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Comparison of patterns of genetic variability in wild and supportively bred stocks of brown trout, *Salmo trutta*

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The use of local wild spawners as broodstock for stocking programmes and supportive breeding (SB; when only the first generation of offspring is used for supplementation) has become increasingly common, especially in salmonids (Wang & Ryman 2001). It is typically employed as a method to avoid stocking domesticated or exogenous fish into wild populations that could compromise the naturally high levels of genetic variability typically observed in brown trout, *Salmo trutta* L., populations and any local adaptations that could be associated with them (Ferguson 1989; Hindar *et al.* 1991; Ryman *et al.* 1995; García-Marín *et al.* 1999; Almodóvar *et al.* 2006; Fraser *et al.* 2008; Hansen *et al.* 2009). However, SB is not without its difficulties; initially broodstock must be selected from the appropriate population to avoid the introduction of exogenous material (which could require considerable effort given the sub-divided nature of brown trout populations; Ryman & Stahl 1981). Additionally, there is the risk that genetic diversity is lost through accelerated genetic drift in small hatchery broodstock (e.g. Allendorf & Phelps 1980; Ryman & Stahl 1980; Hansen *et al.* 1997; Machado-Schiaffino *et al.* 2007; Fraser 2008), or that supportive releases could cause reductions in genetic diversity in wild populations (Ryman & Laikre 1991; Tessier *et al.* 1997; Hansen *et al.* 2000; Wang & Ryman 2001).

A recent policy review from the Environment Agency (the statutory body in England and Wales) highlighted the scarcity of research into brown trout stocking practices in this region (Ferguson 2006), an issue this paper aims to begin to address. A collection of 1225 trout from 22 sample sites across the River Dart, in southwest England, has previously been genotyped with a suite of nine microsatellite markers (Griffiths *et al.*, in press): Str15, Str60, Str73 (Estoup *et al.* 1993), Str85 (Presa & Guyomard 1996), SsoSL417, SsoSL25 (Slettan *et al.* 1995), Strutta58 (Poteaux 1995) SsoSL438 (Slettan unpublished; GenBank Z49134) and SsHaeIII (Goodier unpublished; GenBank U10050). In 2002, an additional sample of 30 first-generation (F1) brown trout was obtained. Broodstock fish comprised approximately 20 adults caught on the mid-reaches of the main river at Dart Bridge (N50:29:13, W3:46:16) in 2001. The F1 generation was then used to supplement trout for angling at a number of sites in the catchment, as well as the basis for a local hatchery strain to be used for supplementation in subsequent years. This represents a departure from previous stocking practices that, historically, used a series of hatchery strains of generally unknown,

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but probably of both domesticated and exogenous, origin (Finnegan & Stevens 2005). The SB stock of fish has now been genotyped with the same loci as used in the catchment-wide study of Dart populations (Griffiths *et al*., 2009) to facilitate comparison with the wild samples.

The SB sample was tested for conformity to Hardy–Weinberg equilibrium (HWE; Guo & Thompson 1992) and linkage disequilibrium using GENEPOP 4.0 (Raymond & Rousset 1995b). GENEPOP was also used to compare allele frequencies between the SB and wild samples with Fisher's exact test (Raymond & Rousset 1995a), and the program FSTAT 2.9.3 (Goudet *et al*. 1996) was used to calculate pairwise F_{ST} values (and their significance). A neighbour-joining (NJ) phylogram was also constructed using the Chord distance (D_{CE}; Cavalli-Sforza & Edwards 1967) and confidence intervals on tree topology were estimated by bootstrap resampling of loci 1000 times using the programs Powermarker 3.23 (Liu & Muse 2002), Consense (Phylip 3.6; Felsenstein 1995) and Treeview 1.6 (Page 1996). Where appropriate, sequential Bonferonni corrections were applied (Rice 1989).

To quantify changes in the allele frequencies that have potentially occurred during the hatchery process, samples are ideally required from the broodstock. However, no such tissue samples were available for this study. In the previous catchment-wide study of genetic variation of brown trout in the River Dart, the closest sample to Dart Bridge, where the broodstock originated, was collected from Holy Brook (the confluence of the Holy Brook is 800 m upstream of Dart Bridge). Previous analysis (Griffiths *et al*., 2009) has shown there is a strong pattern of isolation by distance in the population structure of trout within the Dart, which makes this the best available wild sample for comparison with the SB stock as it should have allele frequencies that mostly closely match those of the original broodstock. The number of alleles and the allelic richness (the number of alleles per locus, corrected for sample size calculated by the FSTAT software) were compared using a Wilcoxon signed ranks test. Individual levels of multilocus heterozygosity [attained by scoring individuals as heterozygous – 1 or homozygous – 0 and averaged across all loci (Blanchet *et al*. 2008)], were also compared between the two groups with a Mann–Whitney test. Finally, for the SB sample the effective population size (*Ne*) of the broodstock was calculated in NeEstimator 1.3 (Peel *et al*. 2004) using the linkage disequilibria method (Bartley *et al*. 1992). Both multi-allelic data and di-allelic data were used to generate *Ne* estimates, by pooling all but the most common allele into a single composite allele (which may be the more valid method; Waples 1991). In addition, BOTTLENECK 1.2 software (with the Wilcoxon test recommended by the developers, Cornuet & Luikart 1996) was used to test whether bottleneck effects (i.e. a drastic reduction in the number of effective breeders) had occurred at the hatchery. The bottleneck software operates by testing for whether heterozygosity is temporarily inflated relative to the observed number of alleles at mutation-drift equilibrium (Cornuet & Luikart 1996).

There was no evidence of linkage disequilibrium in the SB sample and of the nine loci only Str58 showed significant deviation from the expectations of HWE (which could be related to limited numbers of broodstock). The results from the Fisher exact tests (Table 1) were highly significant for every comparison of the 22 wild samples, demonstrating how different the allele frequencies are in the SB sample. The phylogram of genetic distance (Fig. 1) illustrates a comparable picture with the SB sample separated from the wild trout by the longest branch length on the tree. Similarly, the pairwise F_{ST} values (Table 1) are all significant and relatively high, showing how differentiated the SB sample has become from the wild trout samples.

Comparison of the indices of genetic variability between the SB and Holy Brook (Hol) showed that the average number of alleles in the Hol sample was 7.00, compared with 4.67 in the SB sample. This difference was significant when comparing the number of alleles across the loci (Wilcoxon signed ranks test; $P = 0.031$). The allelic richness was also significantly higher (Wilcoxon signed ranks test; $P = 0.008$) in the Hol sample (mean = 6.23) than the SB sample (mean = 4.40). The levels of multi-locus heterozygosity were also lower in the SB sample (mean = 0.59) than in the Hol sample (mean = 0.70), although not significant (Mann–Whitney ranked sum test; $P = 0.059$). Estimates of the effective population size in the SB sample from the linkage disequilibria method were 19.56 [95% confidence limits (CL) = 14.54–27.80] with the multi-allele approach and 13.52 (95% CL = 7.95–26.77) with the bi-allelic approach. Finally, there was no evidence of population bottlenecks and associated heterozygosity excess in the SB sample using the stepwise mutational model (Wilcoxon test; $P = 0.410$), or the two-phase mutational model (Wilcoxon test; $P = 0.213$).

The results of this study demonstrate that the SB stock of trout is highly differentiated from, and shows highly significant differences in allele frequencies from, any of the samples of wild trout collected across the River Dart. This suggests that this SB stock may not be suitable for the supplementation of trout across the

Table 1. Pairwise comparison of the supportively bred stock with 22 samples of wild trout sampled from across the River Dart. The *P*-value of Fisher's exact test for comparing allele frequencies is given for all the loci combined

Sample	Site details	Latitude	Longitude	Sample size	Fisher exact test	Pairwise F_{ST} (*P*-value)
Amm	Amm Brook, Amm house weir	50°28′27″ N	3°39′44″ W	32	< 0.001	0.174 (< 0.001)
Ash	Ashburn, Belford Hill	50°31′50″ N	3°45′26″ W	36	< 0.001	0.264 (< 0.001)
Dury	Dury Brook, Dury Farm	50°35′06″ N	3°53′26″ W	33	< 0.001	0.169 (< 0.001)
EDar	East Dart, Postbridge	50°35′44″ N	3°54′49″ W	21	< 0.001	0.188 (< 0.001)
EWebB	East Webburn, Bagpark Estate	50°35′21″ N	3°48′27″ W	28	< 0.001	0.179 (< 0.001)
EWebD	East Webburn, Dunstone Bridge	50°34′12″ N	3°48′40″ W	57	< 0.001	0.170 (< 0.001)
EWebV	East Webburn, Veton Bridge	50°34′32″ N	3°48′25″ W	49	< 0.001	0.181 (< 0.001)
EWebW	East Webburn, Wooder Manor	50°35′08″ N	3°48′26″ W	77	< 0.001	0.198 (< 0.001)
Gata	Gatacombe River	50°27′08″ N	3°37′49″ W	34	< 0.001	0.239 (< 0.001)
Har	River Harbourne, Hatcheries Fish Farm	50°25′55″ N	3°46′54″ W	41	< 0.001	0.127 (< 0.001)
Hem	River Hems	50°27′45″ N	3°40′08″ W	40	< 0.001	0.158 (< 0.001)
Hol	Holly Brook	50°30′15″ N	3°49′47″ W	29	< 0.001	0.162 (< 0.001)
LChe	Cherry Brook, Lower Bridge	50°33′27″ N	3°55′55″ W	59	< 0.001	0.178 (< 0.001)
RudB	Ruddycleave, Bowden Farm	50°32′55″ N	3°47′46″ W	96	< 0.000	0.226 (< 0.001)
RudC	Ruddycleave, Ruddycleave Cottage	50°32′39″ N	3°48′05″ W	36	< 0.001	0.204 (< 0.001)
RudP	Ruddycleave, Pudsham Down	50°33′28″ N	3°47′11″ W	98	< 0.001	0.285 (< 0.001)
Swin	River Swincombe, Wydemeet	50°32′33″ N	3°54′36″ W	80	< 0.001	0.132 (< 0.001)
UChe	Cherry Brook, Upper Bridge	50°34′38″ N	3°55′58″ W	70	< 0.001	0.155 (< 0.001)
WDar	West Dart, Cockern Tor	50°33′47″ N	3°57′53″ W	119	< 0.001	0.168 (< 0.001)
Web	River Webburn, Mistresses Piece	50°31′35″ N	3°47′43″ W	32	< 0.001	0.159 (< 0.001)
WWeb	West Webburn, Pondsworthy Bridge	50°33′03″ N	3°50′03″ W	125	< 0.001	0.165 (< 0.001)
WWebL	West Webburn, Lower Cator Bridge	50°34′26″ N	3°51′02″ W	33	< 0.001	0.176 (< 0.001)

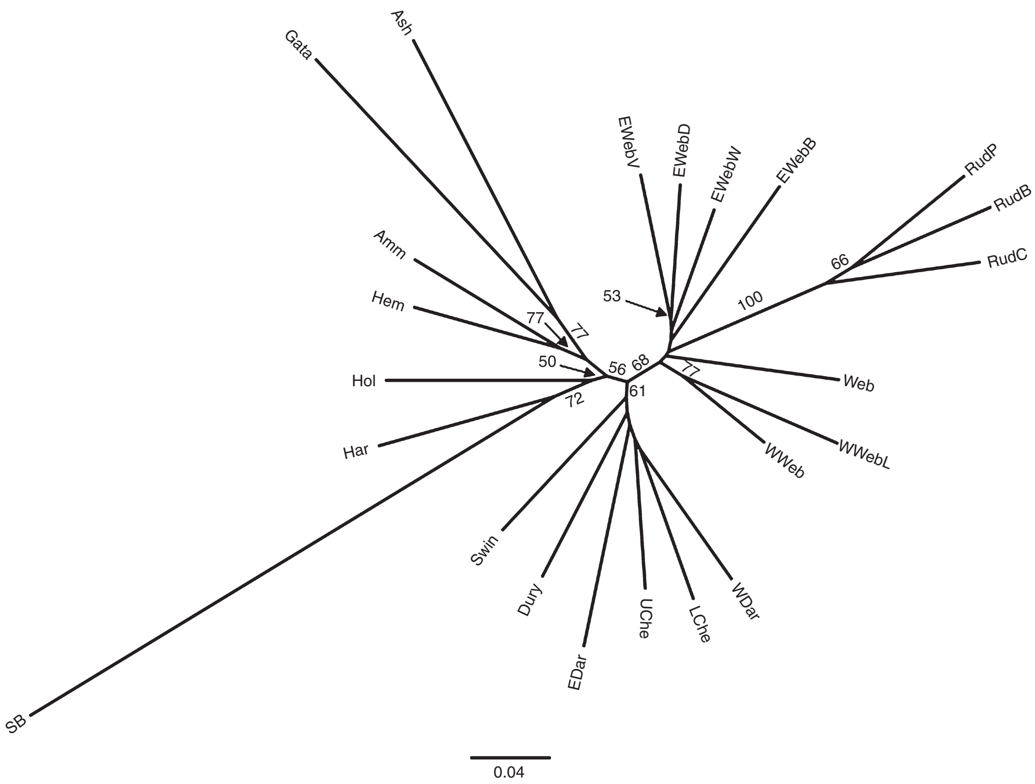

Figure 1. Neighbour-joining phylogram based on pairwise D_{CE} distances between samples. Percentage bootstrap support values > 50% are shown, based on 1000 replicates. Sample abbreviations match Table 1; SB is the supportively-bred population.

catchment, especially if one of the aims of such stocking is to conserve the natural genetic diversity, although it should be noted that microsatellite markers are generally assumed to be selectively neutral and do not directly infer the action of natural selection or local adaptation (Schlotterer & Wiehe 1999). Furthermore, the genetic distances and F_{ST} values that separate the SB sample from the wild samples are generally of much greater order than those observed between any of the wild samples (Griffiths *et al.*, in press). This suggests that the hatchery process may have altered the genetic composition of the stock, leading to inflated values of genetic differentiation.

Comparison of the SB sample with the most closely collected wild sample from the Hol tributary showed a significantly lower number of alleles and allelic richness in the SB stock, along with a near significant difference in levels of multi-locus heterozygosity between the samples. This is despite the fact that main river samples and those closer to the river mouth, generally demonstrating higher levels of genetic variability than samples from within more isolated tributaries (Neville *et al.* 2006; Primmer *et al.* 2006). This suggests that the hatchery process has led to a reduction of genetic diversity in the SB trout stock (Ryman & Stahl 1980; Hansen *et al.* 1997, 2001; Was & Wenne 2002); similar findings have been reported in Atlantic salmon (e.g. Cross & King 1983; Verspoor 1988; Norris *et al.* 1999; Horreo *et al.* 2008). The estimates of the effective population size of the trout broodstock support this assertion, as they are generally below the levels required to avoid undesirable genetic consequences in supportive breeding programmes (e.g. Ryman & Stahl 1980; Theodorou & Couvet 2004; Favé *et al.* 2008). However, bottleneck tests did not demonstrate a significant decline in effective population size, perhaps reflecting the statistical power of the tests (Luikart & Cornuet 1998), or the possibility that the original effective population size of the source population for the broodstock was itself small (Cornuet & Luikart 1996). These results agree with suggestions that a suite of different statistical tests is the most powerful way of detecting the consequences of SB (Hansen *et al.* 2000).

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