MHC-mediated spatial distribution in brown trout (*Salmo trutta*) fry

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Abstract

MHC class I-linked microsatellite data and parental assignment data for a group of wild brown trout (*Salmo trutta* L.) provide evidence of closer spatial aggregation among fry sharing greater numbers of MHC class I alleles under natural conditions. This result confirms predictions from laboratory experiments demonstrating a hierarchical preference for association of fry sharing MHC alleles. Full siblings emerge from the same nest (redd) and a passive kin association pattern arising from limited dispersal from the nest (redd effect) would predict that all such pairs would have a similar distribution. However, the present work demonstrates a strong, significant trend for reduced distance between pairs of full sibling fry sharing more MHC class I alleles reflecting their closer aggregation (no alleles shared, 311.5±(SE)21.03m; one allele shared, 222.2±14.49m; two alleles shared, 124.9±23.88m; $P<0.0001$). A significant trend for closer aggregation amongst fry sharing more MHC class I alleles was also observed in fry pairs which were known to have different mothers and were otherwise unrelated (ML-r=0) (no alleles: 457.6±3.58m; one allele (422.4±3.86m); two alleles (381.7±10.72m); $P<0.0001$). These pairs are expected to have emerged from different redds and a passive association would then be unlikely. These data suggest that sharing MHC class I alleles plays a role in maintaining kin association amongst full-siblings following

50 emergence. The present study demonstrates a pattern consistent with
51 MHC-mediated kin association in the wild for the first time.

Keywords MHC, brown trout, kin association,

Introduction

Kin selection theory suggests that an individual may improve its inclusive fitness by behaving in a manner which enhances the reproductive success of relatives (Hamilton, 1964; Keller and Ross, 1998). A prediction of the theory is that the level of behavioural interactions between individual organisms should be influenced by the degree of relatedness between the individuals. The magnitude of the interaction can be represented by Hamilton's rule; rb-c>0, where r is the coefficient of relatedness, b is the benefit in fitness for the recipient and c is the fitness cost to the actor. Following Hamilton's rule, the effectiveness of kin-mediated cooperative social behaviour should be determined by the capacity of animals to accurately direct such behaviour towards *bona fide* relatives.

Ancillary kin bias may occur in circumstances where interactions tend to be with related rather than unrelated individuals (Sherman and Holmes, 1985). This may be an effect of limited dispersal from natal sites or shared microhabitat preferences amongst siblings (Barnard et al., 1991; Hepper, 1991). This is a form of passive kin association lending itself to altruistic behaviour. Other discrimination mechanisms include prior association and

phenotype matching (Holmes and Sherman, 1983). Kin recognition by prior association refers to a requirement for a period of familiarisation between the discriminator and the recipient, normally prior to weaning or fledging. Phenotype matching entails the learning of phenotypic cues from self or from kin that allows for kin discrimination where the target has not been previously encountered. A number of studies have been conducted to identify which of these two theories applied (Frederickson and Sackett, 1984; Grau, 1982; Halpin and Hoffman, 1987; Holmes and Sherman, 1982; O'Hara and Blaustein, 1982; Waldman, 1981). The bulk of evidence supports recognition by prior association (Boyd and Blaustein, 1985; Dewsbury, 1982; Gavish et al., 1984; Halpin and Hoffman, 1987; Holmes and Sherman, 1982; Porter et al., 1981) though there is also evidence of phenotype matching (Blaustein and O'Hara, 1982; Blaustein and O'Hara, 1981; Grau, 1982).

The nature of cues used in kin recognition is of considerable interest to kin selection. A survey of cues used for kin discrimination found that visual cues were rare and that olfactory or auditory cues were more common (Halpin, 1991). The reaction to these cues can be discrete, identifying that the individual is related to the discriminator or not. The reaction can also be proportional, in response to the degree of perceived relatedness, and

should be context dependent (Harris et al., 2003). In the latter case, it is likely that the number of cues and the number of variants thereof is critical to fine discrimination in the degree of relatedness. Hamilton (1964) hypothesised the existence of a set of "recognition alleles" which entailed the same or closely linked genes to both produce a phenotype that can be recognised by the bearer of the same set of genes and to cause the individual concerned to act altruistically to other bearers of the gene. The theory was popularized by Dawkins (1976) who termed it the 'green beard effect'. A 'green-beard' gene produces a perceptible trait (e.g. the green beard); allows recognition of other individuals with the same gene; and also leads to preferential treatment towards other individuals sharing the gene. The gene provides for direct recognition of copies of itself without concern for average relatedness. These genes can act as 'selfish genes' – genes which are selected for without consideration of their broader adaptive value to the individual. For instance, it is feasible that otherwise unrelated individuals which share 'green-beard' genes could recognise each other as "kin" and that this would affect their behaviour towards one another. These 'green beard' genes were first presented as hypothetical mediators of altruism and kin selection. 'Green beard' genes have now been identified in the red imported fire ant (*Solenopsis invicta*) (Keller and Ross, 1998) and in yeast (*Saccharomyces cerevisiae*) (Smukalla et al., 2008).

The underlying gene(s) that could facilitate cues that can be used to assess relatedness need to be highly variable. Attention was soon drawn to the Major Histocompatibility Complex (MHC) gene family which is critical to determining self from non-self in immune system function in vertebrates. Classical MHC Class Ia molecules are found on the surface of all the nucleated cells of the body. These are composed of a heavy and light chain encoded by polymorphic MHC Class Ia genes and the invariant β2-microglobulin gene. MHC Class II genes, in contrast, are only expressed in a reduced set of cells, e.g. the antigen-presenting cells such as dendritic cells, B cells and macrophages. MHC Class I and II function in the presentation of self and non-self peptides derived from endogenously (i.e. mutated, misfolded or viral) and exogenously (e.g. bacterial or macroparasitic) derived proteins, to cytotoxic T lymphocytes (CTL) or helper T cells (Th), respectively.

MHC diversity is considered crucial for the ability of populations to resist disease challenges (Bernatchez and Landry, 2003; Kurtz et al., 2004; Muirhead, 2001; O'Brien and Evermann, 1988). MHC may also be used as "recognition alleles" for helping to identify related from unrelated individuals (Dawkins, 1976; Hamilton, 1964; Manning et al., 1992). This process may be used in discriminatory behaviour in kin and sexual

136 selection.

137

138 The ability of organisms to detect MHC types provides a mechanism for
139 differential behaviour between MHC types. Identification of olfactory-
140 based kin recognition mediated by MHC was first demonstrated in rodents
141 (Brown et al., 1987; Yamazaki et al., 1976). Humans also appear to have the
142 capacity to discriminate amongst conspecifics based on MHC (Chaix et al.,
143 2008; Ober et al., 1997; Wedekind et al., 1995). Olfaction appears to be
144 important in behaviour in freshwater fish (Burnard et al., 2008). There has
145 also been extensive evidence for MHC-based mate selection (Agbali et al.,
146 2010; Boehm and Zufall, 2006; Consuegra and Garcia de, 2008; Eizaguirre
147 et al., 2009; Forsberg et al., 2007; Milinski et al., 2005; Milinski et al., 2010;
148 Miller et al., 2009; Neff et al., 2008; Reusch et al., 2001; Turner et al., 2009)
149 and kin discrimination (Gerlach et al., 2008; Olsen et al., 1998; Olsen et al.,
150 2002; Rajakaruna et al., 2006) in fish. However, kin discrimination has not
151 been demonstrated in the wild to date.

152

153 MHC class I (*UBA*) and class II (*DAA/DAB*) have only one expressed locus
154 each in salmonids and these loci are not linked (Aoyagi et al., 2002;
155 Grimholt et al., 2002; Shum et al., 2001; Stet et al., 2002). In this respect,
156 these genes are not a “complex”, as in other vertebrates, and are referred

157 to as “Major Histocompatibility” (MH) genes in salmonids. However, we will
158 use the more commonly used acronym “MHC” to refer to them in this
159 paper. In the laboratory setting, salmonids have been shown to prefer to
160 associate with individuals with which they share more MHC alleles (Olsen
161 et al., 1998; Olsen et al., 2002; Rajakaruna et al., 2006). We hypothesize
162 that in the wild, kin discriminatory behaviour should manifest itself as
163 spatial association based on the hierarchical sharing of MHC alleles.
164
165 The breeding behaviour of salmonids, which involves the construction of
166 nests (redds) in gravel in freshwater, suggests that young salmonids are
167 likely to be found in relatively highly related groups (Carlsson et al., 1999;
168 Hansen et al., 1997; Vera et al., 2010). This has been previously considered
169 a purely passive association due to limited dispersal from natal redds
170 (Elliot, 1987; Hansen et al., 1997) which is driven by stochastic factors
171 (Gowan et al., 1994). However, trout (*Salmo trutta* L.) were found to
172 demonstrate kin-biased distribution in both juveniles and older trout
173 (Carlsson et al., 2003). These authors suggested that the persistence of kin
174 association in older age groups implied active association although this
175 could not be proven with the data they had available to them. However,
176 Vera *et al*. (2010) found no evidence of association amongst older trout in
177 Spain.

178

179 We tested the hypothesis of active spatial association based on the
180 hierarchical sharing of MHC alleles in wild populations of trout fry,
181 separate from any passive association caused by a redd effect.

182

183 **Materials and Methods**

184 We examined spatial association of trout fry based on the sharing of MHC
185 class I-linked microsatellite marker alleles over a ~1.2km stretch of the
186 Srahrevagh River.

187 The Srahrevagh is an oligotrophic river (Dineen et al., 2007) in Co. Mayo,
188 Ireland, which has traps operated by the Marine Institute of Ireland, which
189 allow fish moving upstream and downstream to be counted and sampled
190 (Figure 1). On the 26th August 2002, 242 trout fry were obtained by
191 electrofishing the trapped stretch of the Srahrevagh River. The river was
192 divided into sections which were approximately 200m apart (range 110-
193 230m). The start and end of each section was mapped with GPS, and
194 marked with stakes. Sections were fished once in an upstream direction, by
195 one person with a backpack electrofisher, followed by one person with a
196 net. All stunned trout were netted and held in buckets. Each section was
197 fished up to a natural barrier at the top of the section (e.g. small fall) to
198 minimise fish movement out of the section. Distances between sites were

199 taken as the distance between the top of each section. Fry which were
200 found in the same site were given a distance of zero. Finer geographic
201 sampling may have improved resolution power but this was not possible in
202 the context of the current study. The distance data were tested for
203 normality using a Kolmogorv-Smirnov test. Tissue samples were stored in
204 absolute ethanol.
205
206 DNA extractions were conducted by dissecting small pieces of tissue (1-
207 5µg) from the samples and added to 0.5ml tubes containing 300µl of 10%
208 (w/v) Chelex™ solution. The mixture was heated at 99ºC for 1 hour.
209 Samples were centrifuged at 3000 rpm for 3 min and then stored at -20ºC.
210 All 242 individuals were screened at the selectively neutral microsatellite
211 loci *Str73* (Estoup et al., 1993), *Ssa85* and *Ssa197* (O'Reilly et al., 1996),
212 *Ssa2216* (Paterson et al., 2004), SsOsl417 and SsOsl85 (Slettan et al., 1995),
213 F43 (Sanchez et al., 1996) and *Str543* (Presa and Guyomard, 1996), and a
214 microsatellite locus embedded in the untranslated 3' end of MHC class I
215 locus (*Satr-UBA*) (Grimholt et al., 2002). This dinucleotide microsatellite
216 marker is located in the 3' untranslated region (*UTR*) of the MHC class I
217 locus and has been demonstrated to be tightly linked to class I locus in
218 Atlantic salmon (*Salmo salar* L.) (Grimholt et al., 2002). It has been
219 successfully employed previously in studies in salmon (Consuegra et al.,

220 2011; de Eyto et al., 2007) and trout (Coughlan et al., 2006; Hansen et al.,
221 2007). It was not feasible to do *Satr-UBA* sequencing of the fry as *Satr-UBA*
222 has a 14,000 bp intron between exons II and III which code for the α1 and
223 α1 domains. This means that cDNA must first be derived from RNA before
224 PCR and sequencing could be conducted. The tissue samples available were
225 stored in ethanol meaning that this approach could not be undertaken.
226 *Satr-UBA* sequencing of 30 Srahrevagh trout identified 21 alleles, where
227 the linked microsatellite locus had only 13 alleles ($p<0.0001$) (Coughlan et
228 al., 2006). The marker itself is not involved in any putative kin recognition.
229 Allele sharing at the marker is a reasonable and conservative measure of
230 allele sharing at *Satr-UBA*, as demonstrated in simulation tests below.
231
232 PCR amplifications were carried out under the following conditions in a
233 10µl reaction volume: 95°C 3min; (95°C for 30s, 56°C for 30s, and 72°C for
234 30s) X 30 cycles. Alleles were resolved on 18cm or 25 cm 6%
235 polyacrylamide gels, using a Li-Cor 4200 DNA sequencer. Allele sizes were
236 determined by reference to a 50-350bp size ladder and locus-specific allele
237 size standards. These allele size standards were constructed in the
238 laboratory using the full complement of allele sizes observed in pilot
239 studies, to enable consistent scoring amongst batches of individuals
240 screened for each locus. When initial genotyping was unclear due to gel

electrophoresis problems or weak amplification (~85 genotypes out of a total of 2178), trout fry samples were re-extracted and re-screened. Large allele dropout was identified as an occasional problem but large alleles could usually be reliably scored after re-screening. The estimated error rate was ≤0.5% of composite genotypes per individual (Coughlan et al., 2006).

Statistical Analysis

A rarefaction analysis (Altmann et al., 1996; de Ruiter and Geffen, 1998) was conducted using the web-based program RE-RAT (Schwacke and Rosel, 2005). This tested the robustness of relatedness estimates (Lynch and Ritland, 1999) using the eight neutral microsatellite loci. Initially, pairwise matrices of relatedness values were calculated from a single random locus. After 1000 simulations, the procedure provided mean differences in relatedness values with standard deviations as each additional locus was added.

Each unique pair of fry was classified according to whether zero, one or two MHC class I marker alleles were shared. The eight neutral microsatellite markers were used to determine relatedness between fry pairs. The program ML-RELATE (Kalinowski et al., 2006) was used for maximum likelihood relatedness value (ML-r) estimation between pairs of

262 individuals. Full sibling pairs were identified as those showing ML-r≥0.5.
263 These full sibling pairs are expected to have emerged from the same nest
264 (redd).
265
266
267 Parental assignment data was available from sampling adults moving
268 upstream to spawn in the autumn of the previous year (2001). Parental
269 assignment data were then used to identify those pairs of fry (amongst
270 assigned fry) which did not share a mother. These fry were expected not to
271 have emerged from the same redd. Fry pairs in this sub-group with ML-r of
272 0 were then identified and the remaining pairs (Ml-r>0) removed from the
273 analysis. These fry were then also unlikely to be paternal half siblings. This
274 was our “unrelated” group. It should be noted that the large number of
275 uncollected parents in the parental assignment data available meant very
276 few full siblings were identified, necessitating using the relatedness-based
277 approach to identify full siblings instead.
278
279 Separate analyses were then conducted for the full sibling and unrelated
280 groups. The mean distances between pairs sharing different numbers of
281 MHC alleles were calculated, Kruskal-Wallis tests and, subesquent, pairwise
282 Mann-Whitney tests between groups conducted. However, because fish

283 separated by very large distances may be equally unlikely to interact with
284 each other, we wanted to account for this through a second analysis where
285 pairs were binned by distance to “within section”, “neighbouring section”
286 (one section away) and “distant sections” (more than one section away).
287 The number of pairs sharing zero, one and two MHC alleles within each
288 distance category for the observed data were incorporated into a
289 contingency table. A bootstrap procedure was then implemented in Python
290 scripts wherein each pair of trout fry had assigned to it a random value
291 from the observed distribution of MHC marker allele shared values, with
292 replacement. The proportion of 10,000 bootstrap values which exceeded or
293 were less than the observed value in each cell of the contingency table
294 were used to determine if observed values differed significantly from
295 random expectations. The bootstrap procedure was preferable to a
296 parametric approach such as chi square test in that it provided for any
297 imbalance in the experimental design which might result in unequal
298 variances in the different groups.
299
300 Simulation study of microsatellite-*UBA* correlation for full sibling groups
301 We tested the validity of using the microsatellite marker as a proxy for
302 *UBA* allele sharing by simulating full sibling families based on the available
303 microsatellite data from this study and the *Satr-UBA* data from the same

304 river (Coughlan et al., 2006). The *Satr-UBA* data were generated from 27
305 resident brown trout adults selected to represent all microsatellite alleles
306 identified in a sample of 107 fish in 2004 from the Srahrevagh river, Co.
307 Mayo. For each microsatellite allele, the set of *UBA* alleles observed in
308 association with each microsatellite allele were compiled. The 110bp and
309 the 136bp microsatellite alleles (136bp was most common) were always
310 found with a particular *UBA* allele, *Satr-UBA**1701 and *Satr-UBA**1201,
311 respectively.
312
313 At each iteration of the procedure, an independent full sibling data set was
314 simulated and analysed. A set of parental genotypes were then simulated
315 based on the observed MHC microsatellite data for the Srahrevagh 2002
316 trout fry sample. The numbers of males and females were independently
317 selected, a random number between 40 and 50 in both cases. Each parent
318 has a microsatellite genotype drawn at random from the observed
319 microsatellite allele frequency distribution available for the Srahrevagh
320 population. For each microsatellite allele in the parent's genotype, a
321 random *UBA* allele is drawn from the observed set of *UBA* alleles associated
322 that particular microsatellite allele (Coughlan et al., 2006). Each parent
323 then has a pair of MHC "chromosomes" with a particular combination of a
324 *UBA* and a microsatellite allele in each.

A maximum family size was selected at random between 80 and 100. We then created 125 full sibling families by randomly “mating” two simulated parents. The number of fry in each family was randomly selected between 10 and the maximum family size for each iteration. For each fry simulated, a random MHC “chromosome” is inherited from the father and one from the mother.

Following the generation of every full sibling family, the MHC “chromosome” genotype of each fry in the family was compared with that of every other fry in the family. In each pairwise comparison, the number of microsatellite alleles shared amongst the pair was compared with the number of *UBA* alleles shared amongst the pair. If the number is the same, a “correct” score is logged under the appropriate category of microsatellite alleles shared (zero, one or two). If the number of *UBA* alleles shared differs, an “incorrect” score was logged under the appropriate category. At the end of each iteration, the proportion of “correct” to “incorrect” scores were calculated for the zero, one and two alleles shared categories. The procedure was repeated across 1,000 bootstraps. These values were then summarised across the 1,000 iterations of the simulation study. The simulations mirrored the approach taken in our key analysis of full sibling

groups identified in the real data from relatedness values. The simulation results gave us an idea of how well the microsatellite allele sharing patterns fitted those at *UBA* within the full sibling groups simulated from the Srahrevagh data. This also gave us an estimation of how well we might expect the microsatellite marker to perform for identifying *UBA* allele sharing in our real data.

Results

Distance data amongst all pairs of fry were found to be normally distributed (Kolmogorv-Smirnov test, N=29,161, Z=22.187, p = 0.000). The rarefaction analysis demonstrated that resolution power with any seven of the eight neutral loci provided robust relatedness estimation. The total numbers of alleles identified in the 242 fry for each locus were as follows: *Str73* (total alleles: N_A=3, expected heterozygosity: H_E= 0.580), *Ssa85* (N_A=5, H_E= 0.730), *Ssa197* (N_A=8, H_E= 0.704), *Ssa2216* (N_A=6, H_E= 0.673), *SsOsl417* (N_A=21, H_E= 0.772) and *SsOsl85* (N_A=18, H_E= 0.883), *F43* (N_A=13, H_E= 0.823) and *Str543* (N_A=12, H_E= 0.809). The MH class I-linked microsatellite had 11 alleles and an expected Heterozygosity of 0.825.

<u>Full sibling analysis</u>

A total of 749 full sibling pairs were identified. Both individuals in each of these full sibling pairs would have emerged from the same nest (redd). A passive kin association pattern, arising from limited dispersal from the nest (redd effect) would predict that all such pairs would have a similar distribution. However, the present work demonstrates a strong, significant and hierarchical trend for closer aggregation amongst full-sibling fry sharing more MHC class I marker alleles, as shown by a reducing mean distance between pairs sharing more alleles (no alleles shared: mean=311.5±21.03m, median=170m, mean ranks=422.02, n=248; one allele shared: 222.2±14.49m, median=150m, mean ranks=362.01, n=407; two alleles shared: 124.9±23.88m, median = 0m, mean ranks=295.80, n=86; Kruskal-Wallis test (H (df=2) =27.537, df = 2, $p<0.0001$, Figure 2). Details of all tests (including pairwise Mann Whitney U tests) can be found in Table 1.

Full sibling pairs that shared two MHC alleles were more frequent than expected within a section (Bootstrap test, p=0.0029), not significantly different from expectation at neighbouring sections (p=0.1787), and less frequent than expected at distant sections (p=0.0001) (Table 2). Those which shared one MHC allele were more frequent than expected within a section (p=0.0262), but not significantly different from expectation at neighbouring sections (p=0.1699), or distant sections (p=0.3276). Full

388 sibling pairs sharing no MHC alleles were significantly fewer than expected
389 within section (p=0.0001), not significantly different from expectation at
390 neighbouring sections (p=0.4679), and more frequent than expected at
391 distant sections (p=0.0000). Full sibling pairs which shared two MHC alleles
392 were rarely found more than two sections away from each other while full
393 sibling pairs which shared no MHC alleles were unusually common in these
394 "distant sections" (See Table 2).
395
396 Therefore, full sibling fry which emerged from the same redd not only
397 tended to be found closer to fry with which they share more MHC alleles,
398 but to have dispersed less than those sharing fewer alleles. Full siblings
399 who share two MHC alleles demonstrate a particularly marked tendency for
400 association while those that share no MHC alleles show much poorer
401 association. This suggests that sharing MHC class I alleles plays a role in
402 maintaining kin association amongst these full-siblings following
403 emergence.
404
405 Unrelated fry analysis
406 Parental assignment data was available from genotyping adults sampled
407 moving upstream to spawn in the previous year. This parental assignment
408 data was based, primarily, on complete exclusion as the only situation

409 where successful allocation of offspring is difficult to question (Jones and
410 Ardren, 2003). Offspring of the sampled males were found over a mean
411 range of ~250m while those of sampled females were found over a mean
412 range of ~400m. The “unrelated” group consisted of 15,006 pairs. We know
413 that these pairs did not emerge from the same redd as they do not share a
414 mother. Redd effect is the most likely source of passive association. A
415 paternal effect on fry spawned in adjacent sections by the same father may
416 be possible but our exclusion of fry pairs with Ml-r>0 should help exclude
417 half siblings.
418
419 A significant trend for closer aggregation amongst fry sharing more MHC
420 class I alleles was also observed in these unrelated pairs (zero alleles,
421 mean=457.6±3.58m, median=430m, mean ranks=7677.22; one allele,
422 mean=422.4±3.86m, median=350m, mean ranks=7341.41; two alleles,
423 mean=381.7±10.72m, median=345m, mean ranks=7198.54; Kruskal-Wallis
424 test, H (df=2) = 25.503, P<0.0001, See Figure 3). However, no significant
425 difference was found in the pairwise Mann-Whitney test for unrelated fry
426 sharing one allele and two alleles (U=2551245, z=-0.851, P=0.395).
427
428 Unrelated pairs sharing two MHC alleles (p=0.0197) and one MHC allele
429 (p=0.0001) were significantly more likely to be found in the same section

(Table 2). Conversely, those sharing no alleles were significantly less likely to be found in the same section (p=0.0000). No significant pattern was observed for fry sharing different numbers of MHC alleles in neighbouring sections (~100m) (Table 2). Fry sharing one MHC allele were significantly less likely to be found in distant sections (≥200m) (p=0.0041) but, unexpectedly, there was no such significant pattern for fry sharing two alleles (p=0.0722). Fry sharing no MHC alleles were significantly more common in distant sections (p=0.0009). Overall, these data suggest that MHC also significantly affects the distribution of these unrelated brown trout fry. This then also appears to suggest an active association based on the sharing of MHC class I alleles, given a passive association is unlikely.

Simulation study of microsatellite-*UBA* correlation for full sibling groups

Our simulation of full sibling groups, based on the available microsatellite and *UBA* data, appeared to support the efficacy of the microsatellite marker in identifying allele sharing at the *UBA* locus. Over 1,000 bootstrap iterations of the simulation, full siblings which shared no microsatellite also shared no *UBA* alleles 90.0% of the time (SE 0.24%), those which shared one microsatellite allele shared one *UBA* allele 84.9% of the time (SE 0.14%) and those which shared two microsatellite alleles also shared two *UBA* alleles 81.7% of the time (SE 0.27%). This implies that, when applied to

full sibling groups, the microsatellite marker is a good indicator of *UBA* allele sharing amongst pairs of fish. The marker predicts pairs sharing two alleles somewhat less accurately. If there is any bias caused by this, there is no obvious reason why this would lead us to identify *UBA*-mediated kin association where there is none. It seems more likely that inaccurate inclusion of pairs sharing two marker alleles which do not share two *UBA* alleles, for instance, would weaken the power of our analysis to identify an underlying trend of association based on *UBA* allele sharing. This could occur wherein pairs with no behavioural preference for one another and which might be expected to be found a random distance apart, are treated the same as those which share two *UBA* alleles and may demonstrate a strong behavioural preference for each other.

Discussion

To our knowledge, this is the first time kin association based on the sharing of MHC alleles has been demonstrated in the wild. Previous laboratory studies had found evidence for this hierarchical preference for fry sharing more MHC alleles (Rajakaruna et al., 2006) but that study had examined MHC class II. The authors did suggest that their data implied that another locus, perhaps MHC class I, was also affecting behavioural preferences. Our data would appear to suggest that MHC class I is a factor

472 in kin recognition in these fish. Unrelated fry also showed signs of greater
473 association based on the sharing of MHC alleles. This could also be a
474 consequence of the use of MHC in kin recognition. Unrelated salmonids
475 were found to prefer non-kin sharing two MHC class II alleles over non-kin
476 with which they did not share alleles in the laboratory studies (Rajakaruna
477 et al., 2006). Evidence for an influence of a MHC-based kin recognition
478 system in unrelated fry, both in their behaviour and in their distribution in
479 the wild, may be indicative of a form of "green beard effect" (Dawkins,
480 1976) occurring in salmonids fish.
481
482 Laboratory studies of kin-mediated cooperative social behaviour in
483 salmonids (Brown and Brown, 1992; Brown and Brown, 1993; Brown and
484 Brown, 1996; Hedenskog et al., 2002; Olsen, 1989; Quinn and Busack, 1985;
485 Quinn and Hara, 1986) would suggest the benefits of active kin association
486 in these fry would be in the acquisition and maintenance of territories
487 following emergence from the redd. Trout which do not succeed in
488 maintaining a territory are assumed to die, with over 90% mortality during
489 this critical period (Elliot, 1994). This constitutes a powerful selective
490 pressure.
491
492 Altruistic behaviour will be most effective when directed exclusively at full

493 sibling relatives and less so when directed against half-siblings (Hamilton,
494 1964). Thus, the ability for trout fry to discriminate between full sibling
495 relatives and half-sibling relatives would be valuable. Any kin recognition
496 system will only be as effective as the resolution power achievable by the
497 genetic loci and/or phenotypic cues used. In this respect, the employment
498 of the highly polymorphic MHC loci is particularly valuable.
499
500 The resolution power of highly polymorphic loci such as MHC depends not
501 just on the number of alleles within the population at large, but on the
502 relative frequencies of each allele. The number of alleles may vary from
503 one population to the next with varying degrees of skew in the frequencies
504 of each allele. The individuals which share common alleles may identify a
505 greater proportion of conspecifics as related when they are not, with
506 fitness consequences. Conversely, individuals which encounter each other
507 and which share rare alleles may be more likely to be *bona fide* relatives.
508
509 A “rare allele advantage” for kin recognition systems has been suggested by
510 Grafen (1990). In populations which exhibit very skewed allele frequencies
511 or low polymorphism in general (perhaps due to an epizootic or population
512 bottleneck), the resolution ability of kin recognition is greatly reduced for
513 any individual possessing one or more common MHC alleles. In contrast,

514 individuals with rarer alleles enjoy a relative selective advantage arising
515 from very effective cooperative behaviours. Such an advantage is inversely
516 proportional to the frequency of the bearer's allele(s) in the population and
517 may continue to accrue over several generations. A prediction of this
518 theory would be that accurate kin recognition breaks down where MHC
519 variation is reduced in a population. Empirical evidence of kin association,
520 using a genome wide measure such as relatedness, may not be found
521 where kin recognition breaks down. However, if kin recognition is based on
522 additional genes, then kin recognition, albeit less accurate, could still
523 operate following a bottleneck event which reduced MHC variability. This
524 may provide an additional explanation for the retention of high levels of
525 polymorphism at MHC loci due to balancing selection. Heterozygosity and
526 the presence of rare MHC alleles in offspring genotypes may be beneficial
527 to survival even in the absence of direct disease challenge.
528
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Captions

Figure 1 - Map of the Srahrevagh River in Mayo, Ireland. Numbers indicate the top of each section from which fish were sampled.

Figure 2 –Box plot of full sibling fry distribution data. Outliers (values that are between 1.5 and 3 times the interquartile range) are represented by circles while extreme outliers (values that are over 3 times the interquartile range) are marked with asterisks. The whiskers represent the minimum and maximum values which are not outliers.

Figure 3 - Box plot of unrelated fry distribution data.

Table 1 –The results of Kruskal-Wallis tests and pairwise Mann Whitney tests on distance amongst pairs of fry sharing different numbers of MHC alleles, for the full sibling and unrelated analyses.

Table 2 – Results of the bootstrap tests on the respective contingency tables for the full sibling and unrelated fry

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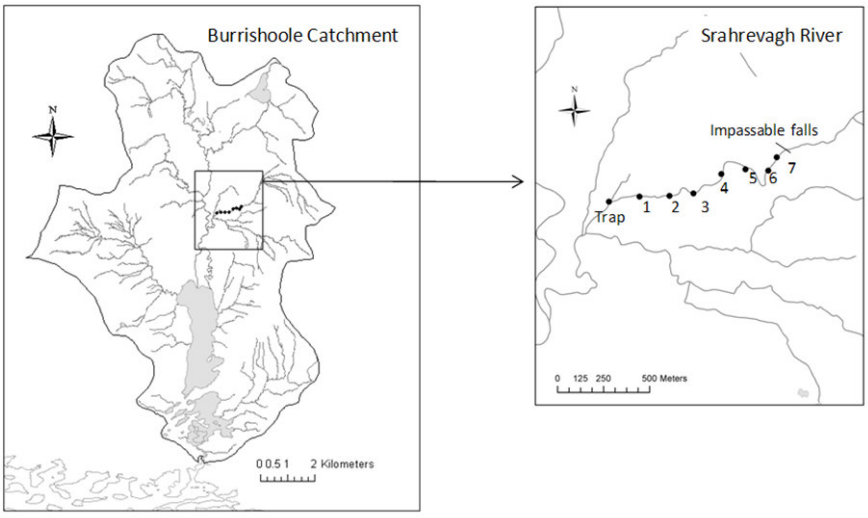

Figure 1

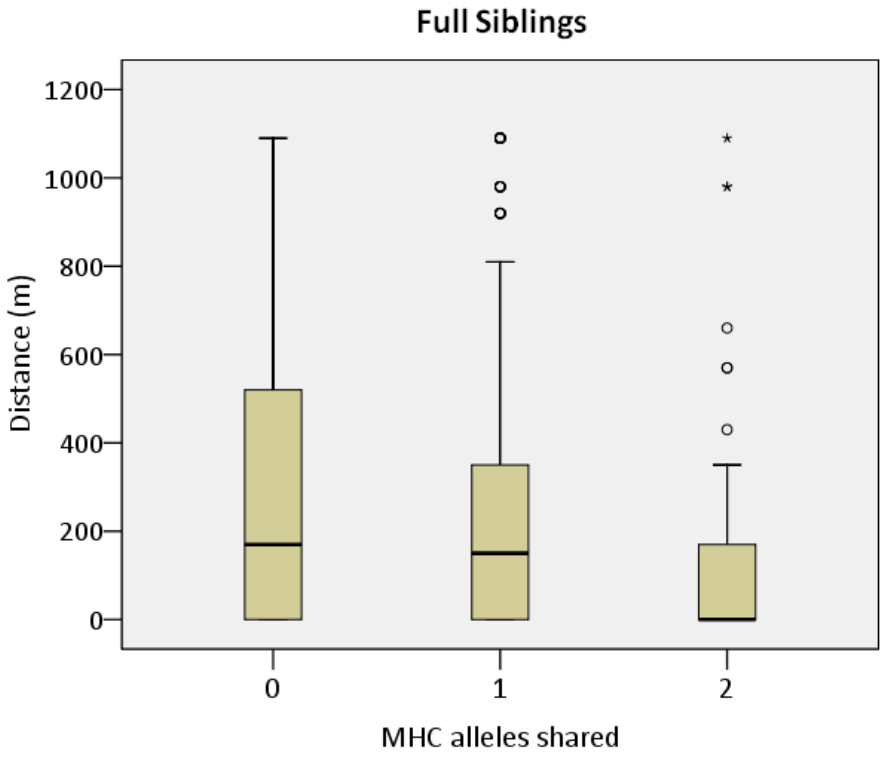
Full Siblings
1200
1000
800
600
400
200
0
Distance (m)
0
1
2
MHC alleles shared

Figure 2

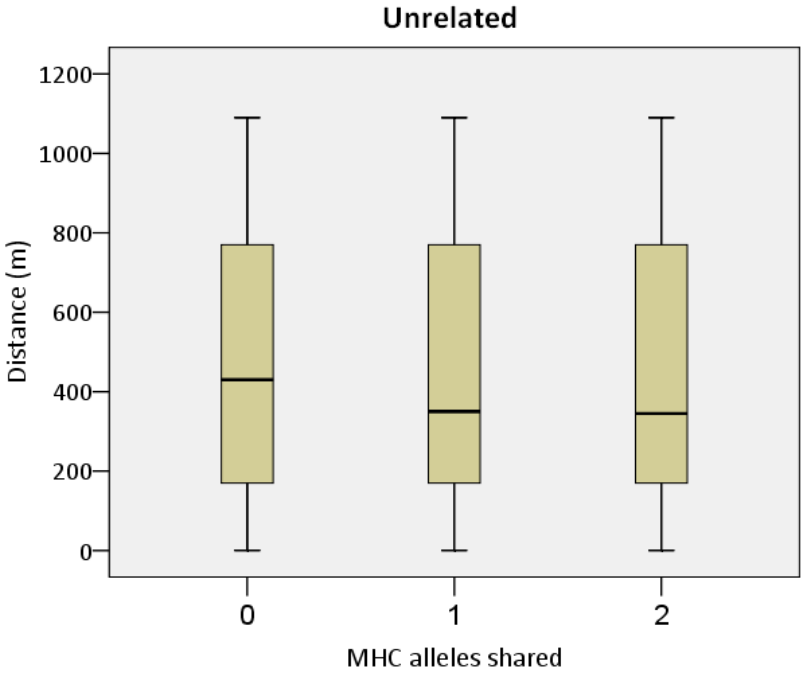

Figure 3

Table 1

MHC alleles shared		MHC alleles shared	Full Sibling U	Z	p	Unrelated U	Z	p
0	vs	1	43848.5	-3.606	0.001	24,060,000	-4.619	0.000
0	vs	2	7289	-4.896	0.000	2,771,857	-2.970	0.004
1	vs	2	14408.5	-2.714	0.006	2551245	-0.851	0.395
			Kruskal-Wallis p<0.0001			Kruskal-Wallis p<0.0001		

Table 2

Bootstraps = 10,000	MHC alleles shared	Full Sibling Observed (proportion)	Bootstraps less than observed	p	Unrelated Observed (proportion)	Bootstraps less than observed	p
Distant sections (≥200m away)	0	100 (0.435)	10000	0.0000	4,923 (0.521)	9,991	0.0009
	1	120 (0.522)	3276	0.3276	4,059 (0.430)	41	0.0041
	2	10 (0.043)	1	0.0001	461 (0.049)	722	0.0722
Neighbouring section (~100m)	0	66 (0.355)	5321	0.4679	1,686 (0.492)	570	0.0570
	1	94 (0.505)	1699	0.1699	1,555 (0.453)	8,886	0.1114
	2	26 (0.140)	8213	0.1787	189 (0.055)	8,002	0.1998
Within section	0	82 (0.252)	1	0.0001	967 (0.453)	0	0.0000
	1	193 (0.594)	9738	0.0262	1034 (0.485)	9,999	0.0001
	2	50 (0.154)	9971	0.0029	132 (0.062)	9,803	0.0197