (fed versus food deprived), there was no significant effect on the probability of being a satellite rather than a caller (estimate \pm SE = -1.16 \pm 0.95; Wald *z* test: $P = 0.222$). Moreover, we found a strong quadratic effect of the start time of the test on the probability of being a caller. Indeed, males were more likely to be satellites at the beginning and at the end of the night than during the middle of the night (Table 2, Fig. 2c, d).

When we used SVL rather than mass before treatment as an indicator of size we found a negative but not significant effect (estimate \pm SE = -1.10 \pm 0.70; Wald *z* test: $P = 0.117$). When we used the condition index (SMI) computed on the mass before the treatment rather than a size indicator we found a far weaker and not significant effect (estimate \pm SE = -0.59 \pm 0.70; Wald *z* test: $P = 0.396$), suggesting that the effect of the body mass before treatment actually reflects an effect of the size of the male and does not indirectly reflect an energetic constraint effect through body condition.

Table 1

Factors affecting the probability of responding to the test (being a caller or a satellite), rather than not responding to the test, estimated through generalized linear mixed models with a binomial error ($N = 200$)

	Estimate	SE	<i>z</i>	<i>P</i>
(Intercept)	-0.179	0.264		
Date	-0.603	0.222	-2.714	0.007
Competitor	-0.731	0.325	-2.246	0.025

For the competitor factor, the reference is the attractive caller.

Table 2

Factors affecting the probability of being a satellite rather than a caller, when responding to the test, estimated through generalized linear mixed models with a binomial error ($N = 82$)

	Estimate	SE	z	P
(Intercept)	0.722	0.838		
Competitor	-3.394	1.431	-2.372	0.018
Body mass	-1.762	0.934	-1.886	0.059
Start time	0.120	12.800	0.009	0.993
(Start time) ²	32.639	15.059	2.167	0.030

For the competitor factor, the reference is the attractive caller.

DISCUSSION

Males were more likely to respond to the test (i.e. to be caller or satellite) at the beginning of the experimental period than at the end. This is not surprising because the reproductive season for this population lasts from early April to mid-May, whereas our experiment was conducted from 20 April to 16 May. Hence the first males were tested at mid-season whereas the last ones were tested at the end of the season, when treefrogs are less sexually active. Given that the males were randomly chosen in the field and randomly assigned to a treatment (fed or food deprived) and that the date factor did not interact with the other factors, this date effect did not impact the validity of our results. The males were also more likely

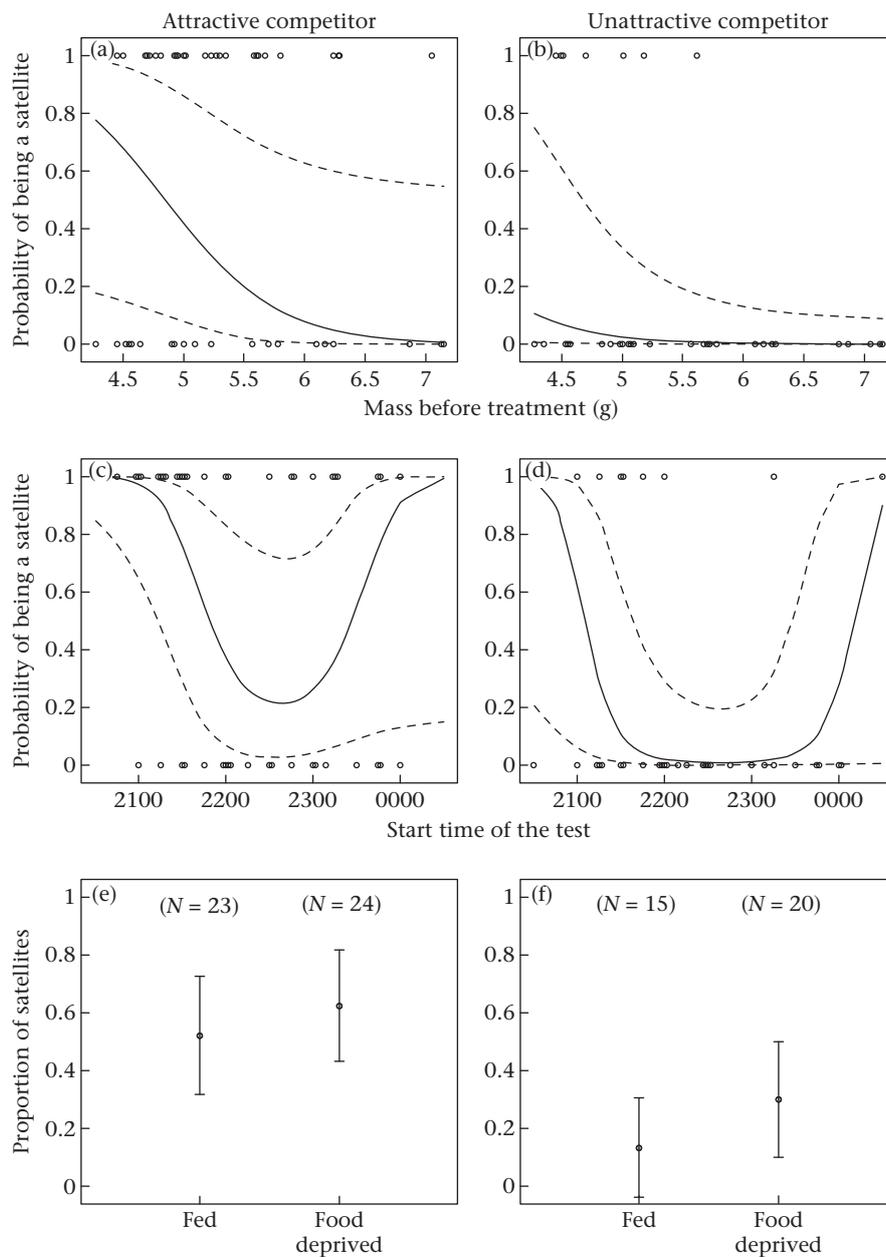


Figure 2. Predicted probability (solid lines) and 95% confidence interval (dashed lines) of being a satellite rather than a caller, as computed using tests with responding males ($N = 82$) and according to (a, b) the mass of the tested male before treatment, (c, d) the start time of the test and whether the male was confronted with an attractive or unattractive competitor. Circles represent the observation data. Predictions according to the body mass are given for the mean start time, whereas predictions according to the start time are given for the mean mass. (e, f) The observed proportion (and 95% confidence interval) of satellites among responding males in the fed and food-deprived groups when confronted with an attractive or unattractive competitor.

to respond to the test if confronted with an attractive competitor than if confronted with an unattractive one. This result could be because callers are expected to call when confronted with both competitors, whereas satellites are expected to be a satellite of an attractive competitor (e.g. Humfeld 2008; Castellano et al. 2009; Berec & Bajgar 2011) but may look for a better male to parasitize if confronted with an unattractive competitor, that is they do not respond to the test. Indeed, among the tests with responding males, callers were more frequently observed than satellites. This competitor effect could lead to an underestimation of the proportion of satellites among the males responding to the test. However, it did not interfere with our predictions and thus did not impact the validity of our results.

Predictions 1 and 3 are verified by our experiment: the males were more likely to be satellites if confronted with an attractive competitor than if confronted with an unattractive one, and small males were more likely to be satellites than large males. However, prediction 2 is not verified by our experiment: the food-deprived males were not more likely to be satellites than the fed males. These results support the inherent disadvantage hypothesis and are consistent with both field observations of *H. arborea* (Berec & Bajgar 2011) and most of the observations and experiments conducted using anurans (reviewed in Halliday & Tejedo 1995; Zamudio & Chan 2008) and other taxa (e.g. Kodric-Brown 1986; Kiflawi & Gray 2000; Oliveira & Schlindwein 2010). In our case, small males were probably physically constrained and unable to produce advertisement calls that were as attractive as those of larger males (e.g. with a low dominant frequency). Within such a context, the expression of alternative noncalling mating tactics may allow a proportion of small males to intercept females attracted by the attractive calling males. When we used SVL rather than mass before treatment as an indicator of size we found a negative but not significant effect probably owing to a lack of statistical power regarding the weak interindividual variability of SVL compared to body mass. When we used the condition index (SMI) computed on the mass before the treatment rather than a size indicator we found a far weaker and not significant effect. Hence, we are confident that the described effect of the body mass before treatment actually reflects an effect of the size of the male and does not indirectly reflect an energetic constraint effect through body condition.

In our experiment, we found no effect of 1 week of food deprivation (an appropriate food deprivation period considering the field behaviour of these animals) on male reproductive tactic. Even when considering the change in the SMI during the treatment instead of the binary factor (fed versus food deprived), there is no significant effect on the probability of being a satellite rather than a caller. Hence, for the first time, we showed experimentally that the energetic constraint hypothesis poorly explains the switch from a bourgeois tactic to a parasitic tactic in comparison to the inherent disadvantage hypothesis. Although the acoustic activity of *H. arborea* is among the costliest measured in anurans (Grafe & Thein 2001), males may not be subject to metabolic constraints that predispose them to use less energetically demanding tactics. Hence, we did not find a linear increase in the probability of being a satellite during the course of the night, which could be explained by the depletion of energetic reserves. Accordingly, a satellite tactic is probably not used by males at the end of a calling period, just before the beginning of a foraging period. The lack of support for the energetic constraint had been suggested by several studies that compared the body condition of callers and satellite males in anurans and found no difference (Arak 1988; Lance & Wells 1993; Leary et al. 2008a; Castellano et al. 2009; but see Leary et al. 2004; Humfeld 2008). This is the first time that body condition has been experimentally manipulated, instead of being only estimated through indices, to assess its effect on tactic use in anurans.

However, another experimental design consisting of within-subjects tests comparing behaviour before and after food deprivation, as well as before and after supplemental feeding, could be even more powerful to test the energetic constraint hypothesis and should be considered for future studies.

In our experiment, the lack of a food deprivation effect on the probability of using a satellite tactic might be caused by too short a food deprivation period. However, our purpose here was not to wipe out males' energetic stores, but rather to diminish these stores significantly to investigate the effect of this diminution on the decision-making process. To do so, 1 week of food deprivation seemed to be appropriate since it corresponds to the average chorus attendance observed in the field for this species (Grafe & Meuche 2005). Indeed, we observed a significant effect of this treatment on males' body condition (see *Methods*). Nevertheless, it remains possible that the energetic constraint acts only after a critical threshold of energetic stores is reached and that our treatment was not strong enough to reach this threshold. However, one might question the relevance of a longer food deprivation period knowing the behaviour of these animals in the field. In addition, given that they have been investigated in the same experiment, on the same individuals, with the same sample size and on the same response variable, the effects of the energetic constraint and the inherent disadvantage hypotheses are rightly comparable. Hence, we can assert that, with an appropriate food deprivation period considering field observations, the energetic constraint has a negligible effect on the probability of using a parasitic tactic compared to the effect of the inherent disadvantage of being small in this species. Therefore, even if our results do not rule out the energetic constraint hypothesis, they provide little support to this hypothesis in comparison with the inherent disadvantage hypothesis.

Moreover, our results strongly suggest that, when switching from one tactic to another, males use the same acoustic cues as those used by females to select mates. This result has also been reported in several previous studies in anurans (e.g. Leary et al. 2006; Humfeld 2008; Castellano et al. 2009; Berec & Bajgar 2011). Nevertheless, it is known that *H. arborea* females also use visual cues based on carotenoid pigments during mate choice (Richardson et al. 2009; Gomez et al. 2010). Thus, it would be highly interesting to test whether males can also use visual cues when switching from one tactic to another.

The start time of the test had a strong effect on the probability of being a satellite or a caller but not on the probability of responding to the test. Therefore, all things being equal, males had a greater probability of being a caller during the middle of the night than at the beginning or end of the night. This result should be paralleled with studies in anurans that investigate the timing of female arrival to the pond. Indeed, synchronization between female arrival and maximum chorus intensity during the middle of the night is often found (Passmore et al. 1992; Bevier 1997; Murphy 1999). Hence, a moderately attractive male should have a very weak probability of mating if calling when females are rare and attracted by the few most attractive males (i.e. at the beginning and the end of the night), and could increase his success by adopting a satellite tactic near an attractive male. Moreover, when both the caller density and chorus noise are high (i.e. in the middle of the night), females are less discriminating and the mating probability of moderately attractive calling males increases (Wollerman & Wiley 2002; Richardson & Lengagne 2010). This relationship between the operational sex ratio and the proportion of males using an ART has previously been highlighted in several studies but generally at the scale of the entire breeding season or by comparing several populations (e.g. Lodé et al. 2005; Gao & Kang 2006; Mendoza-Cuenca & Macias-Ordóñez 2010; see also Rowell & Cade's 1993 modelling

work). Our results suggest that, as the inherent disadvantage, the nightly timing of female availability could be important in determining which tactic a male will choose at a given time to maximize his reproductive success. To our knowledge, only Leary et al. (2008a) dealt with nightly variation of the proportion of satellites in anurans. Leary et al. (2008a) clearly showed that the proportion of satellites was positively correlated with the density of males in the chorus at the reproductive season scale. However, this pattern was not so clear when considering measures made within nights. It is thus possible that, at the night scale, the correlation between the proportion of satellites and density of males in the chorus is hidden by other important factors such as nightly timing of female availability.

To conclude, in this study we showed experimentally that very short-term phenotypic plasticity as observed in some ARTs might be more dependent on long-term intrinsic factors such as body size than on short-term intrinsic factors such as nutritional status. In such systems, extrinsic factors such as social context (including for instance the challenger's competitiveness, male density and operational sex ratio) thus seem to play a major role for short-term switches between tactics. Future studies should therefore try to test experimentally and compare quantitatively potential extrinsic factors acting on the switch decision between tactics, in a sexual selection context but also on short-term phenotypic plasticity in other contexts (e.g. in a foraging context with producer/scrounger tactics). Such experiments would greatly improve our knowledge of the dynamics of switches between tactics and their associated selection pressure in changing environments.

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DISCUSSION

Along this work, we showed that both static and dynamic call components may be reliable indicators of male's condition in *H. arborea*. In addition, the condition-dependence of dynamic call components may be maintained through a stabilising cost due to a higher production cost for low condition males than for high condition ones (articles [1,2]). Our results also suggest that, according to their condition, males can use different strategies of energy allocation between dynamic call properties and the amount of call produced, potentially masking the condition-dependence of some dynamic signal components (article [2]). The call dominant frequency, a static call property seems to be an indicator of past condition since it is correlated to body size, but the variability of this signal component on relatively short-term may hinder, at least partly, its honesty (article [3,4]). Visual components of the sexual signal in *H. arborea* seem also linked to male's condition in the field but they do not mirror short-term variations in male's nutritional status (article [5]). At last, we showed that the decision to switch from a classical reproductive tactic to a parasitic one is condition-dependent and relies more on an inherent disadvantage linked to male's past condition than on an energetic constraint linked to its current condition (article [6]).

- Condition-dependence & multiple messages hypothesis

We tested condition-dependence of 5 sexual signal components known to be under selection through mate choice in *H. arborea*. Among these components, there were 3 acoustic ones and 2 visual ones. The 3 acoustic components included 2 dynamic and 1 static call properties. Here, I summarize and confront the results obtained for all these tested components.

Dynamic acoustic components

We tested condition-dependence of two dynamic acoustic components, within-bout call rate (WCR) and call duration, by manipulating food availability of 36 males during 10 consecutive days (article [2]). By investigating trade-offs between maintenance of attractive dynamic components and maintenance of nightly calling activity (*i.e.* the total number of calls produced by a male during a night), our analysis revealed that WCR was condition-dependent. Indeed, males deprived of food for 10 consecutive days experienced a trade-off between maintaining WCR and nightly calling activity, while fed males did not (article [2]). However, such trade-off was not revealed between call duration and nightly calling activity.

These results are consistent with our investigation of call production costs (article [1]). Indeed, by investigating male oxygen consumption during call production, we found that this measure of energetic cost was strongly positively influenced by both the number of calls emitted and

WCR but not by call duration (article [1]). Note that call duration is highly negatively correlated with WCR in this species (Friedl & Klump 2002); its condition-dependence and its relation to production costs may therefore be masked by a stronger effect of WCR. Moreover, call duration attractiveness to females has never been tested by correcting for WCR (Richardson *et al.* 2010b). This acoustic component may thus bring little information to females, in comparison to WCR, when assessing male condition.

Our investigation of condition-dependence also revealed that, depending on their initial condition, all males do not face the same way the trade-off between maintenance of WCR and maintenance of nightly calling activity. Hence, the highest condition males (measured both through body size and a body condition index) mainly maintained their nightly calling activity to the detriment of WCR while low condition males mainly decreased their nightly calling activity and maintained their WCR. As suggested by Castellano's theoretical work (2009), the fact that males of low condition invest proportionally more on call properties selected by females and less on calling activity than high-condition males may reflect different strategies adopted by males to optimise their mating success according to their condition. In Castellano's study (2009) males of different condition are assumed to differ in the amount of energy they can allocate to signal production. In *H. arborea*, this assumption seems to be verified since we showed that, for a given number of calls produced and for a given WCR, the cost of call production is proportionally higher for small males than for large ones (higher mass-specific oxygen consumption, article [1]).

Given that receivers cannot assess the nightly calling activity of each signal emitter, the different investment strategies adopted by males can disturb the honesty of the information transmitted through WCR. This result can thus be paralleled with studies showing that a sexual signal can be dishonest at a given time if the way to balance current and future reproductive success differs between males (Kokko 1997; Candolin 1999, 2000a). The signalling system in such cases is maintained if, on average, the receiver still benefits from trusting any signal (Johnstone & Grafen 1993; Kokko 1997). In the case of *H. arborea*, mate choice on WCR may be maintained because, by mating with a male emitting calls at high WCR, a female probably have a sufficiently great probability to mate with a high-condition male since low-condition males are not able to call as often as high-condition ones with such selective pressure on WCR (article [2], Castellano 2009).

Here we showed that WCR appears to be a signal component dependent upon current male condition in *H. arborea* (article [2]). This is in agreement with several empirical studies in diverse taxa (*e.g.* Scheuber 2003a; Velando *et al.* 2006, Gibson & Uetz 2012) which showed that dynamic signal components are dependent upon current condition of the emitter. However, this study is the first to show that a manipulation of male condition can impact dynamic call properties in anurans on short term, probably because previous studies did not consider potential trade-offs between dynamic call

properties and the amount of call produced (Green 1990; Murphy 1994; Grafe 1996; Marler & Ryan 1996; Judge & Brooks 2001; Gordon 2004; Meuche & Grafe 2009). By using such methodology, our study also reveals that the honesty of the information conveyed through this dynamic signal component may be partly hampered by different investment strategies adopted by males.

Static acoustic component

Call dominant frequency is a static acoustic component (Gerhardt 1991), generally negatively correlated with body size in anurans (reviewed in Gerhardt 1994). This call property is generally considered as an index (*sensu* Maynard-Smith & Harper 1995) of emitter's size in anurans because it depends physically on vocal cords mass, which is highly correlated with body size (Martin 1971). We can also consider that the reliability of this signal component is due to developmental costs (*sensu* Searcy & Nowicki 2005) and thus that it indicates emitter's condition during the development of the vocal apparatus. Both points of view agree on the fact that dominant frequency is, theoretically, a reliable indicator of past condition (condition during individual's ontogeny). Given that past condition is not currently variable by definition, call dominant frequency of an individual should be highly constant over a short time scale.

In *H. arborea*, call dominant frequency is also negatively correlated with male's body size (Richardson *et al.* 2010b; article [3]) and thus appears to be an indicator of past condition. However, our study of within individual variability at the scale of the night reveals that dominant frequency is much more variable than previously believed and that this variability can significantly decrease the relevance of mate choice based on this signal component (article [3]). Such high variability in call dominant frequency can occur when males are able to modulate this call property by changing vocal cords tension and/or the shape of arytenoid cartilages as it is the case in some anuran species (*e.g.* *Leptodactylus albilabris*, Lopez *et al.* 1988; *Acris crepitans*, Wagner 1989; *Rana clamitans*, Bee & Perrill 1996; *Rana virgatipes* Given 1999; *Rana catesbiana*, Bee & Bowling 2002).

Theoretically, short term variability in dominant frequency could also provide information to the receiver. Indeed, there is a mismatch between the small size of radiating structures (mainly the vocal sac) and the principal wavelengths of the call in anurans. Thereby, when possible, lowering call dominant frequency supposes lowering efficiency in converting metabolic energy into acoustic energy during call production (Ryan 1985; Prestwich *et al.* 1989). Hence, all other call components being equal, it should be costlier for a male to produce lower frequency calls (supported by Wagner 1989). Lowering dominant frequency on short term would therefore inescapably increase the cost of signal production, making signal component a short-term condition dependent signal component. It seems to be the case in the Blanchard's cricket frog, where changes in dominant frequency appear to function as reliable signals of size-independent fighting ability (Wagner 1992).

Our data showed that increasing call amplitude or WCR inevitably results in an increase in dominant frequency, suggesting a trade-off between lowering dominant frequency and increasing quality of other signal components (article [3]). Moreover, there is a negative correlation between nightly calling activity and within-individual variability in dominant frequency, suggesting a trade-off between varying dominant frequency and increasing calling activity. Nevertheless, we failed to show any relationship between within-individual variability and body size or body condition index revealing differential abilities to shift dominant frequency relative to male condition (article [3]). In addition, results of food manipulation experiments (article [2]) show that dominant frequency is not dependent upon nutritional status on short term and not involved in an energy allocation trade-off during signal production. So the variations in call dominant frequency in *H. arborea* are unlikely to provide information on current condition of the emitter. Hence, call dominant frequency appears to be a condition-dependent signal component transmitting information about past condition, even if this information is partly hindered because of short-term condition-independent variations during signal production.

Visual components

We investigated condition-dependence of two visual signal components known to be involved in mate choice in *H. arborea*: vocal sac brightness and vocal sac chroma (Gomez *et al.* 2009, 2010). The promptness at which phenotypic plasticity can generate changes in these signal components was unknown in this species before our study. We thus investigated their potential condition-dependence both on long term, through correlational field observations, and on short-term, through manipulation of male condition over 12 days (article [5]). Moreover, since these components involve the use of specific carotenoid pigments (Richardson *et al.* 2009), we manipulated both food availability and carotenoid availability to investigate the effect of several aspects of condition on signal production (article [5]).

Coloration properties of the vocal sac preferred by females during mate choice (*i.e.*, a lower brightness and a higher chroma, Gomez *et al.* 2010) are associated with a better body condition (measured through a body condition index, but not correlated with body size) of males in the field (article [5]). Hence these signal components appear to be condition-dependent. However, the link between coloration and condition is weak and there are probably several genetic and/or environmental factors that strongly affect coloration properties independently from body condition as measured in this study. According to our results, brightness and chroma seemed to be plastic over the 12 days of the experiment, but the observed changes in coloration were independent of male condition. Indeed, males from the starved group have lost on average 15 % of their initial mass during the treatment while males from the control group and the supplemented group did not.

Hence, starvation did have a strong negative effect on male current condition. Nevertheless, starved males have not lost more coloration than males from the control group. Moreover, males fed on a diet supplemented in β -carotene did not increase or maintain vocal sac coloration better than males from the control group (article [5]). Hence, these two signal components do not seem to be reliable indicators of current nutritional status.

The fact that vocal sac coloration is linked to body condition in the field but does not respond to food and carotenoid availabilities during the breeding season may reflect that this visual signal is a long-term condition indicator. However, since our experiment did not reveal condition-dependent changes in signal component expression, we cannot conclude whether these signal components rely mainly on energy, carotenoid or other limiting resources provided by food. Nevertheless, a recent carotenoid supplementation experiment conducted on the red-eye treefrog (*Agalychnis callidryas*) revealed that carotenoid availability during larval and post-metamorphic development had a strong impact on adult coloration while carotenoid availability on a 3-months period during adulthood did not influence coloration anymore (Ogilvy *et al.* 2012). This study suggests that this kind of coloured signals in anurans may be mainly dependent upon carotenoid availability and that it may provide information about past condition rather than current condition. Conversely, experiments of food availability manipulation in birds have shown that carotenoid-based ornaments located in fleshy tissues are sensitive to variations in nutritional status on short term and thus potentially reliable indicators of current condition (*e.g.* the feet colouration of the blue-footed booby Velando *et al.* 2006; the eye ring colouration of the red-legged partridge Perez-Rodriguez & Vinuela 2008).

Taking into account all our results, we can only assert that visual components of the sexual signal in *H. arborea*, although correlated to male condition in the field, are not sensitive to the same condition aspects as acoustic components. Indeed, they are not impacted by a starvation period of 10 days as WCR does and they are not correlated to body size as dominant frequency does. In this study (article [5]) we focused on nutritional aspects of condition, however, even if it has never been shown in anurans to date, it is possible that this kind of coloured signal components are more sensitive on short term to health aspects of condition. Indeed, several studies suggest that coloured signals in birds and fishes are linked to the parasitic load of males and/ or their immune capacities. For instance, Baeta *et al.* (2008) have experimentally shown that carotenoids are traded off between response to parasites and secondary sexual traits in the blackbird and thus that coloured sexual ornaments may be reliable indicators the parasitic load of a male. Similarly, an experimental increase of the parasitic load in the red grouse induces a decrease of male carotenoid-based colouration (Mougeot *et al.* 2010). Moreover, in some cases carotenoid-based colouration seems to be a reliable indicator of both parasite exposure and parasite resistance since males have to expose themselves to some pathogens to obtain carotenoid pigments and must be resistant to them to express the

colouration (e.g. in sticklebacks Folstad *et al.* 1994, in sandpipers Sanchez *et al.* 2009). In addition, males' coloured signals may also be linked to their degree of oxidative stress. For instance, when dietary carotenoid intake is experimentally limited, male sticklebacks attempt to maintain their sexual ornament at the expense of body carotenoids and suffer from an increased susceptibility to oxidative stress, suggesting an allocation trade-off between sexual signals and antioxidant functions (Pike *et al.* 2007). In line with this result, Alonso-Alvarez *et al.* (2008) have shown that when inducing an oxidative challenge, through the artificial increase of circulating testosterone, carotenoid pigments seem to be re-allocated from coloured signals to antioxidant functions in the red-legged partridge. Conversely, supplementation experiments with nonpigmentary antioxidant can induce an increase of the size of the bill red spot in the yellow-legged gull (Perez *et al.* 2008). Further experiments are thus needed in *H. arborea* to determine which aspects of condition are related to vocal sac colouration.

Taken together, our results support the condition-dependence and the multiple messages hypotheses. Indeed, several acoustic and visual signal components are related to the condition of the emitter and seem to be dependent upon distinct aspects of this condition. This may explain the fact that, in the field, visual and acoustic signal components are not correlated in *H. arborea* (Gomez *et al.* 2011a). Moreover, as previously suggested in other taxa, like field crickets (Scheuber *et al.* 2003a,b) and wolf spiders (Gibson & Uetz 2012), our study of acoustic signal components in *H. arborea* supports the idea that dynamic components reflect current condition of the emitter while static ones are indicators of its past condition. However, the relationships between signal components and condition aspects are, in the 3 aforementioned cases, relatively weak. Hence, the apparent little information transferred to the receiver could be insufficient to maintain an evolutionarily stable signalling equilibrium where both the emitter and the receiver benefit from their interaction. Our results thus highlight the necessity to also consider recent insights in the evolutionary dynamics of signalling systems to understand the existence of such multicomponent signals.

- Dynamics of sexually antagonistic coevolution and honest signalling

While the main paradigm to explain the evolution of animal communication relies on signalling equilibrium situations with honest information transfer from the emitter to the receiver, a great body of theoretical work focuses on dynamics of signalling systems and shows that observed situations in the wild do not necessarily involve such honest information transfer (Bro-Jorgensen 2010). In *Hyla arborea*, the present study has shown that several signal components like call dominant frequency, WCR and vocal sac colouration are condition-dependent. These components seem to rely on different aspects of male condition but are weakly correlated with them. Our results

thus partly support the multiple messages hypothesis. However, the amount of information that females can acquire on male condition seems relatively low and unlikely to maintain the observed female preferences in a signalling equilibrium. The dynamic signalling system evolution proposed van Doorn & Weissing (2006) could provide us a powerful complementary explanation for this apparent lack of information transfer at a given time.

Indeed, several empirical studies have shown that signal components originating from traits that do not provide honest information about the emitter's condition (*e.g.* Fisher traits or traits exploiting sensory biases, see introduction) could evolve toward honest condition indicators (*e.g.* Ryan & Rand 1993; Garcia & Ramirez 2005). Hence, it appears that models of sexually antagonistic coevolution and those of honest signalling are not mutually exclusive. Based on this idea, van Doorn & Weissing (2006) built a model reconciling these two theoretical insights. They showed that the sexual conflict over the information content of the signal components used in mate choice is a potential source of instabilities that can generate non-equilibrium situations with the maintenance of several signal components which are more or less reliable indicators of male condition. Their model rests on standard good genes-models of condition-dependent signalling but is more realistic in the sense that both males and females are allowed to respond optimally (*i.e.* invest more or less resources in each signal component for male, be more or less selective on each signal component for females) by introducing adaptive dynamics. Their results highlight that several condition-dependent signal components can coexist even if multiple female preferences entail significant costs, since females may be continually forced to direct their preferences to novel signal components. Indeed, selective pressures on males should tend to lower slowly the information content of any component and lead to the continual change of sexual preferences and male signal component expression. Moreover, in this model the different signal components can be related to different aspects of condition but can also be redundant and reflect the same aspect of condition. Therefore, by only considering two distinct aspects of condition, this model shows that the joint evolution of up to 20 ornaments and preferences is possible.

These theoretical insights may be of great importance for interpreting the existence of multicomponent signals and are in agreement with empirical findings suggesting weak correlations between signal components and condition aspects in signalling systems involving multicomponent signals (*e.g.* Møller & Pomiankowski 1993; Ligon & Zwartjes 1995; Omland 1996a, b). Moreover, these are compatible with recent fields of research investigating within-individual consistency and between-individual variability in behaviour (*i.e.* behavioural syndromes or personalities, Sih *et al.* 2004). Indeed, in the case of male traits and female preferences, such patterns of variability may highlight a dynamic selection transition from one component to another as proposed in van Doorn & Weissing's model (2006). In *Hyla arborea*, considering the dynamics of such sexually antagonistic

coevolution potentially offers a powerful complement to the multiple messages hypothesis to explain the evolution of signals emitted by males despite the apparent little information transferred to females. Moreover, this explanation is compatible with recent findings on mate choice in this species. Indeed, it has been experimentally shown that, when given contradictory visual signal components (Gomez *et al.* 2010) or contradictory visual and acoustic signal components (Richardson *et al.* 2010a), all females do not choose the same male. These results may highlight different rules for component prioritisation in mate choice among females that can be due to a dynamic selection transition from one component to another.

Besides providing a general explanation to the existence of sexual signals composed of more or less informative components, this model also gives a potential explanation for the apparent frequent turnover and loss of sexually selected traits among closely related species (Wiens 2001). Indeed, such dynamics of signalling system could lead to rapid divergence among sexually isolated individuals and could play a crucial role in speciation events (van Door & Weissing 2006). In agreement with this hypothesis, *Hyla meridionalis*, a species closely related to *H. arborea*, has a strikingly different call which is not organised in call bouts and in which the component WCR is thus absent (Schneider 1974). Accordingly, *Hyla cinerea* has a white vocal sac (Martof *et al.* 1980) probably not involved in sexual selection for its condition-dependent colouration. These observations suggest that such kind of traits and their associated preferences can evolve rapidly and that results on mate choice and condition-dependence of signal components could be better interpreted in the light of dynamics of signalling systems.

- Signalling and male-male competition

In the two previous parts we have mainly discussed sexual signals in a mate choice context. However, multicomponent signals can also be involved in communication from one male to other males (*e.g.* Bro-Jorgensen & Dabelsteen 2008). In those cases, signals are typically used to defend a resource by providing information about emitter's condition and particularly aspects of condition linked to its resource holding potential, like fighting ability (for a review see Searcy & Nowicki 2005). In the case of lek mating systems, the defended resource generally consists merely in an exposed place to emit signals (Andersson 1994). In *H. arborea*, as in other lekking anurans, the signal emitted by males to court females is probably also directed to other males in order to defend a displaying place (Murphy & Floyd 2005; Richardson & Lengagne 2010). Nevertheless, other males not only use the information provided by signals to assess resource holding potential of their competitors, but also to exploit the investment made by these competitors to attract females (article [6]).

Parasitic reproductive tactics rely on the exploitation of the investment in reproduction made by other individuals of the same sex (Taborsky *et al.* 2008). Some of those consist in the exploitation

of signals emitted by competitors. This is for instance the case of satellite parasitic tactics in lekking animals (e.g. van Rhijn 1973, Gosling & Petrie 1990; Castellano *et al.* 2009). In *H. arborea*, we demonstrated that the decision to switch from a classical tactic to a satellite one is condition-dependent (article [6]), and mainly driven by past condition (body size) rather than current condition (nutritional status). Thus, not only some signal components are condition-dependent but also signal emission in itself. This may be due to the fact that small males are unable to produce attractive calls (*i.e.* with a low dominant frequency, Richardson *et al.* 2010b) and/or have a lower resource holding potential (e.g. Davies & Halliday 1978), balancing costs and benefits of signal emission to the disadvantage of such males.

Moreover, males using this kind of parasitic tactics not only exploit the investment made by other males emitting signals but can also exploit the information provided by these signals when deciding to parasitize or not a given competitor. Hence, in several anurans, the attractiveness the signal emitted by a male influences its probability to be parasitized by satellites (article [6], Leary *et al.* 2006; Humfeld 2008; Castellano *et al.* 2009; Berec & Bajgar 2011). Such a strategy probably increases the benefits obtained by males using the satellite tactic by increasing their probability of intercepting a female. However, it implies a cost imposed by receivers on signal emitters in addition to intrinsic costs linked to development, maintenance and production of the signal (cf. Table1 in introduction) since emitting a more attractive call implies an increased probability of being parasitized by a satellite male. This imposed cost probably also plays a role in the maintenance of signal honesty in the sexual signalling system of *H. arborea*. Indeed, it has been shown in other male-male competition contexts that such cost imposed by competitors can increase the honesty of sexual signals, for instance if emitting a dishonest signal involves the risk of fighting with a male of higher condition, as in three-spined sticklebacks (Candolin 200b). Moreover, this situation is comparable to those where signal preferences occurring in females are also involved in prey/host choice by predators/parasites, involving costs imposed by eavesdroppers (e.g. parasitoid preferences for song properties in field crickets Wagner 1996; predator preferences for male ornaments in swordtails Rosenthal *et al.* 2001; see also Zuk & Kolluru 1998 for a review). It would therefore be highly interesting to test independently the influence of each signal component (acoustic but also visual ones) on the probability that a male have to be parasitized, to investigate if such imposed costs can act on all female selected signal components or rather only on those linked to male resource holding potential, like dominant frequency. At last, as for female preferences, we should also consider the dynamics of tactic switches and there relation to the information provided by each signal component.

PERSPECTIVES: towards a more integrative approach of the signalling system

The results presented here constitute a first step towards the comprehension of the evolution of multicomponent signalling systems. However, the apparent lack of information transferred from emitters to receivers in this kind of systems calls for further studying on at least 3 crucial, and often overlooked, points: apprehending the complexity of condition, taking into account signal efficiency and investigating dynamics signalling systems.

- Apprehending the complexity of condition to better estimate the information transferred

The condition, defined as the total pool of resources acquired by an individual and available for allocation to the production and maintenance of fitness-enhancing traits (Rowe & Houle 1996; Hunt *et al.* 2004; Tomkins *et al.* 2004), is a very wide concept which includes many aspects rarely considered together. Indeed, condition depends both on the amount of every limited resources acquired by an individual and on every resource demanding functions of its organism. Traditionally, studies of condition-dependence of sexual signals consider only a small subset of resources (*e.g.* energy or vitamins), and most of the time neglect their potential interdependence (for instance when a particular balance between several resources is needed). In the same way, only some resource-demanding functions (*e.g.* the immune system or antioxidant defences) are considered and generally trade-offs between them are overlooked. Recently, several works have brought new insights by considering simultaneously several resources and their interdependence (*e.g.* McGraw *et al.* 2005; Bertrand *et al.* 2006; Catoni *et al.* 2008), and by taking into account several resource-demanding functions (see Simons *et al.* 2012 for a meta-analysis on carotenoid-based signals and their link to the immune system and antioxidant defences in birds). Moreover, as suggested by our study (article [2]), according to one aspect of their condition, males can use different allocation strategies between components of a signal. Future studies of condition-dependence should therefore consider simultaneously (1) a subset of acquired resources as large and integrative as possible; (2) several relevant resource-demanding functions and potential trade-offs between them; (3) all identified signal components emitted by an individual and the allocation trade-offs between them.

In *H. arborea*, in addition to energetic resources needed for call production, we are conducting analysis both on vocal tissues and blood samples to determine precisely the type of pigments involved in male colouration but also further correlates, potentially revealing other limited resources, like some antioxidant molecules (Bertrand *et al.* 2006). Moreover, from a more reductionist perspective, we are conducting stoichiometric analysis on different body tissues to determine which elements (C, N, P) are limiting during individual ontogeny (Elser *et al.* 2003) and particularly during the development of the vocal apparatus. In addition to nutritional aspects of

condition, we are also investigating health aspects of males *H. arborea* by considering both their oxidative balance (by measuring oxidative damages and antioxidant defences, see Cote *et al.* 2010) and their immune system (through measures of the parasitic load and phytohaemagglutinin injections, see Smits *et al.* 1999). Future studies of signal component condition-dependence in the European treefrog should therefore take into account simultaneously both the different aspects of condition mentioned above and their link to all signal components known to have an impact on female choice, to get a more realistic view of the amount of information transferred through this complex signal.

- Taking into account signal efficiency in addition to signal content

Signals are not only subject to a selective pressure on their content (i.e. what information is being transmitted) but also to a selective pressure on their efficiency to travel from the emitter to the receiver through a transmission channel, and to be received and integrated by the receiver. In addition to the investigation of information content of signal components, it may therefore be crucial to take into account aspects of signal efficiency to explain the existence of multicomponent signals. Indeed, producing several components together can improve detection, discrimination and learning of the signal by the receiver and may thus provide benefits for both the emitter and the receiver ("receiver psychology hypothesis", Guilford & Dawkins 1991; Rowe 1999; Candolin 2003). In *H. arborea*, it has been experimentally shown that the use of visual signal components in addition to acoustic ones can help females to choose a mate by reducing their latency to choose and by improving mate choice accuracy (Gomez *et al.* 2011b), providing support to this hypothesis. Nevertheless, the benefits acquired by emitters and receivers through enhanced signal efficiency might be even higher if considering the multiple sensory environments in which communication occurs (Candolin 2003). Indeed, the different signal components, especially those occurring in different sensory modalities, may be used by the receiver at different distances from the emitter because the sensory environment changes as the receiver goes up to the receiver (*e.g.* Backwell & Passmore 1996; Candolin & Reynolds 2001; Lopez & Martin 2001). In *H. arborea*, given that the calls are very loud and that reproduction occurs by night, it seems probable that females first orient their choice using acoustic components and then, when arriving closer to displaying males, use visual signal components to choose a mate. This hypothesis could be tested by conducting long distance experiments where females get access, first to acoustic, then to visual signal components and by observing their choice (*i.e.* choice target and latency to choose) when experimentally varying the attractiveness of each type of component. Quantifying the benefits brought by enhanced signal efficiency could be a cornerstone in the comprehension of the evolution of multicomponent

signalling systems, particularly when each component seems to convey little information as in *H. arborea*.

- Investigating the dynamics of multicomponent signalling systems

The models of signalling system dynamics, as that developed by van Doorn & Weissing (2006), seem to bring a powerful explanation to the existence of sexual signals composed of more or less informative components. However, these models lead to hardly testable predictions. In *H. arborea*, when given contradictory visual signal components (Gomez *et al.* 2010) or contradictory visual and acoustic signal components (Richardson *et al.* 2010a), all females do not choose the same male. These results may highlight different rules for component prioritisation in mate choice among females that can be due to a dynamic selection transition from one component to another. However, further tests are necessary to confirm this hypothesis. First of all, within-individual choice consistency should be investigated by performing repeated tests with the same individuals. Secondly, different magnitude in the manipulation of each signal component should be investigated to test if females, when assessing male condition, merely rank signal components in their prioritisation rules or rather if they assign relative weights to each component to compute a final score (Castellano *et al.* 2012). As suggested above, males also use signal components of their competitors to obtain information about their condition and then take decisions (*e.g.* act as a satellite or not). It is thus possible that an inter-individual variability in rules for component prioritisation also occurs in males. Investigating this potential variability in males and females, in the light of the information content and the efficiency brought by each component, both within and across populations should provide us precious knowledge about the dynamics of such signalling system. Such integrative investigation would allow us to confront reality to theoretical models of signalling system dynamics and help us to get a broader understanding of the existence of multicomponent signals despite the apparent little information transfer.

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RESUME SUBSTANTIEL

Introduction :

La sélection sexuelle est un sous-ensemble de sélection naturelle où les individus sont en compétition pour l'accès à une ressource particulière : les individus de l'autre sexe. Ce sont les individus du sexe dont l'investissement parental est le plus faible, généralement les mâles, qui entrent en compétition pour l'accès aux individus de l'autre sexe, généralement les femelles. Cette forme de sélection se traduit par deux mécanismes principaux : une compétition par interférence entre les mâles (sélection intrasexuelle) et un choix du partenaire sexuel chez les femelles (sélection intersexuelle). Ces deux mécanismes reposent sur des interactions entre individus du même sexe et/ou de sexes opposés et impliquent donc, la plupart du temps, une communication entre ces individus.

La communication est une interaction entre un individu délivrant une information et un individu utilisant cette information pour prendre une décision. Certains traits évoluent pour cette tâche particulière de transmission d'information, on les appelle « signaux ». Ces signaux peuvent transférer une information concernant l'individu émetteur du signal ou tout autre objet ou individu faisant partie de son environnement. Pour qu'un système de communication soit évolutivement stable, il faut à la fois que l'émetteur et le récepteur tirent bénéfice de cette interaction. La condition indispensable pour que le récepteur tire bénéfice de cette interaction est que l'information transmise soit honnête afin qu'il puisse prendre la meilleure décision en conséquence. L'un des plus vastes champs de recherche dans l'étude de l'évolution de la communication consiste à comprendre ce qui maintient l'honnêteté de l'information transmise par les signaux lorsque les intérêts de l'émetteur et du récepteur sont en conflit. Le paradigme le plus largement accepté à l'heure actuelle stipule que, dans de telles situations, l'honnêteté des signaux est maintenue par des « coûts stabilisants ». Ces coûts peuvent prendre diverses formes suivant les situations mais ont en commun de peser plus lourd sur les individus émettant des signaux malhonnêtes que sur les individus émettant des signaux honnêtes.

Dans le cadre de la sélection sexuelle, il existe un conflit d'intérêt entre les mâles qui cherchent à maximiser leur nombre de partenaires sexuels et les femelles qui cherchent à optimiser la qualité des partenaires avec qui elles vont s'accoupler. Cependant, un grand nombre de caractères sexuels secondaires développés par les mâles sont considérés comme étant des signaux honnêtes transmettant une information sur leur condition, à l'attention des femelles, mais également des autres mâles avec qui ils sont en compétition. Dans un tel système de signalement, l'information transmise peut théoriquement être fiable si certains coûts stabilisants maintiennent une condition-dépendance des signaux sexuels émis par les mâles.

Les signaux sexuels sont souvent complexes et composés de plusieurs éléments qui peuvent jouer un rôle dans la communication. On les appelle alors des « signaux multi-composantes ». Plusieurs hypothèses ont été émises pour expliquer le développement et le maintien de multiples composantes au sein d'un signal. L'une d'elles, l'hypothèse des messages multiples, suggère que chaque composante transfère une information concernant un aspect précis de la condition de l'émetteur. En effet, la condition est généralement définie comme étant l'ensemble des ressources acquises et disponibles pour être investies dans des traits conférant une meilleure aptitude phénotypique. La condition est donc un concept multifactoriel à la fois parce qu'elle dépend de plusieurs aspects nutritionnels (différentes ressources acquises) et sanitaires (fonctions qui sont consommatrices en ressources, *e.g.* lutte contre pathogènes, stress oxydant, etc...) mais aussi parce qu'elle est susceptible de varier au cours du temps.

Au cours de ce travail de thèse nous avons étudié la condition-dépendance des signaux sexuels au sein du système de signalement multiple de la rainette verte, *Hyla arborea*. Chez cette espèce les mâles émettent un chant puissant tout en exposant la coloration vive de leur sac vocal. Différentes composantes acoustiques et visuelles de ces signaux sont connues pour avoir un impact sur les décisions que prennent les femelles lors du choix de leurs partenaires sexuels. Cette parade sexuelle est donc non seulement multi-composantes mais aussi multimodale, car l'information est

transmis dans différentes modalités sensorielles. Par ailleurs, ces différentes composantes sont susceptibles d'être sensibles à différents aspects de la condition des mâles. Le système de signalement sexuel de la rainette verte nous permet ainsi de tester l'hypothèse des messages multiples afin de mieux comprendre les forces évolutives conduisant à l'élaboration de tels signaux.

Nous avons en premier lieu étudié la variabilité du coût métabolique de la production du chant en fonction de ses composantes et de la condition de l'émetteur (article [1]). Nous avons ensuite manipulé expérimentalement l'état nutritionnel des mâles pour étudier la condition-dépendance des différentes composantes du chant (article [2]). Nous avons examiné la fiabilité d'une composante statique du chant (la fréquence dominante) comme indicateur de la condition passée de l'émetteur (articles [3]), et une méthodologie permettant d'évaluer correctement sa variabilité (article [4]). Puis, nous avons manipulé plusieurs aspects de la condition des mâles par des expériences de nourrissage / jeûne / supplémentation en caroténoïdes pour étudier la condition-dépendance des composantes visuelles du signal (article [5]). Enfin, nous avons comparé expérimentalement l'importance de deux aspects de la condition (passée et actuelle) dans la décision de contourner le système de signalement en adoptant une tactique de reproduction alternative (article [6]).

Article 1 : **Energetics of calling in the male treefrog *Hyla arborea*: when being large means being sexy at low cost**

Voituron, Y., Brepson, L., Richardson, C., Joly, P. & Lengagne, T.
Behaviour, 149 (2012), 775-793.

Problématique :

La condition-dépendance des signaux dans un contexte de sélection sexuelle implique des coûts stabilisants supportés par l'émetteur du signal lorsque celui-ci envoie un signal malhonnête. Chez les anoues, ces coûts semblent être maintenus essentiellement par un coût métabolique élevé associé à la production du chant. L'existence de coûts stabilisants pourrait être révélée si (1) l'augmentation de l'attractivité du signal implique une augmentation du coût de production ; (2) pour un niveau de signalement donné, les mâles en mauvaise condition supportent un coût de production plus élevé que les mâles en bonne condition. Cependant la variabilité interindividuelle du coût de production du chant a rarement été étudiée jusqu'à présent.

Hypothèses :

- Augmenter l'attractivité de certaines propriétés du chant implique une augmentation du coût de production.
- Pour un niveau de signalement donné, les mâles en mauvaise condition supportent un coût de production plus élevé que les mâles en bonne condition.

Méthodes :

La production de chant et la consommation d'oxygène (estimation du coût de production) de mâles de différentes conditions ont été mesurées simultanément.

Résultats :

- La consommation d'oxygène est principalement expliquée par le nombre de chants émis par le mâle durant l'enregistrement.
- Pour une quantité de chant donnée, augmenter le taux de chant intra-bout (WCR, *i.e.* augmenter l'attractivité du signal vis-à-vis des femelles) implique une augmentation de la consommation d'oxygène.
- Pour un niveau de signalement donné (*i.e.* un nombre de chant donné et un WCR donné), les gros mâles montrent une consommation d'oxygène rapportée à la masse plus faible que les petits mâles.
- Les gros mâles émettent des chants avec une fréquence dominante plus grave, plus attractifs pour les femelles.

Conclusion :

Augmenter l'attractivité du signal, notamment par la modification de propriétés dynamiques du chant comme le WCR, implique une augmentation du coût de production. Pour un niveau donné de signalement, les mâles en bonne condition semblent supporter un coût de production moindre que les mâles en mauvaise condition. De plus, les gros mâles produisent des chants plus attractifs, indépendamment de leur investissement au moment de la production du chant, car ils produisent des chants dont la fréquence dominante est plus grave (propriété statique du chant). L'ensemble de ces résultats suggère qu'à la fois les propriétés dynamiques et statiques du chant peuvent être des indicateurs honnêtes de la condition du mâle. De plus, la condition-dépendance des propriétés dynamiques peut être maintenue grâce à des coûts stabilisants comme un coût de production plus élevé pour un mâle en mauvaise condition que pour un mâle en bonne condition, toutes choses égales par ailleurs.

Article 2 : **Condition-dependent ways to manage acoustic signals under energetic constraint in a treefrog**

Brepson, L., Voituron, Y. & Lengagne, T.
Behavioral Ecology, in press.

Problématique :

Les composantes dynamiques des signaux sexuels sont censées être condition-dépendantes sur le court terme. Pour étudier la condition-dépendance de signaux multi-composantes comprenant plusieurs composantes dynamiques, il faut prendre en compte les potentiels compromis entre ces diverses composantes ainsi que les compromis entre ces composantes et la quantité de signal émise. De plus, il faut également tenir compte de la possibilité que, en fonction de leur condition, les mâles utilisent différentes stratégies pour gérer ces compromis. Cependant, les études menées jusqu'à présent ont rarement considéré ces potentiels compromis et encore moins les différences interindividuelles dans la façon de les gérer ; particulièrement chez les anoues, où les manipulations expérimentales de la condition ont conduit à des résultats contradictoires.

Hypothèses :

- Une contrainte énergétique peut générer un compromis d'allocation entre les différentes composantes dynamiques d'un signal et/ou entre ces composantes et la quantité de signal émise.
- En fonction de leur condition, les mâles adoptent différentes stratégies pour gérer ces compromis.

Méthodes :

Nous avons étudié la condition-dépendance des composantes du chant et les potentiels compromis associés en manipulant la contrainte énergétique supportée par 36 mâles *H. arborea* grâce à des expériences de nourrissage / privation alimentaire. Au cours de ces expériences nous avons enregistré la totalité des chants émis par chaque mâle sur une période de 10 nuits consécutives.

Résultats :

- Même en prenant en compte à la fois plusieurs composantes du chant et la quantité totale de chant émise, la contrainte énergétique subie par les mâles ne semble pas impacter la production du signal
- Lorsqu'on s'intéresse aux réponses individuelles, on constate que les mâles sous contrainte énergétique ne peuvent pas maintenir à la fois le WCR et la quantité totale de chant émise pendant la nuit.
- Alors que les mâles en bonne condition affectent principalement leur WCR, les mâles en mauvaise condition réduisent principalement la quantité de chant qu'ils produisent par nuit.
- Les mâles qui ont le plus maintenu leur quantité de chant émise par nuit sont ceux qui ont le plus perdu de condition durant l'expérience.
- Quand ils sont re-nourris, les mâles privés de nourriture pendant 10 jours augmentent uniquement la quantité de chant émise par nuit.

Conclusion :

Ces résultats suggèrent qu'à la fois le WCR et la quantité de chant émise par nuit sont condition-dépendants à court terme. La quantité de chant émise par nuit semble plus coûteuse à maintenir que le WCR mais pourrait conférer un meilleur succès reproducteur aux mâles. Cette situation pourrait alors induire l'utilisation de différentes stratégies d'allocation parmi les mâles de condition différente. La condition-dépendance des composantes du signal peut alors être masquée si les éventuels compromis et les différentes façons de les gérer ne sont pas pris en compte.

Article 3 : **Within-individual variability in call dominant frequency and female choice in anurans**

Brepson, L., Gomez, D., Voituron, Y. & Lengagne, T.
Soumis à la revue *Behavioral Ecology*.

Problématique :

La fréquence dominante, une propriété du chant impliquée dans le choix du partenaire sexuel chez les anoures, est considérée comme une composante statique du signal, avec une faible variabilité intra-individuelle. Cependant, ce constat est majoritairement basé sur des études portant sur de petits échantillons de chants. Les quelques études basées sur des enregistrements répétés des mêmes individus montrent une variabilité plus élevée. Cela suggère que la variabilité intra-individuelle est sous-estimée lorsqu'on se base sur de petits échantillons. Il semble donc nécessaire (1) d'estimer la variabilité intra-individuelle de cette composante du signal sur une échelle de temps plus grande ; (2) d'estimer l'impact de cette variabilité sur la force sélective conduisant au choix du partenaire basé sur cette composante du signal.

Hypothèses :

- La variabilité intra-individuelle de la fréquence dominante du chant est plus élevée qu'on ne le pense.
- Cette variabilité peut impacter la force sélective conduisant au choix du partenaire basé sur cette composante du signal.

Méthodes :

Nous avons enregistré la totalité des chants produits par 36 mâles *H. arborea* au cours d'une nuit et mesuré la fréquence dominante de chacun de ces chants. Nous avons ensuite simulé des choix de partenaires (grâce à un programme conçu à cet effet) basés sur des règles de décision simples pour estimer la proportion de bon choix fait par les femelles dans différentes conditions d'échantillonnage et de variabilité de la fréquence dominante.

Résultats :

- En considérant l'échelle de la nuit entière, la variabilité intra-individuelle de la fréquence dominante est plus élevée que lorsqu'elle est estimée à partir de petits échantillons.
- Une grande variabilité intra-individuelle peut aller jusqu'à diminuer de 30% la proportion de bon choix fait par les femelles.
- En augmentant le nombre de chants échantillonnés par la femelle de 10 à 10 000, la proportion de bon choix augmente de 10% dans le meilleur cas considéré ici.

Conclusion :

La variabilité intra-individuelle de la fréquence dominante est plus élevée qu'on ne le pensait et peut impacter le signal transmis aux femelles chez cette espèce. Etant donné le faible avantage apporté par l'augmentation du nombre de chants pris en compte dans leurs choix, les femelles utilisent probablement d'autres sources d'information (*e.g.* autres composantes du signal) dans de petits échantillons plutôt que d'augmenter de manière disproportionnée le temps passé à écouter les mâles. Cette étude apporte donc un support indirect à l'hypothèse selon laquelle plusieurs composantes d'un même signal peuvent améliorer l'estimation de la condition de l'émetteur et peut expliquer le manque d'observation sur le terrain de préférences des femelles pour des composantes isolées.

Article 4: **Do we correctly assess the (un)variability of call dominant frequency in anurans? Towards a new methodology**

Brepson, L., Gomez, D., Josserand, R., Voituren, Y. & Lengagne, T.
Soumis à la revue *Amphibia-Reptilia*.

Problématique :

La fréquence dominante, une propriété du chant impliquée dans le choix du partenaire sexuel chez les anoures, est considérée comme une composante statique du signal avec une faible variabilité intra-individuelle. Cependant, ce constat est majoritairement basé sur des études portant sur de petits échantillons de chants. Lorsqu'elle est mesurée sur une nuit entière, la variabilité intra-individuelle de la fréquence dominante est plus élevée qu'on ne le pensait. Cela suggère que la variabilité intra-individuelle est sous-estimée lorsqu'on se base sur de petits échantillons. Il est donc nécessaire de développer une méthodologie permettant une bonne estimation de cette variabilité tout en gardant un effort d'échantillonnage raisonnable.

Hypothèse :

- L'estimation de cette variabilité devrait être meilleure en utilisant plusieurs échantillons répartis au cours de la nuit plutôt qu'en se basant sur des échantillons composés de chants consécutifs.

Méthodes :

Nous avons enregistré la totalité des chants produits par 36 mâles *H. arborea* au cours d'une nuit et mesuré la fréquence dominante de chacun de ces chants. Nous avons ensuite conçu un programme nous permettant de tirer des sous-échantillons dans cette base de données afin d'estimer la capacité de différentes méthodes d'échantillonnage à capturer la variabilité observée sur la nuit entière.

Résultats :

- L'estimation de la variabilité intra-individuelle de la fréquence dominante est meilleure en utilisant 2 ou 3 courts échantillons bien répartis sur la nuit (début, milieu, fin de nuit) qu'en se basant sur des échantillons composés de chants consécutifs.
- Ce résultat est valable même si l'on considère un très grand nombre de chants consécutifs.

Conclusion :

Nous recommandons d'utiliser plusieurs échantillons bien répartis au cours de la nuit (début, milieu, fin de nuit) plutôt que d'augmenter le nombre de chants consécutifs analysés pour capturer correctement la variabilité intra-individuelle de la fréquence dominante du chant chez les anoures.

Article 5 : **Fading of a condition-dependent coloured signal irrespective of food and carotenoid availability**

Brepson, L., Bellvert, F., Desprat, J., Josserand, R., Voituron, Y. & Lengagne, T.
En préparation.

Problématique :

Sous l'hypothèse de la condition-dépendance des signaux sexuels, une variabilité inter et intra-individuelle dans l'expression des signaux est attendue en fonction des variations de la condition des mâles. Les signaux visuels faisant intervenir des pigments caroténoïdes peuvent être plus ou moins rapidement plastiques selon que les coûts impliqués dans leur expression sont principalement des coûts développementaux ou des coûts de maintenance. Par exemple, ces signaux devraient répondre rapidement à des changements dans la condition du mâle s'ils sont localisés dans des tissus charnus et que les caroténoïdes sont susceptibles d'être remobilisés pour d'autres fonctions, comme c'est le cas chez *H. arborea* pendant la période de reproduction.

Hypothèses :

- Les composantes visuelles du signal sélectionnées par les femelles sont associées à la condition des mâles sur le terrain.
- Les changements dans la condition des mâles sont rapidement reflétés par ces composantes visuelles.

Méthodes :

Nous avons capturé des mâles *H. arborea* sur le terrain et avons manipulé leur condition grâce à des expériences de privation alimentaire et de supplémentation en caroténoïdes pour étudier le lien entre leur condition et la coloration de leur sac vocal.

Résultats :

- Les composantes visuelles du signal sélectionnées par les femelles sont associées à la condition des mâles sur le terrain.
- Ni la disponibilité en nourriture ni celle en caroténoïdes n'ont affecté les composantes visuelles du signal au cours des 12 jours de traitement.
- La coloration du sac vocal est devenue plus pâle (augmentation de la clarté, diminution du chroma) et donc moins attractive pour les femelles chez tous les mâles, quel que soit leur traitement.

Conclusion :

Les composantes visuelles du signal sont peut-être des indicateurs à long terme de la condition plutôt que des indicateurs à court terme. Le palissement du sac vocal observé chez tous les mâles peut être dû à des changements hormonaux condition-indépendants au cours de la saison de reproduction.

Article 6 : **Cheating for sex: Inherent disadvantage or energetic constraint?**

Brepson, L., Troïanowski, M., Voituron, Y. & Lengagne, T.
Animal Behaviour, 84 (2012), 1253-1260.

Problématique :

Chez de nombreuses espèces, certains individus contournent le système de signalement honnête en exploitant les signaux émis par leurs congénères sans émettre de signal eux-mêmes. Dans un contexte de sélection sexuelle, c'est souvent le cas des mâles adoptant des tactiques de reproduction parasites. L'adoption de telles tactiques est souvent déterminée par la condition du mâle ; les mâles en mauvaise condition adoptant plus souvent des tactiques parasites que les mâles en bonne condition. Cependant, on connaît très peu de choses sur les aspects de la condition (*e.g.* actuelle ou passée) et leur importance relative dans la décision de passer d'une tactique classique à une tactique parasite.

Hypothèses :

- La probabilité d'adopter une tactique parasite dépend de la condition passée du mâle, qui a influencé des traits conditionnés par l'ontogenèse comme sa taille corporelle ou la taille de certaines parties de son corps (hypothèse du désavantage intrinsèque).
- La probabilité d'adopter une tactique parasite dépend de la condition actuelle du mâle, qui limite l'énergie disponible pour la production du signal (hypothèse de la contrainte énergétique)

Méthodes :

Nous avons expérimentalement testé et confronté ces deux hypothèses en manipulant la disponibilité en nourriture de mâles supportant différents degrés de désavantage intrinsèque (*i.e.* de tailles corporelles différentes) dans le système chanteur/satellite d'*H. arborea*.

Résultats :

- Les petits mâles ont une plus grande probabilité d'adopter une tactique parasite que les gros mâles.
- Les mâles privés de nourritures n'ont pas une plus grande probabilité d'adopter une tactique parasite que les mâles nourris.
- Toutes choses égales par ailleurs, les mâles ont une plus grande probabilité d'adopter une tactique parasite quand ils sont confrontés à des compétiteurs attractifs (*vis-à-vis* des femelles) que quand ils sont confrontés à des compétiteurs non-attractifs.
- Les mâles ont une plus grande probabilité d'adopter une tactique parasite en début et fin de nuit qu'en milieu de nuit.

Conclusion :

Nos résultats supportent l'hypothèse du désavantage intrinsèque mais ne supportent pas celle de la contrainte énergétique. La condition passée semble donc avoir une plus grande influence sur la décision d'adopter une tactique parasite que la condition actuelle. De plus, nos résultats suggèrent que, lorsqu'ils passent d'une tactique classique à une tactique parasite, les mâles utilisent les mêmes composantes du chant que les femelles lorsqu'elles choisissent un partenaire. Enfin, le fait que la probabilité d'adopter une tactique parasite change au cours de la soirée indique possiblement une synchronisation entre les tactiques adoptées par les mâles et la disponibilité en femelle attendue sur le terrain.

Discussion et perspectives :

Dans leur ensemble, nos résultats supportent l'hypothèse des messages multiples dans le cas du système de signalement d'*H. arborea*. En effet, plusieurs composantes des signaux sexuels émis par les mâles semblent conditions-dépendantes et liées à différents aspects de cette condition. Ainsi, comme cela a déjà été suggéré pour d'autres types de signaux, une composante statique du signal comme la fréquence dominante du chant porte une information concernant la condition passée de l'émetteur car elle est corrélée à sa taille à l'âge adulte. A l'inverse, le WCR, une composante dynamique du chant, semble indiquer la condition actuelle de l'émetteur car elle est impliquée dans un compromis d'allocation de ressources avec la quantité de signal émise et parce que, à nombre de chant égal, l'augmentation du WCR suppose un investissement énergétique plus élevé lors de la production du chant. Par ailleurs, la clarté et le chroma du sac vocal, deux composantes visuelles du signal impliquées dans le choix des femelles, sont corrélés à la condition des mâles sur le terrain. Cependant, elles ne semblent pas indiquer les variations de condition à court terme (12 jours) et pourrait donc être des indicateurs de condition sur le long terme et/ou d'autres aspects de la condition non pris en compte.

Bien que plusieurs composantes du signal puissent être des indicateurs de la condition du mâle, les corrélations entre expression de la composante et condition de l'émetteur sont, dans chacun des cas cités ci-dessus, relativement faibles. Cela suggère que la quantité d'information transmise *via* ces composantes est ténue et, peut-être, insuffisante pour maintenir un équilibre de signalement où les femelles obtiennent suffisamment de bénéfices au travers de leur choix de partenaire pour que leurs préférences soient évolutivement stables. L'une des clés pour comprendre ce faible niveau d'information transmise se trouve probablement dans la dynamique des systèmes de signalement. En effet, certains développements théoriques suggèrent que ce type de signalement ne doit pas nécessairement atteindre un équilibre évolutivement stable pour exister. Ces travaux considèrent généralement un roulement entre différentes composantes des signaux chez les mâles et leurs préférences associées chez les femelles. Ainsi la quantité d'information transmise par une composante et la force de sélection associée à sa préférence chez les femelles peuvent varier au cours du temps, donnant successivement plus d'importance à l'une ou l'autre des composantes et générant des situations où plusieurs composantes avec des degrés variables d'information portée peuvent être maintenues à un instant donné.

Nos résultats suggèrent par ailleurs que les signaux émis par les mâles chez *H. arborea* sont aussi utilisés par les autres mâles pour estimer la condition de leurs compétiteurs et décider de la tactique à adopter dans une situation donnée (*e.g.* utiliser une tactique de reproduction de type satellite ou non). L'exploitation de l'information contenue dans le signal par d'autres mâles peut alors constituer un coût stabilisant supplémentaire maintenant une certaine honnêteté du signal, dans la mesure où émettre un chant attractif augmente la probabilité d'être parasité par un mâle satellite. Dans le cas de la communication entre mâles comme dans le cas de la communication entre mâles et femelles, la dynamique du système est probablement un élément majeur permettant d'expliquer le développement et le maintien de signaux dont les multiples composantes ne semblent transmettre qu'une faible quantité d'information sur la condition de l'émetteur à un instant donné.