Table 1. Effect of various carbohydrates on the fusion of mouse sperm to mouse zona-free eggs in vitro

Carbohydrate	Conc. (mM)	Pronuclear formation* (%)	Carbohydrate	Conc. (mM)	Pronuclear formation* (%)
None	–	56.2 ± 4.4	None	–	69.3 ± 5.1
L-Fuc	20	54.1 ± 10.0	GalN	20	50.3 ± 22.4
D-Man	20	47.1 ± 15.1	GlcN	20	21.4 ± 11.5**
D-Gal	20	51.7 ± 16.0	ManN	10	60.2 ± 5.9
D-Glc	20	52.9 ± 12.9			
GalNAc	20	55.0 ± 11.5			
GlcNAc	20	59.5 ± 9.2			
ManNAc	20	48.5 ± 8.7			

* Mean ± SE of 4 independent tests (more than 45 eggs were examined in each group) significantly different from control; ** $p < 0.01$.

Table 2. Reversible inhibition of pronuclear formation of mouse eggs by glucosamine in vitro

	GlcN (mM)	No. of experiments	No. of eggs examined	Pronuclear formation[a] (%)	Sperm bound index[b]
Exp. 1: eggs were not transferred during the procedure					
Control	0	4	95	56.5 ± 7.4	9.0 ± 1.9
Test	20	4	79	17.0 ± 5.7**	16.5 ± 1.4*
Exp. 2: eggs were transferred after 1 h of incubation to a GlcN-free medium					
Control	0	4	67	55.2 ± 6.7	Not counted
Test	20	4	59	86.6 ± 6.3*	Not counted

[a] Mean ± SE of 4 independent tests. [b] The index was based on the number of sperm bound to eggs. Those eggs which had more than 20 sperm on their surface were scored as 20. The index was expressed as a mean ± SE of these scores. Significantly different from control; * $p < 0.05$, ** $p < 0.01$.

Results and discussion. When epididymal sperm were introduced to zona-free eggs, all the eggs were covered by sperm within 1 h. However, after 5 h of incubation, many sperm had detached from the fertilized eggs. Interestingly, eggs which remained unfertilized retained sperm on their plasma membrane (fig., A). The result indicated that extra sperm were no longer able to remain on the surface of a fertilized egg. In order to avoid the various carbohydrates tested affecting sperm capacitation (and/or acrosome reaction), sperm preincubated in m-KRB for 60 min were used in the following experiments. When preincubated sperm were added to eggs in the presence of various sugars, binding to the egg membrane was not inhibited by any of the carbohydrates tested. However, sperm-egg fusion was significantly inhibited by the addition of GlcN, whereas GalN and ManN were not similarly effective (table 1). The effect was not based on the inhibition of sperm-egg binding. More sperm were observed on the egg surface when GlcN was added to the medium (fig., B) compared to control eggs (fig., C). (The number of bound sperm remained high even after 5 h of incubation in the GlcN-added group. The photo was taken after 1 h of incubation to clarify the effect of GlcN on binding only, because many sperm detached from the eggs after 5 h in the control group when fertilization had been accomplished.) These data clearly demonstrated that the effect of GlcN was due to the block of sperm-egg fusion. It should be noted that the inhibition of fusion by GlcN was a reversible effect. When eggs, heavily covered by sperm in the GlcN-added medium, were transferred after 1 h of incubation to a GlcN free medium and incubated another 4 h, fusion of the gametes occurred at an even higher rate than in the control group. Reflecting the number of sperm on the egg surface, a majority of the eggs became polyspermic in the GlcN-added group, while many eggs were monospermic in the control group (table 2).

The data shown in this article demonstrated that sperm-egg fusion is a two-step reaction; binding and fusion. Shur et al., claiming an involvement of GalTase in sperm-zona binding, recently postulated that the enzyme might also play a role in sperm-egg binding [9]. If we interpret our data to indicate that binding was enhanced so strongly by GlcN that fusion was inhibited, the result, together with Shur's model, may provide a deeper view of sperm-egg fusion.

Abbreviations. GlcN: glucosamine; GalN: galactosamine; ManN: mannosamine; GalTase: galactosyltransferase.

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Sex linkage of malic enzyme in *Xenopus laevis*

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Summary. Genetic analysis of mME variants (mitochondrial malic enzyme, E.C. 1.1.1.40) in *Xenopus laevis* revealed sex linkage of the mMe locus and indicated a WZ/ZZ type of sex determination. Codominant mMe alleles occur on both W and Z chromosomes, with a recombination frequency of 6.1 % ± 1.5 % between mMe and the sex-determining locus (or region).
Key words. Xenopus laevis; sex-linked genes; sex determination; amphibian genetics.

In contrast to mammals, a great majority of anuran amphibians analyzed cytologically do not show heteromorphic sex chromosomes. However, genetic analysis of several frog species (genus *Rana*) revealed sex linkage of certain enzyme loci [1–4]. The mode of inheritance of these loci indicated an XX/XY type of sex-determining mechanism (i.e. male het-

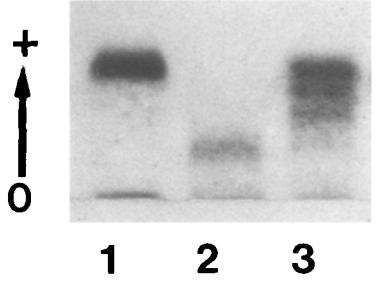
erogamety). Interestingly, recombination was observed between some of the sex-linked genes and the locus (or region) controlling sex, suggesting that male- and female-determining factors are carried on a pair of homologous chromosomes, of which a large part is not involved in the sex-determining mechanism[1-4].

In *Xenopus laevis*, breeding of sex-reversed males[5], germ cell transfer[6], and experimental gynogenesis[7] indicated female heterogamety (♀ WZ, ♂ ZZ). However, there is no evidence of sex chromosome heteromorphism in this species. The present paper reports the sex linkage of the mMe locus (mitochondrial malic enzyme) in *Xenopus laevis*, and confirms the model of a WZ/ZZ sex-determining mechanism.

Methods. A series of crosses was made between laboratory-bred animals of the following subspecies: *Xenopus laevis laevis* (LL) homozygous for the periodic albinism mutation[8], *X. laevis victorianus* (LV), *X. laevis* subspecies nova I (LI) originating from Malawi, and *X. laevis poweri* (LP). Ovulation was artificially induced and fertilization was done in vitro following the procedure described in Gurdon[9]. First generation hybrids between LL and LV, LI or LP were backcrossed to LL or to the other parental subspecies. A sample of each backcross progeny was reared through metamorphosis. Subadult frogs were killed, dissected, and examined for gonadal sex. Tissue samples were either stored at − 70 °C or processed immediately. For separation of mitochondrial and soluble fractions, fresh tissues (kidney or liver) were briefly homogenized with 3 volumes of sucrose buffer (0.28 M sucrose, 5 mM $MgCl_2$, 25 mM KCl, 25 mM Tris-HCl, pH 7.4). All operations were done at 4 °C. The homogenate was centrifuged at 700 × g for 10 min. The supernatant was collected and centrifuged at 12,000 × g for 5 min. The resulting pellet, which contained the mitochondria, was incubated on ice for 30 min in 3 volumes 5 mM $MgCl_2$, 25 mM KCl, 25 mM Tris-HCl, pH 7.4, containing 1% Triton X-100 and 0.01% 2-mercaptoethanol. The suspension was subsequently centrifuged at 12,000 × g for 5 min, and the supernatant (i.e. the mitochondrial fraction of the tissue extract) was subjected to horizontal starch gel electrophoresis using an amine-citrate buffer[19] at pH 6.0. The gels were stained in a solution for malic enzyme activity[10].

Results and discussion. In *Xenopus laevis laevis*, malic enzyme occurs in various organs (e.g. liver, kidney, muscle, stomach) in the form of two electrophoretically distinct isozymes[11]. When the mitochondrial fraction of a tissue homogenate is subjected to electrophoresis, only one band of ME activity is revealed, indicating that the isozyme with the highest anodal mobility (in amine-citrate or tris-citrate, pH 6.0) corresponds to the mitochondrial form (mME), whereas the band closer to the origin represents the soluble form (sME)[11]. Electrophoretic comparison of mitochondrial ME from various *X. laevis* subspecies revealed differences of mobility. For instance, the mME of *X. l. laevis* has a higher anodal mobility than the mME of *X. l. poweri*, and first-generation hybrids between these subspecies display a smear in which diffuse intermediate bands (presumably heterotetramers[10]) appear in addition to the two parental bands (fig.). This heterozygous mME phenotype was found in male and female hybrids, indicating expression of both parental alleles.

The segregation of alleles at the mMe locus was analyzed in four backcross families. It is noteworthy that hybrids between *X. laevis* subspecies show normal fertility[12,13] and normal pairing of homologs in meiosis[14]. The results of the segregation analysis are presented in the form of a contingency table testing the association of the mMe genotype with sex (table). When females heterozygous for mMe were crossed with homozygous males (crosses 1, 2, 5), the frequencies of the four genotypic classes of offspring showed a highly significant deviation from the expected 1:1:1:1 ratio (table). In contrast, the cross involving a heterozygous male and a homozygous female (cross 4) produced offspring showing no association between sex and mMe genotypes. These results clearly indicate that the mMe locus is sex-linked and that the female is the heterogametic sex (♀ WZ, ♂ ZZ). In cross 1, the female parent transmitted to 92.3% of her female offspring the mMe[b] allele inherited from her mother, and to 94.3% of her male offspring the mMe[a] allele inherited from her father. This implies that the W chromo-



Electrophoretic patterns of mitochondrial malic enzyme. Slot 1: *X. laevis laevis* (LL), genotype a/a. Slot 2: *X. laevis poweri* (LP), genotype c/c. Slot 3: hybrid (LP × LL), genotype a/c. Mitochondrial fractions of kidney homogenates were electrophoresed in starch gel with amine-citrate buffer (pH 6.0)[19].

Segregation of mMe alleles and sex in crosses involving hybrids between various *Xenopus laevis* subspecies

Cross[a]	Parents' mMe genotype[b] Female	Male	Offspring mMe genotype[b]	Female	Male	χ^2[c]		R. F. (%)[d]
1	b/a	a/a	b/a	36	3	73.83	$p < 0.001$	6.5 ± 2.6
			a/a	3	50			
2	b/a	a/a	b/a	34	2	49.87	$p < 0.001$	6.7 ± 3.2
			a/a	2	22			
4	b/b	b/a	b/a	22	20	2.20	$p > 0.5$	
			b/b	22	14			
5	c/a	a/a	c/a	50	2	78.18	$p < 0.001$	5.3 ± 2.2
			a/a	3	40			
							mean	6.1 ± 1.5

[a] In each of the four backcrosses, the heterozygous parent originated from hybridization between different subspecies. The composition of the backcrosses was as follows (female listed first, abbreviations explained in 'Methods'): Cross 1, [LV × LL] × LL; cross 2, [LI × LL] × LL; cross 4, LV × [LV × LL]; cross 5, [LP × LL] × LL. [b] The allele inherited from the mother is always listed first. [c] χ^2 values are calculated with an expected 1:1:1:1 ratio for the combination of sex with mMe genotype. [d] Recombinant frequency (in percent).

some of the female of cross 1 carried the mMe^b allele, whereas her Z chromosome carried the mMe^a allele. Following meiosis, about 6% of the ova produced by that female carried a W or Z chromosome that had undergone crossing-over between the mMe locus and the locus (or region) controlling sex. Similarly, the female parent of cross 5 had a W chromosome carrying the mMe^c allele and a Z chromosome carrying the mMe^a allele; 5.3% of the offspring showed recombination between mMe alleles and the sex-determining genes.

The mode of inheritance of the sex-linked mMe locus in *Xenopus laevis* suggests that the dominant female-determining factors (W) and the recessive male-determining factors (Z) are carried on a pair of homologous chromosomes that behave like autosomes. This pattern of sex linkage is quite different from that found in mammals, in which the sex-linked genes are carried almost exclusively on the X chromosome, resulting in hemizygous male genotypes, and crossing-over between the X and Y is suppressed along much of their lengths[15]. It is consistent, however, with the mode of inheritance of sex-linked genes observed in other amphibians, i.e. *Pleurodeles waltlii*[16], *Rana clamitans*[1,2] and *Rana pipiens*[3,4]. Whether or not the mMe locus is sex-linked in these species has so far not been determined. However, comparison of genetic maps among North American *Rana* species suggests that the linkage relationship of sex-determining genes to other loci is evolutionarily unstable even within the same species group[2,4].

Cytogenetic and molecular evidence indicates that *Xenopus laevis* has a tetraploid origin. As a matter of fact, many of the protein loci tested in electrophoretic surveys show multiple banding patterns resulting from duplicate gene expression[11,17]. On the basis of electrophoretic phenotypes and segregation analysis, it appears that mitochondrial malic enzyme is encoded in a single locus in *X. laevis*. The second copy of the mMe locus has probably been silenced or lost as an effect of diploidization, as reported, for example, of the major histocompatibility complex[17]. Quite similarly, sex determination seems to conform to a model with two alleles (i.e. W and Z) segregating at a single locus. In support of this model, it has been shown that the presence of additional sex-determining genes in artificial *Xenopus* polyploids had the effect of disturbing the normal course of genetic sex determination[18].

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Comparative variation at four enzyme loci in ten Melanopline grasshoppers

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Summary. Previous investigations of the migratory grasshopper, *Melanoplus sanguinipes*, demonstrated geographical variation at visible loci but not at enzyme loci. Electrophoretic analysis of 3 other Melanoplines and an Oedipodinid also revealed uniform spacial distributions. Investigation of allelic patterns among these and 6 other Melanoplines suggests that selective rather than stochastic factors are operative.
Key words. Melanoplus sanguinipes; Camnula pellucida; grasshoppers; polymorphism; allozyme.

The migratory grasshopper, *Melanoplus sanguinipes*, is polymorphic for a number of heritable color and electrophoretic characters[2,3]. Visible traits display spacial variation, as exemplified by hind tibia color. In the province of Saskatchewan, there is a dramatic shift in red/blue frequency between parkland and prairie regions. For electrophoretic traits, however, frequencies are more or less the same between and within these areas. Geographical uniformity can result from 1) an interaction of genetic drift and migration; 2) some form of selection in which selection coefficients are independent of the external environment and migration effects are superfluous; or 3) homogenizing effects of gene flow that override local adaptive differences. This paper examines these hypotheses by looking at patterns of electrophoretic variation in several other Melanopline species and an Oedipodinid, *Camnula pellucida*, the clear-winged grasshopper. Since species are reproductively isolated, possible confounding effects of gene flow that may exist within species are obviated. Comparisons of allelic configurations between species should, therefore, be particularly illuminating[4].