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Genetic differentiation within and away from the chromosomal rearrangements characterising hybridising chromosomal races of the western house mouse (*Mus musculus domesticus*)

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Abstract The importance of chromosomal rearrangements for speciation can be inferred from studies of genetic exchange between hybridising chromosomal races within species. Reduced fertility or recombination suppression in karyotypic hybrids has the potential to maintain or promote genetic differentiation in genomic regions near rearrangement breakpoints. We studied genetic exchange between two hybridising groups of chromosomal races of house mouse in Upper Valtellina (Lombardy, Italy), using microsatellites. These groups differ by Robertsonian fusions and/or whole-arm reciprocal translocations such that F_1 hybrids have a chain-of-five meiotic configuration. Previous studies showed genetic differentiation in two chromosomes in the chain-

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of-five (10 and 12) close to their centromeres (i.e. the rearrangement breakpoints); we have shown here that the centromeric regions of the other two chromosomes in the chain (2 and 8) are similarly differentiated. The internal chromosomes of the chain (8 and 12) show the greatest differentiation, which may reflect pairing and recombination properties of internal and external elements in a meiotic chain. Importantly, we found that centromeric regions of some non-rearranged chromosomes also showed genetic differentiation between the hybridising groups, indicating a complex interplay between chromosomal rearrangements and other parts of the genome in maintaining or promoting differentiation and potentially driving speciation between chromosomal races.

Keywords chromosomal hybrid zone · introgression · microsatellites · Robertsonian fusion · speciation

Abbreviations

CHPO	Poschiavo chromosomal race of the house mouse
cM	centiMorgan
F_{CT}	Among group fixation index
ILVA	Lower Valtellina chromosomal race of the house mouse
IMVA	Mid Valtellina chromosomal race of the house mouse
IUVA	Upper Valtellina chromosomal race of the house mouse

Introduction

One of the most enduring interests in chromosomes is their possible role in speciation. Among eukaryotes, there are many examples of closely related species differing from one another in karyotype, posing the possibility that chromosomal rearrangements may have promoted reproductive isolation between them (King 1993). Meiosis is critical to this. During meiosis in diploid organisms, homologous chromosomal regions should pair, potentially recombine, and segregate. This process is error-prone, even when the homologues are structurally the same. If the homologues are structurally different, as they are in the hybrids between karyotypically different taxa, then there can be serious anomalies (as we outline below), and the presence of these anomalies may potentially promote reproductive isolation (and therefore speciation) in various ways; this is true for all sexually reproducing eukaryotes, including mammals (King 1993; Searle 1993; Rieseberg 2001; Brown and O'Neill 2010; Faria and Navarro 2010). This does not necessarily mean speciation was driven by chromosomal rearrangements in all cases where closely related species differ in karyotype. However, as long as chromosomal rearrangements can overcome the hurdle to become fixed, and particularly if they can accumulate within taxa, they have the potential to be potent causal agents of speciation (Rieseberg 2001).

Mammals are karyotypically variable, and sister species that are chromosomally different often occur. There is a long history of studies in mammals pertaining to the role of chromosomes in speciation, including detailed meiotic research on chromosomal races—geographic forms within species that differ in karyotype—and hybrids between them (Searle 1993; Garagna et al. 2014). Pure race individuals are karyotypic homozygotes and hybrids are karyotypic heterozygotes; it is meiosis in such heterozygotes that is expected to be particularly error-prone. Errors of meiotic pairing associated with karyotypic heterozygosity create unpaired regions that typically lead to germ cell death in mammals, through well-documented pathways (Turner et al. 2005). One way that cells with unpairing can be rescued from germ cell death is through non-homologous pairing (Turner et al. 2005), but this also has implications since homologous recombination, by definition, is not possible in the non-homologously paired regions (nor, of course, in regions where there is unpairing). Unpairing, non-homologous pairing and lack of recombination all occur in the vicinity of chromosomal breakpoints (the sites of the chromosomal rearrangements) in karyotypic heterozygotes (Searle 1993). In the case of short inversions where the breakpoints are close together on the same chromosome, there may be non-homologous pairing between the two breakpoints and an associated absence of recombination (Hale 1986). This is interesting, because it prevents the cell from producing unbalanced gametes that would arise if there were recombination in the region of the inversion. Larger inversions may behave more 'normally' by pairing homologously, leading to occasional within-inversion recombination and 'abnormal', unbalanced, gametes (Rieseberg 2001).

In mammals, as in other diploids, unbalanced gametes can be produced either through recombination between the chromosomal breakpoints in inversion

heterozygotes or through malsegregation (= nondisjunction) of tandem fusion, reciprocal translocation or Robertsonian fusion heterozygotes (Daniel 1988). While malsegregation can occur in karyotypically standard (homozygous) individuals, it is more likely to occur in heterozygotes for chromosomal rearrangements (Searle 1993). Unbalanced gametes in mammals survive (Ford and Evans 1973) but lead to embryos that show abnormalities, with greater impact of monosomy of chromosomal regions than trisomy (Epstein 1986). Monosomy of large chromosomal regions leads to preimplantation lethality, and trisomy to later, but usually prenatal, death (Epstein 1986).

With regards speciation, both germ cell death and embryonic lethality may contribute to reproductive isolation. In the extreme, two chromosomal forms may produce hybrids that are completely sterile, and therefore the two forms would be completely reproductively isolated. But a lesser degree of chromosomally induced infertility may still promote speciation. The chromosomal breakpoints, or region between chromosomal breakpoints in the case of inversions, may be considered unfitness loci (Panithanarak et al. 2004). Such unfitness loci act as centres of 'genomic islands of speciation' (Harrison 1990; Feder et al. 2012). Gene flow will be reduced in such regions, and therefore pre-existing genetic differentiation may be retained, and potentially added to, in them. Regions of recombination suppression will also be areas where gene flow will be reduced between the hybridising forms and therefore regions where genetic differentiation will be retained and potentially expanded (Rieseberg 2001). Therefore on this model of speciation, at the contact of two chromosomal races, it is to be expected that genetic differentiation between the two races will be limited to the genomic regions around the rearrangement breakpoints, and it is the evolution of incompatibilities or alleles that promotes assortative mating within this genomic region that leads to reproductive isolation (Piálek et al. 2001; Rieseberg 2001; Brown and O'Neill 2010; Faria and Navarro 2010).

However, the expectation at the contact of chromosomal races may not be as simple as this, and there may be differentiation away from the rearrangement breakpoints as well (Giménez et al. 2013). If the races became differentiated genome-wide in allopatry, and if the erosion of that differentiation after meeting and hybridising over multiple generations (Endler 1977) is incomplete, regions away from rearrangements may retain differentiation at the current time. A second possibility is that the genetic differentiation close to the breakpoints of the chromosomal rearrangements may maintain differentiation elsewhere through epistasis (Giménez et al. 2013). Thirdly, there may be a more non-specific effect that could cause the unfitness loci represented by the chromosomal rearrangements and other unfitness loci in the genome to maintain their differentiation in concert (Barton and Hewitt 1985). In other words, a whole group of unlinked alleles at unfitness loci specific to one race may not be able to spread across the area of contact and hybridisation (the 'hybrid zone') between the races; this also applies to new alleles that arise at unfitness loci after the hybrid zone is formed.

Because of the differing possible outcomes in terms of genetic differentiation at the contact between chromosomal races, there is a need to examine genetic differentiation both close to rearrangement breakpoints but also elsewhere in the genome. Here we describe such studies relating to a chromosomal hybrid zone in the house mouse. The hybrid zone in question is found in Valtellina, an alpine valley of the Province of Sondrio, Lombardy, Italy (Hauffe and Searle 1993; Hauffe et al. 2004, 2012). While the standard karyotype of the house mouse consists of 40 telocentric chromosomes, there are four races in Upper Valtellina which have different combinations of metacentric chromosomes (Table 1). For the purposes of this paper, the Poschiavo race (CHPO; 2n = 26) and Mid Valtellina race (IMVA; 2n = 24) characterised by metacentric 8.12 and telocentrics 2 and 10 are collectively known as the '8.12 group', while the Upper Valtellina race (IUVA; 2n = 24, Fig. 1) and Lower Valtellina race (ILVA; 2n = 22) characterised by metacentrics 2.8 and 10.12 are collectively known as the '10.12 group'. Hybrids between the 8.12 and 10.12 groups are known as 'complex heterozygotes' because they produce meiotic configurations of greater than three elements, which are typically associated with substantial infertility in house mice (Searle 1993). In this case, they produce a chain-of-five meiotic configuration (2–2.8–8.12–12.10–10) and have been shown to suffer high levels of germ cell death and malsegregation (Hauffe and Searle 1998).

Previous studies on genetic differentiation in the Upper Valtellina system have concentrated on chromosomes 10 and 12 (Panithanarak et al. 2004; Giménez et al. 2013). These studies have demonstrated differentiation close to the centromere of both chromosomes, extending further along chromosome 12 than chromosome 10, and have also indicated the presence of

Table 1 Chromosomal characteristics of the chromosomal races of house mouse in Upper Valtellina (CHPO, IMVA, IUVA, ILVA) partitioned into the '8.12' and '10.12' groups

'8.12' group														
CHPO	1.3	**2**	4.6	5.15	**7**	**8.12**	9.14	**10**	11.13	16.17	**18**	19	XX/XY	2n = 26
IMVA	1.3	**2**	4.6	5.15	**7.18**	**8.12**	9.14	**10**	11.13	16.17		19	XX/XY	2n = 24
'10.12' group														
IUVA	1.3	**2.8**	4.6	5.15	**7**		9.14	**10.12**	11.13	16.17	**18**	19	XX/XY	2n = 24
ILVA	1.3	**2.8**	4.6	5.15	**7.18**		9.14	**10.12**	11.13	16.17		19	XX/XY	2n = 22

Chromosomes given in the format x.y are metacentrics; single chromosomes are telocentrics. In bold are the chromosomes that differ among the races. An example karyotype (IUVA) is shown in Fig. 1

differentiation at foci away from the centromere. We concluded that the pattern of differentiation could be explained by the centromeres of these chromosomes acting as unfitness loci but with the possible additional impact of recombination suppression, selective sweeps and/or epistasis (Giménez et al. 2013). The occurrence of recombination suppression near the centromeres of the chain-of-five configuration has been supported by synaptonemal complex analysis (Merico et al. 2013).

We here compare genetic differentiation in the centromeric regions of all the chromosomes which differ between the 8.12 and 10.12 groups (i.e. 2, 8, 10, 12; Table 1) with that seen in other autosomes and the X chromosome. By this wider coverage than has been attempted hitherto, this study reflects well the fascinating interplay of processes acting on chromosomal rearrangements and those acting elsewhere in the genome and shows that the role of chromosomes in speciation is complex.

Methods

Following the microsatellite typing methods described in Panithanarak et al. (2004) and Giménez et al. (2013), we analysed 97 of the 99 mouse samples listed in these papers for the autosomes 1–6, 8, 9, 11, 13–17 and 19 and the X chromosome, selecting three loci per chromosome arm as close as possible to the centromere based on the centiMorgan (cM) distance provided by Dietrich et al. (1996). For subsequent analysis, we only used results from microsatellite loci that amplified reliably and were polymorphic. The analysed loci and their cM distance from the centromere are given in Table 2. Among those loci selected for analysis, certain individuals failed to yield results; ESM 1 lists all the data obtained. Regarding those chromosomes not included in the microsatellite typing here, chromosomes 10 and 12 were analysed in the previously published work (Panithanarak et al. 2004; Giménez et al. 2013) and differentiation at chromosomes 7 and 18 relates to chromosomal variation between races *within* the 8.12 and 10.12 groups (Table 1) and therefore would be confounding for an analysis of differentiation *between* those groups.

Analyses of molecular variance (AMOVAs) were carried out separately for each locus using Arlequin 3.5.2.2 (Excoffier and Lischer 2010) to provide the among group fixation index (F_{CT}) which relates to differentiation between the 8.12 and 10.12 groups. As with Giménez et al. (2013), we carried out four separate AMOVAs named *W–Z* because the data were complex and we considered that there were several equally valid approaches for analysis.

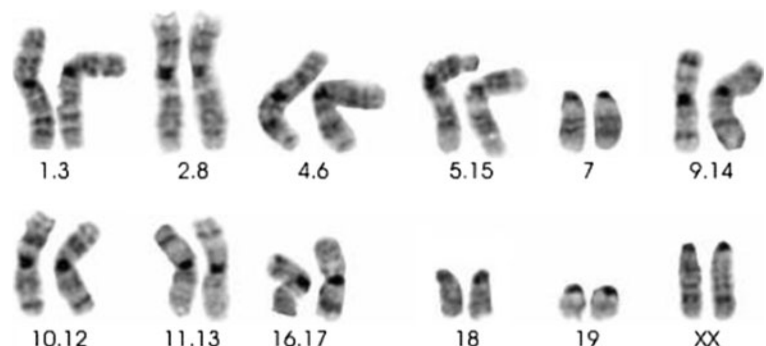

Fig. 1 Example karyotype of one of the chromosomal races within the '10.12' group, the IUVA race. The two metacentrics 2.8 and 10.12 are the chromosomes that differentiate the '10.12' group from the '8.12' group

Table 2 Among group fixation index (F_{CT}), values for analyses *W*–*Z* (see 'Methods') using centromeric microsatellite locus data from Upper Valtellina house mice

Locus	cM	*W*	*X*	*Y*	*Z*
Chromosome 1					
64	0.0	−0.025	−0.026	−0.056	−0.178
316	3.3	−0.073	−0.116	−0.101	−0.186
Chromosome 2					
1	2.2	0.131	**0.234***	0.184	0.064
312	2.2	0.013	−0.029	−0.012	−0.034
425	2.2	−0.123	−0.117	−0.087	−0.220
Chromosome 3					
149	2.2	0.064	0.039	0.050	**0.114***
267	6.6	−0.012	−0.036	−0.044	−0.059
Chromosome 4					
149	0.0	**0.457***	**0.588***	**0.560***	**0.400***
103	2.2	0.035	0.061	**0.064***	0.005
315	2.2	0.010	0.056	0.061	0.040
Chromosome 5					
145	0.0	−0.079	−0.007	−0.012	−0.128
146	0.0	0.012	−0.008	−0.002	−0.085
178	0.0	−0.002	**0.092***	**0.119***	**0.094***
Chromosome 6					
86	0.0	0.153	0.171	0.128	**0.295***
138	2.2	**0.234****	**0.310****	**0.277****	**0.180***
296	2.2	−0.089	−0.113	−0.095	0.195
Chromosome 8					
58	0.0	−0.095	0.119	0.117	0.193
155	0.0	0.209	**0.213***	0.175	**0.294***
157	1.1	**0.591****	**0.634***	**0.618****	**0.420****
Chromosome 9					
43	2.2	0.000	−0.070	−0.047	−0.185
218	2.2	−0.065	−0.096	−0.068	−0.134
219	3.3	−0.011	−0.011	−0.008	−0.015
Chromosome 11					
74	2.2	0.060	−0.008	−0.020	−0.122
Chromosome 13					
55	0.0	−0.103	−0.123	−0.094	−0.147
153	2.2	−0.051	−0.038	−0.018	−0.132
Chromosome 14					
220	1.1	0.016	0.174	0.136	−0.010
179	2.2	0.157	0.198	**0.177***	0.138
250	2.2	0.085	0.163	0.155	−0.020
Chromosome 15					
12	0.0	−0.088	−0.092	−0.083	−0.150
13	0.0	−0.109	−0.077	−0.063	−0.240
174	0.0	**0.392****	**0.397****	**0.397***	0.233
Chromosome 16					
32	0.0	−0.027	−0.017	−0.005	−0.147
107	3.3	−0.040	−0.064	−0.101	−0.200
182	3.3	−0.041	−0.011	−0.018	−0.163
Chromosome 17					
19	0.0	−0.018	0.016	0.047	0.001
164	1.1	−0.061	−0.034	−0.010	−0.031
Chromosome 19					
32	0.0	0.032	0.006	0.023	−0.123
43	0.0	−0.100	−0.136	−0.104	−0.235
29	1.1	−0.069	−0.118	−0.096	−0.181
Chromosome X					
89	0.0	0.035	**0.096***	**0.108***	0.055
55	1.1	**0.076***	0.074	0.082	−0.030
101	2.2	0.133	**0.233***	**0.234***	0.084

Locus names have been simplified; locus '64' of 'chromosome 1' refers to D1Mit64, etc. (see ESM 1). Values significantly different from zero are in bold

cM centiMorgan distance from the centromere, following Dietrich et al. (1996)

*$P<0.05$, **$P<0.01$

There were some villages dominated by 8.12 individuals, others dominated by 10.12 individuals and the villages of Sondalo and Sommacologna that were mixed with 8.12 and 10.12 mice (Fig. 2 and ESM 1). Among those localities dominated by one group (8.12 or 10.12), there could be individuals that were chromosomally homozygous for that group ('pure group chromosomal homozygotes' in ESM 1) and individuals that were not (due to introgression). The analyses *W* and *X* were limited to pure group chromosomal homozygotes: analysis *W* included those individuals within Sondalo and Sommacologna that were pure group chromosomal homozygotes; analysis *X* excluded individuals from these two populations. Analyses *Y* and *Z* were not so selective: all individuals within a village dominated by mice carrying the chromosome 8.12 were categorised as '8.12' even when some of the mice were not pure group chromosomal homozygotes (likewise for populations dominated by the 10.12 group). In analysis *Y*, individuals from Sondalo and Sommacologna were excluded from the analysis; in analysis *Z*, individuals from these two populations were grouped into a third category. These analyses consider gene flow in different ways. Analyses *W* and *X* address to what extent an individual that is chromosomally of one group

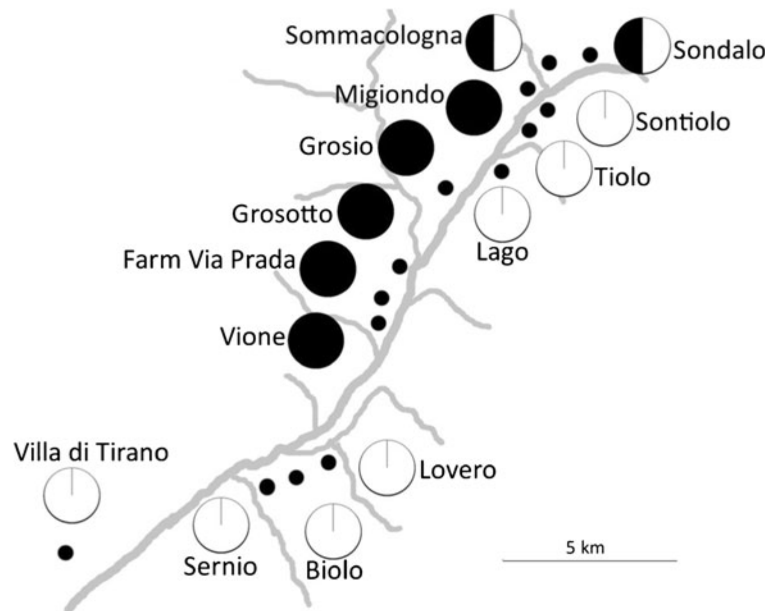

Fig. 2 Locations of villages along the River Adda in Upper Valtellina and allocation of the house mouse populations in those villages into the '8.12' and '10.12' groups, with *black circles* indicating 8.12 populations, *white circles* indicating 10.12 populations and *black + white circles* indicating 8.12 + 10.12 populations (see text and ESM 1)

(either a 8.12 mouse or a 10.12 mouse) is genetically of that group or genetically of the other group, locus-by-locus. Analyses *Y* and *Z* consider genetic exchange on a population basis, elucidating to what extent a population that can be categorised as either '8.12' or '10.12' shows introgression from the other group, again locus-by-locus.

Karyotypes of the mice studied here were determined by Hauffe and Searle (1993). The karyotype information needed for the analyses is given in ESM 1. In each village, all or nearly all individuals karyotyped by G-banding were pure group chromosomal homozygotes. Under these circumstances, we assumed other individuals from the same population with the same diploid number determined by conventional-staining also to be pure group chromosomal homozygotes. For individuals treated as pure group chromosomal homozygotes in the analyses, it is indicated in ESM 1 whether an individual's karyotype was determined by G-banding or inferred from the diploid number without banding.

Results

A total of 42 microsatellite loci were retained for the analysis of differentiation between the 8.12 and 10.12 groups of house mice in Upper Valtellina (Table 2). Five loci showed a significant F_{CT} value in analysis *W*, nine in analysis *X*, nine in analysis *Y* and seven in analysis *Z*. In general, there was consistency between the different analyses. Considering the ten examples of loci where one or both of the *W* and *X* analyses yielded a significant F_{CT} value, for nine of these the F_{CT} value was higher in the *X* analysis. The *W* analysis includes mice from Sondalo and Sommacologna, the 8.12/10.12 mixed populations, while *X* excludes these mice. This indicates that although there may be mice that chromosomally are homozygous 8.12 or 10.12 in Sondalo and Sommacologna, those mice often have a genetic contribution from the other group. This is consistent with successful interbreeding between the 8.12 and 10.12 mice where they occur together and the production of fertile hybrids (both in the field and in the laboratory; Hauffe and Searle 1998). Likewise, the *Y* analysis, that excludes Sondalo and Sommacologna at a population level, tends to generate higher, more frequently significant F_{CT} values than the *Z* analysis that includes them. Of the 12 loci where one or both of the *Y* and *Z* analyses yielded a significant F_{CT} value, for nine of these, the F_{CT} value was higher in the *Y* analysis. Therefore, the combination of allopatry and chromosomal difference enhances the differentiation at centromeric loci.

On a chromosome-by-chromosome basis, the highest level of differentiation was shown by chromosome 8, one of the group-specific chromosomes (Table 2). Locus D8Mit157 (i.e. microsatellite locus 157 of chromosome 8) has the highest F_{CT} values in all categories (*W*, *X*, *Y* and *Z*) of any chromosome, all significantly different from zero. The other two centromeric loci on chromosome 8

also had high (>0.1) F_{CT} values in all categories *W*, *X*, *Y* and *Z* (except the *W* analysis for D8Mit58), with analyses *X* and *Z* for D8Mit155 significantly different from zero. The other group-specific chromosome, 2, did show evidence of differentiation but not to the same degree: only one of the three loci, D2Mit1, showed mostly high F_{CT} values, and only one of the analyses (*X*) generated an F_{CT} value significantly different from zero.

Of the remaining elements, chromosome 6 is notable for two centromeric loci with high and/or significant F_{CT} values for all four analyses, with D6Mit138 showing mostly highly significant results. Chromosomes 4 and 15 also had single loci with notably high and significant F_{CT} values. The X chromosome had significant values associated with all three loci. Chromosome 5 had a single locus with mostly significant results, and chromosome 14 had a single locus with a single significant F_{CT} value but high and non-significant values over all loci.

Chromosomes 1, 3, 9, 11, 13, 16, 17 and 19 showed little or no evidence of differentiation. The data for chromosomes 11 and 13 confirmed previous results from Panithanarak et al. (2004) who looked at single loci on these chromosomes as well as their more extensive studies on chromosomes 10 and 12. For chromosome 11, the locus examined here was the same as that scored in Panithanarak et al. (2004).

Discussion

The previous studies by Panithanarak et al. (2004) and Giménez et al. (2013) showed that the centromeric regions of chromosomes 10 and 12 were significantly differentiated between the 8.12 and 10.12 groups of house mice in Upper Valtellina. There was also evidence that single centromeric loci of two chromosomes that did not differ between these groups (11 and 13) did not show significant differentiation. In Giménez et al. (2013), it was further shown that most loci farther from the centromere of chromosomes 10 and 12 were not significantly differentiated between the 8.12 and 10.12 groups. Förster et al. (2013) obtained similar results for house mouse chromosomal forms on Madeira. They showed that a group of chromosomal races characterised by chromosome 3.8 were highly differentiated from a group of chromosomal races with 3.14 and 8.11 at loci close to the centromere (i.e. proximal loci) on chromosomes 3 and 8 but not at interstitial or distal loci. As is the case for the Valtellina hybrid zone, hybrids between these groups are complex heterozygotes characterised by meiotic chain configurations of at least five elements. Franchini et al. (2010) also showed greater differentiation close to the centromeres than close to the telomeres of rearranged chromosomes at the contact between a metacentric chromosomal race and standard 40-telocentric house mice.

In the present study of Upper Valtellina mice, we have extended the analysis of centromeric regions to include the two other chromosomes that differ between the 8.12 and 10.12 groups, chromosomes 2 and 8, together with other autosomes and the X chromosome, which do not systematically differ between the groups. This allows us to further test the expectation of genetic differentiation close to the chromosomal breakpoints when comparing hybridising chromosomal forms. But it is also of interest to examine whether there is differentiation close to the centromeres of chromosomes not involved in chromosomal rearrangements, which has not been adequately explored in the house mouse before in the context of chromosomal hybrid zones.

Considering first chromosome 8, it showed notable centromeric differentiation reminiscent of that seen for chromosomes 10 and 12. All three centromeric loci examined showed evidence of differentiation. Of all the chromosomes examined in this study, this was the one that showed the greatest centromeric differentiation, supporting the important role of chromosomal rearrangements in genetic differentiation in the Valtellina hybrid zone.

The results for chromosome 2 were very different. There was a signature of centromeric differentiation but only at one of the loci. Also, there were several other chromosomes that are identical in both the 10.12 and 8.12 groups yet showed genetic differentiation of similar or greater magnitude than chromosome 2, namely autosomes 4, 5, 6, 14 and 15 and the X chromosome.

In terms of the chain-of-five meiotic configuration (2–2.8–8.12–12.10–10) expected in hybrids in the Upper Valtellina hybrid zone, chromosome 2 is one of the end elements, while chromosome 8 is an internal element next to it. It is the internal element (chromosome 8) which is most differentiated close to the centromere. For the other two chromosomes, at the other end of the chain (10 and 12), again the internal element (chromosome 12) is more differentiated than the end element (chromosome 10)(Giménez et al. 2013). Thus, for chromosome 12, all six loci screened in the interval 0–8.7 cM (0, 2.2, 5.5, 7.7, 7.7, 8.7) showed significant differentiation for all loci and

all tests (*W*, *X*, *Y*, *Z*) except for the locus at 8.7 cM, where the tests for *W* and *Y* were not significant. However, for chromosome 10, only the most proximal three out of the seven loci in this interval (0, 2.2, 3.3, 5.5, 5.5, 7.7, 8.7) showed significant differentiation for all tests. For the other loci, there was only one instance where an F_{CT} value was significant (the locus at 7.7 cM for test *Z*).

These results are intriguing. Although based on a limited dataset, they may indicate a systematic difference between internal and end elements of a chain. Internal chromosomes in meiotic chain and ring configurations of more than three elements often show centromeric unpairing in synaptonemal complex preparations. This is the case for the chain-of-five produced in hybrids between the 8.12 and 10.12 groups (Merico et al. 2013). However, that unpairing may not be associated with germ cell death in the same way that centromeric unpairing of the end elements is. There is not the same chromosomal condensation associated with internal unpairing as seen with unpairing of the end elements of meiotic chains (Searle 1993) nor is meiotic silencing of unsynapsed chromatin (MSUC) revealed immunologically (see studies of Matveevsky et al. 2012 on shrews). It has been suggested that the centromeric unpairing of the internal chromosomes seen in meiotic chains and rings of more than three elements is an artefact associated with chromosome spreading (Matveevsky et al. 2012), yet, on the basis of the chain-of-five configuration of the hybrids between the 8.12 and 10.12 groups, we have shown there is less genetic exchange for the centromeric regions of the internal chromosomes than the centromeric regions of the end chromosomes. If the unpairing of the internal chromosomes is actually real, this could explain our results with the genetic markers here. Unpairing does not allow recombination (only when homologous regions are paired can there be homologous recombination) and given that there has to be recombination in hybrids to get genetic exchange between the 8.12 and 10.12 groups, this may explain the greater differentiation observed for the centromeric regions of the internal chromosomes. It may be that unpairing of internal elements is somehow permissible, while unpairing of end elements leads to germ cell death. Obviously further studies are needed to examine these ideas. The results are potentially important with regards the role of Robertsonian fusions and whole-arm reciprocal translocations (WARTs) in speciation. In the case of chromosomal races characterised by Robertsonian fusions and WARTs that differ such that complex heterozygous hybrids are formed, the chromosomes that form internal elements of the long meiotic configurations may be particularly likely to show differentiation close to the centromere, including at genes which may have relevance to reproductive isolation. It can be speculated that such differentiation results, at least in part, from reduced recombination due to permissive unpairing.

The second interesting result is the differentiation detected around the centromeres of some of the chromosomes that are identical in the hybridising groups (autosomes 4, 5, 6, 14 and 15 and the X chromosome). Clearly, explanations relating to karyotypic heterozygosity cannot be the basis of this finding. It has been suggested that the 8.12 and 10.12 groups accumulated genome-wide genetic differences in allopatry which have decayed since they have come into contact and hybridised (Giménez et al. 2013). Therefore, it is possible that there has not been sufficient time for decay to be complete in some regions of the genome. However, for regions with free segregation and recombination, it is unlikely that this is the case. In the previous studies examining markers away from the centromeres of chromosomes 10 and 12, there has quite clearly been free genetic exchange between the 8.12 and 10.12 groups (Giménez et al. 2013). Other possibilities are that the non-rearrangement centromeric regions showing differentiation are sites of unfitness loci or loci that show epistatic interactions with centromeric genes of the rearranged chromosomes. However, further studies are desirable to see to what extent genetic differentiation is limited to the centromeric regions. Here we only looked at centromeres. Our previous studies of chromosomes 10 and 12 indicated that while in general regions away from the centromere are undifferentiated, there are some instances of small regions of differentiation in these non-centromeric areas (Giménez et al. 2013). Clearly genomic level studies are desirable to examine this further, i.e. to assess differentiation in fine detail along the full length of all the chromosomes.

One interest in comparing centromeric regions with other regions of the genome is that the former may have special properties. In genetic terms, the centromeric regions are inherently regions of low recombination and there are several population genetic predictions why these regions may have a tendency to become differentiated (Nachman and Payseur 2012; see also Niehuis et al. 2010 and references therein) and may therefore represent 'genomic islands of speciation'

(Weetman et al. 2012). This reduced recombination near centromeres is found in the house mouse (Froenicke et al. 2002), and a tendency for reduced recombination near centromeres is greater still in association with the presence of Robertsonian fusion metacentrics, even in the homozygous state (Bidau et al. 2001; Dumas and Britton-Davidian 2002; Merico et al. 2013; see also Bidau 1990 and Colombo 1993 for earlier studies on grasshoppers). It is also important to think of centromeres as cytogenetic structures. It is evident that different mouse populations are characterised by centromeres of different sizes and different segregational properties at female meiosis (Chmátal et al. 2014). Again, it could be speculated that there is some tendency for co-segregation of the centromeres according to parental origin in hybrids that could explain the differentiation at centromeric loci between the hybridising groups.

The fact that there is differentiation involving the X chromosome is also significant. In studies of speciation in many organisms, there is known to be a 'large X-effect' (Garrigan et al. 2014): the X chromosome carries unfitness loci important in reproductive isolation disproportionately compared with other chromosomes. This has been demonstrated in *Mus*, both in laboratory crosses between species and between subspecies of *Mus musculus* (Oka and Shiroishi 2012). In relation to this study, again it is of interest to ask if the effect is limited to regions near the centromere and, considering the chromosome as a whole, if the effect is larger than other chromosomes that are the same between the 8.12 and 10.12 groups.

Much still needs to be learnt about the role of chromosomal rearrangements in promoting genetic differentiation in hybridising chromosomal races and therefore the importance of chromosomal rearrangements in speciation. However, it is clear that when there are multiple chromosomal differences between hybridising races, each of those chromosomes does not necessarily contribute to an equal extent, even when those chromosomes contribute to the same meiotic configuration. Also, differentiation need not be limited to the chromosomal rearrangement, and there is an important challenge in explaining the basis of this. It appears that the genetic differentiation between hybridising chromosomal groups of house mice in Upper Valtellina requires an explanation involving a complex mix of factors, extending beyond a mere difference in presence or not of chromosomal rearrangements. Genomic studies will be exceptionally valuable to fully understand the basis of this. Although our microsatellite studies have been informative, the number of loci is relatively small and therefore the results are subject to the vagaries of locus-by-locus variation. A next-generation sequencing strategy examining many loci both regionally and throughout the genome will improve our statistical power and will allow a more comprehensive perspective.

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Compliance with ethical standards

Ethical standards All institutional and national guidelines for the care and use of field-collected and laboratory-maintained animals were followed.

Conflict of interest The authors declare that they have no competing interests.

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