Supporting information

Table S1. Published estimates of assortative mating between different threespine stickleback ecotypes as illustrated in Fig. 3 of main paper. For comparability, simplicity and more rigorous inference of assortative mating, only studies using standard "no-choice" or "choice" (e.g. in absence of additional experimental manipulations) experiments and studying nest inspection, nest entry or spawning (i.e. excluding more subtle indicators of mate preference, such as head up posture or orientation) are included. Evidence for assortative mating is classified as Positive (both ecotypes show preference of own type), Asymmetric (one of the ecotypes shows preference of own type) or No (no evidence for assortative mating by ecotype). Traits identified (or assumed, in brackets) as important for mate choice in a given study are given (na=no information available). Ecotypes are indicated as: AN = anadromous, FW= freshwater, RD = red, BL = black, BE=benthic, LI= limnetic, LA= lake, ST=stream, LV=Lava, NI=Nitella and HYB = hybrid. (Note, hybrid combinations are excluded from Fig. 3). Subscripts refer to modifications in the ecotypes based on allopatry ($_{ALL}$) or sympatry ($_{SYM}$), and Japan Sea ($_{JA}$) /Pacific Ocean ($_{PA}$) forms of anadromous stickleback, or inlet ($_{IN}$) and outlet ($_{OUT}$) stream stickleback, respectively. Experiment type is indicated by three categories depending on type of fish used (Wild caught or Lab reared = Wild/Lab) and type of experiment conducted (Choice or no-choice experiment = choice/no-choice; lab vs. semi-natural set up = Lab/Nat) (e.g. artificial ponds). Total number of mating trials (N) in each experiment is reported (for numbers of within and between combination trials see original references). N.B. This overview is merely intended to allow comparisons with findings in our study and does not represent a formal meta-analysis. For some study systems (especially the BE-LI comparisons), several experiments have been reported partially on same data sets, and their interdependency is not here accounted for. Where values were not explicitly provided in text or tables, they were extracted visually from graphs. In Fig. 3, studies are separated to those using A) nest examination and those B) using either nest entry, spawning or genetic mating success as specified below.

Ecotype assortative mating	*Ecotype pairs*	*Female types tested*	*Male types tested*	*Experiment type (N)*	*Response measure*	*Assortative mating*	*Trait affecting choice*	*Reference*
YES	AN - FW	AN FW	AN FW	Wild/Choice/Lab (268)	Nest entry	Positive	na	[1]
		$AN_{JA, PA}$ FW_{PA}	$AN_{JA, PA}$ FW_{PA}	Wild/No choice/Lab (226)	Nest inspection, Nest entry	Positive	Body size	[2]
		AN ST (multiple systems)	AN ST	Wild/No-choice/Lab (850)	Nest inspection	Positive	Body size	[3]
	$AN – AN_{SYM}$	$AN_{JA, PA}$	$AN_{JA, PA}$	Wild/Choice/ Lab	Nest inspection	Asymmetric	(Body size, courtship	[4]

			(18)			behavior)	
BL - RD	BL RD Contact zone	BL RD	Wild/Choice/Nat (52)	Nest entry	Positive	Color	[5]
BE - LI	BE LI	BE LI HYB	Lab/No Choice/Lab (96)	Nest inspection, Spawning	Positive	na	[6]
	BE LI	BE LI	Wild/No choice/Lab (151)	Spawning	Positive	Body size	[7]
	$BE_{ALL, SYM}$	BE LI	Wild/No choice/Lab (231)	Nest inspection,	Positive	na	[8]

					Spawning			
		$BE_{ALL, SYM}$ $LI_{ALL, SYM}$	BE LI	Wild/No choice/Lab (753)	Spawning	Positive	na (Body size)	[9]
		BE LI	BE LI	Wild/Choice/Semi (31)	Spawning	Positive	Body size	[10]
		BE LI	BE LI	Wild/No choice/Lab (318)	Nest inspection, Spawning	Positive /none	(Morphology)	[11]
	LV - NI	LV NI	LV NI	Wild/No choice/Lab (77)	Spawning	Positive	Nest	[12]
NO	AN - FW	AN FW	AN FW	Wild/Choice/Nat (72 mating events)	Mating success (genetic)	None	Body size	[13]

LA-ST	LA ST HYB	LA ST HYB	Lab/Choice/Lab (146)	Nest inspection Nest entry	None	Behavior, (Nest)	[14]
	LA $ST_{IN, OUT}$	LA $ST_{IN, OUT}$	Lab/No choice/Lab (117)	Nest inspection, Nest entry	None	Courtship behavior, (Body size)	This study

References for Table S1

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Table S2. Mixed model analyses of variance on male courtship behavior (display and aggression) in Misty Inlet, Outlet and Lake stickleback testing for the effects of male ecotype (meco) and female ecotype (feco) with relative (RSD) or absolute (ASD) body size difference as covariate. Significant effects are highlighted in bold, marginally significant in italics.

Trait	**Display**						**Aggression**					
	with RSD			with ASD			with RSD			with ASD		
Source												
Random	*Var±S.E.*	*Z*	*P*	*Var±S.E.*	*Z*	*P*	*Var±S.E.*	*Z*	*P*	*Var±S.E.*	*Z*	*P*
Male family (meco)	67.2±22.6	2.98	**0.002**	74. 5±24.6	3.03	**0.001**	24.5±14.5	1.68	**0.046**	26.2±14.9	1.77	**0.039**
Residual	76.9±13.7	5.61	**< 0.001**	80.1±14.3	5.59	**< 0.001**	80.0±15.6	5.64	**<0.001**	88.2±15.7	5.63	**< 0.001**

Fixed	*ndf*	*ddf*	*F*	*P*	*ddf*	*F*	*P*	*ddf*	*F*	*P*	*ddf*	*F*	*P*
Meco	2	47.9	7.87	**0.001**	40	4.47	**0.018**	46.1	2.35	0.107	40.1	6.83	**0.003**
Feco	2	79	2.10	0.130	66.8	2.27	0.111	85.2	0.94	0.394	70.9	0.31	0.731
Meco × Feco	4	66.9	0.43	0.783	66.3	0.39	0.812	70.1	0.53	0.714	70.3	0.52	0.719
RSD	1	98.8	8.46	**0.005**	-			91.8	1.39	0.241	-		
ASD	1	-			83.2	2.83	*0.096*	-			96.7	0.05	0.822

Figure S1. Räsänen et al.

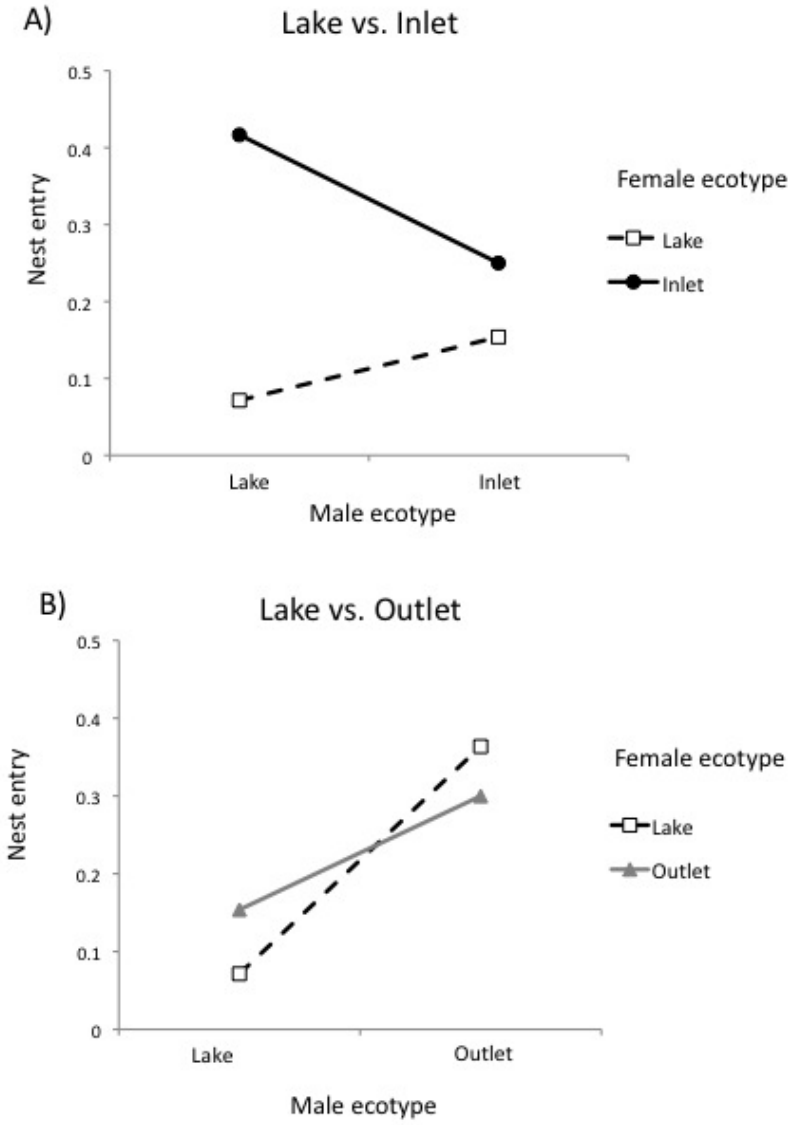

Figure S1. Mating patterns in the A) Lake and Inlet and B) Lake and Outlet combinations of threespine stickleback in the Misty system (using same source data as in Fig. 1C of main paper). The lines present nest entry frequencies in each combination. Noteworthy is that in no case does the pattern reflect positive assortative mating between ecotypes. In A: Inlet females prefer Lake males, whereas Lake females prefer Inlet males, in B: Both Lake and Outlet females prefer Outlet males.