Can female preference drive intraspecific diversity dynamics in brown trout (*Salmo trutta*, L.)?

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Abstract – Intraspecific diversity can be strongly impacted by the dynamics of reproductive isolation during secondary contacts. The high levels of hybridisation and introgression between Atlantic and Mediterranean lineages in contemporary populations of brown trout in the Northern part of the French Alps are a good case in point. After a long period of allopatry, which one assumes has facilitated their divergent phenotypic and genetic evolution, man has removed a geographical reproductive barrier via stocking the Atlantic lineage in the Mediterranean area, thereby potentially enabling gene flow between native and non native populations. We investigate how much a prezygotic reproductive behaviour, that is female preference for male phenotype, can influence hybridisation in natural environments, using subaquatic video recording of reproduction. Our statistical model indicates that female preference appears to be largely heterogamous: females tend to select dissimilar males with respect to their own phenotype; thus, rather than acting as a barrier to gene flow, female preference is favouring gene flow between lineages that have been artificially placed in sympatry. This finding based on observational data is in agreement with previously reported genetic data, high levels of hybridisation between the lineages. We suggest that a knowledge of reproductive behaviour in natural environments is an essential tool for biodiversity managers to assess the potential risks associated with the introduction into recipient populations of non-native lineage fish at the intraspecific level.

Key words: female preference; secondary contact; intraspecific biodiversity; reproductive isolation; hybridisation

Introduction

Evolution of reproductive isolation is central to the understanding of possible routes to speciation (Schluter 2000). An expected reinforcing mechanism for reproductive isolation lies in the preference for locally adapted phenotypes (Fisher 1930; Lande 1981). The rationale is that through natural selection, local individuals have a fitness advantage over migrants and consequently should be preferred as sexual partners (Williams 1966). Yet, a number of recent experimental or theoretical studies point to the absence of a correlation between evolution of phenotype and mate preference (Martin & Mendelson 2013; Räsänen et al. 2014; Servedio & Bürger 2014), while *in natura* there is little evidence to support this

idea (Raeymaekers et al. 2010). This question is equally crucial for the management of biodiversity at both inter- and intraspecific levels (Crandall et al. 2000), where lack of reproductive isolation can lead to dramatic loss of diversity following human mediated introductions or population reinforcements.

Salmonids species are largely impacted by these practices (Hansen et al. 2009) and they often show high levels of local adaptation (Fraser et al. 2011). The brown trout (Salmo trutta, L.) itself displays a high level of intraspecific variation, with potentially five genetic distinct lineages that have evolved independently following their separation an estimated 0.5–2 Ma (Bernatchez 2001). As a result, phenotypic differences have arisen between the lineages. For example, Atlantic (ATL) and Mediterranean (MED) lineages show signifi-

cant differences in ornamentation (Lascaux 1996; Mezzera & Largiadèr 1997; Aparicio et al. 2005). Over the last century, however, these two lineages have been brought into sympatry through intensive stocking in the Mediterranean area (Barbat-Leterrier et al. 1989). The outcome of these practices is heterogeneous, but many rivers now shelter introgressed populations with a large numbers of viable hybrids (HYB). The reasons behind this incomplete reproductive isolation between lineages remain largely unexplored.

In salmonids, female preference is a putative candidate for prezygotic reproductive isolation. Some phenotypic traits are known to be preferred by females like body size (Quinn & Foote 1994), body size ratio (Labonne et al. 2009) and adipose fin size (Petersson et al. 2009). The honesty of some signals has also been demonstrated (i.e., signals that may inform the partner about potential direct or indirect benefits). For instance, in arctic charr (*Salvelinus alpinus, L.),* increased male ornamentation signals lower susceptibility to parasite infection (Skarstein et al. 2005), and in ATL brown trout, paternal melanin-based coloration is positively correlated to offspring survival (Wedekind et al. 2008).

And yet, very few studies have directly investigated the influence of female preference and its mechanisms on reproductive isolation directly in wild populations. Studies undertaken under natural conditions allow the actual strength of mechanisms at work to be measured (Qvarnström et al. 2006). Here, we investigate possible reproductive barriers related to female preference for male phenotype *in natura*, using video recording, lineage-related phenotypic indicators and a behavioural model to describe female preference. Three different versions of the model are evaluated to explain observed data: a null model, where preference is based on body size and OSR only (Labonne et al. 2009); a model with preference based on phenotypic dissimilarity; a lineage dependent preference model. Our results are interpreted with regard to the published introgression patterns in this species and with regard to the general expectation of female preference evolution following a secondary contact. We finish by discussing the relevance of our findings and approach for other similar salmonids conservation issues.

Methods

Data sampling

During two spawning seasons (winters 2011–2012 and 2012–2013), we monitored the mating behaviour of brown trout *in natura* using subaquatic videos in four streams located in the Northern French Alps (River Borne, River Dranse d'Abondance, Chevenne creek, Serve creek) (46.55′57.68N'';6°24′07.40E''/ 46°16′52.94N'';6°42′39.55E''/46.17′54.04N'';6°47′2 2.09E''/46.16′49.60N'';6°42′40.83E'') known for sheltering an array of variably introgressed trout (Caudron et al. 2012).

We defined as an observation unit (OU) every video sequence longer than 15 min in which an active female (i.e., digging a nest) was seen in the presence of at least one male. The OU was stopped if the female left the redd for more than 20 min. The camera was installed 50 cm from the redd, a distance sufficient to distinguish and analyze fish phenotypes. A ruler was placed behind the nest to measure fish. Sixty OUs were thus obtained for analysis. Fish were individually identified using body size and a set of four traits, which could be unambiguously associated with either MED or ATL lineages: presence of stripes; absence of spot aureole; absence of lateral line; and number of opercular spots (Lascaux 1996; Aparicio et al. 2005; see Appendix S1). The first two traits are categorical variables with two modalities: presence of stripes and absence of spot aureole are both MED characters. The lateral line trait is a categorical variable with three modalities: absence (MED), partial (HYB) and present (ATL). The number or opercular spots is a quantitative trait ranging between 0 and 26 in our data; a high number of opercular spots indicate MED origin and *vice versa*. We had individual identities assigned to 58 males and 27 females. In some cases, one of the traits could not be satisfactorily assessed (3.5% of individualised fish). Operational sex ratio (OSR) was calculated as the number of males observed in attendance to females in the OU. Using JWatcher freeware (Blumstein & Daniel 2007), we noted the occurrence of the following behaviours: arrival and departures from the scope of the camera by either male or female, and digging movements by the female. A total of 499 behavioural events were recorded.

Calculation of a phenotypic score

Based on the four discriminant documented phenotypic traits, we calculated a general phenotypic score P as the mean of the score at each trait (Table 1) as follows:

Table 1. Modalities for the various phenotypic traits, the origin they can be unambiguously linked to and their value in the calculation of a phenotypic score.

Phenotypic trait	Modalities	Origin associated	Value for calculation
Presence of stripes	present/absent	MED/ATL	1/0
Lack of spot aureole	present/absent	MED/ATL	1/0
Lack of lateral line	present/partial/absent	MED/HYB/ATL	2/1/0
Number of opercular spots	count	#	#

$$P = (\text{Stripes} + \text{Lack aureole spot}$$
$$+ (\text{Lack lateral line} - 1)/2$$
$$+ (\text{Nb opercular spots})/26)/4$$

The P score equals 0 for phenotypically pure ATL individuals and 1 for phenotypically pure MED individuals. Additionally, the phenotypic dissimilarity between a pair of male and female (D) was calculated as the absolute value of difference between male and female scores.

Preference model

The behavioural model is directly derived from a previous published study (Labonne et al. 2009) (Fig. 1). It assumes different outcomes following the encounter of a male and a female trout on the redd. First, the female can leave the redd (probability 1-s). If the female stays on the nest, she can be inactive (no digging) or active (probability a). We defined female preference as the product of s and a, that is the probability that a female stays on the nest and is active following an encounter with a male. We then fitted three models by inferring the effect of explanatory variables on s and a. The first model (M1) was considered as our null hypothesis and integrated the body size ratio (BSR) and OSR (Labonne et al. 2009), as well as random effects to handle male and female pseudoreplication in our observations. As an example, the inference on s probability is described as follows:

$$M1 : \text{logit}(s)$$
$$= \alpha_{s,j} + \beta_{s,k} + \gamma(s) \times \text{BSR}_i + \delta(s) \times \text{OSR}_i$$
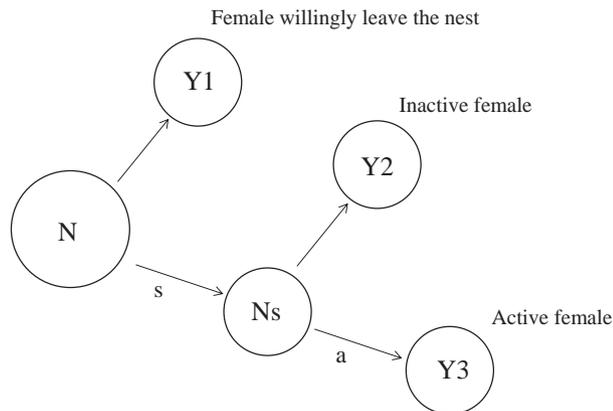
Fig. 1. Behavioural model describing the possible behavioural answers of a female to the presence of a male on the redd. s is the probability for the female to stay on the red and a is the probability for the female to be active conditional on being present. Y1, Y2 and Y3 are the three possible outcomes of female/male interaction in this model.

The second model (M2) additionally integrated the effect of phenotypic dissimilarity D:

$$M2 : \text{logit}(s) = \alpha_{s,j} + \beta_{s,k} + \gamma_{(s)} \times \text{BSR}_i + \delta_{(s)}$$
$$\times \text{OSR}_i + \zeta_{(s)} \times D_i$$

The third model (M3) integrated the score of the male (Pj), the score of the female (Pk) and the interaction between both, thus assuming that female preference could be lineage dependent.

$$M3 : \text{logits} = \alpha_{s,j} + \beta_{s,k} + \gamma_{(s)} \times \text{BSR}_i + \delta_{(s)}$$
$$\times \text{OSR}_i + \eta_{(s)} \times Pj + \theta_{(s)} \times P_k$$
$$+ \lambda_{(s)} \times P_j \times P_k$$

γ, δ, ζ, η, θ and λ are hyperparameters, α_j and β_k are male and female random effects respectively, i, j and k are the male, female and observation identifiers respectively. Full details for the model code, random effects and noninformative prior distributions are provided in Appendix S2.

Statistical inference was conducted in the Bayesian framework using OpenBugs software (3.2.2). The joint values of hyperparameters were obtained by MCMC samplings (10,000 draws, thinning = 10) after checking its convergence using the Gelman–Rubin diagnostic (Gelman & Rubin 1992) based on a 5000 draws undertaken as part of a burning-in period. The three models were compared using the deviance information criterion (DIC, Spiegelhalter et al. 2002). For the best model, we evaluated the percentage of correct predictions made from the model posterior compared to the observed data.

Results

Observation data summary

The distribution of observed phenotypes on redds shows that males often present an intermediate phenotypic score P (around 0.5, Fig. 2a) with fewer individuals presenting phenotypically pure values of 0 or 1. Female scores are more evenly distributed between 0 and 1, although intermediate scores still represent the majority of individuals. As a result, some combinations of male and female with extreme phenotypes are less represented. The distribution of behavioural items (Fig. 2b) shows an important variation between observed couples, that is the number of behavioural items recorded per observed couple can range between 1 and 40 (mean = 8.32).

Model selection

Among the three models, the M2 model (preference based on phenotypic dissimilarity) had the lowest DIC

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(a)

	0–0.09	0.1–0.19	0.2–0.29	0.3–0.39	0.4–0.49	0.5–0.59	0.6–0.69	0.7–0.79	0.8–0.89	0.9–1	
0–0.09	1				1	1					3
0.1–0.19		1				2					3
0.2–0.29		1		1		3			1		6
0.3–0.39	2		1			3		1	2		9
0.4–0.49					1	3	1	1			6
0.5–0.59			2	1		3	2	1	2		11
0.6–0.69			2			5	1	1			9
0.7–0.79						3	1	1			5
0.8–0.89		1		1			1	2		1	6
0.9–1						1		1			2
	3	3	5	3	2	24	6	8	4	2	

(columns headed "Male phenotypic score"; rows "Female phenotypic score")

(b)

	0–0.09	0.1–0.19	0.2–0.29	0.3–0.39	0.4–0.49	0.5–0.59	0.6–0.69	0.7–0.79	0.8–0.89	0.9–1	
0–0.09	1				2	8					11
0.1–0.19		7				27					34
0.2–0.29		2		10		14			16		42
0.3–0.39	23		7			8		6	21		65
0.4–0.49					3	17	4	5			29
0.5–0.59			5	8		59	8	2	20		102
0.6–0.69			26			51	1	1			79
0.7–0.79						25	8	7			40
0.8–0.89		9		3			7	12		22	53
0.9–1						11		33			44
	24	18	38	21	5	220	28	66	41	38	

(columns headed "Male phenotypic score"; rows "Female phenotypic score")

Fig. 2. (a) Occurrence of paired phenotypes in all observation units and (b) number of behavioural items sampled per paired phenotypic association in all observation units.

value, showing the best compromise between data fit and model parsimony (Table 2). We therefore retained this model, indicating that preference was neither random nor lineage dependent with respect to phenotype. This model predicted 50.8% of exact responses. Under the M2 model, BSR had a positive effect on both s ($\gamma_{(s)} = 0.6839$, SD = 0.3111) and a probabilities ($\gamma_{(a)} = 0.325$, SD = 0.2422), females expressing preference for high BSR values. OSR had a positive effect on s ($\delta_{(s)} = 0.0857$, SD = 0.0736) and a ($\delta_{(a)} = 0.0786$, SD = 0.0559), with females leaving the redd less often and being more active for increased OSR values. The phenotypic dissimilarity D also had positive effects on s ($\zeta(s) = 2.68$, SD = 1.114) and a ($\zeta(a) = 1.105$, SD = 0.7322) indicating that females were preferring dissimilar males with respect to their own phenotype,

whereas they were leaving the redd more often and were less active with similar males. Predictions from the M2 model show that preference for dissimilar males can be from 28.9% to 39.6% higher than for similar males conditional on OSR and BSR (Fig. 3).

Discussion

We found direct evidence of a female preference based on mate dissimilarity in introgressed populations of brown trout. Specifically, females tend to prefer males with phenotypes that are different from their own, thereby potentially negatively discriminating against males from their own lineage. The magnitude of this preference gradient is remarkable if we consider that it has been estimated under natural conditions, where several other mechanisms may also be involved and interact. We further investigated whether this preference could be different between ATL and MED females, but the M3 model did not improve the fit despite a higher number of parameters. The M2 model also seems to confirm that results from previous experiments regarding OSR and BSR effects still hold (Labonne et al. 2009). A general heterogamous model can therefore be

Table 2. DIC and estimated number of parameters for each model.

Model	DIC	Estimated number of parameters
M1: null model	882.9	35.07
M2: dissimilarity	874.9	30.99
M3: lineage dependent	880.4	41.03
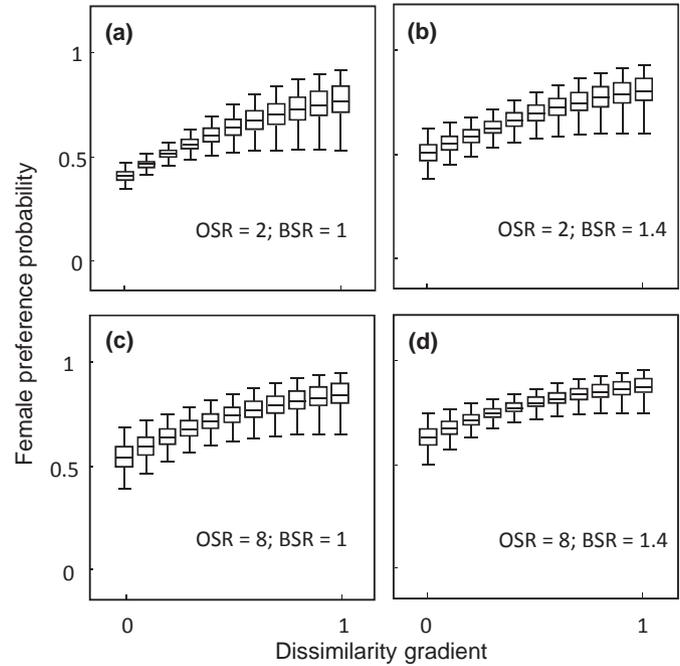
Fig. 3. Predictions for general female preference ($s \times a$) for an average female, when (a) OSR = 2, BSR = 1; (b) OSR = 2, BSR = 1.4; (c) OSR = 8, BSR = 1; (d) OSR = 8, BSR = 1.4.

accepted for female preference in these populations. Additionally, in brown trout, male–male competition may override female preference (Petersson et al. 1999) and therefore mitigate the role of preference in reproductive isolation. We checked this hypothesis by comparing aggressiveness between males with respect to phenotype, and we found no effect of phenotype on male intrasexual competition (Gil, J., Caudron, A., Labonne, J.).

Admittedly, our sampling does not provide a complete picture of all possible pairs of phenotypes: this situation implies that predictions made on the boundaries (for instance, when phenotypic dissimilarity D equals 1) must be interpreted carefully. However, the steepest part of the gradient in preference is actually for relatively small values of D (0–0.5, see Fig. 3), indicating that females are choosy even when males are not entirely dissimilar with respect to their own phenotype. One would also points out that despite having some phenotypically pure individuals in our data, similar phenotypes were rarely observed together on the redd, which could indicate a different mechanism for reproductive isolation. This question would require additional years of sampling in habitats where the admixture between the two lineages is variable. Still, the rarity of the pure phenotypes matches our knowledge of what is known of the population structure of trout in the rivers study (Caudron et al. 2012).

While the two lineages have evolved separately and developed different phenotypes, we failed to find any female preference for similarity, or any lineage dependent preference, that would have been indica-

tive of a possible anterior coevolution of preference and phenotype. It would be a presumption to conclude that the currently observed preference, estimated on a wide range of differentially introgressed females, correctly represents the preference of each initially isolated lineage. The secondary contact between the two lineages can be traced back to the beginning of the 20th century (Caudron et al. 2012), the sympatry may then have reshuffled the genetic bases for female preference. At the very least, female preference was initially not so homogamous that it prevented hybridisation between lineages. In recent populations management experiments, the generation of extensive hybrid pools following an introduction of pure Mediterranean individuals into an Atlantic dominated population was quick (10–20 years, Caudron et al. 2012), a pattern compatible with our findings; female preference appears to facilitate rapid gene flow between lineages.

If we assume that heterogamous preference is the rule in *S. trutta*, it is tempting to speculate as to the possible genetic basis of the observed mate choice preference. Garner et al. (2009) found female MHC-based preference in chinook salmon (*Oncorhynchus tshawytscha,* W.), indicating a possible female aversion for males with similar MHC genotypes. Similarly, Forsberg et al. (2007) showed that intermediately dissimilar MHC matings were more frequent than expected in brown trout. To our knowledge, no relationship between MHC type and the phenotypic traits used here has been published, so it remains difficult to hypothesise for an actual link between our finding and the MHC dissimilarity

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hypothesis. Alternative explanations can be evoked such as sensory bias (Ryan 1990), which could lead to a preference for dissimilar males. Alternatively, females could be deceived by a trait that would be adaptive for local males and overexpressed, yet uncorrelated to fitness (dishonest) in allopatric males. In both cases, the mechanism has to evolve in an antagonistic way in each lineage before the secondary contact to produce the general heterogamous pattern observed. Lastly, the preference for dissimilarity can be considered as a mechanism to prevent inbreeding in small populations, a frequent situation in mountain areas such as Haute-Savoie. The panel of possible evolutionary causes for this heterogamous female preference model in brown trout offers several avenues and some of them could now be easily explored.

Our observations somehow echo recent data and predictions (Räsänen et al. 2014; Servedio & Bürger 2014) nuancing the strength and possible pathways for coevolution of female preference and male phenotypes. From an applied point of view, this has direct consequences on our expectations of intraspecific diversity dynamics long allopatric evolution will not necessarily prevent hydridisation in case of secondary contact. Population managers have therefore a direct impact and control on intraspecific diversity, through the effects of their management practices on the genetic admixture between lineages. The precautionary approach must be to preclude any contact between lineages in Mediterranean areas that have not yet been stocked with Atlantic fish. For those areas where introgression has already occurred, one can assume that there will be potential fitness variation among newly generated hybrid combinations and presume that natural selection will act on the new genetic admixture to achieve new, but different adaptive peaks. For instance, introgression patterns show some local variations, the pure ATL sometimes disappearing in some areas, and the pure MED persisting in others (Largiadèr & Scholl 1996), which could be the result of environmentally controlled postzygotic selection. But in most areas, hybrids represent a large part of the population even many years after stocking have ceased (Gil et al. Submitted).

The MED versus ATL conservation challenge is a typical case in salmonids: the introgression of a native population arising from contact between lineages or species facilitated by man (Scribner et al. 2001; Gozlan et al. 2010). As an example, Young et al. (2001) underlined the lack of knowledge on the mechanisms generating reproductive isolation in the cut-throat versus rainbow trout conservation issue. Rubidge & Taylor (2004b) also pointed at the lack of study focusing on prezygotic isolation. Mating systems have been tentatively inferred from

a posteriori molecular analyses (Rubidge & Taylor 2004a; Metcalf et al. 2008), but generally, no direct evidence is available to validate the assumptions used. Interestingly, Weigel et al. (2002) identified throat slash pattern or spot shape as being discriminant phenotypic traits during reproduction between westslope cut-throat trout and rainbow trout; subaquatic video recording could therefore be successfully applied in this case to evaluate the relative influence of prezygotic barriers on total reproductive isolation by performing observations of mating systems under various environmental set-ups and conditions. This should help understanding why ecological factors may sometimes constrain gene flow between gene pools (Weigel et al. 2003) while they do not seem to matter in other occasions (Hitt et al. 2003).

In a more general context, the method used in this study could be transposable to other conservation issues in salmonids, where the phenotypic recognition for fish of different origin is possible. *In natura* observations contribute actively to the detection of mechanisms controlling for reproductive isolation between gene pools of major interest for biodiversity conservation.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Phenotypic traits used in the calculation of the phenotypic score.

Appendix S2. OpenBugs model code.