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The context dependence of assortative mating: a demonstration with conspecific salmonid populations

Z. GAUTHEY*, A. P. HENDRY†, A. ELOSEGI‡, C. TENTELIER* & J. LABONNE*

*ECOBIOP, INRA, University of Pau & Pays Adour, Saint-Pée sur Nivelle, France
†Redpath Museum and Department of Biology, McGill University, Montreal, QC, Canada
‡Faculty of Science and Technology, University of the Basque Country UPV/EHU, Bilbao, Spain

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Abstract

Assortative mating is thought to play a key role in reproductive isolation. However, most experimental studies of assortative mating do not take place in multiple natural environments, and hence, they ignore its potential context dependence. We implemented an experiment in which two populations of brown trout (*Salmo trutta*) with different natural flow regimes were placed into semi-natural stream channels under two different artificial flow regimes. Natural reproduction was allowed, and reproductive isolation was measured by means of parentage assignment to compare within-population vs. between-population male–female mating and relative offspring production. For both metrics, reproductive isolation was highly context dependent: no isolation was evident under one flow regime, but strong isolation was evident under the other flow regime. These patterns were fully driven by variance in the mating success of males from one of the two populations. Our results highlight how reproductive isolation through assortative mating can be strongly context dependent, which could have dramatic consequences for patterns of gene flow and speciation under environmental change.

Introduction

A key mechanism generating reproductive isolation is positive assortative mating: the tendency of individuals of one type to mate with individuals of the same type rather than individuals of some other type (Andersson, 1994; Maan & Seehausen, 2011; Bolnick & Kirkpatrick, 2012; Servedio & Kopp, 2012; Langerhans & Makowicz, 2013). Positive assortative mating is frequently observed within populations, among populations and among species (Coyne & Orr, 2004; Nosil, 2012; Jiang *et al.*, 2013). Among these levels, conspecific populations are especially interesting because they facilitate the assessment of reproductive barriers that act early in speciation, as opposed to accumulating after the fact (Thibert-Plante & Hendry, 2009; Nosil, 2012; Servedio & Kopp, 2012). Potential barriers contributing to assortative mating at this stage include habitat preference, reproductive timing and mate choice (Nosil, 2012).

Many experiments have assessed assortative mating by measuring the relative success of within-type vs. between-type male–female pairings (e.g. Rundle *et al.*, 2000; Nosil *et al.*, 2002; Schwartz *et al.*, 2010). However, most such studies have several limitations with regard to the question at hand. First, they are usually conducted in carefully controlled laboratory environments, which renders uncertain their applicability to reproductive isolation in nature. Second, many experiments employ only crude proxies for assortative mating, such as the relative amount of time females spend with same-type vs. different-type males, which might not translate into overall reproductive isolation. Third, most experiments do not use multiple environments and therefore cannot assess the context dependence of assortative mating.

Indeed, the strength of assortative mating between any two populations should be context dependent. As one example, sensory drive predicts that the

Correspondence: Jacques Labonne, ECOBIOP, INRA, University of Pau & Pays Adour, 64310 Saint-Pée sur Nivelle, France.
Tel.: +33 559515980; fax: +33 559545152;
e-mail: labonne@st-pee.inra.fr

preferences of females for same-type males will be greatest in the specific environment where the male traits and female preferences evolved (Boughman, 2001, 2002). As another example, female preferences should depend on social factors, such as the preferences of other females (e.g. mate choice copying: Kirkpatrick & Dugatkin, 1994; Briggs *et al.*, 1996; Servedio & Kirkpatrick, 1996) and the number of other males (e.g. females should be pickier when more males are present: Kokko & Monaghan, 2001). As yet another example, female choice should be stronger when the risks associated with carefully assessing males, such as exposure to predators or parasites, are lower (Crowley *et al.*, 1991). This context dependence of assortative mating is critical to evaluate because it dictates the consistency of reproductive barriers and, therefore, the likelihood they will persist in Variable environments. For instance, changes in water clarity that alter the perception of male colours by females have caused speciation reversal in cichlids (Seehausen *et al.*, 1997) and perhaps also in threespine stickleback, *Gasterosteus aculeatus* (Lackey & Boughman, 2013). We designed an experiment with goal of reducing or eliminating the above-mentioned inferential limitations while also assessing context dependence in assortative mating.

Our experiment

Brown trout (*Salmo trutta*) are known to express divergence among populations in a number of traits (colour, size, morphology: Jonsson & Jonsson, 2011) that influence mate choice (Petersson *et al.*, 1999; Labonne *et al.*, 2009; Gil *et al.*, 2016). We therefore might expect mating isolation between conspecific populations; yet, explicit tests for such isolation have been rare (Gil *et al.*, 2016). In hopes of detecting ecologically relevant reproductive isolation, we selected two populations from quite different environments: the River Bastan has relatively low and predictable variation in water flow, whereas the River Urumea has higher and less predictable variation in water flow (see Materials and methods). Although these two populations do not differ dramatically in external characters, their different environments suggest the potential for divergence in a number of important ecological traits that might then influence reproductive isolation.

Our experiment was conducted in artificial stream reaches that were similar to natural spawning environments (see Materials and methods), increasing the chances that our results would be applicable to reproductive isolation in nature. Further, the use of these semi-natural reaches allowed us to generate two different environmental conditions so as to assess the context dependence of assortative mating. Specifically, we maintained a constant flow regime in one channel and a variable flow regime in the other channel. We expected this contrast might influence assortative mating because flow regime can influence uncertainty, perceived risk, and the ability and cost to assess and monopolize mates (Allouche & Gaudin, 2001; Petersson & Järvi, 2001; Labonne *et al.*, 2009).

A robust method of assessing assortative mating would ideally integrate the various stages at which this barrier could arise. For instance, although many studies focus only on visual observations of male–female interactions, cryptic female choice (e.g. biased use of sperm) could also be important (Birkhead & Pizzari, 2002). We therefore used genetic assignment of offspring among putative parents to quantify assortative mating based on (i) male–female pairings, and (ii) the number of offspring produced conditional on the observed male–female pairings. In our study system, these metrics would not be influenced by intrinsic genetic incompatibilities unrelated to cryptic female choice (or sperm competition) because such incompatibilities are lacking among conspecific populations of salmonids.

Materials and methods

Our study populations were the River Bastan (France, +43°16′2.51″, −1°22′32.46″) and the River Urumea (Spain, +43°14′31.81″, −1°55′28.98″). These rivers have similar annual mean discharge (about 6 m^3 s^{-1}), watershed areas and land use, but the River Bastan has more predictable flows (daily discharge data for 31 years for Bastan and 17 years for Urumea): several indicators for flow variation have been estimated using IHA software (Richter *et al.* 1998). For instance, River Bastan has a lower coefficient of variation for annual discharge (1.21 vs. 1.56), generally lower coefficients of dispersion for monthly discharge, and fewer high (9 vs. 10) and low (7 vs. 19) flow events per year (see Appendix S1, details of calculation in Colwell, 1974; Poff & Ward, 1989). The reason for this difference is that the River Bastan is a snow/rain driven system, whereas the River Urumea is a perennial run-off-driven system.

Adults were collected from the rivers between 21 November and 13 December 2012 by electrofishing and brought back to the laboratory at the Lapitxuri channel (details below), where they were acclimated in separated tanks for 48 h without food. After acclimation, the fish were individually anesthetized (0.3 mL L^{-1} of 2-phenoxyethanol), measured to the nearest mm, weighed to the nearest g and photographed using a Pentax K-R digital camera with dimmed light (Ricoh Imaging Company, Tokyo, Japan). The photographs allowed us to identify individual fish at the beginning and end of the experiment through the position and shape of red and black spots (Appendix S2). This method allowed us to avoid the use of visual or internal tags that might influence mating behaviour and individual condition. Sexual maturity was assessed by gentle squeezing of the belly to reveal the presence of

sperm for males and ripe eggs for females. Only mature fish were selected for the experiment.

The experiment

The experiment was conducted from November 2012 to April 2013 in a controlled channel beside the Lapitxuri Stream (+43°16′59″, −1°28′54″), a tributary to the Nivelle River in south-western France. The channel has a 2% slope and is fed with natural river water and therefore provides ample food. Temperature variation follows the natural river pattern. It has been used in a number of experiments of reproductive behaviour in salmonid fishes (e.g. Thomaz *et al.*, 1997; Martinez *et al.*, 2000; Hendry & Beall, 2004; Gauthey *et al.*, 2015, 2016) and allows to finely control water flow using valves and a real-time indicator of discharge. The width of the channel (2.80 m) is comparable to tributaries of either River Urumea or River Bastan.

Separate reaches of 30 m each were used to generate two distinct environments with different water flows: constant flow (Constant environment) and variable flow (Variable environment). In the Constant environment, flow was maintained at 210 m^3 h^{-1} (SD = 3.4 m^3 h^{-1}) throughout the experiment. In the Variable environment, rapid discharge variations were implemented in cycles of three consecutive modalities: high (360 m^3 h^{-1}), intermediate (210 m^3 h^{-1}) and low (80 m^3 h^{-1}). The duration of each modality for each cycle was drawn randomly from a discrete uniform distribution (1–4 days), which was reasonably comparable to natural conditions. However, the *rate* of water level change was much faster (about 1–3 min) than in nature. Within each of the two environments, the channel was divided into three sequential 10–m-long sections. The middle section was optimized for spawning, with 5–20 mm substrate sizes and 10–20 cm water depths at intermediate discharge. By contrast, the upstream and downstream sections were optimized for hiding and resting, with 40–80 mm gravel, up to 60 cm water depth and visual obstacles. The fish were free to move between the three sections within each environment but could not move between environments. Upstream passage was prevented by an impassable obstacle. Downstream passage was prevented by a net trap. Whenever an adult fish was caught in the net trap, it was released back into its environment.

Between 21 November and 13 December, fish were released into the channels as they matured, with similar body sizes, numbers and sex ratios released into each environment on each release date. Thirty-three females and 19 males were released in the Constant environment, and 31 females and 19 males were released in the Variable environment (phenotypic data available in Appendix S3). The relative number of males and females were chosen so to maximize the number of potential matings (thus more females) while still providing ample phenotypic variation in both sexes. No significant body size difference was detected between males of both origins released in Constant (Wilcoxon, $P = 0.05212$) or Variable (Wilcoxon, $P = 0.7696$) environments, as well as between females of both origins in Constant (Wilcoxon, $P = 0.2291$) or Variable (Wilcoxon, $P = 0.9367$) environment. The fish were then removed from the channel by electrofishing on 13 February 2013, 2 weeks after the last observed reproductive activity (digging or antagonistic behaviours). All fish were anesthetized, measured and weighed (as above) and were then stripped to assess any remaining eggs or sperm. In addition, a small piece of caudal fin was removed to perform genetic analysis (details below). Each fish was identified based on the pictures taken before reproduction and was then released into its original river after 48 h.

Seventy-five days after the first reproduction (corresponding to 800 degree days), we began collecting daily drifting juveniles in the net traps. A subsample was kept for genetic analysis. Specifically, up to 20 juveniles were taken randomly from the traps each day per environment (irrespective of the total number of juveniles trapped). Ninety days after the last observed reproductive activity, the few remaining juveniles were electrofished, and 20% were also kept at random. This subsampling represented 98.8% (Constant) and 84.3% (Variable) of the total number of caught juveniles. Juveniles were killed with a lethal dose of 2-phenoxyethanol and placed individually in a tube of 90° ethanol for later genetic parentage analysis.

Parentage analysis

DNA was extracted using NaCl/chloroform, and eight microsatellites previously developed for salmonids were amplified: Str60INRA, Str73INRA (Estoup *et al.*, 1993), Ssa85 (O'Reilly *et al.*, 1996), SsoSL438 and SsoSL417 (Slettan *et al.*, 1995), SSsp2216 (Paterson *et al.*, 2004), and Ssa410Uos and Ssa408Uos (Cairney *et al.*, 2000). We used a multiplex protocol allowing amplification of the eight loci in one polymerase chain reaction (multiplex PCR), following (Lerceteau-Köhler & Weiss, 2006). Fragments were sized on an ABI 3100-Avant (Thermo Fisher Scientific, Waltham, MD, USA) using a GeneScan 500 LIZ internal size standard (Thermo Fisher Scientific, Waltham, Maryland, USA). They were then scored using STRand software (Toonen & Hughes, 2001), and raw allele sizes were binned into discrete allele classes using the MSatAllele package (Alberto, 2009) for R version 2.13.0 (R Core Team 2011).

We used the 'parents pair analysis, sexes known' option in Cervus (version 3.0.3, Kalinowski, 2002) to assign parents to each sampled offspring based on allele frequencies computed from genotypes of the candidate parents. The following simulation parameters were used: 10 000 cycles, 33 candidate mothers and 19

candidate fathers in the Constant environment, 31 candidate mothers and 19 candidate fathers in Variable environment, a mistyping error rate of 1%, and a genotyping error rate of 1%. All juveniles with more than one locus missing (1.4% of the total) were removed from the analysis. We accepted parentage assignment at a confidence level of 95% and only when the juvenile was assigned to both parents, which excluded 0.3% of the total. Finally, the genetic distance between the populations was calculated using Weir & Cockerham's (1984) θ on adult genotypes, with a bootstrap method to determine significance ($N = 10\,000$, Geneland Software, Guillot *et al.*, 2005).

Assortative mating

Reproductive success calculated from parentage assignment resulted in a matrix of non-negative integers for the number of offspring assigned to all possible pairs of males and females in each environment (Appendix S4). From this matrix, two indexes were calculated separately for each environment based on Sobel & Chen (2014). The first index estimated assortative mating based on mating success (indirect measure: at least one offspring detected for a given male–female pair):

$$\mathrm{AM_{ms}} = 1 - 2 \times \frac{(M_{\mathrm{between}})}{(M_{\mathrm{between}} + M_{\mathrm{within}})}$$

where M_{between} represents the number of matings between populations, and M_{within} represents the number of matings within populations. The second index estimated assortative mating based on relative reproductive success *conditional on having mated* (number of offspring detected per successful pair):

$$\mathrm{AM_{rs}} = 1 - 2 \times \frac{(O_{\mathrm{between}})}{(O_{\mathrm{between}} + O_{\mathrm{within}})}$$

where O_{between} represents the number of genotyped offspring produced by pairs from different populations, and O_{within} represents then number of offspring produced by pairs from the same population. For both metrics, a -1 value would indicate total negative assortative mating between populations, a 0 value would indicate a random mating, and a 1 value would indicate total positive assortative mating between populations. This approach is especially powerful when population size and sex ratio are known, as they were in the present case, because we can precisely calculate the expected outcome under a panmictic gene flow scenario (Martin & Willis, 2007).

To determine whether $\mathrm{AM_{ms}}$ and $\mathrm{AM_{rs}}$ were greater or lesser than expected by chance in each environment, we calculated expected values under a panmictic scenario using a bootstrap approach. For $\mathrm{AM_{ms}}$, we generated 10 000 new matrices of mating success by randomizing pairs of males and females and attributing an observed mating success to each new pair. That is, the observed matings (number of offspring per pair) were assigned to new pairs of parents chosen at random from all possible pairs in a given environment. For each simulated matrix, this randomized $\mathrm{AM_{ms}}$ was calculated and compared to the observed $\mathrm{AM_{ms}}$. The same approach was used for $\mathrm{AM_{rs}}$, although we calculated the expected random reproductive success conditioned by the observed matings: pairs of individuals that actually mated were randomly assigned observed reproductive success values. This last calculation allowed us to check more precisely if observed $\mathrm{AM_{rs}}$ values were solely driven by variation in mating success (i.e. $\mathrm{AM_{ms}}$) or if they instead could include some cryptic mate choice or additional post-zygotic isolation.

Results

Adults from the two populations were strongly genetically differentiated at the examined microsatellite loci ($\theta = 0.147$, $P < 0.00001$, see Appendix S4), which suggests the potential for reproductive barriers to have evolved and for our methods to reveal them. A total of 1305 juveniles were captured and genotyped, with only 18 being excluded owing to missing data at more than one locus (Constant environment = 13; Variable environment = 5). In the Constant environment, 555 juveniles were successfully genotyped and 552 could be assigned to both parents (95% confidence level). In the Variable environment, 732 juveniles were successfully genotyped and 731 juveniles could be assigned to both parents. In the Constant environment, three Bastan adults (two males and one female) escaped at the beginning of the experiment and one Bastan female died. Only one offspring was assigned to each of the females (none to the males) and so these four individuals (and the two juveniles sired) were excluded from further analyses.

Parentage analysis revealed at least 40 successful mating pairs in the Constant environment and 55 successful mating pairs in the Variable environment (Table 1). Out of the 63 total females, only five (Constant environment: one from Urumea population; Variable environment: three from Bastan population and one from Urumea population) were still at least partially ovigerous at the end of the experiment, indicating that they had not deposited all (or any) of their eggs.

Table 1 The number of inferred mating pairs between (M_{between}) and within (M_{within}) populations, the number of inferred offspring between (O_{between}) and within (O_{within}) populations, and the associated assortative mating indexes ($\mathrm{AM_{ms}}$ and $\mathrm{AM_{rs}}$).

	M_{between}	M_{within}	$\mathrm{AM_{ms}}$	O_{between}	O_{within}	$\mathrm{AM_{rs}}$
Constant environment	17	23	0.15	246	302	0.11
Variable environment	17	38	0.38	210	521	0.43

Only seven offspring were assigned to these females – all to the Urumea female in the Constant environment.

The total number of matings inferred per male ranged between 0 and 11 in the Constant environment (mean was 2.83 for Bastan males and 2.09 for Urumea males) and between 0 and 15 in the Variable environment (mean was 5.5 for Bastan males and 0.91 for Urumea males). The total number of matings inferred per female ranged between 0 and 4 in the Constant environment (mean was 1.29 for Bastan females and also 1.29 for Urumea females) and between 0 and 5 in the Variable environment (mean was 2.06 for Bastan females and 1.5 for Urumea females, Appendix S5).

The total number of offspring inferred per male ranged between 0 and 201 in the Constant environment (mean was 43.75 for Bastan males and 26.45 for Urumea males) and between 0 and 270 in the Variable environment (mean was 73.75 for Bastan males and 6.27 for Urumea males). The total number of offspring inferred per female ranged between 0 and 86 in the Constant environment (mean was 11.35 for Bastan females and 25.5 for Urumea females) and between 0 and 112 in the Variable environment (mean was 24.62 for Bastan females and 18.93 for Urumea females, Appendix S6).

In the Constant environment, assortative mating based on mating pairs was low and not significantly different from zero ($AM_{ms} = 0.15$, $P = 0.87$), implying random mating between individuals from the two populations (Fig. 1). In the Variable environment, however, assortative mating was more than twice as high as in the Constant environment and was significantly different from zero ($AM_{ms} = 0.38$, $P = 0.002$), implying positive assortative mating. The reason for this assortment was that Bastan males achieved higher than random mating success with females from Bastan but not with females from Urumea. By contrast, males from Urumea had lower than random mating success with females from both populations (Fig. 2).

A similar pattern was obtained when considering relative numbers of offspring produced per mating pair: in the Constant environment, $AM_{rs} = 0.11$ and in the Variable environment, $AM_{rs} = 0.43$ (Table 1). However, after accounting for realized mating pairs (offspring produced conditional on a mating having occurred), neither values of AM_{rs} was significantly different from zero (Constant: $P = 0.3189$; Variable: $P = 0.168$). This result shows that variation in AM_{rs} was fully driven by variation in AM_{ms} (whether or not a pair mated) as opposed to differential numbers of offspring produced per mating pair, and it therefore also implies lack of cryptic mate choice.

Discussion

Our experiment revealed the context dependence of assortative mating between two brown trout populations (Fig. 1). In one environment, assortative mating was absent with respect to successful male–female pairings and with respect to total offspring production. In the other environment, assortative mating was strong with respect to both of these metrics, with no added contribution from differential offspring production conditional on the type of pairing. This assortative mating was presumably driven by behavioural interactions because spatial and temporal isolation were absent, intrinsic genetic incompatibilities are lacking, factors typically involved in intrasexual competition and intersexual preference (body size, OSR) did not differ, and cryptic mate choice did not make a substantial contribution. This strong context dependence in mate choice was closely associated with variation in male mating success. In the first environment, males of both origins had similar mating success and males of neither population showed a preference for females from their home population. In the second environment, males from one population had much lower mating success than did males from the other population, with the latter showing strong assortative mating (Fig. 2).

Our study thus demonstrates that assortative mating is strongly context dependent in semi-natural environments; yet, key elements of the experimental design dictate that some inferences about causality and generality remain speculative. First, our use of only two

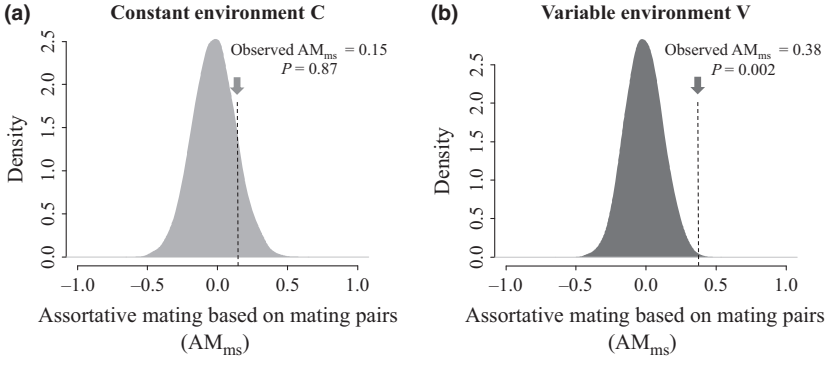

Fig. 1 Assortative mating based on mating pairs (AM_{ms}) in (a) Environment C (constant water flow) and (b) Environment V (variable water flow). The shaded areas represent expected distribution of AM_{ms} under a panmictic scenario. Arrows show the observed values for AM_{ms}.

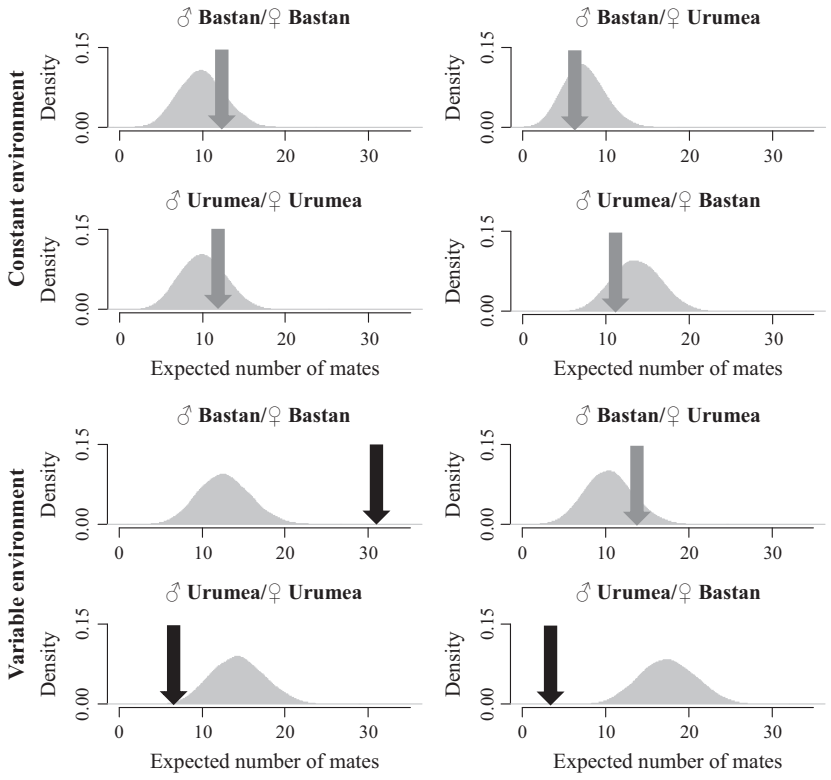

Fig. 2 Observed numbers of mates (arrows) and expected numbers of mates (density probability) calculated under a panmictic scenario between Bastan and Urumea populations in (a) Constant environment and (b) Variable environment. Grey arrows indicate that the observed number of mates falls within expected values for a panmictic scenario, whereas black arrows indicate a significant difference between observed and expected number of mates.

populations and only two experimental environments means that inferences are specific to those two populations and those two environments. That is, the lack of replication of each source population type (i.e. high flow variation vs. low flow variation) and each experimental treatment (constant flow vs. variable flow) means that we cannot confidently infer whether (i) adaptation to different flow regimes causes assortative mating or (ii) whether the context dependence was driven specifically by variation in flow regimes. Second, the use of wild-caught individuals means that assortative mating could reflect some unknown combination of genetic differences, plasticity and prior experience. Of course, each of these design elements is typical in many previous studies asserting inferences about assortative mating in fishes and other taxa. Their presence in our study reflects the trade-offs associated with the logistical challenges of allowing near-natural reproductive behaviour in relatively large and long-lived fish.

Context dependence of assortative mating within the experiment

Much of available knowledge on assortative mating in salmonids focuses on body size as the primary variable driving mate choice (Blanchfield & Ridgway, 1999; Petersson *et al.*, 1999; Labonne *et al.*, 2009); but, as previously mentioned, body size was similar between our populations and experimental treatments. Assortative mating, and its context dependence in our experiment, must therefore be shaped by other mechanisms. Although it is difficult to conclusively infer what these mechanisms might be, we can at least rule out some typical expectations. First, although intrasexual competition in females influences mating patterns in salmonids (Quinn, 1999; Adkinson *et al.*, 2014), nearly all females in our experiment laid eggs and mated in both environments. Second, although intrasexual competition in males can be even more important (Petersson *et al.*, 1999), it does not explain why mating success of Bastan males is higher with Bastan females than it is with Urumea females in the Variable environment.

Evidence against the importance of intrasexual selection leads naturally to a consideration of intersexual selection, which is usually thought to play a lesser role in mating success variation in salmonids (Petersson *et al.*, 1999). On the male side, choosiness is assumed to be weak because dominant males can most effectively maximize their overall reproductive success by simply by moving from female to female depending on their readiness to release eggs (Kokko & Monaghan, 2001). Indeed, in our experiment, no evidence emerged that males of either population actively discriminated against females of either population in either environment (Fig. 2). On the female side, choosiness is more documented and may in fine play a significant role in

the mating patterns (Labonne *et al.*, 2009; Gil *et al.*, 2016). In our experiment, we suggest that female-driven intersexual selection could be a primary driver of assortative mating and its context dependence. For instance, Bastan females mated almost exclusively with Bastan males, whereas Urumea females seem to be considerable less discriminant and these differences were most dramatic in the Variable environment (Fig. 2). This hypothesis is further supported by the fact that female reproductive investment differed between the two populations only in the Variable environment (both weight variation and energetic plasma metabolites variations during reproductive season were lower in Urumea than Bastan Females: Gauthey *et al.*, 2016).

Why would female preference for same-population males be context dependent? Work on other taxa suggests that such preferences are strongly influenced by various aspects of the local environment (Crowley *et al.*, 1991; Candolin, 1997; Seehausen *et al.*, 1997; Evans *et al.*, 2002; Atwell & Wagner, 2015). In the present context, we cannot be certain why flow regime might drive variation in choosiness that then shapes assortative mating. However, it is certainly true that variation in this environmental variable seems likely to influence a number of factors, such as predation risk (Allouche & Gaudin, 2001), female aggressiveness and maternal care (Labonne *et al.*, 2009), and possibly female deception (Petersson & Järvi, 2001), because the energetic cost of these behaviours is probably dependent on flow intensity. Further work will be needed to assess these, and other, possibilities.

Conclusions and implications

Our finding that two conspecific trout populations show positive assortative mating in one environmental context (experimental treatment) but not in another has several important implications. First, it confirms that assortative mating can be present between closely related and geographically proximate populations, which complements previous studies of salmonids that used genetic markers (e.g. Hendry *et al.*, 2000; Pearse *et al.*, 2009). Unlike those previous studies, however, we have here uncovered a specific reproductive barrier: assortative mating likely driven by female choice. Second, the striking context dependence of the assortative mating means that the strength of a reproductive barrier can depend critically on the specific local conditions. Although this outcome was not unexpected (see Introduction), it nevertheless highlights the need for experimental studies of assortative mating to employ multiple, ecologically relevant environments (see also Berdan & Fuller, 2012). Such context dependence might explain a lot of the variation in reproductive isolation in natural systems, although it remains difficult in our system – and others – to pinpoint the most important causal factor(s).

The context dependence of mating isolation could have important implications in relation to environmental change. If, for example, flow regimes really are the reason for context dependence in our experiment, we might be concerned with how such regimes will change in the future, with a frequent prediction being an increase in stochasticity (Milly *et al.*, 2002; IPCC 2013). Depending on the specific populations and environments, our results imply that mating isolation could increase or decrease, which could have a number of potential consequences. As one example, an increase in mating isolation should reduce gene flow which could then reduce the potential for adaptation to changing conditions (e.g. Bell & Gonzalez, 2011). Decreasing gene flow might also increase the potential for inbreeding, which could then depress population fitness in a number of ways (Kirkpatrick & Barton, 1997; Verhoeven *et al.*, 2011). By contrast, a *decrease* in mating isolation could reduce the distinctiveness of unique populations, thus comprising among-population diversity. Indeed, just such effects have been observed for cichlids (Seehausen *et al.*, 1997) and stickleback (Boughman, 2001). The key point is that we should be not only assessing the effects of environmental change on evolution within populations but also on interactions between them, specifically the degree of mating isolation and therefore gene flow.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1 Synthetic indicators of hydrologic alterations as calculated by IHA software (Richter *et al.* 1998).

Appendix S2 Examples of pictures taken before and after reproduction (a 2 months span), showing robes and specific patterns that allows for an individual recognition without tagging.

Appendix S3 Characteristics of fish (sex, weight, body size and offspring number and origin) used for the two experimental treatments (constant and variable flow).

Appendix S4 Estimated θ genetic distance and distribution of 10 000 bootstrapped values for θ obtained by random permutation of individuals between populations, using GeneLand R package (Guillot *et al.*, 2005).

Appendix S5 Matrices of mating success between pairs of mates for Constant (above) and stochastic (below) environments.

Appendix S6 Matrices of reproductive success between pairs of mates for Constant (above) and stochastic (below) environments.

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