

## Supplemental Information for:

# Widespread intersex differentiation across the stickleback genome – the signature of sexually antagonistic selection?

by Mirjam BISSEGGER, Telma G. LAURENTINO, Marius ROESTI, and Daniel BERNER

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**Figure S1.** Intersex differentiation along an exemplary chromosome based on reduced-representation SNP data from Lake Constance stickleback (the ROM and UNT sample sites in Roesti et al. 2015). The underlying sequence data were derived from individual-level restriction site-associated DNA (RAD) involving digestion with the *Nsi1* enzyme (details given in Roesti et al. 2015). Intersex differentiation is quantified by the allele frequency difference (AFD), considering only SNPs with a read depth of at least 20 within each sex, and a minor allele frequency of at least 0.25 across the sexes pooled. The chromosome displayed was chosen because it contains the SNP exhibiting the strongest intersex AFD (0.71) observed in this analysis (blue dot). Note that this genome scan for intersex differentiation is highly noisy due to low sample sizes (12 females and 13 males).



**Figure S2.** Distribution of the magnitude of allele frequency differentiation between the sexes across 1.63 million simulated SNPs, considering sampling stochasticity as the only driver of intersex differentiation. At all simulated SNPs, the expected frequency of the two alleles was invariably 0.5 within both sexes. For the analysis presented in the upper panel, a sparse read depth of just 50 nucleotides per sex pool was assumed. The lower panel is based on a read depth of 120x, which is more representative of our empirical analysis (see Materials and Methods). Since in the latter situation allele frequencies within each sex are estimated with greater precision, the range of observed intersex AFD values is narrower.

Figure S3



**Figure S3.** Magnitude of genetic differentiation between females and males (a), and between two population samples with sex bias in opposite directions (b), along the segment of chromosome XI characterized in Fig. 2. The two graphs are analogous to the panels (a) and (d) in Fig. 2, except that differentiation is here quantified by Nei's 1973  $G_{ST}$  estimator of  $F_{ST}$  (Nei M. 1973 *Analysis of gene diversity in subdivided populations*. Proc. Natl. Acad. Sci. USA 70: 3321-3323).





### **Figure S4 continued**



**Figure S4.** Magnitude of intersex differentiation at SNPs along all stickleback chromosomes, including the sex chromosome XIX. Note that the first approximately 2.5 Mb of the sex chromosome represents the pseudoautosomal region (PAR), to which crossover between the X and Y is restricted. All chromosomes are drawn to the same physical (megabase) scale.



**Figure S5.** Regions of strong differentiation between female and male stickleback also emerge when using SNPs identified after sequence alignment to a *de novo* stickleback genome assembly based on an individual from a population very closely related to the study population. Shown are three representative 50 kb windows on different scaffolds of the *de novo* assembly, each containing a region of high intersex differentiation (bottom row). The corresponding chromosome regions within the standard stickleback reference genome are shown in the top row. The gray vertical lines indicate the alignment positions of the 151 bp sequences used to establish the positional link between the two genome assemblies.

### Figure S6



**Figure S6.** Magnitude of intersex differentiation (AFD) in relation to the strength of selection in simulations of a single locus under divergent SAS. The dots represent mean values across 200 generations of SAS.