Linkage Groups of Protein-Coding Genes in Western Palearctic Water Frogs Reveal Extensive Evolutionary Conservation

Hansjürg Hotz,* Thomas Uzzell† and Leszek Berger‡

**Zoologisches Museum, Universität Zürich-Irchel, CH-8057 Zürich, Switzerland, †Academy of Natural Sciences, Philadelphia, Pennsylvania 19103 and ‡Research Centre for Agricultural and Forest Environment, Polish Academy of Sciences, PL-60-809 Poznań, Poland*

Manuscript received October 14, 1996
Accepted for publication April 21, 1997

ABSTRACT

Among progeny of a hybrid (*Rana shqiperica* × *R. lessonae*) × *R. lessonae*, 14 of 22 loci form four linkage groups (LGs): (1) *mitochondrial aspartate aminotransferase, carbonate dehydratase-2, esterase 4, peptidase D;* (2) *mannosephosphate isomerase, lactate dehydrogenase-B,* sex, *hexokinase-1, peptidase B;* (3) *albumin, fructose-biphosphatase-1, guanine deaminase;* (4) *mitochondrial superoxide dismutase, cytosolic malic enzyme, xanthine oxidase. Fructose-biphosphate aldolase-2* and *cytosolic aspartate aminotransferase* possibly form a fifth LG. *Mitochondrial aconitate hydratase, α-glucosidase, glyceraldehyde-3-phosphate dehydrogenase, phosphogluconate dehydrogenase,* and *phosphoglucomutase-2* are unlinked to other loci. All testable linkages (among eight loci of LGs 1, 2, 3, and 4) are shared with eastern Palearctic water frogs. Including published data, 44 protein loci can be assigned to 10 of the 13 chromosomes in Holarctic Rana. Of testable pairs among 18 protein loci, agreement between Palearctic and Nearctic Rana is complete (125 unlinked, 14 linked pairs among 14 loci of five syntenies), and Holarctic Rana and *Xenopus laevis* are highly concordant (125 shared nonlinkages, 13 shared linkages, three differences). Several Rana syntenies occur in mammals and fish. Many syntenies apparently have persisted for 60–140 × 10^6 years (frogs), some even for 350–400 × 10^6 years (mammals and teleosts).

A growing body of recent evidence suggests that some linkage groups (LGs) and syntenies of protein-coding genes have been highly conserved during the evolution of vertebrate animals (*e.g.*, MORIZOT 1983, 1990, 1994; STALLINGS and SICILIANO 1983; GRAF 1989a; MORIZOT *et al.* 1991; O'BRIEN 1993b). New molecular techniques have greatly facilitated the identification of genetic markers, many of them anonymous, allowing rapid construction of genetic maps for many organisms (*e.g.*, BERNATZKI and TANKSLEY 1986; BARKER *et al.* 1987; LANDRY *et al.* 1987; MICHELMORE *et al.* 1991; OSTRANDER *et al.* 1992; TANKSLEY *et al.* 1992; COPELAND *et al.* 1993; WILLIAMS *et al.* 1993; GYAPAY *et al.* 1994; POSTLETHWAIT *et al.* 1994; RABBITTS *et al.* 1995; MA *et al.* 1996). Nevertheless, it is important to map specific genes of known coding function and relatively easily determinable homology among distantly related taxa (for example, using protein electrophoresis), because it permits reconstruction of ancestral gene arrangements, and because a comparison between protein-coding genes and functionally different genome regions is essential for an eventual understanding of whether long-term LG conservation has an adaptive significance. While searching for genetic markers to study an unusual reproductive mode in a group of frogs, we have found strong supporting evidence for such evolutionarily conserved LGs.

Corresponding author: Hansjürg Hotz, Zoologisches Museum, Universität Zürich-Irchel, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland. E-mail: hotz@zoolmus.unizh.ch

Hemiclonal reproduction characterizes the widespread and abundant natural interspecies hybrid lineages of western Palearctic water frogs (*Rana esculenta* group, Amphibia; GRAF and POLLS PELAZ 1989 provided a review). These hybrids (*AB; AA* × *BB*) exclude one parental genome (*B*) in the germ line, endoreduplicate the other (*A* → *AA*), and produce haploid, unrecombined gametes (*A*), whether ova or sperm; hybridity (*AB*) is restored in the following generation because hybrids mate with parental species *BB* (hybridogenesis: SCHULTZ 1969). Inter- as well as intraspecific genetic variation in traits connected with the hemiclonal gametogenesis of hybrids has been detected in Mendelian species of these frogs (HOTZ and UZZELL 1983; HOTZ *et al.* 1985; BUCCI *et al.* 1990; GUERRINI *et al.* 1997). Hemiclonal reproduction precludes direct genetic analysis of hybridogenesis, because the clonally transmitted genome acts as a single recombination unit, but the intraspecific variation found can be used to circumvent this difficulty and analyze the genetic basis of hemiclonal gametogenesis. As part of such an analysis, we have begun to determine LGs in western Palearctic water frogs, using backcross progeny of highly heterozygous, nonclonal hybrids of several parental combinations. The genetic markers assigned to LGs can later be tested for association with traits affecting hemiclonal gametogenesis in progeny of F_1 hybrids that are genomically heterozygous for presence/absence of such traits.

We here present linkage data obtained by protein

electrophoresis of backcross progeny using the species pair *R. lessonae/R. shqiperica*. We found four LGs containing 14 protein loci and sex, and a possible fifth LG with two additional enzyme loci. Virtually all testable linkages are shared with eastern Palearctic water frogs and with Nearctic Rana. Utilizing published data, at least 44 protein loci can now be plausibly assigned to 10 of the $n = 13$ chromosomes in Holarctic Rana. This comprehensive summary of Rana linkages reveals that for protein-coding genes there is complete agreement between Palearctic and Nearctic species, and that a majority of testable linkages are shared between Rana and distantly related pipid frogs (Xenopus). Several syntenies are even shared with mammals and with teleost fish. An extensive set of linkages thus has apparently been conserved for 60–140 million years in anurans; some syntenies may have persisted since the early divergence of the vertebrates, over 400 million years ago.

MATERIALS AND METHODS

A female *R. shqiperica* from Virpazar, Skadarsko Jezero, Crna Gora, Yugoslavia was crossed with a *R. lessonae* male from near Poznań, Poland (cross 7/83c), and a male hybrid from this cross was backcrossed to *R. lessonae* from Poznań (cross 8/84a). We analyzed 73 progeny of this backcross family. Crossing of adults and rearing of larval and metamorphosed progeny followed standard procedures (BERGER 1988). Ploidy of progeny was assessed by erythrocyte size (UZZELL and BERGER 1975; GÜNTHER 1977; POLLS PELAZ and GRAF 1988).

Frogs were anesthetized with 3-aminobenzoic acid ethyl ester (MS222) before removal of tissue samples, which were stored at −80°. Protein electrophoresis followed standard methods (WRIGHT *et al.* 1980; HOTZ and UZZELL 1982; HOTZ 1983). Liver or muscle samples were crushed in homogenizing buffer (WRIGHT *et al.* 1980) and applied to horizontal 11–12% starch gels on filter paper tabs. We scored 21 informative protein loci that were segregating in the hybrid parent (Table 1; APPENDIX A). Products of the 20 enzyme loci were assayed on 1 or 2 mm thick gel slices, using three continuous buffer systems (Table 1); all except AAT, EST, GAPDH, and XO were stained using 1% agar overlays rather than in solutions. Plasma proteins (ALB) were separated on vertical discontinuous polyacrylamide gels (Table 1).

For pairs of enzyme loci encoding proteins with alternative subcellular compartment localizations (AAT, ACO, IDH, MDH, ME, SOD), the translation products located in mitochondria were identified by comparing the observed electrophoretic patterns with those obtained from a tissue fraction enriched in mitochondrial proteins. Oocytes, livers, hearts, and kidneys of two *R. lessonae* and of two *R. esculenta* from Switzerland were each homogenized in STE buffer (0.25 M sucrose, 0.03 M Tris, 0.1 M EDTA pH 7.4) and centrifuged at 3.5 krpm (SS34 rotor) for 5 min; the supernatant was centrifuged at 12 krpm (SS34) for 20 min and the resulting pellet was resuspended in 2–3 vol STE and subjected to a 0.9 M/1.5 M sucrose step gradient (26 krpm, SW41 rotor, for 1 hr). The fraction at the 0.9 M/1.5 M interphase containing mitochondria was collected and centrifuged in 20 ml of 25 mM Tris, 5 mM $MgCl_2$, 25 mM KCl pH 7.4 at 12 krpm (SS34) for 20 min. The resulting pellet was resuspended and the organelles lysed in 1 vol of the same solution containing 1% Triton X-100 and 0.01% β-mercaptoethanol (GRAF 1989b). After centrifugation at 12 krpm (Sarstedt MH2-K) for 10 min, the supernatant containing mitochondrial proteins was stored at −80° for later electrophoresis. To assess locus homologies between our data and those on Nearctic Rana, we also used mitochondrial proteins from three commercially obtained (Nasco, Wisconsin) *R. pipiens*. One kidney and $^1/_2$ heart and, separately, $^2/_3$ liver from each individual were pooled, and mitochondrial proteins were enriched similarly, except that two rounds of low followed by high speed SS34 spins were used rather than a sucrose step gradient. Loci coding for cytosolic and mitochondrial enzymes are designated with the prefix s and m, respectively.

Goodness of fit tests of single-locus segregations for conformity to the expected Mendelian ratios used chi-square statistics. For two-point linkage data analysis we tested all pairs of informative loci, including sex, for conformity to independent assortment by contingency table chi-square statistics. Because in our data set linkage phase of the informative parent is known in each case, chi-square is a maximum likelihood statistic for detection of linkage (MATHER 1957). An excess of parental over recombinant genotypes is expected for linked locus pairs, so one-tailed tests for significant deviations from random assortment are appropriate (DOERGE 1995). Because of the multiple comparisons made, we used a sequential Bonferroni test (RICE 1989) to evaluate significance of chi-square over the set of 231 tests. For constructing linkage maps we used the program MAPMAKER version 1.9 (LANDER *et al.* 1987).

Homologies of the loci that we and others have studied (APPENDIX A) were established using several criteria, including subcellular compartment localization, substrate specificity, tissue distribution, tissue-specific relative activity, inferred subunit structure, and relative mobility of translation products (APPENDIX B).

RESULTS

All 73 backcross progeny analyzed were diploid judged by erythrocyte size (data not shown). Electrophoretic phenotypes for products of seven (*sAAT, ALB, ALD-2, LDH-B, MPI, PGDH, PGM-2*) of the 21 informative protein loci scored in this family (Table 1) have been described earlier (HOTZ and UZZELL 1982; HOTZ 1983). Notes on electrophoretic phenotypes, especially particulars relevant to assessing homologies of the loci scored by us to those reported in other studies (APPENDIX A), are presented in APPENDIX B.

Segregation of individual loci: Goodness of fit tests for conformity to the expected Mendelian 1:1 segregation ratio of backcross-parental (*R. lessonae*) to hybrid genotypes in the B_1 progeny are presented in Table 2 for each of the 21 informative protein loci and for sex. Because sex determination in western Palearctic water frogs is by a male-heterogametic *XX-XY* mechanism (BERGER *et al.* 1988) and the male hybrid used stems from a *R. shqiperica* female × *R. lessonae* male cross, female progeny are assumed to have inherited *R. shqiperica* sex determinants from the hybrid father, male progeny, *R. lessonae* ones. For most loci, segregation was in good agreement with the expected Mendelian values; the sex ratio conformed to the 1:1 expectation. For two of 21 protein loci (10%), however, segregation deviated significantly from the expected 1:1 ratio: *ALD-2* showed a deficit of hybrid (heterozygous) genotypes ($0.01 > P$

TABLE 1

Protein loci used for linkage analysis of B_1 progeny of the species pair *R. lessonae/R. shqiperica*

Protein[a]	No. of loci		Locus	Segregating alleles[b]		Buffer system[c]	Tissue
	Scored	Informative		*lessonae*	*shqiperica*		
AAT	2	2	*sAAT*	*g*	*e*	TEB, TC7, TC6	Muscle, liver
			mAAT	*a*	*b*		Liver, muscle
ACO	2	1	*mACO*	*b*	*c*	TC7	Liver
ALB	1	1	*ALB*	*a*	*b*	PAGE	Plasma
ALD	2	1	*ALD-2*	*a*	*b*	TC7, TEB	Muscle
CA	2[d]	1	*CA-2*	*d*	*f*	TEB	Liver
EST	3[e]	1[d]	*EST4*	*a**	*b**	TEB	Liver
FDP	2	1	*FDP-1*	*b*	*c*	TEB	Liver
GAPDH	1	1	*GAPDH*	*a*	*d*	TC7	Muscle
GDA	1	1	*GDA*	*a*	*b*	TEB	Liver
αGLU	1	1	*αGLU*	*b**	*a**	TEB	Liver
HK	2	1	*HK-1*	*a**	*b**	TEB	Liver
LDH	2	1	*LDH-B*	*e*	*d*	TC7, TEB, TC6	Liver, muscle
MDH	2	0[f]				TC7, TEB, TC6	Muscle, liver
ME	2	1	*sME*	*a**	*b**	TC7, TEB, TC6	Muscle, liver
MPI	1	1	*MPI*	*h*	*d*	TC7, TC6	Muscle
PEP	5	2[g]	*PEPB*	*b**	*a**	TEB, TC7	Liver, muscle
			PEPD	*a**	*b**		Liver
PGDH	1	1	*PGDH*	*c*	*h**	TEB, TC7	Liver, muscle
PGM	2	1	*PGM-2*	*c*	*b*	TC7	Muscle
SOD	2	1[h]	*mSOD*	*b**	*a**	TEB	Liver
XO	1	1	*XO*	*a**	*b**	TEB	Liver

[a] Abbreviations are listed in APPENDIX A.

[b] Alleles are designated by lowercase letters (HOTZ and UZZELL 1982; HOTZ 1983; BEERLI 1994); newly designated alleles are marked with an asterisk (*a* and *b* in sequence of decreasing anodal mobility).

[c] PAGE = polyacrylamide gels (stacking gel, 2.5% acrylamide + 0.6% bisacrylamide; separating gel, 7.5% acrylamide + 0.2% bis; electrode buffer, 5 mM Tris + 38 mM glycine pH 8.4); starch gels: TC6 = low pH Tris-citrate (gel buffer, 8 mM Tris + 3 mM citric acid; electrode buffer, 220 mM Tris + 90 mM citric acid pH 6); TC7 = Tris-citrate (gel buffer, 9 mM Tris + 3 mM citric acid; electrode buffer, 130 mM Tris + 43 mM citric acid pH 7); TEB = Tris-EDTA-borate (gel buffer, 50 mM Tris + 65 mM boric acid + 1.6 mM EDTA; electrode buffer, 500 mM Tris + 650 mM boric acid + 16 mM EDTA).

[d] *CA-2* products also show up on stains for esterases (*"EST 3"*).

[e] A mixture of α-naphthyl acetate, α-naphthyl butyrate, and α-naphthyl propionate was used as substrate.

[f] The locus *sMDH* was used for linkage analysis in another taxon pair, *R. lessonae*/a related Sicilian taxon (H. HOTZ, T. UZZELL and L. BERGER, unpublished data).

[g] Substrates used are L-leucyl-L-glycylglycine for PEPB, L-leucyl-L-proline for PEPD (proline dipeptidase).

[h] Also scored on GDA stains.

> 0.001), *GDA* an excess of hybrid genotypes ($0.05 > P > 0.01$). It is possible that preferential scoring of homozygotes (heterozygotes being more likely to be omitted from analysis as "uncertain") accounts in part for the large hybrid deficiency for *ALD-2*.

Linkage: All 231 possible pairs of informative protein loci and sex were tested for linkage (in contrast to mammals, sex in Rana is determined by a small region, possibly a single locus, of the sex chromosomes that in meiosis otherwise behaves like a homologous pair of autosomes; Table 3; *e.g.*, ELINSON 1983; WRIGHT and RICHARDS 1983; HOTZ *et al.* 1993). Because the allelic differences between the parental species are known, linkage phase in the hybrid parent of the backcross progeny is known for each pair of loci, and all offspring genotypes can unambiguously be identified as "parental" or "recombinant." Among the 231 possible pairs, 33 (14%) deviated significantly from random assortment at $P < 0.01$ in individual one-tailed tests with an excess of parental (individuals with two backcross-parental or two hybrid genotypes) over recombinant genotypes (Table 3); an additional pair (*HK-1/PGM-2*) had a significant excess of recombinant genotypes. Over the entire set of 231 tests, using the conservative sequential Bonferroni test (RICE 1989), 20 (59%) of these 34 pairs deviated from random assortment at $P < 0.001$, and two (6%) at $0.01 > P > 0.001$ (Table 3); the remaining 12 pairs (35%) did not deviate significantly from independent assortment ($P > 0.05$ for each comparison). We interpret the 22 pairs with significant excess of parental genotypes in table-wide tests as classically linked. These linked pairs contain 14 protein loci and sex (68% of the tested loci, including sex) and form four LGs, arbitrarily numbered 1–4, containing

TABLE 2

Segregation of 21 informative protein loci and of sex in the hybrid parent, inferred from all scorable individuals among 73 B_1 progeny of a male hybrid (*R. shqiperica* × *R. lessonae*) backcrossed to *R. lessonae*

Locus	Offspring genotype		χ^2 1:1 (1 d.f.)
	R. lessonae	Hybrid	
sAAT	43	27	3.7
mAAT	26	33	0.8
mACO	6	7	0.1
ALB	30	43	2.3
ALD-2	36	13	10.8**
CA-2	33	37	0.2
EST 4	32	38	0.5
FDP-1	27	43	3.7
GAPDH	12	18	1.2
GDA	25	45	5.7*
αGLU	32	30	0.1
HK-1	36	34	0.1
LDH-B	37	31	0.5
sME	37	28	1.2
MPI	25	34	1.4
PEPB	36	32	0.2
PEPD	32	38	0.5
PGDH	38	31	0.7
PGM-2	30	38	0.9
mSOD	12	7	1.3
XO	38	31	0.7
Sex	38	34	0.2

* Significant at $0.01 < P < 0.05$. ** Significant at $0.001 < P < 0.01$.

four, five, three, and three marker loci, respectively (Figure 1). The 22 linked pairs comprise each of the possible pairs among loci assigned to individual LGs.

Of the remaining 12 pairs deviating from random assortment at $P < 0.01$ in individual tests but at $P > 0.05$ in table-wide tests (Table 3), one (*sAAT/ALD-2*) possibly represents a fifth LG: in the individual test, the parental genotype excess is significant at $P < 0.001$, and the deviation from random segregation among progeny scored for *ALD-2* products (Table 2) may indicate biased omission of heterozygotes.

The five remaining loci (*mACO, GAPDH, αGLU, PGDH,* and *PGM-2*) were not linked to any other locus of the set at $P < 0.001$ in individual tests and at $P < 0.05$ in table-wide tests.

Linkage maps: Linkage maps for the four LGs of which we are confident are presented in Figure 1. The locus orders given for LGs 3 and 4, each containing three markers, are those having maximum likelihood (generated by MAPMAKER using two-point map data) among the six possible orders for each; the likelihoods of these orders are 1000 or more times the highest likelihood for any other arrangement. For LGs 1 and 2, which have four and five member loci, respectively, the order of some loci is ambiguous. We considered the members of each LG in sets of three. Using as a criterion that the likelihood for the best three-point map for each set is 1000 times greater than for any of the five other possible three-point maps for that set, we accepted the order *mAAT-CA-2-PEPD* for LG 1, *MPI-*Sex-*PEPB* for LG 2. Alternative placements are thus possible for three marker loci: *EST 4* of LG 1 may fall between *CA-2* and *PEPD,* or between *mAAT* and *CA-2,* but not distal to *mAAT* or to *PEPD. HK-1* and *LDH-B* of LG 2 may each fall in three places: between *MPI* and Sex, between Sex and *PEPB,* or distal to *PEPB,* but not distal to *MPI.* Double-crossovers occur for the two LGs with more than three markers: each of the two alternative marker orders for LG 1 requires one double-crossover event for one of two central markers in one meiosis; each of the 12 alternative marker orders for LG 2 requires one double-crossover event for each of three central markers in three meioses. None of the central markers of LGs 3 and 4 requires a double-crossover.

DISCUSSION

Linkage groups detected in western Palearctic water frogs: Heterozygosity and fertility of F_1 hybrids both depend on genetic distance between the parental taxa, but are negatively correlated with each other. Among the taxon pairs of western Palearctic water frogs that we have used for linkage studies, *R. lessonae* and *R. shqiperica* so far give the best combination of a high number of loci with allelic differences and sufficient viability of first backcross generation progeny. The two species are genetically quite distinct: using electrophoresis of 51 protein loci, CAVALLI-SFORZA and EDWARDS' (1967) chord distance between our samples is 0.65, NEI's (1972) standard genetic distance, 0.72 (H. HOTZ and T. UZZELL, unpublished results). The two species have fixed or major differences in allele frequency in 25 of the 51 loci; 21 of these plus Sex were scored and informative for linkage analysis. That each of these loci evidenced segregation in the hybrid parent, most of them in good agreement with the expected 1:1 genotype ratio (Table 2), is concordant with results from cytogenetic examination of meiotic chromosomes and electrophoresis of oocytes I (HOTZ *et al.* 1985); it confirms that hybrids between *R. lessonae* and *R. shqiperica* have a Mendelian rather than a hemiclonal gametogenesis, which would preclude linkage analysis.

Of the 22 informative loci, 14 protein loci plus sex were assignable to LGs. This large proportion (68%) is probably related to the informative hybrid parent of the backcross analyzed being male. The recombination rate in hybrid water frogs was found to be substantially lower in males than in females for at least two locus pairs of two LGs in other crosses: we observed reduced recombination, in male hybrids between *R. lessonae* and a related Sicilian taxon, between two protein-coding loci each of our LGs 2 and 4, but not among four loci of

TABLE 3

List of 34 locus pairs deviating from random assortment ($P < 0.01$ in individual one-tailed tests) in all scorable individuals among 73 progeny of a male *R. shqiperica* × *R. lessonae* hybrid backcrossed to *R. lessonae*

Locus pair[a]	No. of parentals[b]		No. of recombinants[c]		r[d]	χ^2 (1 d.f.)	P[e]	Table-wide significance[f]
	LL	HH	LH	HL				
mAAT sAAT	22	14	17	4	0.368 ± 0.064	5.80	0.016	NS
mAAT CA-2	25	29	1	4	0.085 ± 0.036	41.09	0.000	***
mAAT EST 4	24	29	2	4	0.102 ± 0.039	37.50	0.000	***
mAAT PEPD	21	26	5	7	0.203 ± 0.052	20.69	0.000	***
sAAT ALD-2	26	10	3	8	0.234 ± 0.062	11.35	0.001	NS
sAAT EST 4	23	19	19	6	0.373 ± 0.059	6.04	0.014	NS
sAAT MPI	19	18	15	5	0.351 ± 0.063	6.56	0.010	NS
sAAT PEPB	26	17	14	8	0.338 ± 0.059	6.72	0.010	NS
sAAT PEPD	23	19	19	6	0.373 ± 0.059	6.04	0.014	NS
sAAT Sex	28	18	8	15	0.333 ± 0.057	7.66	0.006	NS
ALB FDP-1	27	42	1	0	0.014 ± 0.014	65.93	0.000	***
ALB GDA	25	42	3	0	0.043 ± 0.024	58.33	0.000	***
ALD-2 EST 4	17	11	18	1	0.404 ± 0.072	6.12	0.013	NS
ALD-2 PEPD	17	11	18	1	0.404 ± 0.072	6.12	0.013	NS
CA-2 EST 4	32	37	1	0	0.014 ± 0.014	66.09	0.000	***
CA-2 GAPDH	8	14	4	4	0.267 ± 0.081	5.93	0.015	NS
CA-2 PEPD	29	34	4	3	0.100 ± 0.036	44.73	0.000	***
EST 4 GAPDH	8	15	3	4	0.233 ± 0.077	7.75	0.005	NS
EST 4 PEPD	28	34	4	4	0.114 ± 0.038	41.47	0.000	***
FDP-1 GDA	25	43	2	0	0.029 ± 0.020	61.93	0.000	***
HK-1 LDH-B	34	29	0	2	0.031 ± 0.021	57.43	0.000	***
HK-1 MPI	23	29	3	1	0.071 ± 0.034	41.22	0.000	***
HK-1 PEPB	35	31	1	1	0.029 ± 0.020	60.21	0.000	***
HK-1 PGM-2[g]	10	13	22	20	0.646 ± 0.059	5.63	0.018	NS
HK-1 Sex	35	32	2	0	0.029 ± 0.020	61.43	0.000	***
LDH-B MPI	25	28	4	0	0.070 ± 0.034	43.00	0.000	***
LDH-B PEPB	34	27	2	0	0.032 ± 0.022	55.40	0.000	***
LDH-B Sex	36	30	1	1	0.029 ± 0.020	60.18	0.000	***
sME mSOD	11	6	0	0	0.000 ± 0.000	17.00	0.000	**
sME XO	35	27	1	0	0.016 ± 0.016	59.06	0.000	***
MPI PEPB	23	27	1	3	0.074 ± 0.036	39.35	0.000	***
MPI Sex	25	30	4	0	0.068 ± 0.033	44.88	0.000	***
PEPB Sex	35	30	2	0	0.030 ± 0.021	59.42	0.000	***
mSOD XO	11	7	1	0	0.053 ± 0.051	15.24	0.000	**

NS, not significant ($P > 0.05$); **significant at $0.001 < P < 0.01$; ***significant at $P < 0.001$.

[a] For sex, males are treated as "lessonae," females as "hybrid" genotypes, in accordance with an *XX-YY* male heterogametic sex determining mechanism.

[b] Parentals: both loci homozygous for *R. lessonae* alleles (LL) or both loci heterozygous (hybrid; HH).

[c] Recombinants: one locus homozygous for *R. lessonae* alleles, the other heterozygous (hybrid; LH and HL).

[d] Recombination fraction; values are ±SE.

[e] The P values are associated with individual chi-square tests; linkage phase is known, so the criterion for inclusion in the table is set as a significance level of $2\alpha = 0.02$ (one-tailed tests).

[f] One-tailed evaluations of table-wide significance were made using a sequential Bonferroni test (RICE 1989).

[g] Excess of recombinant over parental genotypes.

LG 1 (H. HOTZ, T. UZZELL and L. BERGER, unpublished data). Reduced recombination rates in males compared to females also occur in eastern Palearctic water frogs (NISHIOKA and SUMIDA 1994a) and in the brown frog *R. japonica* (SUMIDA and NISHIOKA 1994). The large proportion of detectable linkages may also be related to the informative parent being an interspecies hybrid; in females of eastern Palearctic water frogs, crossover frequencies of hybrids were lower than those of the parental species (NISHIOKA and SUMIDA 1994a). Translation of the centimorgan units (Figure 1) into physical distances thus requires caution.

Among western Palearctic water frogs, linkages of protein loci have been tested using another species pair (*R. lessonae* and an unnamed related Sicilian taxon; H. HOTZ, T. UZZELL and L. BERGER, unpublished data). All seven linkages that are comparable (among *mAAT, CA-2, EST 4, PEPD* of LG 1, and between *HK-1* and *MPI* of LG 2) occur in both data sets.

Comparisons with other Holarctic Rana: Within an-

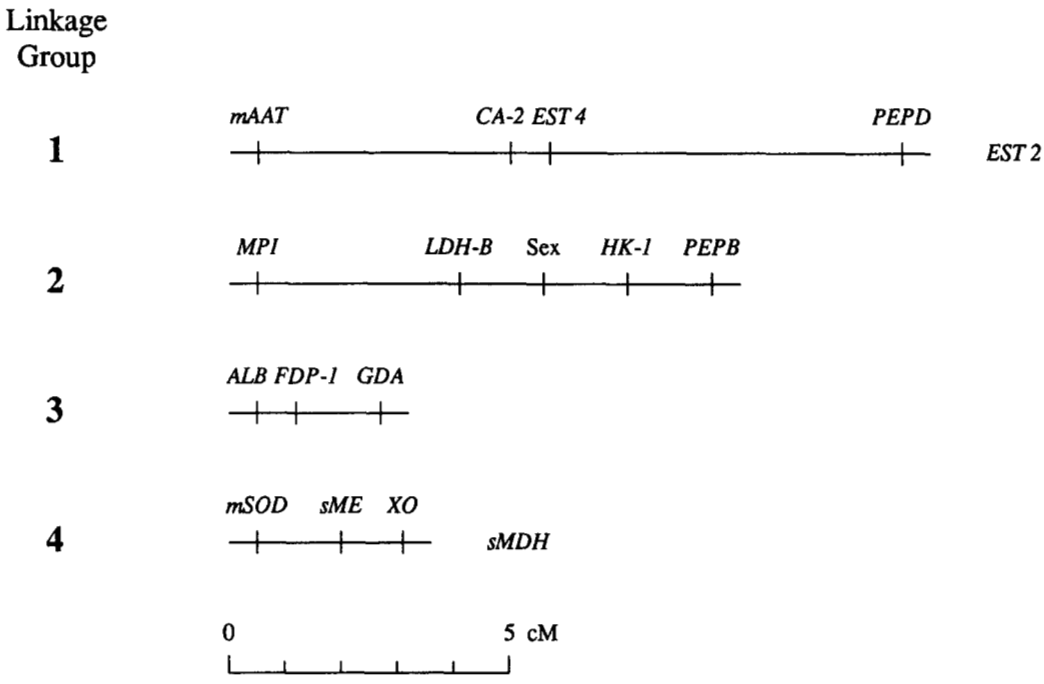

FIGURE 1.—Tentative linkage maps for 14 protein loci and sex detected in progeny of a male *R. shqiperica* × *R. lessonae* hybrid backcrossed to *R. lessonae*. Numbering of the four linkage groups is arbitrary. Distances in cM, calculated using HALDANE's (1919) mapping function, are maximum likelihood estimates generated for each pair of adjacent marker loci by MAPMAKER (LANDER *et al.* 1987). *EST 2* and *sMDH* are unmapped additional loci of LG 1 and 4, respectively, as revealed in backcross progeny of hybrids between *R. lessonae* and an unnamed Sicilian taxon (H. HOTZ, T. UZZELL and L. BERGER, unpublished data). Homology of *EST 4* and *EST 2* of LG 1 to *EST* loci in other studies is unknown. Of the seven additional informative loci that each appear unlinked to any other locus of the set, *sAAT* and *ALD-2* (marked *) possibly form a fifth LG, with a distance of 13.8 cM between them.

urans, linkage has been investigated mainly in frogs of the Holarctic radiation of Rana and in the pipid frog *Xenopus laevis;* limited data are available for discoglossid frogs, genus Bombina (SZYMURA 1995). Among Rana, linkage has been extensively analyzed in both the Nearctic *R. pipiens* group (WRIGHT *et al.* 1980, 1983; WRIGHT and RICHARDS 1993) and the eastern Palearctic *R. nigromaculata* group (NISHIOKA *et al.* 1980, 1987). The striking similarities in LGs revealed by these and our studies are summarized in Table 4, which includes shared linked locus pairs and syntenic groups built from them.

For the eastern Palearctic water frogs (EPWF), the sister group (UZZELL 1982, unpublished data; NISHIOKA and SUMIDA 1992) of western Palearctic water frogs (WPWF), seven LGs of electrophoretic marker loci have been established, and 24 protein loci have been assigned to nine individual chromosomes (NISHIOKA *et al.* 1980, 1987) of the $n = 13$. Of these loci, eight to 10 (*ALB, ALD-2, HK-1, LDH-B, sME, MPI, PEPB, PEPD;* and possibly one to two *EST* loci) are also used in the present paper (involving all four of our LGs; Figure 1); an additional locus (*sMDH*) can be compared because in WPWF it belongs to LG 4 (Figure 1). Of the minimum of 36 locus pairs thus available for comparison, there is complete agreement between the two data sets: seven pairs (six among *HK-1, LDH-B, MPI* and *PEPB,* our LG 2; and one between *sMDH* and *sME,* our LG 4) are linked in both species groups; all others are unlinked in both. Moreover, the group of four *EST* loci syntenic with *PEPD* on chromosome *10* in the *R. nigromaculata* group may well contain our *EST 4* and/or *CA-2* loci (APPENDIX B), both linked to *PEPD* in our LG 1, which contains an additional *EST* locus (*EST 2;* Figure 1). It is thus likely that our LG 1 is also shared with the *R. nigromaculata* group.

Combining our data on WPWF with those reported for EPWF results in a total of seven LGs comprising 30–34 protein loci, of which five to eight are analyzed for WPWF only, 12–16 for EPWF only, and nine to 12 for both (the ranges indicate uncertain homology for *EST* loci and the possibility that *sAAT* and *ALD-2* form a fifth LG in WPWF). Based on chromosome assignments of one to several loci in them, each of these seven LGs

has been assigned to a different chromosome in EPWF; two additional loci (*ADA* and *PEPA*) have been assigned to two additional chromosomes in EPWF. We have summarized the resulting nine synteny groups of Palearctic water frogs, together with data on Nearctic Rana, in Table 4, which provides a comprehensive summary of Rana linkages. This data set comprises 51–57 protein loci, of which 44–46 can plausibly be assigned to 10 of the 13 chromosomes (the ranges indicate uncertain homology of *EST* loci); 15–26 of these loci, depending on homology, are representatives of the 321 anchored reference loci proposed for comparative vertebrate gene mapping (O'BRIEN *et al.* 1993).

In Nearctic Rana, 18 protein loci have been studied that have clear homologies with loci studied in Palearctic water frogs (*mAAT, sAAT, ADA, ADH-2, ALB, FDP-1, HB I, HB II, HK-1, sIDH, LDH-B, sME, MPI, PEPB, PEPC, PEPD, PGM-2, sSOD;* Table 4). For 123 of the 153 pairs among these, both loci have been examined in Nearctic Rana and either WPWF or EPWF or both and can thus be directly compared; for another 16, comparisons can be made by inference because linked loci have been examined. Twelve locus pairs cannot be tested because one member of the pair (and all other members of its LG) has been examined only in WPWF, the other (and all other members of its LG) only in EPWF. These pairs consist of *sAAT* (examined in WPWF) and *ADA, HB I, HB II, sIDH, PEPC,* and *sSOD* (examined in EPWF); and of *PGM-2* (examined in WPWF) and the same six loci. Two pairs (*sAAT/mAAT* and *sAAT/PEPD*) are omitted from the comparison because they are not certainly linked in WPWF and were not directly tested in Nearctic Rana; it is plausible that they are syntenic but too distantly located for detection of linkage (Table 4). Thus, 139 pairs are comparable between Palearctic and Nearctic Rana. Among these pairs, agreement is complete: 14 linkages as well as 125 nonlinkages are shared in both groups. The 14 shared linked pairs involve 14 loci situated on five chromosomes (Table 4): chromosome *1* (three pairs among the three loci *ADH-2, ALB, FDP-1*), chromosome *2* (one pair: *PEPC* and *sSOD*), chromosome *4* (six pairs among the four loci *HK-1, LDH-B, MPI, PEPB*), chromosome *6* (three pairs among the three loci *HB I, HB II, sIDH*), and chromosome *10* (one pair: *mAAT* and *PEPD*). The *MPI/LDH-B* linkage (Nearctic *R. pipiens* and Palearctic *R. esculenta* and *R. nigromaculata* groups; Table 4) also occurs in *R. clamitans* of the Nearctic *R. catesbeiana* group (ELINSON 1983). In sum, there is very high concordance of linkages among and between Palearctic and Nearctic Rana; the only two possible inconsistencies in LG assignment are discussed in APPENDIX C. Data are generally insufficient to compare gene order within LGs; the single comparison possible suggests a gene rearrangement among *MPI, HK-1,* and *PEPB* that possibly occurred within the *R. pipiens* group (APPENDIX C).

Inconsistent location of sex determining region: In contrast to the generally consistent linkages detected among protein loci, the LG assignment of sex in Rana shows major inconsistencies. Although in each case tested it has been readily possible to assign sex to a particular LG when using male hybrids, there is considerable variation, even among closely related species, as to which LG this is. In Holarctic Rana, sex has been assigned to a minimum of four linkage groups, apparently comprising the four largest chromosomes (*1-4;* Table 4): in the *R. catesbeiana* group, sex is assigned to chromosome *1* (*R. clamitans*) or *4* (*R. catesbeiana*); in the *R. pipiens* group, it has variously been assigned to chromosomes *2, 3* and *4,* depending on the species tested; in the *R. nigromaculata* group, it has been assigned to chromosomes *3* and *4;* and in the *R. esculenta* group, to chromosome *4* in the present study, but to some other chromosome in *R. ridibunda* (H. HOTZ, L. BERGER and T. UZZELL, unpublished results).

Comparisons with Xenopus: Linkage analysis in the distantly related allotetraploid pipid frog *X. laevis* has revealed 10 LGs containing 25 of 33 informative electrophoretic marker loci and sex (GRAF 1889a,b, 1993; GRAF and KOBEL 1991). Omitting two *EST* loci with unknown homology, results for nine informative protein loci of Xenopus (*mACO-1, ALB-1+2, sME, MPI-1+2, PGDH-2, PEPB, PEPD*) can be compared with ours. Four (*mACO, ALB, MPI,* and *PGDH*) reflect the genome duplication that has led to the tetraploidy in *X. laevis,* although for two (*mACO* and *PGDH*) only one of the duplicate loci is informative; each can be compared with the corresponding locus in Rana (*mACO, ALB, MPI, PGDH*). Thus 21 pairs among the seven protein loci in Rana can be compared with 36 pairs among the nine loci in Xenopus. Although the number of comparisons is modest, there is complete agreement between the data sets: one pair (*MPI/PEPB* in Rana; *MPI-1/PEPB* in Xenopus) is linked in both groups, 20 are unlinked in both groups. When using the extended data set on Holarctic Rana (Table 4), 18 protein loci assigned to LGs in Rana (*sACO, mACO, ADH-2, ALB, FH, αGDH, GLO, GPI, IDDH, αMAN, mME, sME, MPI, PEPB, PEPD, PGDH, sSOD, TF*) can be compared with loci in Xenopus. Of the 153 possible pairwise comparisons, 11 are not testable because one locus of the pair (and all other members of its LG) have only be examined in one Rana group, the second (and all other members of its LG) in another. Among the 142 testable pairs, there is again a remarkably high concordance: there are 125 shared nonlinkages, 13 shared linkages that comprise four LGs (corresponding to Rana locus pairs *sACO/ADH-2, sACO/ALB, ADH-2/ALB, αGDH/mME, αGDH/sSOD, GPI/PEPD, IDDH/αMAN, IDDH/MPI, IDDH/PEPB, αMAN/MPI, αMAN/PEPB, mME/sSOD,* and *MPI/PEPB*), and only three differences (*sACO/TF* and *ADH-2/TF* linked in Rana, unlinked in Xenopus; *FH/TF* unlinked in Rana, linked in Xenopus). Only single loci for *sACO, FH,* and *TF* are known in *X. laevis* (GRAF and

TABLE 4

An overview of sources of syntenies and linkage groups for protein loci and sex in frogs of the Holarctic radiation of the genus Rana

Synteny/linkage group[a]	Observed linkages[b]										
			Nearctic Rana[c]								
	Palearctic Rana		*catesbeiana* group		*pipiens* group						
	esculenta group	*nigromaculata* group	cat	cla	pip		pip/ bla	pip/ pal	sph	sph/ ber	sph/ bla
Chromosome *1*[d]											
sACO				6	15, 16, 17	—			17		
*ADH-2**		10								17	
*ALB**	1	9, 10			15, 16, 17	—	4	17		17	17
FDP-1	1							17		17	
GDA	1										
βGLU					—	17				17	
βGUS					15, 16, 17	—					
PGM-1					—	17		17		17	17
Sex	—			6	—	—		—	17	17	—
TF							4				
Chromosome *2*[d]											
*αGDH**		9, 10									
mMDH										17	
*mME**		10									
*PEPC**		10			13, 14			13		17	
Sex					14, 15, 16		17			—	17
*sSOD**		10			13, 14			13		17	17
*TYR**		11									
Chromosome *3*[d]											
*sMDH**	2	10									
*sME**	1, 2	9, 10			11						
Sex	—	8[e]			11						
mSOD	1										
XO	1										
Chromosome *4*[d]											
ENO		7									
*HK-1**	1	10			15, 16					17	
IDDH		7									
*LDH-B**	1	9, 10	5	6				17		17	
αMAN					15						
*MPI**	1	10		6	13, 15, 17			17		17	
*PEPB**	1	10			13, 15, 17					17	
Sex	1[e]	8[e]	5							—	
Chromosome *5*[d]											
*PEPA**		10									
Chromosome *6*[d]											
*HB I**		9			15		3	17			
*HB II**		9					3				
*sIDH**		9			15			17			
Chromosome *9*[d]											
ALD-2		10									
*PROT C**		9, 10									
Chromosome *10*[d]											
mAAT	1				15						
sAAT	—							17			

TABLE 4

Continued

Synteny/linkage group[a]	Observed linkages[b]									
			Nearctic Rana[c]							
	Palearctic Rana		*catesbeiana* group		*pipiens* group					
	esculenta group	*nigromaculata* group	cat	cla	pip	pip/bla	pip/pal	sph	sph/ber	sph/bla
CA-2	1									
EST 1[f]*		10[f]								
EST 2[f]*	2[f]	10[f]								
EST 4[f]*	1[f]	10[f]								
EST 5[f]*		10[f]								
GPI					15		17		17	
*PEPD**	1	10			15				17	
TPI							17			
Chromosome *11*[d,g]										
*ADA**		10								
Chromosome *13*[h]										
*PGM-2**					17					
Unassigned to a chromosome (U1)										
AP-1					16					
AP-2					16					
EST 5[f]					16[f]					
GLO					16					
Unassigned to a chromosome (U2)										
EST 1[f]					16[f]					
EST 4[f]					16[f]					
EST 6[f]					16[f]					
EST 10[f]					16[f]					
Unassigned to a chromosome (U3)										
G6PDH					12[i]					
PGDH					12[i]					
Unassigned to a chromosome (U4)										
FH					15					

References: 1, This article; 2, H. HOTZ, T. UZZELL and L. BERGER, unpublished results (pair *R. lessonae*/Sicilian taxon); 3, DUNLAP 1979; 4, DUNLAP 1982; 5, ELINSON 1981; 6, ELINSON 1983; 7, NISHIOKA and SUMIDA 1994a; 8, NISHIOKA and SUMIDA 1994b; 9, NISHIOKA *et al.* 1980; 10, NISHIOKA *et al.* 1987; 11, TAKASE *et al.* 1992; 12, WRIGHT 1975; 13, WRIGHT and RICHARDS 1982; 14, WRIGHT and RICHARDS 1983; 15, WRIGHT and RICHARDS 1993; 16, WRIGHT *et al.* 1980; 17, WRIGHT *et al.* 1983.

[a] Within syntenies/LGs, loci are listed alphabetically; no mapping sequence is intended. Locus abbreviations are listed in APPENDIX A. In case of different naming conventions, locus names used in the present study or those indicating subcellular localizations are given preference. Loci that have been assigned to a chromosome are marked by an asterisk.

[b] For each LG, numbers indicate studies demonstrating linkage of genetic markers in a taxon. —, markers not linked (in studies indicated in the same column of an LG).

[c] Abbreviations for species: ber, *R. berlandieri;* bla, *R. blairi;* cat, *R. catesbeiana;* cla, *R. clamitans;* pal, *R. palustris;* pip, *R. pipiens;* sph, *R. sphenocephala.*

[d] Chromosome assignment for the *R. nigromaculata* group (NISHIOKA *et al.* 1980, 1987).

[e] Variable among species and populations.

[f] Homology of *EST* loci across studies is not known.

[g] *ADA* has been assigned to chromosome *10* for the *R. pipiens* group (WRIGHT *et al.* 1993), probably a result of different numbering of the similarly sized small chromosomes (a problem occurring even within the *R. esculenta* group; BUCCI *et al.* 1990).

[h] Chromosome assignment for the *R. pipiens* group (WRIGHT *et al.* 1983).

[i] The informative parents used were probably hybrids between *R. pipiens* and either *R. magnaocularis* or *R. forreri.*

KOBEL 1991), but presumably all loci were duplicated in the tetraploidization that gave rise to the *X. laevis* lineage. It is thus possible that duplicate *sACO* or *TF* genes, not yet detected because of silencing mutations or tissue or developmental specificity, show the two linkages seen in Rana.

Conservation of linkage groups: Our results and published data together permit us to identify an extensive set of linkage groups and syntenies that can plausibly be assigned to individual chromosomes in the genus Rana (Table 4). Among protein-coding loci, such LGs or syntenies show a remarkably high degree of evolutionary conservation in anurans: between western and eastern Palearctic water frogs (divergence $\sim 35 \times 10^6$ years ago; UZZELL 1982; T. UZZELL, unpublished results); between Palearctic water frogs and Nearctic Rana (divergence $\sim 60 \times 10^6$ years ago; UZZELL 1982); and even between Rana and the but distantly related pipid frog genus Xenopus (conservative divergence estimate $\sim 140 \times 10^6$ years ago; *e.g.*, CARROLL 1988). Such conservation parallels synteny conservation observed within mammals (*e.g.*, STALLINGS and SICILIANO 1983; LALLEY *et al.* 1989; O'BRIEN 1993b; O'BRIEN *et al.* 1993), but in anurans apparently is even more extensive, despite longer divergence times and greater genetic distances in this group (for example, the genus Rana in the broad sense has been compared to an order of mammals in terms of genetic dispersion; WALLACE *et al.* 1973).

Assessment of evolutionary conservation of LGs and syntenies becomes more difficult with increasing phylogenetic distance of the groups compared: determination of gene homology becomes more difficult, and karyotypic evolution, including chromosome rearrangements such as fusions, fissions, translocations, inversions, and duplications, results in different numbers of chromosomes or chromosome arms. Nevertheless, several of the LGs and syntenies that we discern in anurans are present both in mammals (divergence from Anurans $\sim 350 \times 10^6$ years ago; CARROLL 1988) and in teleost fish (divergence from Anurans over 400×10^6 years ago; CARROLL 1988). A brief synopsis of some shared syntenies that we can see is presented in APPENDIX D. Even with these data, however, it is difficult to formulate statistically testable predictions that are able to discriminate between competing hypotheses on selective conservation of LGs or syntenies and constraints on chromosomal evolution (*e.g.*, MORIZOT 1990), and between plesiomorphy and parallelism. The more loci contained in a synteny group and the more groups of organisms that share a synteny, the more convincing is evolutionary conservation as opposed to linkage shared by chance; among syntenies shared among mammals, amphibians, and fishes, the pairs *GPI/PEPD* (APPENDIX D) and *mIDH/MPI* (not tested in Rana but syntenic in Xenopus; GRAF 1989a) may have persisted since the ancestral vertebrates (*e.g.*, MORIZOT *et al.* 1991). Despite the difficulties in comparisons with distantly related groups, the extensive conservation here documented for linkages or syntenies among protein genes within anurans during time spans of well over 100 million years is certainly impressive, although its causes remain to be determined. The rapid expansion of genetic mapping using recent improvements in DNA techniques may soon lead to answers to some of the questions raised. We expect especially important evolutionary insights from a comparison of the relative amounts of synteny or linkage conservation between protein-coding genes such as reported here and functionally different parts of the genome such as the various types of noncoding regions.

PETER BEERLI (Seattle) and CHRISTINA SPOLSKY (Philadelphia) helped in mitochondrial protein isolation. DAVID A. WRIGHT (Houston) kindly communicated to us unpublished results on Nearctic Rana. Two anonymous reviewers provided constructive criticism. This research was supported by the U.S. National Science Foundation grant BSR 86-14881 (H.H., T.U.) and by the Swiss National Fund grant 31-37579.93 (H.H.). Field work was supported by the National Geographic Society grant 2804-84 (T.U., H.H.).

LITERATURE CITED

ADAMS, M., P. R. BAVERSTOCK, C. H. S. WATTS and G. A. GUTMAN, 1984 Enzyme markers in inbred rat strains: genetics of new markers and strain profiles. Biochem. Genet. **22:** 611–629.

ALLENDORF, F. W., F. M. UTTER and B. P. MAY, 1975 Gene duplication within the family Salmonidae: II. Detection and determination of the genetic control of duplicate loci through inheritance studies and the examination of populations, pp. 415–432 in *Isozymes. IV. Genetics and Evolution,* edited by C. L. MARKERT. Academic Press, New York.

BARKER, D., P. GREEN, R. KNOWLTON, J. SCHUMM, E. LANDER *et al.*, 1987 Genetic linkage map of human chromosome 7 with 63 DNA markers. Proc. Natl. Acad. Sci. USA **84:** 8006–8010.

BEERLI, P., 1994 Genetic isolation and calibration of an average protein clock in western Palearctic water frogs of the Aegean region. Ph. D. Dissertation, Universität Zürich.

BERGER, L., 1988 Principles of studies of European water frogs. Acta zool. cracov. **31:** 563–580 + Plates XXIV-XXVIII.

BERGER, L., T. UZZELL and H. HOTZ, 1988 Sex determination and sex ratios in western Palearctic water frogs: XX and XY female hybrids in the Pannonian Basin? Proc. Acad. Nat. Sci. Phila. **140:** 220–239.

BERNATZKI, R., and S. D. TANKSLEY, 1986 Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics **112:** 887–898.

BUCCI, S., M. RAGGHIANTI, G. MANCINO, L. BERGER, H. HOTZ *et al.*, 1990 Lampbrush and mitotic chromosomes of the hemiclonally reproducing hybrid *Rana esculenta* and its parental species. J. Exp. Zool. **255:** 37–56.

CARROLL, R. L., 1988 *Vertebrate Paleontology and Evolution.* W. H. Freeman and Company, New York.

CAVALLI-SFORZA, L. L., and A. W. F. EDWARDS, 1967 Phylogenetic analysis: models and estimation procedures. Evolution **21:** 550–570.

COPELAND, N. G., N. A. JENKINS, D. J. GILBERT, J. T. EPPIG, L. J. MALTAIS *et al.*, 1993 A genetic linkage map of the mouse: current applications and future prospects. Science **262:** 57–66.

DAWSON, W. D., and D. S. ROGERS, 1993 Genetic linkage map of the deer mouse (*Peromyscus maniculatus*). 2N=48, pp. 4.232–4.235 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

DOERGE, R. W., 1995 Testing for linkage: phase known/unknown. J. Hered. **86:** 61–62.

DUNLAP, D. G., 1979 Hemoglobin phenotypes in the frogs *Rana pipiens, Rana blairi,* their hybrids and a backcross. Comp. Biochem. Physiol. **62B:** 167–173.

DUNLAP, D. G., 1982 Linkage analysis of the transferrin, albumin, and hemoglobin loci in leopard frogs. J. Hered. **73:** 247–248.

ECHARD, G., 1993 The gene map of the sheep (*Ovis aries*). 2N=54, pp. 4.237–4.239 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

ECHARD, G., M. YERLE, D. MILAN, J. GELLIN and A. L. ARCHIBALD, 1993 The gene map of the pig (*Sus scrofa domestica* L.). 2N=38, pp. 4.240–4.249 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

ELINSON, R. P., 1981 Genetic analysis of developmental arrest in an amphibian hybrid (*Rana catesbeiana, Rana clamitans*). Dev. Biol. **81:** 167–176.

ELINSON, R. P., 1983 Inheritance and expression of a sex-linked enzyme in the frog, *Rana clamitans.* Biochem. Genet. **21:** 435–442.

FOX, R. R., 1993 Linkage map of the rabbit (*Oryctolagus cuniculus*) (2N=44), pp. 4.258–4.263 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

GRAF, J-D., 1989a Genetic mapping in *Xenopus laevis:* eight linkage groups established. Genetics **123:** 389–398.

GRAF, J-D., 1989b Sex linkage of malic enzyme in *Xenopus laevis.* Experientia **45:** 194–196.

GRAF, J-D., 1993 Linkage map of *Xenopus laevis.* 2N=36, pp. 4.330–4.331 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

GRAF, J-D., and H. R. KOBEL, 1991 Genetics of *Xenopus laevis.* Methods Cell Biol. **36:** 19–34.

GRAF, J-D., and M. POLLS PELAZ, 1989 Evolutionary genetics of the *Rana esculenta* complex, pp. 289–302 in *Evolution and Ecology of Unisexual Vertebrates,* edited by R. M. DAWLEY and J. P. BOGART. New York State Mus. Bull. Albany, NY.

GUERRINI, F., S. BUCCI, M. RAGGHIANTI, G. MANCINO, H. HOTZ *et al.,* 1997 Genomes of two water frog species resist germ line exclusion in interspecies hybrids. J. Exp. Zool. (in press).

GÜNTHER, R., 1977 Die Erythrozytengrösse als Kriterium zur Unterscheidung diploider und triploider Teichfrösche, *Rana "esculenta"* L. (Anura). Biol. Zentralbl. **96:** 457–466.

GYAPAY, G., J. MORISSETTE, A. VIGNAL, C. DIB, C. FIZAMES *et al.,* 1994 The 1993–94 Généthon human genetic linkage map. Nature Genet. **7:** 246–339.

HALDANE, J. B. S., 1919 The combination of linkage values and the calculation of distances between the loci of linked factors. J. Genet. **52:** 631–635.

HILLYARD, A. L., M. T. DAVISSON, D. P. DOOLITTLE, J. N. GUIDI, L. J. MALTAIS *et al.,* 1993 Locus map of mouse (*Mus musculus/domesticus*). 2n=40, pp. 4.1–4.52 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

HOTZ, H., 1983 Genic diversity among water frog genomes inherited with and without recombination. Ph. D. Dissertation, Universität Zürich.

HOTZ, H., L. BERGER, T. UZZELL, H. G. TUNNER, P. BEERLI *et al.,* 1993 Sex determination, sex linkage, and two kinds of unisexuality among XX and XY genotypes in western Palearctic water frogs, p. 181 in *Evolution 93. Fourth Congress of the European Society for Evolutionary Biology,* edited by F. M. CATZEFLIS, M. GAUTIER, A. ATLAN, J-C. AUFFRAY, P. BOURSOT *et al.* Université Montpellier II, Montpellier.

HOTZ, H., G. MANCINO, S. BUCCI-INNOCENTI, M. RAGGHIANTI, L. BERGER *et al.,* 1985 *Rana ridibunda* varies geographically in inducing clonal gametogenesis in interspecies hybrids. J. Exp. Zool. **236:** 199–210.

HOTZ, H., and T. UZZELL, 1982 Biochemically detected sympatry of two water frog species: two different cases in the Adriatic Balkans (Amphibia, Ranidae). Proc. Acad. Nat. Sci. Phila. **134:** 50–79.

HOTZ, H., and T. UZZELL, 1983 Interspecific hybrids of *Rana ridibunda* without germ line exclusion of a parental genome. Experientia **39:** 538–540.

KAWAMURA, T., and M. NISHIOKA, 1983 Reproductive capacity of male autotetraploid *Rana nigromaculata* and male and female amphidiploids produced from them by mating with female diploid *Rana brevipoda.* Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **6:** 1–45.

KAWAMURA, T., and M. NISHIOKA, 1986 Hybridization experiments among *Rana lessonae, Rana ridibunda* and *Rana esculenta,* with special reference to hybridogenesis. Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **8:** 117–271.

LALLEY, P. A., M. T. DAVISSON, J. A. M. GRAVES, S. J. O'BRIEN, J. E. WOMACK *et al.,* 1989 Report of the committee on comparative mapping. Cytogenet. Cell Genet. **51:** 503–532.

LANDER, E. S., P. GREEN, J. ABRAHAMSON, A. BARLOW, M. J. DALY *et al.,* 1987 MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1:** 174–181.

LANDRY, B. S., R. V. KESSLI, B. FARRARA and R. W. MICHELMORE, 1987 A genetic map of lettuce (*Lactuca sativa* L.) with restriction fragment length polymorphism, isozyme, disease resistance and morphological markers. Genetics **116:** 331–337.

LEVAN, G., K. K. LEVAN, C. SZPIRER and J. SZPIRER, 1993 Gene map of the rat (*Rattus norvegicus*). 2N=42, pp. 4.193–4.204 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

MA, R. Z., I. RUSS, C. PARK, D. W. HEYEN, J. E. BEEVER *et al.,* 1996 Isolation and characterization of 45 polymorphic microsatellites from the bovine genome. Anim. Genet. **27:** 43–47.

MATHER, K., 1957 *The Measurement of Linkage in Heredity.* Methuen & Co., London.

MAY, B., and K. JOHNSON, 1993 Composite linkage map of salmonid fishes (*Salvelinus, Salmo, Oncorhynchus*), pp. 4.309–4.317 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

MEERA KHAN, P., C. BRAHE and L. M. M. WIJNEN, 1993 Domestic dog (*Canis familiaris*) (2n=78), pp. 4.254–4.255 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

MICHELMORE, R. W., I. PARAN and R. V. KESSELI, 1991 Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA **88:** 9828–9832.

MIURA, I., H. OKUMOTO, K. MAKINO, A. NAKATA and M. NISHIOKA, 1995 Analysis of the tyrosinase gene of the Japanese pond frog, *Rana nigromaculata:* cloning and nucleotide sequence of the genomic DNA containing the tyrosinase gene and its flanking regions. Jpn. J. Genet. **70:** 79–92.

MORIZOT, D. C., 1983 Tracing linkage groups from fishes to mammals. J. Hered. **74:** 413–416.

MORIZOT, D. C., 1990 Use of fish gene maps to predict ancestral vertebrate genome organization, pp. 207–234 in *Isozymes. Structure, Function, and Use in Biology and Medicine,* edited by Z-I. OGITA and C. L. MARKERT. Wiley-Liss, New York.

MORIZOT, D. C., 1994 Reconstructing the gene map of the vertebrate ancestor. Anim. Biotech. **5:** 113–122.

MORIZOT, D. C., J. HARLESS, R. S. NAIRN, K. D. KALLMAN and R. B. WALTER, 1993 Linkage maps of non-salmonid fishes, pp. 4.318–4.325 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

MORIZOT, D. C., S. A. SLAUGENHAUPT, K. D. KALLMAN and A. CHAKRAVARTI, 1991 Genetic linkage map of fishes of the genus *Xiphophorus* (Teleostei: Poeciliidae). Genetics **127:** 399–410.

NEI, M., 1972 Genetic distance between populations. Am. Nat. **106:** 283–292.

NIH/CEPH COLLABORATIVE MAPPING GROUP, 1993 A comprehensive genetic linkage map of the human genome, pp. 5.46–5.105 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

NISHIOKA, M., H. OHTANI and M. SUMIDA, 1980 Detection of chromosomes bearing the loci for seven kinds of proteins in Japanese pond frogs. Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **4:** 127–184.

NISHIOKA, M., H. OHTANI and M. SUMIDA, 1987 Chromosomes and the sites of five albino gene loci in the *Rana nigromaculata* group. Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **9:** 1–52.

NISHIOKA, M., and M. SUMIDA, 1992 Biochemical differentiation of

pond frogs distributed in the Palearctic region. Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **11:** 71–108.

NISHIOKA, M., and M. SUMIDA, 1994a The differences in recombination rate between the male and female in *Rana nigromaculata* and *Rana brevipoda*. Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **13:** 99–136.

NISHIOKA, M., and M. SUMIDA, 1994b The position of sex-determining genes in the chromosomes of *Rana nigromaculata* and *Rana brevipoda*. Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **13:** 51–97.

NISHIOKA, M., M. SUMIDA and H. OHTANI, 1992 Differentiation of 70 populations in the *Rana nigromaculata* group by the method of electrophoretic analyses. Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **11:** 1–70.

O'BRIEN, S. J., 1993a Domestic cat (*Felis catus*). 2N=38, pp. 4.250–4.253 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

O'BRIEN, S. J. (Editor), 1993b *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

O'BRIEN, S. J., J. E. WOMACK, L. A. LYONS, K. J. MOORE, N. A. JENKINS *et al.,* 1993 Anchored reference loci for comparative genome mapping in mammals. Nature Genet. **3:** 103–112.

OHNO, S., U. WOLF and N. B. ATKIN, 1968 Evolution from fish to mammals by gene duplication. Hereditas **59:** 169–187.

OSTRANDER, E. A., P. M. JONG, J. RINE and G. DUYK, 1992 Construction of small-insert genomic DNA libraries highly enriched for microsatellite repeat sequences. Proc. Natl. Acad. Sci. USA **89:** 3419–3423.

POLLS PELAZ, M., and J-D. GRAF, 1988 Erythrocyte size as an indicator of ploidy level in *Rana* kl. *esculenta* before and after the metamorphosis. Alytes **7:** 53–61.

POSTLETHWAIT, J. H., S. L. JOHNSON, C. N. MIDSON, W. S. TALBOT, M. GATES *et al.,* 1994 A genetic linkage map for the zebrafish. Science **264:** 699–703.

RABBITTS, P., H. IMPEY, A. HEPPELL-PARTON, C. LANGFORD, C. TEASE *et al.,* 1995 Chromosome specific paints from a high resolution flow karyotype of the mouse. Nature Genet. **9:** 369–375.

RICE, W. R., 1989 Analyzing tables of statistical tests. Evolution **43:** 223–225.

RICHARDSON, B. J., P. R. BAVERSTOCK and M. ADAMS, 1986 *Allozyme Electrophoresis. A Handbook for Animal Systematics and Population Studies.* Academic Press, Sydney.

SANDBERG, K., and L. ANDERSSON, 1993 Horse (*Equus caballus*). 2N=64, pp. 4.276–4.278 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SCHULTZ, R. J., 1969 Hybridization, unisexuality, and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. Am. Nat. **103:** 605–619.

SEROV, O. L., and S. D. PACK, 1993 Gene map of the American mink (*Mustela vison*). 2N=30, pp. 4.279–4.281 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

STALLINGS, R. L., G. M. ADAIR and M. J. SICILIANO, 1993 The Chinese hamster gene map (*Cricetulus griseus*) (2N = 22), pp. 4.224–4.227 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

STALLINGS, R. L., and M. J. SICILIANO, 1983 Evolutionary conservatism in the arrangement of autosomal gene loci in mammals, pp. 313–321 in *Isozymes. Current Topics in Biological and Medical Research, vol. 10 (Genetics and Evolution),* edited by M. C. RATTAZZI, J. G. SCANDALIOS and G. S. WHITT.

STEPHENS, J. C., 1993 Cytogenetic map of human (*Homo sapiens,* 2N=46) genes as of July 1992, pp. 5.1–5.45 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SUMIDA, M., 1980 Electrophoretic study on the hemoglobin of Japanese pond frogs. Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **4:** 239–248.

SUMIDA, M., and M. NISHIOKA, 1994 A pronounced sex difference when two linked loci of the Japanese brown frog *Rana japonica* are recombined. Biochem. Genet. **32:** 361–369.

SZYMURA, J. M., 1995 Inheritance of allozyme loci in *Bombina:* one linkage group established. Biochem. Genet. **33:** 167–172.

TAKASE, M., I. MIURA, A. NAKATA, T. TAKEUCHI and M. NISHIOKA, 1992 Cloning and sequencing of the cDNA encoding tyrosinase of the Japanese pond frog, *Rana nigromaculata.* Gene **121:** 359–363.

TANKSLEY, S. D., M. W. GANAL, J. P. PRINCE, M. D. DE VICENTE, M. W. BONIERBALE *et al.,* 1992 High density molecular linkage maps of the tomato and potato genomes. Genetics **132:** 1141–1160.

UZZELL, T., 1982 Immunological relationship of western Palearctic water frogs (Salientia: Ranidae). Amphibia-Reptilia **3:** 135–143.

UZZELL, T., and L. BERGER, 1975 Electrophoretic phenotypes of *Rana ridibunda, Rana lessonae,* and their hybridogenetic associate, *Rana esculenta.* Proc. Acad. Nat. Sci. Phila. **127:** 13–24.

UZZELL, T., H. HOTZ and L. BERGER, 1980 Genome exclusion in gametogenesis by an interspecific *Rana* hybrid: evidence from electrophoresis of individual oocytes. J. Exp. Zool. **214:** 251–259.

WALLACE, D. G., M-C. KING and A. C. WILSON, 1973 Albumin differences among ranid frogs: taxonomic and phylogenetic implications. Syst. Zool. **22:** 1–13.

WILLIAMS, J. G. K., R. S. REITER, R. M. YOUNG and P. A. SCOLNIK, 1993 Genetic mapping of mutations using phenotypic pools and mapped RAPD markers. Nucleic Acids Res. **21:** 2697–2702.

WOMACK, J. E., S. N. BOLCH and R. FRIES, 1993 Gene map of the cow (*Bos taurus*). 2N=60, pp. 4.264–4.275 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

WRIGHT, D. A., 1975 Expression of enzyme phenotypes in hybrid embryos, pp. 649–664 in *Isozymes. IV. Genetics and Evolution,* edited by C. L. MARKERT. Academic Press, New York.

WRIGHT, D. A., and C. M. RICHARDS, 1982 Peptidase isozymes of the leopard frog *Rana pipiens:* properties and genetics. J. Exp. Zool. **221:** 283–293.

WRIGHT, D. A., and C. M. RICHARDS, 1983 Two sex-linked loci in the leopard frog, *Rana pipiens.* Genetics **103:** 249–261.

WRIGHT, D. A., and C. M. RICHARDS, 1993 Linkage groups in the leopard frog, *Rana pipiens.* 2N=26, pp. 4.326–4.329 in *Genetic Maps. Locus Maps of Complex Genomes,* Ed. 6, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

WRIGHT, D. A., C. M. RICHARDS and G. W. NACE, 1980 Inheritance of enzymes and blood proteins in the leopard frog, *Rana pipiens:* three linkage groups established. Biochem. Genet. **18:** 591–616.

WRIGHT, D. A., C. M. RICHARDS, J. S. FROST, A. M. CAMOZZI and B. J. KUNZ, 1983 Genetic mapping in amphibians, pp. 287–311 in *Isozymes: Current Topics in Biological and Medical Research, vol. 10 (Genetics and Evolution),* edited by M. C. RATTAZZI, J. G. SCANDALIOS and G. S. WHITT. Alan R. Liss, New York.

Communicating editor: N. A. JENKINS

APPENDIX A

Protein loci used for linkage analysis in Holarctic frogs of the genus Rana

Abbreviation	Protein	Enzyme Commission no.	No. of loci	Locus	Synonyms used in other Rana linkage studies
AAT	Aspartate aminotransferase	2.6.1.1	2	*sAAT*	*GOT-1*
				mAAT	*GOT-2*
ACO	Aconitate hydratase	4.2.1.3	2	*sACO*	*ACO-1*
				mACO	*ACO-2*
ADA	Adenosine deaminase	3.5.4.4	1	*ADA*	
ADH	Alcohol dehydrogenase	1.1.1.1	1	*ADH-2*	*ADH-A*
ALB	Albumin	—	1	*ALB*	
ALD	Fructose-biphosphate aldolase	4.1.2.13	1	*ALD-2*	*ALD*
AP	Acid phosphatase	3.1.3.2	2	*AP-1*	
				AP-2	
CA	Carbonate dehydratase	4.2.1.1	1	*CA-2*	*EST?*[a]
ENO	Enolase	4.2.1.11	1	*ENO*	
EST	Carboxylesterases	3.1.1	7[b]	*EST 1* etc.	
FDP	Fructose-biphosphatase	3.1.3.11	1	*FDP-1*	*F16DP-2*
FH	Fumarate hydratase	4.2.1.2	1	*FH*	*FUM*
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	1	*GAPDH*	
GDA	Guanine deaminase	3.5.4.3	1	*GDA*	
αGDH	α-Glycerophosphate dehydrogenase	1.1.1.8	1	*αGDH*	
GLO	Glyoxalase I	4.4.1.5	1	*GLO*	*GLY*
αGLU	α-Glucosidase	3.2.1.20	1	*αGLU*	
βGLU	β-Glucosidase	3.2.1.21	1	*βGLU*	
G6PDH	Glucose-6-phosphate 1-dehydrogenase	1.1.1.49	1	*G6PDH*	
GPI	Glucose-6-phosphate isomerase	5.3.1.9	1	*GPI*	
βGUS	β-Glucuronidase	3.2.1.31	1	*βGUS*	
HB	Hemoglobin	—	2	*HBI*	
				HBII	
HK	Hexokinase	2.7.1.1	1	*HK-1*	*HK-2*
IDDH	L-Iditol 2-dehydrogenase	1.1.1.14	1	*IDDH*	*SORD*
IDH	Isocitrate dehydrogenase	1.1.1.42	1	*sIDH*	*IDH-1, IDH-B*
LDH	L-Lactate dehydrogenase	1.1.1.27	1	*LDH-B*	
αMAN	α-Mannosidase	3.2.1.24	1	*αMAN*	
MDH	Malate dehydrogenase	1.1.1.37	2	*sMDH*	*MDH-1, MDH-B*
				mMDH	*MDH-2, MDH-A*
ME	Malic enzyme[c]	1.1.1.40	2	*sME*	*ME-1, ME-B*
				mME	*ME-2, ME-A*
MPI	Mannose-6-phosphate isomerase	5.3.1.8	1	*MPI*	
PEP	Peptidases	3.4	4	*PEPA*	
				PEPB	
				PEPC	
		3.4.13.9		*PEPD*	
PGDH	Phosphogluconate dehydrogenase	1.1.1.44	1	*PGDH*	
PGM	Phosphoglucomutase	5.4.2.2[d]	2	*PGM-1*	
				PGM-2	
PROT	Plasma proteins	—	1	*PROTC*	
SOD	Superoxide dismutase	1.15.1.1	2	*sSOD*	*SOD-1, SOD-B*
				mSOD	*SOD-2, SOD-A*
TF	Transferrin	—	1	*TF*	
TPI	Triosephosphate isomerase	5.3.1.1	1	*TPI*	
TYR	Tyrosinase	1.10.3.1	1	*TYR*	
XO	Xanthine oxidase	1.2.3.2	1	*XO*	

[a] *CA-2* products are revealed on EST stains (Table 1).
[b] Homology of *EST* loci across laboratories is not known.
[c] NADP-dependent malate dehydrogenase.
[d] Formerly 2.7.5.1.

APPENDIX B

Electrophoretic phenotypes and homology of protein loci used for intergroup comparison: Homology of *ADA, ALB, FH, GAPDH, GDA, αGDH, GLO, αGLU, βGLU, G6PDH, GPI, βGUS, IDDH, αMAN, MPI, PGDH, PROT C, TF,* and *TPI* is obvious because only a single locus is known for Rana. For six locus pairs (*AAT, ACO, IDH, MDH, ME, SOD*), homology is assessed among others by alternative subcellular localization of the products; given functional constraints, it is difficult to imagine that cytosolic and mitochondrial location of such enzymes encoded by independent loci have reversed in two Rana lineages since their common ancestry.

AAT: Products of the *sAAT* locus (our former *AAT-1;* UZZELL *et al.* 1980) are slightly more active than *mAAT* products in skeletal muscle, equally active in liver tissue. Products of the two loci separated best but still overlapped on TEB gels; mAAT phenotypes sometimes could not be reliably determined for *sAAT* heterozygotes. *sAAT* and *mAAT* correspond to *GOT-1* and *GOT-2,* respectively, reported for *R. pipiens* (WRIGHT *et al.* 1980: Figure 3), confirmed by a stronger band in the cathodal "GOT-2" position on TC7 gels for mitochondrial protein-enriched solutions of *R. pipiens.*

ACO: The *mACO* locus refers to the less anodal of two activity zones using liver tissue; *ACO-1* and *ACO-2* reported for the *R. pipiens* group correspond to *sACO* and *mACO,* respectively (WRIGHT *et al.* 1980: Figure 4); using mitochondrial protein solutions of *R. pipiens,* faint bands occurred only in the less anodal activity zone.

ADH: ADH-A of EPWF (NISHIOKA *et al.* 1987) and *ADH-2* of the *R. pipiens* group (WRIGHT *et al.* 1983) probably correspond to the same locus, the cathodalmost and strongest activity zone using liver tissue (NISHIOKA *et al.* 1992: Figure 1; D. A. WRIGHT, personal communication).

ALD: Products of the informative *ALD-2* locus have major activity in skeletal muscle; it corresponds to the *ALD* locus analyzed by NISHIOKA *et al.* (1987: Figure 7 p. 37), and to *ALD-2* reported for the *R. pipiens* group (WRIGHT *et al.* 1983). More anodally moving products of another locus (*ALD-1*) are active in brain and eye tissues, and less anodally moving products of a third locus (*ALD-3*) in liver tissues (WRIGHT *et al.* 1983).

CA-2 and EST: CA was visualized both with CA stain and under UV light using fluorescein diacetate as substrate ("fluorescein deaminase"). *CA-2* products were also scored as one of four esterases with major activity ("EST 3") using the conditions listed in Table 1; this identity is conclusively confirmed, both by shared mobility and inferred monomeric subunit structure and, more importantly, by identical patterns in several water frog species with fixed allelic differences, as well as in individuals from a *R. perezi* population in which two *CA-2* alleles are segregating (H. HOTZ, unpublished results). Homologies of the *EST* loci scored to those reported in other studies (which may well include *CA*) cannot presently be assessed.

FDP: Products of the *FDP-1* locus have major activity in liver tissue; by tissue-specific relative staining intensities it corresponds to *F16DP-2* reported for the *R. pipiens* group by WRIGHT *et al.* (1983); it is this locus that is linked to *ALB* in the *R. pipiens* group (D. A. WRIGHT, personal communication).

HB: Electrophoretic HB phenotypes of adult Rana usually show two bands or sets of bands, the less anodal band or set ("HB I") being more intense. Although sometimes interpreted as products of two codominant alleles at a single locus (NISHIOKA *et al.* 1980), several observations suggest that these patterns reflect products of two distinct loci. The patterns observed in different species often differ in position of each band, and there is wide variation among species in mobility difference between the two (*e.g.,* KAWAMURA and NISHIOKA 1986, Figure 10; NISHIOKA and SUMIDA 1992, Figure 2). Occasional intraspecific allelic variation, in which one of the two bands is monomorphic but the other reveals one- to two-banded variation as expected for products of a segregating locus (*e.g.,* for *HB I: R. nigromaculata* from Beijing; NISHIOKA *et al.* 1992, Figure 1), also supports a multilocus interpretation. The HB phenotypes of hybrids between *R. nigromaculata* and *R. brevipoda* consist of four bands, a summation of the two-banded patterns of each parental species in position and relative intensity; 80 backcross progeny, some to each parental species, showed either the hybrid four-banded or the backcross parental two-banded pattern; none had three-banded combinations (NISHIOKA *et al.* 1980, Figure 17). We assume that on chromosome *6* (NISHIOKA *et al.* 1980; *cf.* Table 4) there are at least two *HB* loci closely linked to each other. All phenotypes pictured for adults of Palearctic water frogs (*e.g.,* SUMIDA 1980, Figure 1; KAWAMURA and NISHIOKA 1983, Figure 12; KAWAMURA and NISHIOKA 1986, FIGURE 10; NISHIOKA and SUMIDA 1992, Figure 2) and of the Nearctic species *R. pipiens, R. blairi,* and their hybrids and backcrosses (DUNLAP 1979) are consistent with HB being encoded by a minimum of two independent loci. An anodal-most phenotype apparently reveals an additional locus expressed in the larval stage (*HB III;* WRIGHT *et al.* 1980, Figure 1).

HK: The *HK-1* locus refers to the main activity zone in liver tissue; in tissue-specific relative staining intensity and mobility its products correspond to those of *HK-2* reported for the *R. pipiens* group (WRIGHT *et al.* 1983, Figure 3; D. A. WRIGHT, personal communication).

IDH: sIDH and *mIDH* correspond to *IDH-B* and *IDH-A,* respectively, reported for EPWF (*e.g.,* KAWAMURA and NISHIOKA 1986: Figure 10), and to *IDH-1* and *IDH-2,* respectively, of the *R. pipiens* group (WRIGHT *et al.* 1980: Figure 3).

LDH: LDH-A and *LDH-B* correspond to the loci primarily expressed in skeletal and heart muscle, respec-

tively (EPWF: KAWAMURA and NISHIOKA 1986, Figure 11; *R. pipiens* group: WRIGHT *et al.* 1983, Figure 10; *R. catesbeiana* group: ELINSON 1981, Figure 2).

MDH: sMDH and *mMDH* correspond to *MDH-B* and *MDH-A,* respectively, reported for EPWF (KAWAMURA and NISHIOKA 1986, Figure 11), and to *MDH-1* and *MDH-2,* respectively, of the *R. pipiens* group: only the less anodal *MDH-2* products in *R. pipiens* yielded bands using mitochondrial protein solutions (*cf.* also WRIGHT 1975, Figure 4D).

ME: The *sME* locus informative in this paper corresponds to *ME-B* of NISHIOKA *et al.* (1987), as shown by the pattern pictured for *R. lessonae* by KAWAMURA and NISHIOKA (1986, Figure 10); and to *ME-1,* sex-linked in *R. pipiens* (WRIGHT and RICHARDS 1993; D. A. WRIGHT, personal communications); only the less anodal *ME-2* products in *R. pipiens* yielded bands using mitochondrial protein solutions.

PEPB and PEPD: Homology of the loci encoding these enzymes to those reported in other studies (*e.g.,* WRIGHT and RICHARDS 1982 for *R. pipiens*) is ascertained by concordant substrate specificity (Table 1), tissue distribution, relative staining intensity, and inferred subunit structure of the enzymes.

PGM: Products of the *PGM-2* locus yield the zone of highest activity in skeletal muscle tissue; it corresponds to *PGM-2* products pictured for *R. pipiens* by WRIGHT *et al.* (1980, Figure 5; 1983, Figure 2).

SOD: mSOD refers to the minor activity zone using liver tissue, near or cathodal to the origin on TEB gels; *sSOD* and *mSOD* correspond to *SOD-1* and *SOD-2,* respectively, of WRIGHT *et al.* (1983: Figure 6), and to *SOD-B* and *SOD-A,* respectively, of KAWAMURA and NISHIOKA (1983, Figure 12; this is consistent with the genotypes of western Palearctic water frog species reported by KAWAMURA and NISHIOKA 1986, Table 14).

XO: This multimeric (probably tetrameric) enzyme, with strongest activity in liver tissue, can be revealed both by using XO stains and using stains for xanthine dehydrogenase that differ from XO stains only by containing NAD (*cf.* ADAMS *et al.* 1984; RICHARDSON *et al.* 1986).

APPENDIX C

Inconsistent linkage assignments and gene orders in Holarctic Rana: Within the Nearctic *R. pipiens* group, one inconsistent assignment of protein loci has been made using different species combinations. In crosses within *R. pipiens, ALB* and *PGM-1* belong to two different LGs (I and VI), whereas they are linked in backcrosses using *R. palustris* × *R. pipiens, R. sphenocephala* × *R. blairi,* and *R. sphenocephala* × *R. berlandieri* hybrids (Table 4; WRIGHT *et al.* 1983, Figure 9). This discrepancy may be explained by LGs I and VI in *R. pipiens* being syntenic (Table 4); *ADH-2* and *ALB,* both linked to *PGM-1* in backcrosses of *R. sphenocephala* × *R. berlandieri* hybrids (WRIGHT *et al.* 1983), have both been assigned to chromosome *1* in EPWF (NISHIOKA *et al.* 1987). Even if LGs I and VI in *R. pipiens* are syntenic, however, these data suggest rearrangements within this chromosome in the *R. pipiens* group (unless the different genome combinations in hybrids cause markedly different recombination rates in the chromosome segment containing these loci).

Comparison between Palearctic and Nearctic Rana reveals one inconsistency for protein loci that is possible but not required (*EST* loci are not considered because their homology across laboratories is not established; Table 4). In the *R. pipiens* group, *GPI, PEPD* and *mAAT* (*GOT2*) are linked in *R. pipiens* (WRIGHT and RICHARDS 1993), and *GPI* and *sAAT* (*GOT1*) are linked in backcrosses of *R. palustris* × *R. pipiens* hybrids (WRIGHT *et al.* 1983), suggesting that *mAAT* and *sAAT* are syntenic (Table 4). In our data, *mAAT* and *sAAT* are not linked at $P < 0.05$ using the sequential Bonferroni test (Table 3), although they may be syntenic but too distant from each other to show linkage. A direct linkage test of this locus pair was not possible in the *R. pipiens* group, and the two *AAT* loci may well be situated on opposite sides of the *GPI* locus. In WPWF, *PEPD* and *mAAT* are also linked (Figure 1); and it is worth noting that *sAAT* compared with each member of our LG 1 had excess of parental over recombinant genotypes. Of the four possible pairs, *sAAT/mAAT, EST 4,* and *PEPD* deviated from random assortment at $0.001 > P > 0.01$ in individual tests (Table 3), and *sAAT/CA-2* at $0.05 > P > 0.01$ (data not shown). If in WPWF sAAT and mAAT are syntenic and if the uncertain linkage of *sAAT* and *ALD-2* is real, however, then an inconsistency between WPWF and EPWF would be revealed, because *ALD-2* and *PEPD* have been assigned to different chromosomes in EPWF (*9* and *10,* respectively; NISHIOKA *et al.* 1987; Table 4).

For only one LG the gene order of the same set of more than two loci has been determined in two different groups of Rana and can thus be compared: *HK-1, LDH-B, MPI,* and *PEPB* of LG 2 in WPWF (Figure 1) and LG V in the *R. pipiens* group (WRIGHT *et al.* 1983). The order *PEPB-MPI-HK* detected in crosses within *R. pipiens* (WRIGHT and RICHARDS 1993) is inconsistent with the order *MPI-HK-PEPB* in WPWF (Figure 1), suggesting that gene rearrangement has occurred within this LG. The inconsistency is not revealed in *R. sphenocephala* × *R. berlandieri* backcrosses (order *MPI-PEPB-LDHB-HK;* WRIGHT *et al.* 1983); the much smaller map distances in this cross, similar to our WPWF values (Figure 1), probably reflect reduced recombination rates in hybrids relative to parental species.

APPENDIX D

A partial list of Rana syntenies shared with mammals or teleost fish

Syntenies compared[a]		Taxa compared[b]	Chromosome or LG	Reference[c]
Rana chromosome *1* (*sACO-ADH2-ALB-FDP1-GDA-βGLU-βGUS-PGM1-TF*)[d]				
GBA (*βGLU*)-*PGM1*	M:	Homo	1	10
ADH1,2,3,4,5-ALB-PGM2		Homo	4	10
Adh1-Alb		Peromyscus	VI	1
ADH3-ALB		Sus	8	3
Adh1,3,5-Gba (*βGLU*)		Mus	3	5
ADH2-PGM2		Bos	6	15
Aco1-Pgm1		Rattus	5	6
Aco1 (*sACO*)-*Pgm2*		Mus	4	5
Alb1-βGus-Pgm1		Mus	5	5
Rana chromosome *2* (*αGDH-mMDH-mME-PEPC-sSOD-TYR*)				
Mod2 (*mME*)-*Tyr*	M:	Mus	7	5
SOD1 (*sSOD*)-*TYR*		Sus	9	3
G3p1 (*αGDH*)-*PepC2*	F:	Salmonidae[e]	2	7
Rana chromosome *3* (*sMDH-sME-mSOD-XO*)				
ME1 (*sME*)-*SOD2* (*mSOD*)	M:	Bos	SG2	15
		Canis	U10	8
		Felis	B2	11
		Homo	6	10
		Mustela	1	13
Rana chromosome *4* (*ENO-HK1-IDDH-LDHB-αMAN-MPI-PEPB*)				
ENO2-LDHB-PEPB	M:	Homo	12	10
LDHB-PEPB		Bos	5	15
		Felis	B4	11
		Mustela	9	13
		Ovis	3	2
Eno2-Ldhb		Rattus	4	6
HK1-PEPB		Cricetulus	1	14
Hk1-Pep2		Mus	10	5
ENO1-HK1		Mustela	2	13
SORD (*IDDH*)-*MANA* (*αMAN*)-*MPI*		Homo	15	10
MANA (*αMAN*)-*MPI*		Felis	B3	11
ENO2-LDHC-MPI	F:	Xiphophorus	II	9
Ldh3-PepB1; Ldh4-PepB2		Salmonidae[e]	7; 8	7
Ldh1-Ldh5-Mpi		Salmonidae[e]	13	7
Rana chromosome *10* (*mAAT-sAAT-CA2-4 ESTs-GPI-PEPD-TPI*)				
Got1 (*AAT*)-*Gpi-Pepd*	M:	Rattus	1	6
GPI-PEPD		Cricetulus	9	14
		Homo	19	10
GOT2 (*mAAT*)-*CA IV, VII-CES1* (*EST*)-*ESB3* (*EST*)		Homo	16	10
Got2 (*mAAT*)-*Ces1* (*EST*)-11 *Es* loci (*EST*)		Mus	8	5
GOT2 (*mAAT*)-*ES* (*EST*)		Equus	U2	12
3 *ES loci* (*EST*)-*GPI-PEPD*		Mustela	7	13
Es8 (*EST*)-*Gpi1-Pep4*		Mus	7	5
mAAT-GPI1-PEPD	F:	Xiphophorus	IV	9
GPI1-PEPD		Poeciliopsis	IV	9
Gpi1-PepD1; Gpi2-PepD2		Salmonidae[e]	3; 4	7
3 *EST* loci-*GP12*		Xiphophorus	II	9
Gpi3-Aat5		Salmonidae[e]	13	7
Rana chromosome *10* (*mAAT-sAAT-CA2-4 ESTs-GPI-PEPD-TPI*) and LG U2 (*4 ESTs*)				
2 *Es* loci (*EST*)-4 *Est* loci	M:	Oryctolagus	VI	4
3 *Es* loci (*EST*); 11 *Es* loci, *Ces 1* (*EST*); 4 *Es* loci		Mus	3; 8; 9	5
3 *ES* loci (*EST*)		Mustela	7	13
2 *Es* clusters (*EST*)		Peromyscus	VIII	1
12 *Es* loci (*EST*)		Rattus	19	6
Rana LG U1 (*AP1-AP2*-an *EST-GLO*)				
GLO-ESD (*EST*)	M:	Cricetulus	1	14
Rana LG U3 (*G6PDH*)-*PGDH*)				
Gpd1 (*G6PDH-Pgd* (*PGDH*)	M:	Mus	4	5
G6PDH-PGDH	F:	Poecilia, Xiphophorus	I	9
		Poeciliopsis	III	9

[a] Loci are listed with their original names used for the mammal or fish taxa compared; for certain loci, in which these names are quite different from those we use, or for which homology is known, the Rana name is indicated in parentheses. Several locus homologies cannot be established with certainty on the basis of data in the sources cited.

[b] M, mammals; F, fish.

[c] References: 1, DAWSON and ROGERS 1993; 2, ECHARD 1993; 3, ECHARD *et al.* 1993; 4, FOX 1993; 5, HILLYARD *et al.* 1993; 6, LEVAN *et al.* 1993; 7, MAY and JOHNSON 1993; 8, MEERA KHAN *et al.* 1993; 9, MORIZOT *et al.* 1993; 10, NIH/CEPH Collaborative Mapping Group 1993, STEPHENS 1993; 11, O'BRIEN 1993a; 12, SANDBERG and ANDERSSON 1993; 13, SEROV and PACK 1993; 14, STALLINGS *et al.* 1993; 15, WOMACK *et al.* 1993. The synteny lists and chromosome assignments for Rana are those of Table 4.

[d] The lists of syntenic Rana loci are alphabetical.

[e] Salmonid fishes are tetraploid-derivative (*e.g.*, OHNO *et al.* 1968; ALLENDORF *et al.* 1975).