

Genetic distance and social compatibility in the aggregation behavior of Japanese toad tadpoles

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Supplementary materials

Table S1

Information for the 18 clutches used in the experimental trials. Mitochondrial lineages, sampling locality, coordinates, identified MHC class II allele type, number of sequencing samples (figures in parentheses indicate TA cloning samples) used to detect the alleles and RFLP enzymes used for genotyping. To genotype one cohort, we separated 50 tadpoles into two sets (1–25 and 26–50) and used combinations of one to four of the enzymes listed in the table, considering the clutches from which the tested tadpoles were derived. Thirteen alleles, BJ1–12, were detected from among the 18 clutches. Allele BJ1 was present across all lineages, whereas BJ6–10 and BJ 3–5, 11 and 12 were derived only from *japonicus* and *formosus*, respectively. Allele BJ13 was eliminated from the analysis (PCR-RFLP) due to polyandry of clutch NK1.

lineage	site	clutch	Locality	Coordinates	MHC allele			sequencing	RFLP enzyme
<i>miyakonis</i>	1*	DT1	Minami-Daitō Is., Okinawa	N25.844°E131.229°	BJ1	BJ2		5(5)	BseRI, BsgI
		DT2	Minami-Daitō Is., Okinawa	N25.844°E131.229°	BJ1	BJ2		6(4)	BseRI, BsgI
		DT4	Minami-Daitō Is., Okinawa	N25.835°E131.236°	BJ1			7(6)	BseRI
<i>japonicus</i>	2	YW1	Yoshiwa, Hiroshima	N34.457°E132.132°	BJ1	BJ6		4(4)	BseRI, BsgI
		YW2	Yoshiwa, Hiroshima	N34.457°E132.132°	BJ1			7(7)	BseRI
		YW3	Yoshiwa, Hiroshima	N34.457°E132.132°	BJ1	BJ7	BJ8	5(3)	BseRI, BmeT1101, XhoII
		YW4	Yoshiwa, Hiroshima	N34.457°E132.132°	BJ1	BJ9		4(3)	BseRI, XhoII
	3	OM	Omogo, Ehime	N33.715°E133.069°	BJ1	BJ10		4(3)	BseRI, BsgI
	4*	KM1	Komaba, Tokyo	N35.659°E139.688°	BJ1			12(10)	BseRI
		KM2	Komaba, Tokyo	N35.659°E139.688°	BJ1	BJ2		4(4)	BseRI, BsgI
		KM3	Komaba, Tokyo	N35.659°E139.688°	BJ1			10(9)	BseRI
<i>formosus</i>	5	NK1	Nikko, Tochigi	N36.751°E139.586°	BJ1	BJ3	BJ13	15(10)	–
		NK2	Nikko, Tochigi	N36.751°E139.586°	BJ1	BJ3		10(6)	BseRI, BmeT1101
	6	CB	Yorii, Saitama	N36.116°E139.220°	BJ3	BJ11	BJ12	4(3)	BmeT1101, BsgI
	7	ES1	Chichibu, Saitama	N35.888°E138.811°	BJ1	BJ5		7(7)	BseRI, BmeT1101
		ES2	Chichibu, Saitama	N35.888°E138.811°	BJ1	BJ5		6(3)	BseRI, BmeT1101
	8	RS1	Hiki, Saitama	N36.037°E132.294°	BJ4			12(7)	BsgI
		RS2	Hiki, Saitama	N36.037°E132.294°	BJ1	BJ4		5(3)	BseRI, BsgI

*Artificially introduced

Table S2

Restriction enzymes used for PCR-RFLP to genotype 12 alleles of the MHC class II gene. Numbers indicate restriction fragment sizes, e.g. *BseRI* cuts the BJ1 allele into fragments of 0.12 kb and 0.20 kb.

Enzyme	Recognition site	identified MHC alleles used for genotyping												
		BJ1	BJ2	BJ3	BJ4	BJ5	BJ5	BJ6	BJ7	BJ8	BJ9	BJ10	BJ11	BJ12
BmeT1101	c/ycgrg			169/151		169/151	169/151		169/151		169/151	169/151		169/151
BseRI	gaggag	120/200		120/200	120/200			120/200	120/200	120/200	120/200	120/200	120/200	120/200
BsgI	gtgcag		96/224		187/133	96/224	96/224	187/133		187/133	96/224	96/224	96/224	96/224
XhoII	r/gatcy									207/113	207/113			

Figure S1

Amino acid alignment of the MHC class II partial exon (324 bp) of the 13 identified alleles.

allele_BJ1	PLLSITDAVDYMI EVKSECHYVNGTQQVRYLQRYSYNQEEFVYFDSEEGR	50
allele_BJ2	PLLSITDAVDYMTESKFECHYVNGTQQVRFLHRCFYNQEEIVYFDSEEGR	50
allele_BJ3	PLLSITAAVDYMT EVKSECHYVNGTQQVRYLDRYFYNQEEIVYFDSEEGR	50
allele_BJ4	PLLSITDAVDYMT EIKSDCHYVNGTQQVRYLQRYSYNQEEFVYFDSEEGR	50
allele_BJ5	PLLSITDAVDYMT EIKNECHYVNGTQQVRYLHRYFYNQEEITLYFDSDKGR	50
allele_BJ6	PLLSITDAVDYMI EVKSDCHYVNGTQQVRYLQRYSYNQEEFVYFDSEEGR	50
allele_BJ7	PLLSITDAVDYMI EMKSDCHYVNGTQQVRYLQRYSYNQEEIVYFDSEEGR	50
allele_BJ8	PLLSITDAVDYMI EVKSDCHYVNGTQQVRYLQRYSYNQEEFVYFDSEEGR	50
allele_BJ9	PLLSITDAVDYMT EIKSDCHFLNGTQQVRFLHRYFYNQEEIVYFDSEEGR	50
allele_BJ10	PLLSITAAVDYMT EEKFECHYVNGTQQVRYLHRCFYNQEEIVYFDSDKGY	50
allele_BJ11	PLLSITAAVDYMT EEKFECHYVNGTQQVRYLHRCFYNQEEIVYFDSDKGR	50
allele_BJ12	PLLSITAAVDYMT EEKFECHYVNGTQQVRYLHRCFYNQEEFVYFDSDKGR	50
allele_BJ13	PLLSITDAVDYMI EVKSECHYVNGTQQVRYLQRYSYNQEEFVYFDSEEGR	50
allele_BJ1	FI AKTEFGKPSAD YWNKN KDF I EDRKSAVERFCKHNYG - VFHSVTADRKG	99
allele_BJ2	Y I AKTEFGKPDAD YWNKN KE I I EQRKSYVET YCKHNYG - VFHSVTADRRG	99
allele_BJ3	Y I AKTELGKPDAD EAWNKN KE I I EDRKSSVET FCKYNYGT ATT V G I T GRRG	100
allele_BJ4	FI AKTEFGKPSAD YWNK D KDF I EQKKS AVET FCKHNYG - VFHSVTADRRG	99
allele_BJ5	Y I AKTELGKPEAEAWNKN KE I I EDRKSAVE ? FCKYNYG - VIHSFTADRRG	99
allele_BJ6	FI AKTEFGKPSAE LWNK D KDF I EQKKS AVET FCKHNYG - VLHSVTADRRG	99
allele_BJ7	FI AKTELGKPDAD YWNKN KDF I EQRKSAVET FCKHNYG - VFHSVTADRRG	99
allele_BJ8	FI AKTEFGKPSAD YWNK D KDL I EQRKSAVET FCKHNYG - VLHSVTADRRG	99
allele_BJ9	Y I AKTELGKPDAD YWNK D KDL I EREKSYVET FCKYNYG - VFHSVTADRRG	99
allele_BJ10	Y I AKTELGKPDAD YWNK D KE I I EHKKSEVET FCKHNYG - VLHGVTGDRRG	99
allele_BJ11	Y I AKTEFGKPDAD EAWNKN KE F I EQKKS AVET FCKHNYG - VLHGVTGDRRG	99
allele_BJ12	Y I AKTELGKPDAD EAWNKN KE I I EQKKS EVET FCKHNYG - VLHGVTGDRRG	99
allele_BJ13	I I AKTEFGKPSAD YWNKN KDF I EDRKSAVERFCRHNYG - LYHSVTADRKG	99
allele_BJ1	ETLYILNY	107
allele_BJ2	ETLYILNY	107
allele_BJ3	ETLYILNY	108
allele_BJ4	ETLYILNY	107
allele_BJ5	ETLYILNY	107
allele_BJ6	ETLYILNY	107
allele_BJ7	ETLYILYY	107
allele_BJ8	ETLYILNY	107
allele_BJ9	ETLYILNY	107
allele_BJ10	EPLYILYY	107
allele_BJ11	ETLYILNY	107
allele_BJ12	ETLYILNY	107
allele_BJ13	ETLYILNY	107