Autosomal differences between males and females in hybrid zones: a first report from *Barbus barbus* and *Barbus meridionalis* (Cyprinidae)

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Narrow hybrid zones are generally subjected to the action of two forces: dispersal, which tends to homogenise the hybridising taxa, and selection against hybrids, which, in contrast, produces steep clines of introgression for diagnostic markers. Although differences between sexes in dispersal abilities or in susceptibility to hybrid counterselection are common in hybrid zones, autosomal genetic differences between males and females have never been reported to our knowledge. *Barbus barbus* and *Barbus meridionalis* (Cyprinidae) form a hybrid zone along the Lergue river. By carrying out a genetic analysis of males and females in six samples from two central stations of the hybrid zone using codomi-

nant markers (six allozymes and four microsatellite loci), we revealed significant multilocus and monolocus differences between the sexes. This could reflect a genetic difference among sexes within a same cohort, caused either by a survival (or fertility) differential among sexes or by a sex-specific pattern of dispersal. Alternatively, this may be due to genetic differentiation between cohorts, since male and female barbel exhibit different maturation, growth and survival patterns leading to different age distributions among sexes, and particularly among reproducers.

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Introduction

Narrow hybrid zones are those whose width is small relative to dispersal. They are often maintained by a dynamic equilibrium between two antagonistic forces: selection against hybrids and gene flow (Barton and Gale, 1993). These two antagonistic forces may act differentially on male and female genotypes. Indeed, it is well known that selection against hybrids is generally stronger against the heterogametic sex than the homogametic sex, termed Haldane's rule (Haldane, 1922; Turelli and Orr, 1995, 2000). Furthermore, dispersal often differs between sexes: for instance, in mammals, dispersal is generally achieved by males, whereas in birds females disperse more (Greenwood, 1980; Johnson and Gaines, 1990). Differences in dispersal may lead to differences in gene flow and therefore to differences in the genetic characteristics of both sexes.

Several studies of hybrid zones reported that markers located in autosomes, sex chromosomes or mitochondrial DNA exhibited different patterns or cline shapes (Vanlerberghe *et al*, 1986; Nancé *et al*, 1990; Sperling

and Spence, 1991; Dod *et al*, 1993; Fel-Clair *et al*, 1996; Boissinot and Boursot, 1997). Surprisingly however, sex differentiation at autosomal molecular markers, a totally different phenomenon, has not, to our knowledge, been reported in hybrid zones. The fact that cline shapes are different between markers located in mitochondrial genome, in autosomes and in sex chromosomes can be explained by simple tension zone models, without involving differences between sexes in selection or dispersal. The location of genes strongly involved in postzygotic isolation and the numbers of copies of each marker are important explanatory factors; mitochondrial DNA and the hemizygote sex chromosome (Y or W) are always transmitted by the same sex and, therefore, can reflect evolutionary forces that accumulate over generations, such as mutation or genetic drift. Conversely, differences in autosomal allele frequency between sexes necessarily arise from short-term effects because half the autosomes were in the opposite sex in the last generation.

In this paper, using four microsatellite, and six allozymic, diagnostic loci, we characterise the genotypes of males and females from six samples taken at two locations at different dates near the centre of the hybrid zone of *Barbus barbus* L. 1758 and *B. meridionalis* Risso 1826 (Cyprinidae). We investigate whether or not there are any differences at these loci between the sexes. We expected to find such differences because (i) an experimental cross between *B. barbus* and *B. meridionalis* (Philippart and Berrebi, 1990) revealed that hybrid

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dysgenesis (ie endogenous selection, independent of environment and attributed to the rupture of coadapted gene complexes) strongly varies among sexes, and (ii) there is direct evidence that dispersal patterns are different between male and female barbel (Baras and Cherry, 1990).

Materials and methods

Barbel and the hybrid zone of the Lergue river (southern France, Hérault basin)

Both species exhibit marked differences in morphology, genetics and ecology. *B. barbus*, the larger species, lives in lowland rivers and is never found in small mountainous streams where it apparently cannot survive. It recently colonised the Hérault river, probably after the last glaciations, whereas the presence of *B. meridionalis* is more ancient (about 5 my) as attested by fossils from the Miocene (Persat and Berrebi, 1990). The much smaller *B. meridionalis* is found in medium-altitude Mediterranean rivers but also lives in lowland rivers, which have not been colonised by *B. barbus* (Berrebi *et al*, 1988). The upstream of the Lergue, a typical Mediterranean river, is inhabited by pure *B. meridionalis* populations, which are progressively introgressed by *B. barbus* downstream until the Lergue reaches the Hérault river, forming a 9-km-long hybrid zone. The sex ratio of adults is strongly biased towards males, but is highly variable between samples (unpublished data). This is at least partly due to a shorter life span of females, which also grow faster, whereas the numbers of male and female zygotes formed at fertilisation are equal (Baras, 1992).

Strong linkage disequilibria detected with allozymic markers (Crespin and Berrebi, 1994; Crespin, 1996), as well as patterns displayed by morphological markers (Crespin *et al*, 1999), suggested that gene flow plays an important role in the functioning of this hybrid zone. There is evidence for both exogenous selection (*B. barbus* is unable to live in typical *B. meridionalis* habitats and a capture-marked-recapture study demonstrated marked differences in survival between upstream and downstream sites (Crespin *et al*, 2002); and endogenous selection (eg more than 50% of experimental F1 hybrids are unviable or sterile; Philippart and Berrebi, 1990; Crespin, 1996). Clines along the hybrid zone were detected using diagnostic allozymic (Crespin and Berrebi, 1994; nine locations) and microsatellite loci (Chenuil *et al*, 2000; five locations). The introgression index was calculated as the percentage of alleles diagnostic of *B. meridionalis* in a given population (100% for a pure *B. meridionalis* population, 0% for a pure *B. barbus*). This hybrid zone appears to be asymmetric both for allozymic markers (some allozymic loci do not reach the 0% introgression at the downstream end where the Hérault river enters the Mediterranean sea; Crespin and Berrebi, 1994; Crespin, 1996) and morphological indices (Crespin *et al*, 1999).

Samples and locations

Two locations of about 1000 m², close to the centre of the hybrid zone, hence with a majority of hybrid genotypes, were sampled by electro-fishing (as in Crespin *et al*, 1999). The upstream station, Cartel, had an allozymic introgression index (defined above, see also below) of

about 60% in 1992 (Crespin and Berrebi, 1994) and an average microsatellite introgression index of about 40% in 1993–1995 (Chenuil *et al*, 2000), whereas the downstream location, Saint-Fréchoux, had a 40% introgression index with allozymes in 1992 and around 20% with microsatellites in 1993–1995. For each station, samples were taken in spring (the spawning season) and in winter, and were analysed with either allozymes or microsatellite loci (Table 1). Samples were named according to their location and, within each location, were ranked by decreasing sizes, for example, Fréchoux1, Fréchoux2, etc. Samples from the different seasons taken at a particular location may not be comparable because (i) concerted population movements have previously been inferred from significant temporal variations in introgression (Chenuil *et al*, 2000) and (ii) migrations for reproduction are suspected in this hybrid zone (unpublished field and microsatellite data from spawning individuals) and were proven for *B. barbus* in other rivers (Hunt and Jones, 1974; Baras and Cherry, 1990; Lucas and Batley, 1996). Therefore, despite small female sample sizes, we were unable to analyse larger samples by pooling different dates for each location.

Genotyping techniques and marker loci

A total of 10 diagnostic allozymic markers distinguish the two hybridising *Barbus* species (Berrebi *et al*, 1993). Only six of them (*AAT2**, *GDA**, *LDH3**, *MDH2**, *PT2**, *6PGDH1**) provide an unambiguous distinction between heterozygote hybrids and parental genotypes (Crespin, 1996) and were used in this study. Five diagnostic or semidiagnostic microsatellite loci were analysed using the laboratory methods described by Chenuil *et al* (1997). One of them, *Barb65*, was omitted from all analyses because of the presence of null alleles. The four other loci, characterised in controlled crosses, appear to be unlinked to sex chromosomes (Chenuil *et al*, 1998). All loci had alleles diagnostic of one or both pure species; some loci were diagnostic loci *sensu stricto*. Locus B62 has two alleles, one of them being absent from *B. barbus* pure populations; locus B59 has 17 alleles, of which three and 12 are respectively diagnostic of *B. barbus* and *B. meridionalis*, whereas one allele is too rare to display a clinal pattern and another allele has its maximum frequency (12%) in a station of the hybrid zone (Saint-Fréchoux); locus B79 has six alleles, respectively one and five alleles diagnostic of *B. meridionalis* and *B. barbus*; and locus B37 has one diagnostic allele for each species (Chenuil *et al*, 1997, 2000). Introgression indices are calculated as the frequency of diagnostic *meridionalis* alleles (ie alleles absent from pure *B. barbus* populations) divided by the sum of the frequency of all alleles diagnostic of either *B. meridionalis* or *B. barbus*, so that even for loci B62 and B59 introgression indices theoretically range from 1 to 0.

Definition of fish sex classes

Five sex classes were defined according to the method used on the sample, namely M+ (sperm-emitting male), M− (male not emitting sperm), M (male), F (female) and F+ (ripe female) and U (undetermined). For the five samples analysed for allozymes, fish were dissected to examine the gonads. In sample Fréchoux1 (Table 1), we

Table 1 Samples ranked by size

Sample	Date	Total N	F	M	U	Markers
Cartel1	May 1995	136	14 (F+)	77 (M+)	45	Microsatellites
Fréchoux1	April 1990	105	17	13 (M–) 75 (M+)		Allozymes
Fréchoux2	Feb. 1992	40	15	25		Allozymes
Cartel2	Feb. 1992	28	10	18		Allozymes
Fréchoux3	May 1986	23	6	17		Allozymes
Fréchoux4	Jan. 1989	21	7	14		Allozymes

Males are designated by M, females by F, and individuals for which sex could not be determined by U. The '+' sign indicates gamete emission at the time of the sampling, the '–' sign indicates the absence of gamete emission for males during sampling in the spawning season.

also distinguished sperm-emitting males (denoted M+) from males that did not emit sperm (M–). For the single sample analysed with microsatellites, Cartel1, dissection was not performed since individuals were released for a capture–recapture survey, after a tissue sample was taken for genetic analysis. Because this sample was collected on spawning days, the sex could be determined by whether sperm or ova were released from the genital papilla by abdominal stripping. Therefore, males with mature sperm, denoted M+, could be identified, as well as mature females, denoted F+. Some individuals, which may have been juveniles, or sterile individuals, or simply adult males or females that had already spawned or whose gametes were not yet mature, were classified as 'undetermined' (denoted U).

Population genetic analyses between and within sex classes

For each sample, global and pairwise tests of genetic differentiation (ie differences in gene frequency) among sex classes, as well as tests of excess and deficit in heterozygotes relative to the Hardy–Weinberg equilibrium within each sex class were carried out. All analyses performed using the 'Genetix' software (Belkhir *et al*, 1998), using Wright's (1969) F statistics F_{IS} and F_{ST} , were also performed by corresponding exact tests, namely 'Hardy–Weinberg equilibrium' and 'population differentiation' tests, using the 'Genepop' software (Raymond and Rousset, 1995). F statistics were obtained using Weir and Cockerham (1984) estimators and their significance level was tested by comparison with a distribution generated by 1000 permuted data sets in which multilocus genotypes are not disrupted (so that significance levels are not affected by any heterozygote deficiency within populations) and randomly allocated to the same number of populations of identical size.

The sequential Bonferroni correction for significance levels (Rice, 1989) was performed for F_{ST} values between sex classes. As there were six samples, the most significant sample must display a significance level of 0.008 (ie 0.05 divided by six samples) to be considered significant at the 0.05 level, the second most significant one must display a significance level of 0.01 (0.05 divided by five remaining samples), and so on.

An additional approach was used to visualise and test genetic differentiation between sex classes. For each sex class, we determined the distribution of individual introgression, which is the array of the numbers of individuals respectively displaying zero, one, two, ... to $2n$ diagnostic alleles of the species *B. meridionalis* (where

n is the number of loci). The histogram of this distribution provides a way of visualising 'parental' genotypes (0 or $2n$ *B. meridionalis* alleles) and hybrid genotypes. We then computed, for each sample, Fisher's exact P -value, using the Genepop software (Raymond and Rousset, 1995), to test the hypothesis that all sex classes had identical distributions of individual introgression.

The mean introgression index, defined as the frequency of *B. meridionalis* alleles (see Materials and methods), was computed for all sex classes in each sample.

Results

The results of F_{IS} and F_{ST} analyses were similar to the results of exact tests. For the sake of simplicity, only F_{IS} and F_{ST} will be presented. Only the two largest samples displayed significant genetic differences between sex classes. Results of F_{ST} significance levels for global and pairwise, multilocus and monolocus analyses are shown in Table 2. Table 3 displays introgression indices for all sex classes as well as F_{IS} values and levels of significance in all six samples.

In Cartel1, allele frequencies in ripe females are significantly different from those in sperm-emitting males (Table 2a) both for multilocus and monolocus analyses (all four loci). Global F_{ST} values (ie considering

Table 2 Multilocus and monolocus tests of genetic differentiation for Cartel1 (a) and Fréchoux1 (b)

(a)					
Sex classes	All	Barb37	Barb59	Barb62	Barb79
F+/M+/U	***	***	**	***	***
F+/M+	***	***	*	*	*
M+/U	***	**	***	*	***
F/U	NS	*	NS	NS	NS
(b)					
Sex classes	All	LDH	MDH	Each of four other loci	
F/M+/M–	**	*	***	NS	
F/M	*	*	*	NS	
M+/M–	*	NS	*	NS	
F/M–	*	*	***	NS	
F/M+	#	NS	**	NS	

Significance level of F_{ST} values are * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. For Cartel1, alleles diagnostic of each species were pooled as synthetic diagnostic alleles (loci B59 and B79). When all alleles were considered, significance levels of F_{ST} between F+ and M+ decreased, and were no longer significant for locus L59.

Table 3 Mean introgression index and multilocus F_{IS} value for each sex class

Sample	Mean introgression index		F_{IS}	
	F	M	F	M
Cartel1	0.550 (F+)	0.273 (M+)	0.238** (F+)	0.047 (M+)
	0.456 (U)		−0.082 (U)	
Fréchoux1	0.249	0.335 (all M)	−0.110	−0.17*** (all M)
		0.453 (M−)		−0.48*** (M−)
		0.314 (M+)		−0.12*** (M+)
Fréchoux2	0.369	0.383	0.174*	0.149*
Cartel2	0.558	0.652	0.239*	0.108
Fréchoux3	0.361	0.422	0.038	−0.170*
Fréchoux4	0.333	0.309	−0.138	0.135

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. **Figure 1** Histograms of individual introgression for the different sex classes of the two largest samples, Cartel1 and Fréchoux1. The vertical axis indicates the number of individuals, the horizontal axis indicates the number of 'meridionalis' alleles. a: Sample Cartel1 (four microsatellite loci), b: sample Fréchoux2 (six allozymic loci). Black bars: sperm-emitting males (M+); hatched bars: females (a: F+; b: F); grey bars: undetermined individuals (U) in a, males not emitting sperm (M−) in b.

all sex classes, including undetermined individuals) also are significant for all monolocus and multilocus analyses. The distribution of individual introgression of females seems skewed towards both extremes (Figure 1), females appearing more 'parental' than males, and the distributions of individual introgression are significantly distinct between each pair of sex classes ($P < 0.01$ for F+ /U and $P < 0.001$ for F+ /M+ and M+ /U). We observed a very significant heterozygote deficiency in females ($F_{IS} = 0.238$) (Table 3), but not in males or undetermined individuals.

In Fréchoux2 also, gene frequencies are significantly different between females and males (pooling sperm-emitting and non-emitting ones) as revealed by the multilocus and two of the six monolocus analyses

(Table 2b). Overall F_{ST} values (considering the three sex classes F+, M− and M+) are significant for the same multilocus and monolocus analyses. Individual introgression does not appear skewed as for Cartel1, but females still appear more 'parental' ('less hybrid') than males, since in this sample the global introgression parameter is lower than 0.5 (individuals are genetically closer to 'barbus' than 'meridionalis') and even lower in females than in males (Table 3). However, differences in individual introgression distributions between sex classes do not reach significance. Heterozygotes are in excess ($P < 0.001$) in each sex class, but in absolute value, females have higher F_{IS} than males, as in Cartel1 (Table 3).

Introgression indices are not systematically higher for one sex: they are higher for females in Cartel1 (although very heterogeneous among females, Figure 1) but higher for males in Fréchoux1 (Table 3).

The other smaller samples did not present significant F_{ST} between sex classes, but a few significant F_{IS} (Table 3). However, when considering the results from all six samples (ie after the Bonferroni correction), differences between males and females as well as global F_{ST} remain significant ($P_{cor} < 0.01$ level for Cartel1 between F+ and M+, and $P_{cor} < 0.01$ for Cartel1 and $P_{cor} < 0.05$ for Fréchoux1 considering all sex classes), meaning that there actually are gene frequency differences among sexes that cannot be attributed to multiple testing. Moreover, the phenomenon does not seem to be due to a particular locus, but is rather general, as revealed by monolocus analyses in which three (when considering all alleles) or four (when creating synthetic diagnostic alleles) of the four microsatellite loci analysed (Cartel1) and two of the six allozymic loci (Fréchoux1) displayed significant F_{ST} despite moderate sample sizes (Table 2).

Many samples displayed significant positive or negative multilocus F_{IS} values (Table 3), most significant values being displayed by the largest samples. Average multilocus F_{IS} value of the six samples (weighted by female and male sample sizes) was much higher for females (0.0829) than for males (0.0297). Despite the smaller size of female samples, more positive F_{IS} values were significant in females than in males or undetermined individuals. In five samples out of six, females have higher F_{IS} values than males (nonsignificant Wilcoxon's signed ranked test). This set of observations may be an indication that the female component of the population had a higher heterozygote deficiency than

males although additional sampling would be necessary to establish this point (eg by obtaining a significant Wilcoxon test).

Discussion

We demonstrated that there were significant genetic differences between sex classes in the Lergue hybrid zone. Pairwise analyses confirmed that there were gene frequency differences between males and females. These differences are more salient when comparing ripe females and sperm-emitting males (Cartel1) than when comparing just males and females (Fréchoux1). Moreover, we show that these genetic differences were observed for both microsatellite and allozymic markers and appeared to be a general phenomenon at the genomic level since monolocus analyses were significant for all microsatellite loci and two allozymic markers, nonsignificant results at four allozymic loci being possibly explained by small sample sizes. This observation allows us to rule out hypotheses involving selective effects affecting particular loci, and to focus on more general explanations. Explanations involving systematic introgression differences between sexes (such as, for instance, the assumption that females genetically closer to *B. barbus* would survive better) can also be eliminated since the most introgressed sex can be either males or females according to sample.

Our results are the first demonstration of gene frequency differences among sexes at apparently neutral autosomal markers in hybrid zones. Several mechanisms may generate genetic differentiation between sexes in hybrid zones, but we will focus on the most likely ones, for the sake of simplicity. Males and females are not separate genealogical entities at the scale of several generations: their autosomal genes are mixed at each generation. Therefore, long-term evolutionary forces such as drift or mutation could not generate autosomal genetic differences between sexes. Selection and migration have short-term (one generation) effects and provide likely explanations. Most hybrid zones can be modelled as tension zones (Barton and Hewitt, 1985), and tension zone models *sensu stricto*, in which selection acts against heterozygotes, highlight two mechanisms that are likely to generate genetic differences among sexes. The shape of introgression clines results from dispersal, which tends to homogenise the two taxa, and selection against heterozygotes, which has the opposite effect and makes clines steeper in the centre of hybrid zones (clines are sigmoid). The stronger the selection, or the lower the dispersal, the steeper the cline and the narrower the hybrid zone (Figure 2).

(1) Assuming a differential viability between sexes, the sex whose hybrids are less viable should display higher heterozygote deficiency (more heterozygotes are eliminated) and steeper and narrower clines since individuals with more parental genotypes are in excess. Except at the centre and at the two boundaries of the hybrid zone, males and females should have different introgression indices at diagnostic or semidiagnostic loci, thus allele frequency should differ between sexes (Figure 2). Controlled crosses between pure *B. barbus* males and *B. meridionalis* females produced only F1 males (females being unviable) whose fertility was not investigated, whereas the reciprocal cross produced sterile males and

Figure 2 Different cline shapes lead to different gene frequency among sexes (except at the centre and boundaries of the hybrid zone), which can be detected by F_{ST} . We can predict that least dispersing or less viable sex (or also less fertile sex, if fertile males and females are compared) will form a steeper cline (thicker line).

fertile females (Philippart and Berrebi, 1990). Thus it seems likely that hybrid females of the Lergue river are more affected than hybrid males by selection on viability, following Haldane's rule since in barbels, females are the heterogametic sex (Castelli, 1994). Fertility and viability generally follow the same pattern; thus, it is highly probable that fertility also is lower in hybrid females, although experimental data are lacking to confirm this. This hypothesis may account for the observation that in Fréchoux1, females (F) are significantly different from males (M) but not from sperm-emitting males (M+): viability selection may have eliminated more hybrid females than males, leading to gene frequency differences between sex classes F and M, whereas M+ were in addition submitted to fertility selection eliminating hybrids, making this class genetically closer to the F sex class than was the M sex class.

(2) Assuming different patterns of dispersal between sexes, the sex that disperses more should display higher heterozygote deficiency at all loci as a result of the Wahlund effect at the centre of the hybrid zone, and clines should be smoother (Figure 2). As for a selective differential between sexes, the position (ie centre) of the cline should remain the same for both sexes if dispersal is symmetric. Radio-tracking data, obtained in a river different from the Lergue, revealed that females of pure populations of *B. barbus*, unlike males, may move over large distances (9 km) for reproduction (Baras and Cherry, 1990).

Thus, genetic and nongenetic data support the possibility that barbel females disperse more or are less viable (probably also less fertile) than males in the hybrid zone (scenario illustrated in Figure 3). Under both nonexclusive hypotheses, higher heterozygote deficiency is expected in females. Our genetic data corroborate this prediction, with a tendency for higher F_{IS} in females. Nonsignificance of this trend may be explained by reduced sample sizes (in particular, the low female sample sizes) and low power of the Wilcoxon's signed ranked test for small numbers of replicates (six samples). Furthermore, the fact that genetic differences are higher when comparing ripe females with sperm-emitting

Step 0: Identical cline for males and females

Step 1: Action of selection or migration differing among sexes

In both cases, Females have higher heterozygote deficiency (F_{IS}) than males

Step 2: Reproduction among males and females differently introgressed

In the newly produced cohort of zygotes, heterozygotes are in excess (negative F_{IS}), but clines are identical for males and females, as in step 0) before selection or migration decreases their proportion, and the F_{IS} value

Figure 3 Simple scenario, involving single-generation effects only, to explain data observed in the barbel hybrid zone by differences in selection or dispersal among sexes. Clines of females are represented by thick lines.

males (Cartel1) than when just comparing viable males and females (Fréchoux1) suggests that an additional selection (on a component of fertility) increased the genetic differences already generated by selection on viability (as expected under Haldane's rule). As shown in Figure 3, comparing male and female clines would allow us to distinguish among these hypotheses, but owing to temporal variation in introgression indices, this would require sampling all stations along the hybrid zone in a very short time span.

(3) Alternatively, genetic differentiation between sexes could be due to the fact that males and females of a sample belong to distinct cohorts that are genetically different (due to temporal genetic differences). Observations support the two assumptions of this hypothesis. First, significant temporal differences in introgression at a given location were reported in this hybrid zone (Chenuil *et al.*, 2000). These fluctuations were correlated with the river flow and at least partly explained by fish movement, and it is likely that smaller hence younger individuals were more strongly affected. Second, males are known to reproduce before females in the wild, and when they are less than 10 cm, whereas females start reproducing after reaching a given size, possibly to avoid male harassment during courtship behaviour (Baras, 1992). Third, this study reports genetic differences between sperm-emitting and non-emitting males (Fréchoux1), which may be caused by genetic differences among cohorts and by the fact that sperm-emitting males are statistically older than the other males, which include juveniles. Therefore, female and male reproducers probably have distinct demographic pyramids.

Given the available data in the barbel hybrid zone of the Lergue, quantifying the relative parts of these three nonmutually exclusive hypotheses is difficult. However, whatever the causes of such differences, reproduction

among differentially introgressed males and females probably has important consequences. For instance, it should produce heterozygote excess in the new cohort before selection and migration partly or totally counter this effect, eventually leading to heterozygote deficiency (Figure 3). Indeed, some of our samples displayed significant heterozygote excesses (Table 3). According to sample (eg whether it contains more or less individuals that were already submitted to selection and dispersal), processes leading to excess and processes leading to deficiency in heterozygotes have a variable relative importance, so that negative or positive F_{IS} values may be observed. Random differentiation between small populations of males and/or females may also contribute to heterozygote excess (Luikart and Cornuet, 1999) in this hybrid zone, since the population of reproducing females at a given time may be very small (sex ratio is strongly biased and the fertility period is very short for a given female). Sex-specific dispersal also can lead to heterozygote excess, as reported by several field studies, theoretical and genetic marker studies (eg Schwartz and Armitage, 1980; Prout, 1981; Webb *et al.*, 1995; Prugnolle and DeMeeus, 2002). Whatever its cause, the production of an excess of heterozygote zygotes, which in tension zone models are counterselected, will lead to an increase in the hybrid sink relative to hybrid zones without sex differentiation.

Conclusion

We observed genetic differences between males and females sampled at given times and places in the barbel hybrid zone. A few different causes could produce autosomal genetic differences among sexes at autosomal loci. Two of them, selection and migration differences among sexes, are commonly observed in nature and occur in the barbel hybrid zone, in the very direction required to explain our data. However, given that barbel are long-lived species with overlapping generations and females reproduce at a much older age than males, it is also possible that differentiation stems from differences among generations. Further investigation is needed to establish the relative contributions of the different causes. This study reports for the first time a phenomenon that is likely to be common, and whose consequences are not currently taken into account by hybrid zone dynamics theory.

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