TRANSFERRIN GROUPS OF TUNAS

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Received September 14, 1967

TRANSFERRIN polymorphism has been demonstrated by gel electrophoresis in many vertebrates. These include men (SMITHIES and HILLER 1959), monkeys (GOODMAN and POULIK 1961), cattle (ASHTON 1958; SMITHIES and HICKMAN 1958), sheep and goats (ASHTON and FERGUSON 1963; EFREMOV and BRAEND 1964), horses (BRAEND and STORMONT 1964), burros (NIECE and KRACHT 1967), pigs (ASHTON 1960a), mice (ASHTON and BRADEN 1961), antelopes (ASHTON and CARR 1965), harp seals (NAEVDAL 1965), doves (MUELLER 1961), chickens (OGDEN, MORTON, GILMOUR and MCDERMID 1962), and gadoid fishes (MØLLER and NAEVDAL 1966; MØLLER 1966). BARRETT and TSUYUKI (1967) have found polymorphisms of transferrin in several scombroid fishes—yellowfin tuna, skipjack tuna, albacore, and bonito.

The animals most thoroughly studied have been domestic cattle. Transferrin phenotypes have been shown to be associated with differences in fertility and fetal mortality (ASHTON 1959), and maternal-fetal incompatibility (ASHTON 1965). Transferrin phenotypes have also been associated with differences in the production of milk and butterfat (ASHTON 1960b; ASHTON, FALLON and SUTHERLAND 1964).

This paper (1) describes transferrin polymorphisms in skipjack tuna (*Katsuwonus pelamis*), southern bluefin tuna (*Thunnus maccoyi*), and yellowfin tuna (*T. albacares*) from the Atlantic and Pacific Oceans; (2) presents evidence of differential adaptive values of phenotypes in skipjack tuna; and (3) discusses possible mechanisms of maintaining transferrin polymorphism in a randomly mating population.

MATERIALS AND METHODS

Tuna serum: Skipjack serum or supernatant (plasma obtained by dialysing whole blood specimen frozen in glycerol solution, FUJINO 1966) specimens include: 3 lots of 213 specimens taken from the tropical Atlantic Ocean (latitude 12° N–19° N, longitude 61° W–68° W) from February through November 1966; 27 lots of 2,257 specimens from Hawaiian waters taken between April 1965 and August 1967; 5 lots of 401 specimens from Japanese waters taken from September through November 1966; and 20 lots of 1,495 specimens taken from Palau from November 1966 through November 1967. These samples were collected from fish caught by the Laboratory's research vessel *Charles H. Gilbert* and commercial fishing boats.

Southern bluefin samples were 302 collected from waters off Australia in 1963 and 1964 by the staff of the Commonwealth Scientific and Industrial Research Organization, Cronulla, N.S.W.

Yellowfin samples were 206 taken from the eastern Pacific Ocean in 1966, 182 taken from Hawaiian waters in 1966 and 1967, and 307 taken from waters of Line Islands in 1964 and 1967.

Techniques of bleeding fish and preservation and preparation of serum and supernatant specimens were the same as those described elsewhere (FUJINO and KANG 1968).

Skipjack and yellowfin specimens representing different transferrin phenotypes were exchanged between our Laboratory and the Laboratory of the Inter-American Tropical Tuna Commission to standardize identification of transferrin bands recognized in both laboratories. Phenotypic incidences obtained by BARRETT and TSUYUKI (1967, Tables 2 and 4) were cited for statistical comparison with our results.

Electrophoresis and staining: Horizontal starch-gel electrophoresis was performed at room temperature (22°–23°C), with the same buffer system and under the same electrical conditions used for serum esterase analysis (FUJINO and KANG 1968). Transferrin bands were identified by labeling with Fe^{59} sulfate, followed by preparation of radioautographs (SMITHIES and HILLER 1959) as well as staining with amido-black (SMITHIES 1955). Results of identification of transferrin bands on each of samples from more than several hundred fishes were always identical between the above two techniques.

RESULTS

Electrophoretic patterns. Through use of Fe^{59} we were able to confirm that the bands that appeared in the β-globulin location were transferrin. Three transferrin bands were recognized in skipjack tuna and yellowfin tuna and two in southern bluefin tuna. Relative mobility of these bands is shown in Figure 1. Details are described by species.

Skipjack tuna[1]: Three bands, named 1, 2, and 3 in descending order of their mobility, occurred. These bands were identical with C, D, and E of BARRETT and TSUYUKI (1967). Five phenotypes 1-2, 2, 2-3, 3, and 1-3, were observed. A gene frequency analysis applied to all specimens taken from Hawaiian waters suggested that the six phenotypes form a system designated T_{SJ} which is determined by three codominant, autosomal alleles $T^1{}_{SJ}$, $T^2{}_{SJ}$, and $T^3{}_{SJ}$ (Table 1). A theoretically possible group, 1, was not observed among 4,366 specimens tested by us. The T_{SJ} system is independent of sex, size of fish, the blood group systems B and Y, and

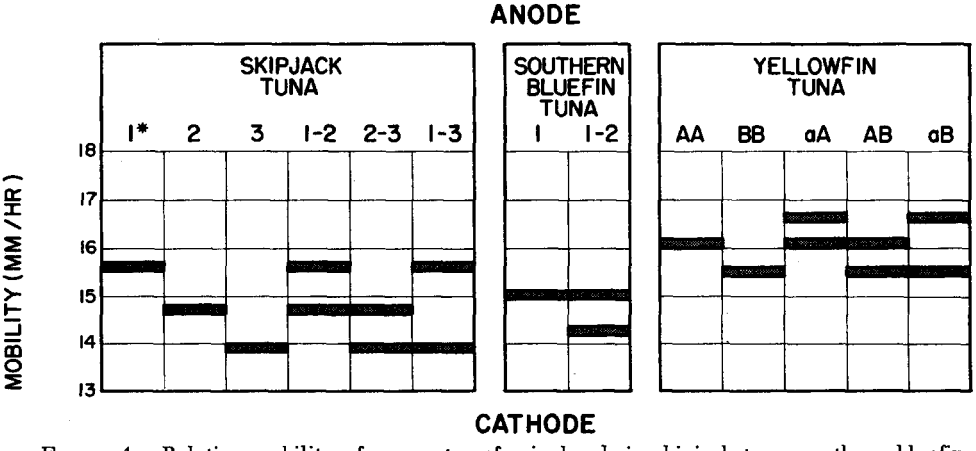

FIGURE 1.—Relative mobility of serum transferrin bands in skipjack tuna, southern bluefin tuna, and yellowfin tuna. * Not observed by us.

[1] The nomenclature of bands and phenotypes of the transferrin system published by FUJINO (1967) was used.

TABLE 1

Comparison of observed phenotypes (O) with expected (E) in the T_{SJ} transferrin system of skipjack tuna in Hawaiian samples

Collected by	No. of lots		Phenotypes 1–2	2	2–3	3	1–3	Total	χ^2	Probability (2 d.f.)
Research vessels	18	O	11	680	598	99	5	1,393	4.51	>0.1
		E	11.8	696.3	565.3	114.8	4.8			
Commercial vessels	9	O	14	422	366	58	4	864	3.50	>0.1
		E	13.8	432.9	343.9	68.2	5.2			

serum esterase system (SPRAGUE and HOLLOWAY 1962; FUJINO and KAZAMA 1968; FUJINO and KANG 1968).

Frequencies of genes of all 55 lots of specimens, together with data obtained by BARRETT and TSUYUKI (1967) for fish from the eastern Pacific, were subjected to intra-area and inter-area heterogeneity tests (chi-square tests for contingency table). No significant intra-area and inter-area heterogeneity was present. Pooled data of phenotype and gene frequencies by area are summarized in Table 2. The ranges, means, and standard deviations of frequencies of gene T^2_{SJ} are shown in Figure 2 by geographical area. Comparison for mean values of frequency of an allele T^2_{SJ} (t-test) between neighboring areas indicated that only one combination —that between Hawaiian and eastern Pacific waters—was significantly different ($t = 2.49$, 30 d.f., $P<0.05$). This result suggests that further data, especially from the eastern Pacific, are required to understand the relation between Ha-

TABLE 2

Phenotype and gene frequencies of T_{SJ} system of skipjack tuna in different areas

Area	No. of lots		Phenotypes 1	1–2	2	2–3	3	1–3	Total	Alleles T^1_{SJ}	T^2_{SJ}	T^3_{SJ}
Atlantic	3	O	0	3	110	80	18	2	213	0.012	0.711	0.277
		E	0.0	3.6	107.7	83.9	16.4	1.4				
*Eastern Pacific	5	O	4	5	70	86	9	1	175	0.040	0.660	0.300
		E	0.3	9.2	76.2	69.3	15.8	4.2				
Hawaii	27	O	0	25	1,102	964	157	9	2,257	0.008	0.707	0.285
		E	0.1	25.5	1,128.2	909.6	183.3	10.3				
Japan	5	O	0	8	184	181	25	3	401	0.013	0.695	0.292
		E	0.1	7.2	193.7	162.8	34.2	3.0				
Palau	20	O	0	10	698	659	119	9	1,495	0.006	0.691	0.303
		E	0.1	12.4	713.8	626.0	137.3	5.4				

* Cited from BARRETT and TSUYUKI (1967).

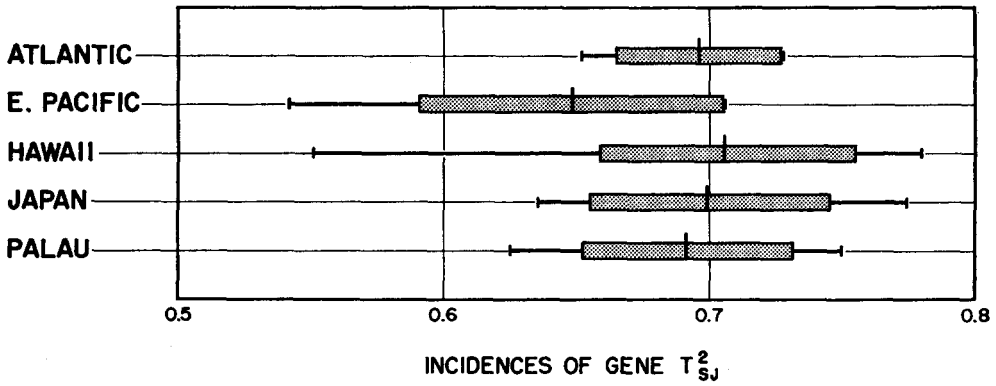

FIGURE 2.—Frequencies of transferrin gene T^2_{SJ} in skipjack tuna from different areas.

waiian and eastern Pacific skipjack tuna. The study of the serum esterase system (FUJINO and KANG 1968) also demonstrated this need.

Southern bluefin tuna: Two bands, named 1 and 2, occurred in the southern bluefin tuna. The combinations of these bands expressed two phenotypes, 1 and 1-2, but not 2, a theoretically possible one. A gene frequency analysis shown in Table 3 suggests that the two bands are determined by two codominant, autosomal alleles of the system (T_{SB} system), T^1_{SB} and T^2_{SB}. The T_{SB} system is independent of sex and size of fish. No significant heterogeneity of gene frequencies was seen between stocks A, B, and C, which were so identified for studies of tagging and growth rate data by the staff of the Commonwealth Scientific and Industrial Research Organization (HYND 1965; G. L. KESTEVEN, personal communication).

Yellowfin tuna: Two bands A and B (BARRETT and TSUYUKI 1967) and another band "a" faster than A and B occurred in yellowfin tuna. The combinations of these bands expressed five phenotypes, aA, AA, AB, BB, and aB. A theoretical type aa was not observed in the 705 specimens tested. A gene frequency analysis of the transferrin system, shown in Table 4, suggests that the three recognized bands are determined by three codominant, autosomal alleles. The system is independent of sex, size of fish, and the esterase system.

TABLE 3

Gene frequency analysis of the T_{SB} *transferrin system of southern bluefin tuna*

		Phenotypes						
		1		1–2		2		
Stock*	No. of lots	O	E	O	E	O	E	Total
A	2	12	12.0	1	1.0	0	0.0	13
B	2	79	79.4	11	10.3	0	0.3	90
C	2	39	39.0	3	2.9	0	0.1	42
UI	4	137	137.6	20	18.8	0	0.6	157

* Identifications of stocks A, B, and C were provided by G. L. KESTEVEN (personal communication); UI—unidentified stock.

TABLE 4

Gene frequency analysis of the transferrin system of yellowfin tuna

Area	No. of lots		Phenotypes aA	AA	AB	BB	aB	Total	χ^2	d.f.	Probability
Hawaii	2	O	2	99	81	9	1	192	3.05	2	>0.2
		E	2.2	102.9	73.1	13.0	0.8				
Line Is.	2	O	.	167	115	25	.	307	0.67	1	>0.3
		E	.	164.1	120.7	22.2	.				
Eastern Pacific	1	O	.	114	75	17	.	206	0.83	1	>0.3
		E	.	111.2	80.3	14.5	.				
Do.*	7	O	.	267	185	42	.	494	1.47	1	>0.2
		E	.	261.8	195.6	36.6	.				

* Cited from BARRETT and TSUYUKI (1967).

Gene frequencies of the samples taken from various areas as well as results obtained by BARRETT and TSUYUKI (1967) were subjected to heterogeneity tests. No significant heterogeneity was observed in either intra-area or inter-area comparisons for the eastern Pacific, Hawaii, and Line Islands.

Adaptive value of phenotypes (1) Excess of heterozygotes and deficiency of homozygotes in skipjack tuna. Comparison of the observed and expected incidences of phenotypes in Tables 1 and 2 indicates that usually an excess of phenotype 2-3 and a deficiency of phenotypes 2 and 3 existed in the samples (Table 5). For the other phenotypes, 1-2 and 1-3, no particular trend was visible. Each of the

TABLE 5

Excessive incidences of a heterozygote (2–3) of T_{SJ} system of skipjack tuna in different areas

Area		Phenotypes 2	2–3	3	Others*	Total	O/E ratio of 2–3 type	HARDY-WEINBERG analysis χ^2	P (2 d.f.)
Eastern Pacific†	O	70	86	9	10	175	1.24	8.46	<0.02‡
	E	76.2	69.3	15.8	13.7				
Hawaii	O	1,102	964	157	34	2,257	1.06	8.00	<0.02‡
	E	1,128.2	909.6	183.3	35.9				
Japan	O	184	181	25	11	401	1.11	5.01	>0.05
	E	193.7	162.8	34.2	10.3				
Palau	O	698	659	119	19	1,495	1.05	4.60	>0.1
	E	713.8	626.0	137.3	17.9				

* Sum of phenotypes 1, 1–2, and 1–3.
† Obtained by BARRETT and TSUYUKI (1967).
‡ Statistically significant.

TABLE 6

Excessive incidences of a heterozygote (2–3) of T_{SJ} system of skipjack tuna in different sexes in Hawaiian sample

Sex	Phenotypes				Total	O/E ratios of phenotypes			
	2	2–3	3	Others*		2	2–3	3	Others
Male									
O	183	175	26	4	388				
						0.96	1.09	0.77	1.05
E	190.1	160.3	33.8	3.8	388.0				
Female									
O	195	179	24	4	402				
						0.95	1.11	0.75	1.00
E	204.4	161.6	32.0	4.0	402.0				

* Sum of phenotypes 1–2 and 1–3.

lots of specimens was also tested for the serum esterase system (FUJINO and KANG 1968) and they did not deviate significantly from the population from which they were drawn. Table 5 shows the observed-expected differences of phenotypes in each geographical area, although the differences do not reach the significance level of 5% for the samples from Japan and Palau. This result was seen in both sexes (Table 6).

(2) Association of phenotypic excess and deficiency with size of fish in skipjack tuna. Phenotypic excess and deficiency were examined for groups of fish of different sizes in the samples from Hawaiian waters (Table 7). Individual values and regressions of the ratio of observed/expected incidences (O/E ratio) were plotted (Figure 3). It can be seen from these relations that the phenotypic excess and

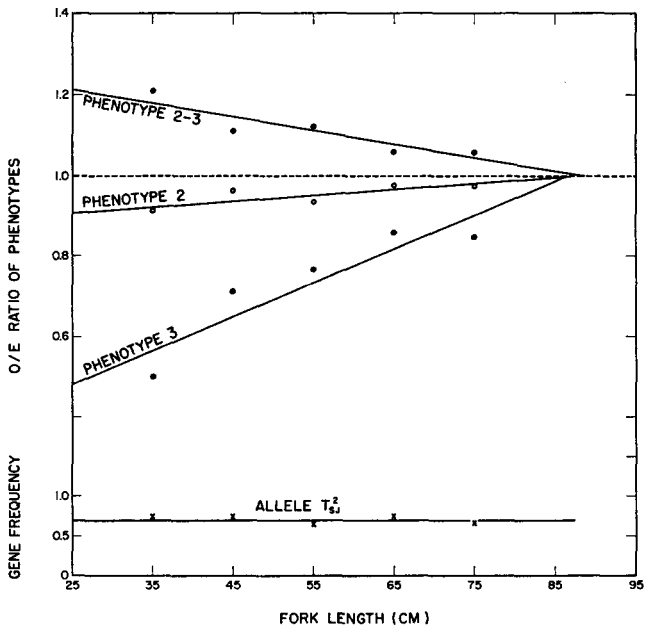

FIGURE 3.—Relation of O/E ratio of three phenotypes and frequency of allele T^2_{SJ} of transferrin system to size of fish in Hawaiian skipjack tuna population.

TABLE 7

O/E ratio of phenotypic incidences of T_{SJ} *system in different size groups of skipjack tuna from Hawaii*

Fork length (cm)	Phenotypes										Total observed	Alleles			O/E ratios of phenotypes		
	1–2		2		2–3		3		1–3								
	O	E	O	E	O	E	O	E	O	E		T^1_{SJ}	T^2_{SJ}	T^3_{SJ}	2	2–3	3
31–40	0	0.0	21	23.0	23	19.0	2	4.0	0	0.0	46	0.000	0.707	0.392	0.91	1.21	0.50
41–50	4	3.5	201	209.3	169	152.9	20	28.0	1	1.3	395	0.006	0.728	0.266	0.96	1.11	0.71
51–60	1	0.6	60	64.3	74	65.9	13	16.9	0	0.3	148	0.003	0.659	0.338	0.93	1.12	0.77
61–70	0	0.0	58	59.5	50	47.2	8	9.3	0	0.0	116	0.000	0.716	0.284	0.97	1.06	0.86
71–80	1	1.4	38	38.8	38	35.9	7	8.3	1	0.6	85	0.012	0.676	0.312	0.98	1.06	0.84

deficiency (1) are associated with size (i.e. age) of the fish, being greater in juveniles than in adults, and (2) disappear at a fork length of approximately 86 cm, which is about the maximum for the species.

(3) Is the T_{SJ} system a balanced polymorphism? FALCONER (1961, Table 2.1 and equation 2.16) discussed the condition for balancing a polymorphism with two alleles. This can be applied to the T_{SJ} system by appropriate modification as follows.

In a system with three codominant alleles, in which the heterozygote 2-3 is overdominant and the phenotypes 1, 1-2, and 1-3 have an equal fitness, the proportionate contribution of each phenotype to the gametes that will form the next generation can be obtained, thus:

Phenotypes	1	1-2	2	2-3	3	1-3	Total
Initial frequencies	p^2	$2pq$	q^2	$2qr$	r^2	$2pr$	1
Fitness	$1-S$	$1-S$	$1-S_2$	1	$1-S_3$	$1-S$	
Gametic contribution	$p^2(1-S)$	$2pq(1-S)$	$q^2(1-S_2)$	$2qr$	$r^2(1-S_3)$	$2pr(1-S)$	A

where p, q, and r are frequencies of genes $T^1{}_{SJ}$, $T^2{}_{SJ}$, and $T^3{}_{SJ}$, respectively, in a parent population and total 1; S, S_2, and S_3 are coefficients of selection for the phenotypes 1 and 1-2 and 1-3, 2, and 3, respectively; and $A = 1-S[1-(q+r)^2] - S_2q^2-S_3r^2$. Changes of gene frequencies in the next generation are:

$$\begin{aligned} \Delta p &= \frac{p}{A}\,[-S(1-p)^2+S_2q^2+S_3r^2] \\ \Delta q &= \frac{q}{A}\,[S(1-p)p-S_2(1-q)q+S_3r^2] \\ \Delta r &= \frac{r}{A}\,[S(1-p)p+S_2q^2-S_3(1-r)r]\ . \end{aligned} \tag{1}$$

When the polymorphism is balanced, $\Delta p = \Delta q = \Delta r = 0$. Then from the equations (1),

$$\frac{S_3}{S_2} = \frac{q}{r} \text{ and } \frac{S}{S_2} = \frac{q}{1-p} \tag{2}$$

$$\text{or } S(1-p) = S_2q = S_3r, \tag{3}$$

where $p+q+r=1$ and none of p, q, r, and A is equal to 0 or 1.

By the relation (3),

$$A = 1 - S. \tag{4}$$

The phenotype frequencies in the balancing populations can be obtained from the proportionate gametic contribution, noted earlier, and equation (4) as follows:

Phenotypes	1	1-2	2	2-3	3	1-3	Total
Frequencies in population (I)	p^2	$2pq$	$q^2\frac{1-S_2}{1-S}$	$2qr\frac{1}{1-S}$	$r^2\frac{1-S_3}{1-S}$	$2pr$	1
Frequencies under no selection (II)	p^2	$2pq$	q^2	$2qr$	r^2	$2pr$	1

Ratio I/II	1	1	$\frac{1-S_2}{1-S}$	$\frac{1}{1-S}$	$\frac{1-S_3}{1-S}$	1
Ratio I/II × (1−S) (=fitness)	$1-S$	$1-S$	$1-S_2$	1	$1-S_3$	$1-S$

where the frequencies I and II are represented by the observed and expected incidences of phenotypes, noted earlier, respectively. Thus fitness of each phenotype can be given as a proportion of ratio of observed/expected incidences (O/E ratio) of each phenotype against O/E ratio of the 2–3 type.

Table 8 summarizes the products of coefficient of selection and gene frequency which are defined by each term of equation (3). Values of fitness (or coefficient of selection) were calculated, as demonstrated above, from O/E ratios shown in Table 5. In Table 8, it is shown that the relations of equation (3) are satisfied except the figures of $S(1-p)$ in each area; the deviation in the figures of $S(1-p)$ seems due to low frequencies of 1–2 and 1–3 phenotypes.

(4) Unchanged gene frequency of T_{SJ} transferrin system in skipjack tuna. Equations (1) through (4) are also applicable for the life-span of a single generation of a population by replacing S, S_2, and S_3 with coefficients of mortality M, M_2, and M_3 for the phenotypes 1–2 and 1–3, 2, and 3, respectively. Then the condition by which gene frequencies remain unchanged regardless of increasing age is,

$$M(1-p) = M_2 q = M_3 r \tag{5}$$

$$\text{or } M : M_2 : M_3 = \frac{1}{1-p} : \frac{1}{q} : \frac{1}{r} \tag{6}$$

where coefficient of mortality is defined as 1 minus survival value, which is proportionate survival rate for each phenotype (where survival value of 2–3 type = 1) at given age of fish. Survival value of each phenotype in relation to the size of fish can be defined in a similar way as obtaining fitness from O/E ratio as demonstrated in section (3). Figure 4 shows the survival value-length relations obtained from the O/E ratio regressions (Figure 3). The applicability of the portion of these curves below 25 cm fork length requires verification.

If the regression of the survival value for each phenotype is actually represented by a straight line throughout the fish's life, as shown in Figure 4, the ratio $M : M_2 : M_3$ has a set of constant values, despite changes of individual values of M, M_2, and M_3 with increasing size of fish.

TABLE 8

Relative fitness of phenotypes and products of coefficient of selection and gene frequency

Area	Fitness			Product		
	$1-S$	$1-S_2$	$1-S_3$	$(1-p)S$	$q.S_2$	$r.S_3$
Eastern Pacific	0.59	0.74	0.46	0.39	0.17	0.16
Hawaii	0.89	0.92	0.81	0.11	0.06	0.05
Japan	0.96	0.85	0.66	0.04	0.10	0.10
Palau	1.00	0.92	0.82	0.00	0.06	0.05

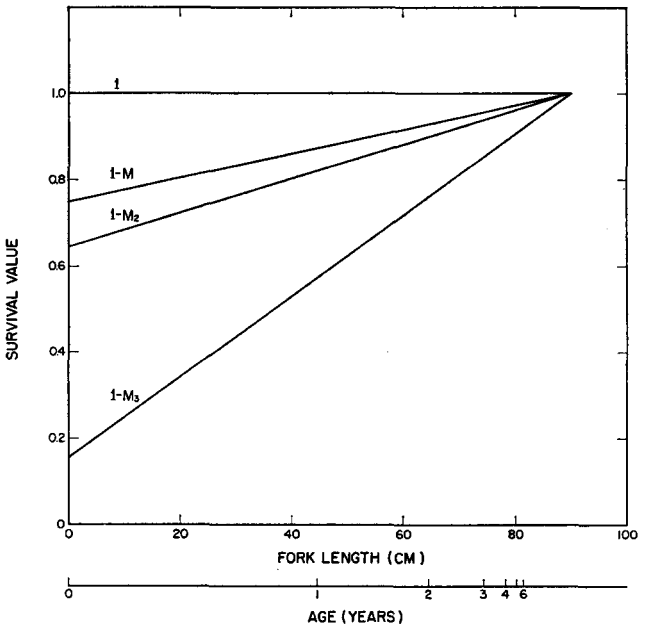

FIGURE 4.—Survival values of phenotypes of T_{SJ} system in relation to size of fish in Hawaiian skipjack tuna population. Ages were estimated from VON BERTALANFFY growth curve (ROTHSCHILD 1966).

It can be said from the above discussion, the equation (6), and $p+q+r=1$ that the condition that a set of gene frequencies p, q, and r remains unchanged throughout the life of fish is satisfied if the regression of the survival value with respect to size of fish is represented by a straight line for each phenotype.

The theoretical conclusions above were supported by the data as follows: (a) Table 9 summarizes product of coefficient of mortality and gene frequency, which is represented by the equation (5), in different size groups in Hawaiian samples. (Owing to low incidences of the phenotypes 1–2 and 1–3, the figure for the first term of formula (5) is not shown.) This table shows that the relation of formula (5) is satisfied in each size group. (b) The observed gene frequencies of Table 7

TABLE 9

Products of coefficient of mortality and gene frequency in different size groups in Hawaiian population

Fork length (cm)	Product	
	$q.M_2$	$r.M_3$
31–40	0.18	0.23
41–50	0.09	0.09
51–60	0.11	0.11
61–70	0.06	0.05
71–80	0.05	0.06

and a regression line of frequency of allele T^2_{SJ} of Figure 3 show the unchanged gene frequencies throughout different size groups.

The four kinds of evidence presented above suggest that (1) adaptive values of phenotypes exist throughout the life of skipjack tuna, (2) the survival values of phenotypes shift with age of fish, and (3) the transferrin system of skipjack tuna is a balanced polymorphism despite existence of adaptive value among phenotypes.

HYPOTHESIS ON MECHANISMS OF MAINTAINING TRANSFERRIN POLYMORPHISM

The results of the above observations and analyses now make it possible to propose a hypothesis with respect to mechanisms of maintaining transferrin polymorphism in a randomly mating population of skipjack tuna. (1) There is a significant differential fertility among different phenotypes in the T_{SJ} system, resulting in excess production of heterozygote 2–3 and deficiency of homozygotes 2 and 3. (2) These biases in phenotypic incidence at the time of fertilization are gradually compensated by differential viabilities among phenotypes with increasing age, and disappear when the fish reach their maximum size. Gene frequencies remain unchanged despite the selective pressures on phenotypes.

To test the hypothesis, 1) an extensive accumulation of data of phenotype frequency for the phenotypes 1–2 and 1–3, 2) verification of applicability of regression of survival value under 25 cm fork length of fish, and 3) an experiment of artificial fertilization (KUME 1962) by rearing fish in captivity (MAGNUSON 1965) are suggested.

G. C. ASHTON, Y. HIRAIZUMI, and M. VENDORF gave us technical assistance and valuable suggestions and comments on the manuscript. Research materials from various geographical areas were collected and shipped by G. L. KESTEVEN, the Commonwealth Scientific and Industrial Research Organization of Australia; A. SUZUKI, Tokai Regional Fisheries Research Laboratory, Tokyo; J. JOSEPH, Inter-American Tropical Tuna Commission, La Jolla; and T. S. AUSTIN, the Bureau of Commercial Fisheries Tropical Atlantic Biological Laboratory, Miami.

SUMMARY

Transferrin polymorphism was found in skipjack tuna, southern bluefin tuna, and yellowfin tuna taken from the Pacific and Atlantic Oceans. No significant heterogeneity of gene frequency distribution was seen between different geographical areas or different stocks in each species. Evidence for differential fertility and viability among transferrin phenotypes of skipjack tuna and their association with age of fish were described. A hypothesis on mechanisms maintaining transferrin polymorphism in a randomly mating skipjack tuna population was proposed.

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