AN ESTIMATE OF THE NUMBER OF HISTOCOMPATIBILITY LOCI IN THE TELEOST XIPHOPHORUS MACULATUS[1]

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A vigorous immune response against tissue homografts is characteristic of nearly all postembryonic vertebrates. The homograft reaction has been demonstrated in all species of teleosts tested (KALLMAN 1964; HILDEMANN and HAAS 1960), and in many amphibians (HILDEMANN and HAAS 1959; VOGEL 1940; HOROWITZ 1937), in at least one reptile (MAY 1923), several birds (HEALEY, RUSSEL, POOLE and OLSEN 1962; LAZZARINI 1960; CANNON, TERASAKI and LONGMIRE 1958) and in a wide variety of mammals (BILLINGHAM and SILVERS 1963; MEDAWAR 1959; SNELL 1957). Very few exceptions to this immune response have been reported. A slow chronic homograft reaction has been observed in several species of urodeles (SQUADRONI and WOLSKY 1962; PIZARELLO and WOLSKY 1960; TEMME 1957). AMBROSIUS (1962) found that in urodeles heterografts of ovary and testis are often permanently accepted. PAPERMASTER, CONDIE and GOOD (1962) have briefly described the prolonged survival of skin homografts in the California hagfish, *Eptatretus stoutii*.

Nothing is known about the origin and the evolutionary significance of the immune response, which appears to be absent from invertebrates. The homograft reaction has so far not been investigated in other chordate groups, but the observations of OKA and WATANABE (1957) that in Ascidians the ability of colonies to fuse with each other is under genetic control, may have some bearing on this problem.

In most vertebrates, homografts exchanged among members of random bred strains or among members of large, freely interbreeding populations, disintegrate within a short time, often within a period of one or two weeks. This reaction has been attributed to tissue antigens, the presence of which is under genetic control (SNELL 1957). Genetic laws of transplantation have been formulated, based largely upon experiments with inbred stocks of mice. They state that grafts survive only if all or almost all of the donor's histocompatibility genes are also present in the host.

The failure of any homografts exchanged among members of large populations to survive has led to the tacit, perhaps unwarranted assumption that many genes are involved in the homograft immunity of most vertebrates. Since each gene may exist in several allelic states, the chance that two individuals selected at

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random will possess compatible genotypes is very small indeed (NEWTH 1961). Although the homograft reaction has been demonstrated in many species, the number of genes involved in this immune response has been determined, with any degree of accuracy, for only two related species, the mouse and the rat.

Since the genetic laws of tissue transplantation have been exclusively derived from experiments with rodents, it was important from an evolutionary and comparative point of view to determine whether the same laws are also applicable to other vertebrate classes. In 1958 KALLMAN and GORDON showed for the first time that the same laws are operative in fishes.

The experiments described in this paper are concerned with an estimate of the number of histocompatibility loci in the teleost, *Xiphophorus maculatus*.

MATERIALS AND METHODS

The method used in these experiments is the same as that successfully used by other authors. It involves crossing an inbred strain, sufficiently homozygous to permit the successful exchange of tissue grafts among its members, with an individual of a second unrelated strain that may or may not be inbred. Some F_1 hybrids are then bred with each other while others are backcrossed to the second strain. In both F_2 and BC generations, a certain percentage of the offspring, depending upon the number of histocompatibility genes involved, will possess one set of all the histocompatibility genes of the homozygous inbred strain. Only in these hosts will grafts from donors of the inbred line survive. From the percentage of accepted transplants, the minimum number of segregating histocompatibility loci can be estimated. If n is the number of histocompatibility loci, the proportion of F_2 hosts that will accept grafts from the inbred strain is $(\frac{3}{4})^n$ and of BC hosts $(\frac{1}{2})^n$.

Five strains of the southern platyfish, *Xiphophorus maculatus* and one strain of the Monterrey platyfish, *X. couchianus*, were used in these experiments. Strains 30 and 163, the Jamapa strains (Jp) of the Genetics Laboratory, are the descendants of a small number of *X. maculatus* collected in 1939 in the Rio Jamapa, Mexico (GORDON 1947). Strain 163 has now been inbred by brother-sister matings for more than 20 generations, and strain 30 for more than 30. Both have become sufficiently homozygous to accept permanently intrastrain fin grafts (KALLMAN 1960; KALLMAN and GORDON 1958). MILLER (1962), however, has recently found that under certain conditions, some tissue incompatibility manifests itself in Strain-30 male to female grafts. The Cp strain arose from a few *X. maculatus* collected in the Rio Coatzacoalcos, Mexico in 1948. The Np strain originated from a single female collected in 1954 in the New River, British Honduras. The Ap strain was obtained through the courtesy of MR. ALBERT GREENBERG, Everglades Aquatic Nurseries Inc., Tampa, Florida, who collected the progenitors of this stock in 1958 near Alvarado, Mexico. The strain of *X. couchianus* was derived from a few fish caught in 1958 near Monterrey, Mexico. In our crosses, e.g. 30 × Ap, the female is always listed first.

Anal, dorsal and caudal fins were used as test grafts. The transplantation of fins, which are composed of epidermis, dermis, blood vessels, nerves and bone, is wholly comparable to full-thickness skin grafting in mammals. The method of fin grafting has been described by KALLMAN and GORDON (1958). It involves the insertion of the suspensorium of the fin transplant into a slitlike pocket cut into the musculature of the caudal peduncle of the host. Muscular contraction around the pocket holds the transplant in place until the graft has healed. As in previous studies, the fin graft was considered to be alive as long as any of its pigment cells were visible. As long as some erythrophores, guanophores or melanophores can be seen in a graft, the surviving portion of the fin can be retransplanted successfully to a second host belonging to the same strain as the original donor (KALLMAN 1960). In grafts which survive for several months homograft rejection is a slow, chronic process lasting several weeks. The first macroscopic sign of incompatibility is a cessation of growth, followed by hemorrhages, disintegration of pigment

cells, opaqueness of the membraneous tissue between fin rays, breakage of fin rays and gradual sloughing of the external portion of the fin. Eventually only a scar remains.

All fin grafts which survived for 11 months or longer were considered "permanent survivals." Although sometimes distorted, such grafts are otherwise identical to fins *in situ*. The pigment pattern that develops in the grafts is always specific for the type of fin and the genotype of the donor. No effect of the genotype of the host upon the pigment pattern of the graft was observed, although a few host pigment cells may invade the proximal portion of the graft, and, conversely, some pigment cells of the transplant may be found in host tissue adjacent to the graft site. All transplanted anal fins regardless of the sex of the donor, differentiate into a gonopodium-like structure in male hosts upon sexual maturity, but in female hosts no such transformation occurs. In all fins scored as "permanent survivals" no sign of graft rejection was observed when the fish were finally sacrificed. The fin grafts were checked twice weekly during the first three weeks after the operation, once weekly during the fourth to eighth week, and thereafter at monthly intervals.

All hosts and donors were between one and three weeks old at the time of transplantation. Two-day old platyfish are fully competent to respond immunologically against homotransplants (KALLMAN and GORDON 1957). No attention was paid to the sex of either host or donor. Temperatures during the experiments averaged 25° to 28°C.

RESULTS

KALLMAN (1960) showed that Strain 30 and Strain 163 were sufficiently inbred to accept permanently intrastrain grafts. From the proportion of accepted transplants in the F_2 and backcross generations it was estimated that the two strains differ from each other at three or four histocompatibility loci. The experiments described below are a direct continuation of these studies.

Jamapa × *Alvarado strains:* 225 F_2 offspring of $(30 \times Ap)^2$ and 216 F_2 offspring of $(163 \times Ap)^2$ were given fin transplants from Strain 30 or 163 donors, respectively. Each F_2 generation represents the combined offspring of four F_1 matings, set up to insure an adequate number of F_2 hosts. In each case, the percentage of accepted grafts of each sibship was quite similar and, therefore, the data have been combined. Of the Strain-30 grafts, 31 (14 percent) survived permanently (Table 1). These results are in best agreement with the theoretical expectation of 13.3 percent takes for seven loci, but they do not differ significantly from the theoretical expectations of 17.8 percent for six or 10 percent for eight loci.

In the corresponding backcross generation $(30 \times Ap) \times Ap$ (produced by two F_1 females, but fertilized by the same male), nine fins (3.8 percent) survived in autograft-like fashion indicating that four or five histocompatibility loci were segregating. The discrepancy between the number of histocompatibility loci identified in the F_2 and BC generation may be due to the fact that the Alvarado strain was not inbred and therefore some F_1 and Ap fish may have differed from Strain 30 at one or two loci less than others. One of the backcross hosts that had accepted its graft permanently, was backcrossed a second time to the Alvarado strain. One out of 46 hosts (2.1 percent) accepted its graft permanently.

Of the strain 163 grafts in $(163 \times Ap)^2$ hosts, 51 (24 percent) survived permanently (Table 1). This result is in perfect agreement with the theoretical

TABLE 1

Survival times of Strain 30 and Strain 163 grafts in F_2 and backcross hosts (Jamapa × Ap strains)

Days after grafting	Hosts: F_2 $(30\times Ap)^2$ Grafts surviving		Backcross $(30\times Ap)\times Ap$ Grafts surviving		F_2 $(163\times Ap)^2$ Grafts surviving		Backcross $(163\times Ap)\times Ap$ Grafts surviving	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
3	225	100	237	100	216	100	185	100
10	200	89	113	45	210	97	138	75
14	138	61	63	27	185	86	79	43
17	98	44	45	19	164	76	44	24
21	64	28	32	14	148	69	32	17
28	54	24	23	10	134	62	25	14
45	43	19	16	7	111	51	14	8
60	38	17	14	6	96	44	12	6
90	37	16	13	5	73	34	11	6
120	36	16	13	5	64†	30	8	4
150	34	15	11	5	59	27	6	3
180	33	15	10	4	57	26	6	3
210	33	15	9	4	54†	25	5	3
240	32	14	9	4	51†	24	5	3
270	31*	14	9	4	51	24	5	3
330 plus	31	14	9	4	51	24	5	3

Theoretical expectation of takes for 4 to 8 loci

Number of loci	Number	Percent	χ^2	Number	Percent	χ^2	Number	Percent	χ^2	Number	Percent	χ^2
4	..	...	....	15	6.2	2.24	68	31.6	6.15	10	6.2	3.85
5	53	23.7	14.86	7	3.1	.43	51	23.7	.001	6	3.1	1.8
6	40	17.8	2.4	4	1.5	8.5	38	17.8	5.3	3	1.5	1.96
7	30	13.3	.04	..	..	..	..	...	..	1.5	.8	7.43
8	23	10	3.58	..	..	..	..	...	..	..	..	...

$\chi^2 = 3.84$, $P = .05$

* One host died with graft intact.
† Two hosts died with grafts intact.

expectation of 23.7 percent takes for five loci. Results with the corresponding backcross generation also indicate that Strain 163 differs from the Alvarado strain at about five loci. The acceptance of 2.7 percent of the grafts in the backcross generation fits best the theoretical expectation of 3.1 percent for five loci, but it also does not differ significantly from the theoretical expectation of 1.5 percent for six loci.

The range of the survival times of the unsuccessful grafts was quite similar, ranging from the 7th to 240th day. However, the Strain 163 grafts survived on the average longer than the Strain 30 transplants. On the 28th day, 12.8 percent of the unsuccessful Strain 30 grafts were still alive, while only 50 percent of the 163 grafts had been rejected. The corresponding figures for the 45th day were 6.2 percent and 36.6 percent alive.

Jamapa × New River strains: 508 F_2 offspring of $(Np \times 30)^2$ and 318 F_2 offspring of $(Np \times 163)^2$ were grafted with Strain 30 or Strain 163 fins, respectively. The $(Np \times 30)^2$ hosts represent the offspring of five different F_1 matings which were set up to insure an adequate number of F_2 hosts. The percentage of takes in these sibships varied from 2 to 7 percent. Since each sibship consisted of 150 or fewer fish, these differences are not statistically significant and, therefore, all data are presented together (Table 2). Only 24 out of 508 hosts accepted their

TABLE 2

Survival times of Strain 30 and Strain 163 grafts in F_2 and backcross hosts (Jamapa × Np strains)

Days after grafting	Hosts: F_2 $(Np \times 30)^2$ Grafts surviving		Backcross $Np \times (Np \times 30)$ Grafts surviving		F_2 $(Np \times 163)^2$ Grafts surviving		Backcross $Np \times (Np \times 163)$ Grafts surviving	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
3	508	100	200	100	318	100	172	100
10	472	93	152	76	282	89	139	81
14	395	78	117	59	252	79	101	59
17	310	61	88	44	196	62	76	44
21	230	45	45	23	165	52	55	32
28	155	31	26	13	136	43	23	13
45	74	15	4	2	117	37	8	5
60	54	11	3	2	105	33	6	3
90	41	8	3	2	96	30	4	2
120	38*	7	2	1	84†	26	3	2
150	35	7	1	1	71	22	3	2
180	32	6	1	1	58†	18	2	1
210	29	6	0	0	46*	14	1	1
240	26	5	.	.	43	14	1	1
270	25	5	.	.	41	13	1	1
300	24	5	.	.	36†	11	0	0
360	24	5	.	.	36	11	.	.

Theoretical expectation of takes for 6 to 12 loci

Number of loci	Number	Percent	χ^2	Number of loci	Number	Percent	χ^2
9	38	7.5	5.63	6	57	17.8	9.0
10	28	5.6	.57	7	42	13.3	1.04
11	21	4.2	.47	8	32	10	.67
12	16	3.2	5.15	9	24	7.5	6.4

$\chi^2 = 3.84$, $P = .05$

* One host died with graft intact.
† Two hosts died with grafts intact.

grafts permanently (4.7 percent). The result fits best the theoretical expectation of 4.2 percent for 11 histocompatibility loci, but the result also does not differ significantly from the theoretical expectation of 5.6 percent takes for ten loci.

The 318 hosts of $(Np \times 163)^2$ comprise the combined offspring of three F_1

matings. Again the percentage of accepted grafts in each sibship was of the same order of magnitude and they have therefore been combined (Table 2). The acceptance of 36 grafts (11 percent) is in best agreement with the theoretical expectation of 10 percent for eight loci, but again the difference from the theoretical expectation of 13.3 percent for seven loci is not significant. Seven hosts died between the 120th and 300th day after the operation with their transplants still in autograft-like condition. In many of the grafts that survived longer than five months the homograft reaction is a long, drawnout process that can be recognized many weeks or months before the graft is completely destroyed. Since no such chronic reaction was visible in these seven hosts, it is felt that they would have survived permanently. In this case the actual result would have been in best agreement with the expected 13.3 percent for seven histocompatibility loci.

As expected, not a single graft survived in the backcross hosts. When eight or ten histocompatibility genes are segregating, only 0.8 or 0.1 percent of the grafts are expected to take.

Jamapa × *Coatzacoalcos strains:* Strain 30 fins were transplanted into 90 (30 × Cp)^2 hosts. No transplant survived for more than 210 days (Table 3). These results indicate that at least nine, but perhaps many more, histocompatibility loci are involved in determining the fate of fin grafts between these two strains. Similarly no fin graft survived for more than two months among the 166 backcross hosts.

Jamapa × X. couchianus *strains:* A total of 124 F_2 hybrids of strain 30 × *X. couchianus* received fin transplants from Strain 30 donors and 125 F_2 hybrids of Strain 163 × *X. couchianus* received grafts from Strain 163 donors. Both F_2

TABLE 3

Survival times of Strain 30 grafts in F_2 and backcross hosts (Strain 30 × Coatzacoalcos)

Days after grafting	Hosts			
	F_2 (30 × Cp)^2 Grafts surviving		Backcross (30 × Cp) × Cp Grafts surviving	
	Number	Percent	Number	Percent
4	90	100	166	100
7	88	98	155	93
10	72	80	120	72
14	53	59	82	49
17	36	40	48	29
21	23	26	40	24
28	18	20	23	14
35	9	10	14	9
45	7	8	4	2
60	4	4	3	2
90	1	1	0	0
.				
.				
210	1	1		
240	0	0		

generations comprise the combined offspring of two different matings. In both F_2 generations, the same percentage of grafts survived (3 percent). These results are in best agreement with the theoretical expectation of 3.2 percent takes when 12 histocompatibility loci are segregating (Table 4). However, the difference between the experimental result and the theoretical expectations of 5.6 percent takes for ten loci or 1.3 percent takes for 15 loci are not statistically significant. This illustrates one of the serious shortcomings of the method, which loses much of its precision when alleles at a large number of loci are segregating. In such cases, only transplants into a large number of F_2 hosts will provide unequivocal results. As expected, none of the grafts in the corresponding backcross generation survived. Only one out of 232 grafts in backcross hosts survived for more than 75 days. The theoretical expectation of takes in the backcross generation when 12 histocompatibility loci are involved is only 0.01 percent.

TABLE 4

Survival times of Strain 30 and Strain 163 grafts in F_2 and backcross hosts
(Jamapa strains × *X. couchianus*)

Days after grafting	Hosts: F_2 $(30\times Xc)^2$ Grafts surviving		Backcross $30\times(30\times Xc)$ Grafts surviving		F_2 $(163\times Xc)^2$ Grafts surviving		Backcross $(163\times Xc)\times Xc$ Grafts surviving	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
3	124	100	66	100	125	100	166	100
7	123	99	61	92	116	93	134	81
10	111	90	53	80	86	69	106	63
14	91	73	43	65	60	48	63	38
17	58	47	21	32	40	32	41	25
21	39	31	11	17	34	27	18	11
28	23	19	4	6	22	18	12	7
45	11	9	1	2	12	10	4	2
60	9	7	1	2	10	8	3	2
90	8	6	0	0	9	7	1	1
120	7	6	.	.	8	6	0	0
150	7	6	.	.	7	6	.	.
180	6	5	.	.	6	5	.	.
210	5	4	.	.	5	4	.	.
240	5	4	.	.	4	3	.	.
270	4	3	.	.	4	3	.	.
330	4	3	.	.	4	3	.	.

Theoretical expectation of takes for 9, 10 and 15 loci

Number of loci	Number	Percent	χ^2	Number	Percent	χ^2
9	9	7.5	3.26	9	7.5	3.33
10	7	5.6	1.31	7	5.6	1.37
15	2	1.3	3.6	2	1.3	3.6

$\chi^2 = 3.84$, $P = .05$

DISCUSSION

The products of at least 12 histocompatibility genes appear to be involved in the rejection of tissue grafts from *Xiphophorus maculatus* donors into *X. couchianus* hosts. This number, however, can only be considered a minimum estimate, since the method employed does not reveal loci when both strains are homozygous for the same allele, and the method also fails to differentiate between loci linked on the same chromosome. The first potential error can be minimized by using strains of independent origin, since they will probably share fewer alleles in common than those of a common genetic background. By the use of strains belonging to different species, maximum genetic diversity is introduced and an estimate based upon interspecific crosses may be quite close to the actual number of histocompatibility genes of a species.

A comparison of the different F_2 generations (Table 5) shows that the smallest percentage of takes was obtained in the interspecific crosses. In only a single intraspecific cross (strain 30 × Cp) was a similar genetic diversity, possibly even surpassing that of the interspecific cross, detected. In all other F_2 generations, the percentage of accepted grafts was higher, indicating that fewer histocompatibility genes are involved in the rejection of transplants between strains of the same species than between fish belonging to different species. As expected, the smallest number of histocompatibility gene differences was observed between

TABLE 5

Estimated minimum number of histocompatibility loci involved in graft rejection between different strains of platyfish, mice and rats

	Donor strain	Host strain	Number of F_2 hosts	Percent graft survival	Number of BC hosts	Percent graft survival	Number of loci
Platyfish							
	Jp 30	Jp 163	617	42.6	139	9.4	3*
	Jp 163	Jp 30	617	44.2	72	9.8	3*
	Jp 30	Ap	225	14	237	3.8	6–8
	Jp 163	Ap	216	24	185	2.7	5
	Jp 30	Np	508	4.7	200	0	10–11
	Jp 163	Np	318	11	172	0	7–8
	Jp 30	Cp	90	0	166	0	⩾10
	Jp 30	Xc	124	3	66	0	10–15
	Jp 163	Xc	125	3	166	0	10–15
Rat							
	Lewis	B.N.	318	1.2	. . .	.	16†
	B.N.	Lewis	329	1.8	. . .	.	14†
Mouse							
	A	CBA	118	1.7	. . .	.	14–17‡
	CBA	A	153	0.7	. . .	.	14–17‡
	BALB/cAn	DBA/2	122	2.4	99	0	13§

* KALLMAN 1960.
† BILLINGHAM, HODGE and SILVERS 1962.
‡ BARNES and KROHN 1957.
§ PREHN and MAIN 1958.

Strain 30 and Strain 163 which are related to each other. They differ only at three or four loci (KALLMAN 1960).

It is interesting that the difference of three histocompatibility loci between Strains 30 and 163 was also reflected in the crosses involving the Ap and Np strains. Strain 30 differs from the Ap and Np strains at three more loci than does Strain 163. However, in the interspecific cross no such difference was observed; the same percentage of grafts from Strain 30 and Strain 163 donors were accepted by the F_2 hosts (Tables 4 and 5). This strongly suggests that Strain 30 and Strain 163 are homozygous for different alleles at these three loci, but that the alleles at the homologous loci of *X. couchianus* are different from those of both Jamapa strains.

In general, the results obtained from the F_2 generation are in good agreement with those obtained from the backcross hosts. In the 30 × 163 crosses the F_2 data indicated that the strains differ at three loci, the backcross data indicated that three or four loci may be involved. Similarly, results from the F_2 and backcross generation of Strain 163 × Ap are in excellent agreement with each other. The only discrepancy between F_2 and backcross data was discovered in Strain 30 × Ap crosses. The F_2 results indicated that the strains differ at six to eight histocompatibility loci while the backcross data showed only a difference of four or five loci. In all other cases where the F_2 data suggested a difference at seven or more loci, no grafts were accepted by the backcross hosts.

There is an additional reason why an estimate of the number of histocompatibility genes as determined by this method can only be considered a minimum. The method is based upon the assumption that the presence of a single histocompatibility allele in the cells of the graft and its absence from the cells of the host always leads to a homograft reaction. Histocompatibility genes, however, can be classified into strong and weak loci according to their effects. When a donor possesses a different allele at a strong histocompatibility locus, the graft will be rejected rapidly, even when host and donor are identical with respect to the remainder of the genome (SNELL 1957). But when host and donor differ at a weak histocompatibility locus, such as the *H-1* and *H-3* loci in mice, the homograft reaction may not manifest itself for several weeks or months after the operation (COUNCE, SMITH, BARTH and SNELL 1956; SNELL and STEVENS 1961). Weak histocompatibility genes apparently occur in most species. In the hamster (HILDEMANN and WALFORD 1960), guinea pig (BAUER 1960), rat (BILLINGHAM, HODGE and SILVERS 1962), mouse (COUNCE *et al.* 1956) and platyfish (as shown in the experiments reported here), a certain number of grafts from donors of the inbred strain to hosts of the F_2 and BC generations survive for four months or longer. Among the hosts of both generations there are, of course, a number of individuals that differ from the parental inbred strain by only a single, or very few histocompatibility genes. I believe these are the hosts in which the grafts survive for long periods of time, since in interstrain grafts, when both strains differ at many loci, or at a single strong histocompatibility locus, no such spread in survival times has been reported. There exists, therefore, the possibility that in some species even "weaker" histocompatibility genes exist which by themselves

are not sufficient to elicit a homograft reaction. In such cases only the additive effect of a combination of weak histocompatibility loci would bring about a homograft rejection.

If genes with different capacities to bring about the homograft reaction exist, the difference between histocompatibility genes and all other genes becomes blurred and artificial. The fate of a graft may then be decided by the entire genome with some genes—those at strong histocompatibility loci—responsible for a disproportionate amount of the total antigenicity. A somewhat similar view has been expressed by PREHN and MAIN (1958).

Evidence is already accumulating for the existence of weak histocompatibility loci which by themselves do not cause homograft rejection. In *X. maculatus*, KALLMAN (1960) demonstrated that heterozygous grafts not only survive much longer than grafts from homozygous donors (which possess twice as many foreign histocompatibility genes), but that in some instances heterozygous grafts survive permanently while homozygous grafts into the same donor are rejected. In mice, additive effects of weak histocompatibility genes have been demonstrated by MCKHANN (1964).

A number of experiments have been reported that deal with an estimate of the number of histocompatibility loci in other species. In 1916 LITTLE and TYZZER showed that at least 12 or 14 histocompatibility loci were involved in determining the fate of tumor grafts transplanted between two unrelated strains of mice. Forty years later, similar minimum estimates were made by PREHN and MAIN (1958) and by BARNES and KROHN (1957). More recently, two unrelated inbred strains of rats have been found to differ in at least 16 histocompatibility loci (BILLINGHAM *et al.* 1962). These estimates which are based upon intraspecific crosses with unrelated strains, are slightly higher than those obtained for *X. maculatus*, based upon interspecific crosses (Table 5). In the rodents still more histocompatibility loci may be uncovered by means of interspecific crosses. Whether the slightly lower minimum of histocompatibility loci in fish has any phylogenetic significance cannot be decided at the present time.

In the guinea pig, the existence of four to six histocompatibility loci has been reported (BAUER 1960), but the strains had a common origin more than 50 years ago (BAUER 1958) and the two strains presumably exhibit only a small fraction of the total genetic variability of the species. In the Syrian hamster, *Mesocricetus auratus*, transplantation experiments involving three inbred stocks have revealed only three histocompatibility loci (BILLINGHAM, SAWCHUCK and SILVERS 1960). The origin of all three strains from a single litter, however, raises some doubts as to whether the paucity of demonstrable histocompatibility genes is not more apparent than real. The situation in the hamster bears a striking resemblance to the genetics of tissue transplantation in small, isolated populations of *X. couchianus*. In these populations, transplants exchanged among sibs of wild-caught gravid females often survive in a high percentage of cases, indicating that there exists little genetic variation (KALLMAN 1964). In the goldfish, *Carassius auratus*, HILDEMANN and OWEN (1956) showed that a minimum of four histocompatibility loci was involved in determining the fate of scale transplants among the

offspring of unrelated parents, but their experimental design did not permit them to set an upper limit. Similarly, MEDAWAR (1945) concluded on the basis of complete cross-grafting with 22 rabbits that at least seven different histocompatibility loci were involved in the homograft reaction. Again the experimental design did not permit the determination of an upper limit. LONGMIRE, STONE, DANIEL and GOON (1947) have placed the number of histocompatibility loci in the human being as high as 23.

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SUMMARY

The minimum number of histocompatibility loci involved in controlling the fate of fin transplants exchanged between members of different strains of the platyfish, *Xiphophorus maculatus*, and between *X. maculatus* and *X. couchianus*, was determined. This number can be estimated from the proportion of accepted P_1 grafts in F_2 and backcross generation hosts, obtained by crossing a rigidly inbred P_1 strain with a second unrelated strain. The inbred stocks of *X. maculatus*, Strains 30 and 163, have been inbred for more than 20 and 30 generations, and fin transplants exchanged within each group survive permanently.

In the F_2 generation of Strain 30 × Ap, 31 out of 225 hosts (14 percent) accepted their transplants, and in the backcross generation, 9 out of 237 hosts (3.8 percent). In the corresponding generations involving Strain 163, the grafts survived permanently in 51 out of 216 F_2 hosts (24 percent) and in five out of 185 BC hosts (2.7 percent). Based upon the F_2 results, Strain 30 appears to differ from the Ap strain at approximately seven, and Strain 163 at five histocompatibility loci.

Strain 30 differs from the Np strain at at least 10 or 11 histocompatibility loci. Only 24 out of 508 F_2 hosts (4.7 percent) accepted their grafts. In the F_2 hosts of Strain 163 × Np, 36 out of 316 grafts (11 percent) survived, indicating that 7 or 8 histocompatibility genes were involved. Not a single transplant survived in 318 backcross hosts.

Only 90 F_2 hosts were available from the cross of strain 30 × Cp. Not a single graft survived for more than 210 days. Similarly, all grafts in 166 backcross hosts were rejected. These results indicate that at least 9 or 10, but possible many more, histocompatibility genes were segregating.

In the F_2 generation obtained by mating Strain 30 and 163 with *X. couchianus* only 3.2 percent of the hosts accepted their grafts. These results are in best agreement with the theoretical expectation of 3.2 percent for 12 loci.

The results of these experiments are compared with similar experiments performed with different species of rodents.

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