

A sex-linked enzyme in a reptile – association with a recent centric fusion in the common lizard

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(Received 20 November 1985 and in revised form 5 March 1986)

Summary

In a series of *Lacerta vivipara* samples from southern France's central mountains, the distribution of genotypes at the mannose phosphate isomerase (MPI) locus has only been accountable as due to sex linkage in female heterogamety, provided the W chromosome carries only one of the two observed alleles in the populations sampled. This is in perfect accordance with cytogenetical data. Reasons are presented that seem to limit acceptable hypotheses for the recent origin of this sex-linked polymorphism to three: a founder effect, a point mutation in Z chromosomes after crossover suppression, or hitch-hiking with the translocation involved.

1. Introduction

Reptiles, consistently the poor relatives among vertebrates in genetic studies, are invaluable in one particular field of genetics: evolution of sex determination (Ohno, 1967; Bull, 1980; Jones, 1984). While in carinate birds (Ohno, 1967; Baverstock *et al.* 1982) and eutherian mammals (Ohno, 1967; O'Brien, 1984) a highly conserved sex chromosome is present throughout the group and seems to have been inherited from a common ancestor, squamata and chelonia offer a large array of cases from complete absence of genotypic sex determination (Pieau, 1971; Bull, 1980; Standora & Spotila, 1985) to various conditions of mild or strong chromosome heteromorphism and simple or multiple systems in either male or female heterogamety: contrary to the situation in fishes and amphibians, where differentiated sex chromosomes are rare (Bull, 1983), optically recognizable sex chromosomes are the rule in major snake families and have independently arisen many times in lizards and twice in turtles (Bull, 1980; Peccinini-Seale, 1981; Bickham, 1984).

Ironically, though, little is still known about the early stages of X–Y or Z–W differentiation (Bull, 1983), as the genetics of sex determination and sex-linked traits has been little investigated in reptiles. (Breeding reptiles is unrewarding, and usually inef-

ficient in terms of genetics even when successful.) This report illustrates early sex linkage in the common Eurasian lizard *Lacerta vivipara*, where we assign a gene to a chromosome for the first time in reptiles.

2. Materials and Methods

One way to circumvent breeding difficulties is statistical analysis of protein electrophoresis data obtained from samples of natural animal populations. If a polymorphic gene is linked with a sex-determining factor, the discrepancy relative to allele frequencies at autosomal loci in the two sexes should readily show up.

Some 149 individuals of the common lizard have been collected in six areas of France's south-central mountain ranges, where *L. vivipara* populations possess a conspicuous Z_1Z_2W sex chromosome system involving 8 percent of genome length in both sexes (Chevalier *et al.* 1979). Among 24 enzyme loci obtained after submitting 14 enzyme systems from liver and kidney homogenates to standard starch gel electrophoresis (see techniques in Pasteur *et al.* 1986), 12 loci were polymorphic, and in all of these except one the genotypic frequencies did not deviate significantly from the usual Hardy–Weinberg proportions. The exception was with mannose phosphate isomerase (MPI, Enzyme Commission No. 5.3.1.8), a monomeric enzyme whose electrophoresis yields straightforward, unambiguous migration pictures. Only two electro-

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Table 1. Observed genotype frequencies at the MPI locus in *Lacerta vivipara* samples from southern France's central mountains. From north to south

Area	Males			Females		
	<i>MPI</i> ^{100/100}	<i>MPI</i> ^{100/120}	<i>MPI</i> ^{120/120}	<i>MPI</i> ^{100/100}	<i>MPI</i> ^{100/120}	<i>MPI</i> ^{120/120}
Adults						
Les Puys	13	3	0	0	21	0
Lac de Charpal	10	5	0	0	18	3
Mt Lozère, NE	1	0	0	0	2	0
Mt Lozère, SW	8	1	0	0	13	0
Espinouse	4	0	0	0	10	0
Pradel	1	0	0	0	1	0
Subtotal	37	9	0	0	65	3
Young						
Lac de Charpal	2	2	0	0	3	1
Mt Lozère, NE	—	—	—	0	1	0
Mt Lozère, SW	7	3	0	0	10	2
Espinouse	1	1	0	0	1	1
Subtotal	10	6	0	0	15	4
Total	47	15	0	0	80	7

morphs, *MPI*¹⁰⁰ and *MPI*¹²⁰, have been observed (hereafter *p* and *q* will stand for the respective frequencies), and frequencies of corresponding genotypes in our *L. vivipara* samples are given in Table 1. Age classes have been distributed into two groups, 'adults' for those lizards where sexual maturity had been reached or was to be reached within the season and 'young' for the remainder (a size gap was present in each sex as the lizards were collected in late spring/early summer and in autumn).

3. Results

Two homozygous genotypes are completely missing, *MPI*^{120/120} in males and *MPI*^{100/100} in females. Three-quarters of males are homozygous for *MPI*¹⁰⁰, while as many as 92% of the females are heterozygotes (Table 1).

(i) *Autosomy and selection*

Under an autosomal inheritance hypothesis, allelic frequencies are very significantly different in the two sexes (*p* = 0.88 in males, *p* = 0.46 in females). Such a result could be explained on the basis of different fitnesses in the two sexes. For instance, we may suppose sex-limited autosomal lethality: in homozygous condition, *MPI*¹⁰⁰ would be lethal in females and *MPI*¹²⁰ would be so in males. In such an unusual kind of balanced lethal system polymorphism would then be maintained by fitness heterosis in the female sex; this might seem a not too far-fetched explanation, as population studies of *L. vivipara* (Avery, 1975; Bauwens & Verheyen, 1980; Pilorge & Xavier, 1982; Heulin,

1984) and other lizards (Barbault, 1976) usually show females surviving better and reproducing longer than males, and supposing that MPI has something to do with it in the populations studied here. However, that in males the *MPI*^{100/100} phenotype should be much more successful than the heterozygous phenotype is hardly consistent with *MPI*¹⁰⁰ switching to lethality in the other sex.

On the other hand, if we compare proportions between young and adults for localities where both are present, either in males or females, no significant difference is found globally, but we can note that, in all these localities, the change of proportions up to the adult stage is always in the same direction, favouring 100/100 in males, 100/120 in females. The probability of random deviations being such is 0.0039 (Fisher's exact test for four male and four female samples). So there is some indication for a mild selection favouring allozyme *MPI*¹⁰⁰ against *MPI*¹²⁰ during postnatal life, and this is incompatible with this allozyme being lethal in one sex. A necessary implication is selection reversal: a counterselection has to take place if the MPI polymorphism is to remain in existence (see Marin-kovic & Ayala, 1975*a, b*; Pasteur, 1977; Goux, 1978).

It is impressive to see that genotypic frequencies in males strictly obey binomial proportions, putting re-

Table 2.

	<i>MPI</i> ^{100/100}	<i>MPI</i> ^{120/100}	<i>MPI</i> ^{120/120}
Adults	37.4	8.1	0.4
Young	10.6	4.9	0.6
All	48.0	13.0	1.0

strictions on possible selection models. The expected numbers among males are, with the hypothesis of random mating, as shown in Table 2.

All these figures are very close to the observed numbers in Table 1.

(ii) Sex linkage

Linkage with sex is strongly suggested, even though the observed genotypic distribution runs contrary to classical-sex linked inheritance, where the heterogametic sex can only be hemizygous. Contrasting with the inconsistencies of explaining the phenotype frequencies through selection at an autosomal locus, the data fit in well with sex linkage of the MPI locus if (1) the female sex is heterogametic, but (2) the odd sex chromosome (W) is not inert or 'empty' at this locus even though the locus is part of a 'differential segment' of the pairing chromosomes where chiasmata no longer occur. However, since MPI^{100/100} daughters are never produced the MPI¹⁰⁰ allele is necessarily absent in the female-limited W chromosome, which is left with MPI¹²⁰ alone. Note that symmetrical reasoning could not account for the lack of MPI^{120/120} males in the samples: Z chromosomes borne by females cannot have only MPI¹⁰⁰ alleles since MPI^{120/120} daughters are produced.

Let us then suppose that all Z chromosomes

Table 3.

Male chromosomes	Female chromosomes		
	Z ₁₀₀	Z ₁₂₀	W ₁₂₀
Z ₁₀₀	♂100/100	♂120/100	♀120/100
Z ₁₂₀	♂120/100	♂120/120	♀120/120

recombine freely and alleles MPI¹⁰⁰ and MPI¹²⁰ are mixed in a Z chromosome pool. Qualitatively, crosses will be as shown in Table 3.

The observed number of Z₁₂₀ chromosomes is 15 out of 124 in males, 7 out of 87 in females. We have the results shown in Table 4.

It is worth pointing out that the assumption of sex linkage gives calculated gene frequencies that are (virtually) equal in the two sexes, i.e. what they should be in populations at the equilibrium.

From the above values we can compute the overall frequencies to be expected in panmictic conditions. Similar operations can be separately done from young and adult observed frequencies. Table 5 shows that the results agree quite satisfactorily with the observed data, to which the goodness-of-fit test with 8 D.F. gives $\chi^2 = 9.113$ ($P \approx 0.34$). All other hypotheses lead to widely different frequencies. So we conclude that the MPI locus is sex linked.

Table 4.

	Males	Females	Combined
<i>q</i>	15/124 = 0.1210	7/87 = 0.805	22/211 = 0.1043
<i>p</i> = 1 - <i>q</i>	0.8790	0.9195	0.8957

Table 5. Average expected frequencies at the MPI locus in the populations of *L. vivipara* sampled, under the hypothesis that the locus is sex-linked but allele MPI¹⁰⁰ is excluded from the W chromosome, and assuming that mating is random. In parentheses: observed frequencies

Sex	Sample size	Phenotypes		
		MPI ^{100/100}	MPI ^{100/120}	MPI ^{120/120}
Young				
Males	16	12.84 (10)	2.99 (6)	0.17 (0)
Females	19	—	17.02 (15)	1.98 (4)
Adults				
Males	46	36.90 (37)	8.59 (9)	0.50 (0)
Females	68	—	60.91 (65)	7.09 (3)
All				
Males	62	49.75 (47)	11.59 (15)	0.67 (0)
Females	87	0 (0)	77.93 (80)	9.07 (7)

4. Discussion

Is the foregoing interpretation consistent with what is known of the sex chromosomes from cytology?

Female heterogamety of recent origin is indicated by the results, since the inferred W chromosomes are endowed with genetic function at the MPI locus rather than being inert, even though crossing over is suppressed (see Charlesworth, 1978). This nicely matches cytogenetical data from *Lacerta vivipara*, whether general or local. The common lizard displays – over a range that covers, from Spain to Kamchatka and Japan, a seventh of all lands – geographical variation in karyotype (Chevalier *et al.* 1979; Kupriyanova, pers. comm.): some populations have a distinctive karyotypic female digamety, others do not show any chromosomal heteromorphism (Chevalier *et al.* 1979; van Brink, 1959; Orlova & Orlov, 1969). It follows that chromosomal sex determination is likely to be at very early stages of evolution in this lacertid species, the more so as related species (see Gorman, 1973) do not have visible sex chromosomes. Concerning populations of south central France in particular, Chevalier *et al.* (1979) have observed that (1) all 18 chromosome pairs in males are telocentric, and (2) telocentrics nos. 11 and 15 remain unpaired in females, where there exists a very large metacentric which is also unpaired and whose two arms have just about the lengths of the unpaired telocentrics. It follows that the large metacentric is a W chromosome resulting from a Robertsonian translocation (centric fusion) between chromosomes 11 and 15, henceforth Z_1 and Z_2 , one of which was bearing a female-determining locus ('sex factor'). Males are $Z_1Z_1Z_2Z_2$ and females are Z_1Z_2W . Since in other species of lizards the MPI locus is autosomal, this locus must be on one of the Z chromosomes, with an allele on the homologous part of the W chromosome.

How then can we explain the formation of a W with MPI^{120} only? One possibility can be dismissed at the outset: an evolution similar to that acknowledged for the main stems of birds and mammals, i.e. both alleles were present in the ancestors of present-day heterochromosomes before crossover suppression between the sex-factor region and the MPI locus, and later MPI^{100} was rendered inactive by deleterious mutations. This would imply, in addition to an inconsistently long evolution, the existence of females heterozygous for MPI^{100} and a null allele, and therefore looking like $MPI^{100/100}$ homozygotes. No female of such a kind has been observed.

Luykx (1981), finding recent association of a particular esterase allele with the heterogametic sex in the termite *Incisitermes schwarzi*, attributes it either to a founder effect, or to hitch-hiking of the gene with the translocation establishing the sex chromosome system (see the Ishii & Charlesworth (1977) review), or to a sex difference in the fitnesses of gene combinations at or near the locus at stake. As far as our material is

concerned, the third hypothesis seems falsified, since the MPI^{100} allele is similarly selected for survival, and consequently submitted to similar endocyclic selection, in the two sexes.

Call A' the telocentric chromosome involved in the centric fusion and A its homologue which has become a Z. If we suppose that A' was carrying an MPI^{120} allele, the apparent absence of recombination between the Z and W chromosomes could be due to close association of the MPI locus and the female sex determiner on the A' chromosome. Then the founder-effect hypothesis would be that this would not preclude rare crossing over, that south central France's populations were founded through females all of which bore an MPI^{120} -carrying W, and there would not have been enough time to reach genetic equilibrium through rare recombination. Another way to look at a linkage disequilibrium situation does not require a colonization event. Suppose only allele MPI^{120} existed until the chromosomal rearrangement occurred. MPI^{100} could have appeared later through mutation (or immigration) in the Z-chromosome subpopulation, where it would have spread to its present-day frequencies.

However, the existence of two or several hundred lizards per hectare in Massif Central (Pilorge, 1982; personal observation), with an absolute number in the 10^6 or 10^7 order of magnitude over the mountain range, makes it hard to admit a significant departure from equilibrium, unless crossing-over-inhibiting factors accumulated in the system, for instance inversions. On the other hand, the hitch-hiking hypothesis (Charlesworth & Charlesworth, 1980) offers the advantage of permitting an equilibrium situation, and is also compatible with the results. Finally, if the absence of 120/120 males proves to be due to such males' being effectively not produced when a much larger sample is investigated, it will then become a serious possibility that allele MPI^{120} is not transmitted from the female. For the time being, a far simpler explanation for the absence of 120/120 males is sampling hazards (see Table 5).

There is little doubt that the various possibilities can be tested when other populations in the region have been explored for MPI and sex-chromosome polymorphisms, the easiest assumption to put to the test being that of an MPI^{100} mutation subsequent to crossover 'suppression'. Pending this, and hopefully the discovery of additional sex-linked polymorphism, at any rate there is sufficient evidence to state that we are dealing here with a chromosomal sex determination system *in statu nascendi*.

Lizard samples were collected by Dr Jean-Paul G. Orsini. We are glad to acknowledge the technical assistance of Anne-Marie Orth, and we thank Drs Nicole Pasteur and Vitaly Volobouev for reading the manuscript. Numerous clarifications were suggested by a referee, to whom we are grateful for his or her help.

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