# Relatedness predicts male mating success in a pond-breeding amphibian

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## ARTICLE INFO

When deciding to mate, it is assumed that females choose males bearing genes that will improve the genetic quality of their offspring, which is affected by both additive and nonadditive genetic variation. In this context, a 'compatible genes' model has been put forward to explain female mating decisions. According to this model, females are assumed to increase the genetic quality of their offspring by choosing mates on the basis of interactions between maternal and paternal genomes. Yet, this model is mainly supported by empirical data in endotherm vertebrates. Few studies have investigated this issue in terrestrial ectotherms like amphibians. These organisms often live in spatially structured populations characterized by small subpopulations and a high degree of philopatry, leading to striking reduction in gene flow, high genetic drift and relatively high inbreeding levels. In such a situation, one might expect that natural selection should favour mating tactics limiting the risk of inbreeding depression. In this paper, using an experimental approach controlling for the reproductive state of males, we examined how genetic compatibility may affect mating behaviour in an anuran, the yellow-bellied toad, *Bombina variegata*. First, our analyses confirmed a high degree of inbreeding in the studied population. Yet, we did not find any mating tactic that reduced the risk of inbreeding depression. Contrary to our expectations, males more closely related to the female had the higher mating success. We discuss the ecological and evolutionary implications of these results.
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In many organisms, females are expected to be highly selective when choosing a mate since they invariably invest more energy in their gametes than males do (Andersson, 1994; Bateson, 1983). Females may choose their mates on the basis of direct benefits (parental care, food, shelter or protection from predators) or indirect (i.e. genetic) benefits provided by males to offspring (Andersson, 1994; Clutton-Brock, 1991). In nonresource-based mating systems, females are expected to select their mates according to genetic benefits (Kirkpatrick & Ryan, 1991; Kotiaho, Simmons, & Tomkins, 2001; Tregenza & Wedell, 2000). In particular, they are assumed to choose the male(s) bearing genes that will improve offspring genetic quality, which is affected by both additive and nonadditive genetic variation (Neff & Pitcher, 2005).

In the context of nonadditive genetic variation, the 'compatible genes' model of sexual selection assumes that females may increase the genetic quality of their offspring by choosing mates on the basis of interactions between maternal and paternal genomes (Neff & Pitcher, 2005; Pryke & Griffith, 2009; Tregenza & Wedell, 2000; Zeh & Zeh, 1996). While the 'good genes' model states that an allele increases offspring fitness regardless of the architecture of the rest of the genome (Neff & Pitcher, 2005; Tregenza & Wedell, 2000), the 'compatible genes' hypothesis postulates that an allele increases fitness when paired with a specific homologue (dominance and overdominance) or an allele at another gene locus (epistasis), i.e. nonadditive genetic variation. In previous studies, three predictions have been made regarding female mate choice: first, due to the risk of inbreeding depression (Charlesworth & Charlesworth, 1987; Crnokrak & Roff, 1999), females might benefit by mating with unrelated males since heterozygous offspring experience lower expression of recessive deleterious mutations (dominance) and are superior per se since they benefit from a great variety of gene products (overdominance; Kempenaers, 2007; Pusey & Wolf, 1996; Szulkin, Stopher,

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Pemberton, & Reid, 2013). Second, it has been hypothesized that females might benefit by mating with closely related males (Edmands, 1999; Szulkin et al., 2013) since 'outbreeding depression' (or 'hybrid breakdown') can reduce offspring fitness due to disruptions in local adaptations (i.e. genotype∗environment interactions; Templeton, 1986), underdominance (Schierup & Christiansen, 1996) or epistatic interactions (Lynch, 1991). Third, since both small and large genomic differences can have negative fitness consequences, it has been hypothesized that females might benefit from selecting males with an optimal level of outbreeding (Bateson, 1983; Puurtinen, 2011).

The compatible gene model is mainly supported by empirical data in endotherm vertebrates. In contrast, there are few studies addressing this issue in terrestrial ectotherms such as amphibians (Bos, Williams, Gopurenko, Bulut, & Dewoody, 2009; Garner & Schmidt, 2003; Luo et al., 2015). These organisms often live in spatially structured populations that are composed of subpopulations occurring in distinct breeding patches that are linked by dispersing individuals (Marsh & Trenham, 2001; Smith & Green, 2005). In these population systems, small subpopulations and a high degree of philopatry lead to drastic reduction in gene flow, high genetic drift and relatively high inbreeding levels (Allentoft & O'Brien, 2010; Beebee, 2005). In such a context, one might expect that natural selection should favour mating tactics that limit the risk of inbreeding depression (the first hypothesis described above).

In this study, we examined whether genetic compatibility may affect mating behaviour in a pond-breeding amphibian, the yellow-bellied toad, Bombina variegata. We investigated this issue in a spatially structured population where small subpopulations and very low dispersal rates among breeding patches are expected to result in both high genetic drift and isolation (Cayuela et al., 2016a; Cayuela, Boualit et al., 2016). First, we verified that individuals experienced a high degree of inbreeding by calculating individual heterozygosity coefficients. Second, we investigated whether male mating success depended on male—female relatedness and/or male heterozygosity. To do so, we conducted breeding trials in which females were offered the choice between males. Male mating success was evaluated twice by considering two successive breeding trials. To minimize the effect of male competition while increasing the difference in male—female relatedness within dyads, dyadic males were selected according to their body size and their pond origin. Moreover, we also accounted for potential differences in male breeding investment by quantifying their plasma testosterone level. Male heterozygosity and the respective relatedness of males with the female were inferred from microsatellite markers that were developed for the study. Because of the high inbreeding level in this population, we expected mating tactics based on male heterozygosity and inbreeding avoidance. We thus predicted a negative relationship between male mating success and female—male relatedness. Furthermore, we expected a higher breeding success in the most heterozygous males.

## METHODS

### Biological Model

The yellow-bellied toad reproduces in small temporary ponds including rocky pools, residual puddles, ruts and ditches (Barandun, Reyer, & Anholt, 1997; Cayuela, Besnard, & Joly, 2013). Fertilization is external and males compete for access to females by producing acoustic courtship signals (Schneider, Hussein, & Akef, 1986). Males are also territorial and use hydrodynamic wave signals as intrasexual territorial advertisements (i.e. wave communication, see Elepfandt & Simm, 1985; Seidel, 1999, Seidal et al.,

2001). The presence of postvitellogenic follicles throughout the breeding season allows females to lay several clutches (up to seven; Barandun et al., 1997; Buschmann, 2002; Guarino, Bellini, Mazzarella, & Angelini, 1998).

### Ethical Note

Toad capture was authorized by the Préfecture de l'Ardèche (arrêté no. 2014-288-002). This study was authorized by the Ethical Committee of Lyon University (DR 2013-09).

### Toad Captivity and Experimental Design

The yellow-bellied toads used in the study were collected in 2013 from a spatially structured population located (called POP1 in Appendix Tables A1, A2) in southern France (Ardèche, GIS coordinates: 4°30′12.2″E, 44°52′ 50.4″N) and monitored since 2009 using capture—mark—recapture (Cayuela et al., 2016b). In this population, the toads breed in a set of 29 breeding patches (an aggregation of small rocky pools along a riverbank) distributed along a 5 km stretch of the river. Individuals were caught in 13 of 29 patches. Just after their arrival at the laboratory, the individuals were measured and weighed, and their DNA was collected by noninvasive buccal swabbing. The males were kept indoors in a licensed animal facility in a climatic room with a light:dark cycle of 18:6 h, corresponding to the natural day:night cycle in the study area. They were individually housed in covered plastic boxes (0.35 × 0.20 m and 0.20 m high) kept inclined and filled with 300 ml of tap water. The males were fed crickets (Acheta domestica) ad libitum, and the tap water was changed three times per week until the beginning of experiments.

To ensure the reproductive state of the females at the onset of the mating experiments, they were kept together in a seminatural outdoor enclosure (4 × 8 m and 0.5 m high) with one side mimicking a nonbreeding aquatic habitat usually used for foraging and resting activities (Cayuela, Pradel, Joly, & Besnard, 2017, i.e. a cool shady area containing terrestrial shelters and covered with wet forest litter) and the other mimicking a breeding site (shallow water bodies). The toads received 150 crickets twice a week, and artificial rainfall was simulated once a day for 1 h since female ovulation is synchronized with rainfall in this species (Barandun et al., 1997). Females were allowed to participate in breeding experiments when found in the breeding site. For each female (N = 30), we formed a male dyad, using one male originating from the female's breeding patch (i.e. local male) and the other originating from another breeding patch (i.e. foreign male) in order to contrast a priori male—female relatedness between dyadic males. Both dyadic males were also chosen to match in size and body condition (i.e. the excess of weight for a given body length) since mating success may depend on body condition in this species (Cayuela, Lengagne, Joly, & Léna, 2017, Cayuela, Lengagne, Kaufmann, Joly, & Léna, 2016). Because call features such as frequency, rate and duration are strongly related to male body size and condition (Hoskin & Goosem, 2010; Humfeld, 2013; Morris & Yoon, 1989; Ziegler, Arim, & Bozinovic, 2016; Ziegler, Arim, & Narins, 2011), we expected relatively similar call attractiveness in the two dyadic males. In addition, as size and body condition have a strong influence on aggressive male interactions (Owen & Gordon, 2005; Reichert & Gerhardt, 2011), we expected the two dyadic males to display a relatively similar level of aggressiveness and territoriality.

All experiments were performed in a set of 10 outdoor seminatural enclosures (2 × 4 m and 0.5 m high), each being divided into two equal subunits (2 × 2 m and 0.5 m high) separated by a fence. Each subunit contained a breeding site (i.e. a small artificial pond) and a shelter (i.e. a half-buried tile). Two egg-laying supports