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Klaus D. Kallman

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DEPARTMENT OF ZOOLOGY AND ENTOMOLOGY, MONTANA STATE COLLEGE, BOZEMAN, MONTANA.

Genetics of Tissue Transplantation in Isolated Platyfish Populations¹ KLAUS D. KALLMAN

Small isolated populations are generally considered to have become homozygous at many gene loci. However, in nearly all cases it is quite difficult to demonstrate experimentally such a decrease in the genetic variability, since suitable genetic markers are usually absent from most species. An attempt has been made to determine by means of tissue transplantation whether small populations are "naturally" inbred. Since the genetic requirements for graft survival are very stringent and since many genes are involved in determining the fate of a graft, the survival of a transplant can be taken as an excellent indication that host and donor possess similar, if not identical, genotypes. Fin transplants were exchanged among the offspring (intrasib grafts) of 15 wild-caught platyfish, Xiphophorus couchianus, collected in 8 different springs or pools. A high percentage (25-69) of the grafts were accepted permanently among sibs whose parents had been collected in springs where the adult population numbered only a few dozen or less. The percentage of successful intrasib grafts declined sharply when the parents came from large populations.

I SOLATED populations are of interest to the biologist, for they provide raw material from which new races, subspecies, or species arise. In small, completely isolated populations, genes spread rapidly and become fixed. Their spread is a random and often nonadaptive process, and different alleles may become established in otherwise similar populations of the same species.

The genetic variability of populations has

¹ This investigation was carried out during the tenure of a postdoctoral fellowship, HF-9500, of the National Heart Institute, and was aided by a research grant, C-4945, of the National Cancer Institute to Dr. Sylvia Greenberg. been studied by determining the frequencies of polymorphic pigment patterns, blood group antigens or chromosomal rearrangements, and differences in meristic and morphometric characters. Some of these traits are modified by the environment and are not good indicators of genetic differences, while others are controlled by a single or by relatively few genes and therefore do not provide enough information to estimate the size of the gene pool. An experimental analysis of the genetic variability of a population ought to involve a character largely unaffected by environmental factors and

produced by the interaction of a relatively large number of genes scattered randomly over the chromosomes and which exist in 2 or more allelic states. And each possible genetic combination has to result in a definite phenotype that can be identified by appropriate methods. With respect to such a hypothetical character, a large population presumably will be more variable than a small one.

A character that meets these requirements is found in the homograft reaction, the now well-recognized ability of postembryonic vertebrates to react antigenically against tissue transplants from donors of the same species which possess different genotypes. The homograft reaction is due to an antibody reaction by the host against tissue antigens present in the graft. The presence of these antigens is under genetic control (Billingham 1959, Medawar 1958, Snell 1957).

The number of histocompatibility loci controlling the presence of tissue antigens has been shown to be at least 16 for the rat (Billingham et al. 1962), 14-17 for the mouse (Barnes and Krohn 1957, Prehn and Main 1958), and more than 12 for the teleost, Xiphophorus maculatus (unpublished). No valid estimate has been presented for any other vertebrate, but the universal rejection of all homotransplants in vertebrates within 1 or 2 weeks strongly suggests that a large number of loci may be involved in most species. If 16 histocompatibility genes are present, each represented by 2 alleles, over 43 million different genetic combinations are possible. Since tissue transplants succeed only when all or almost all of the histocompatibility alleles of the donor are also present in the host, the possibility that 2 individuals selected at random from a large interbreeding population will possess genotypes that permit one of them to accept the other's graft is very small indeed. This has been termed the uniqueness of the individual by Medawar (1957).

In fishes, tissue specificity is as highly developed as in mammals. In fishes, not a single instance is known in which a tissue homotransplant survived for more than 3 or 4 weeks when host and donor were either wild caught, members of a closed colony maintained by mass matings, or obtained from commercial breeders (Goodrich and Nichols 1933; Goss 1961; Hildemann 1957; Hildemann and Haas 1960; Hildemann and

Owen 1956; Kallman 1960, 1962; Nardi 1935; Sauter 1934; Triplett and Barrymore 1960). This contrasts strikingly with the permanent survival of tissue transplants in fishes belonging to highly inbred strains (Kallman 1960, 1962; Kallman and Harrington 1964).

The homograft reaction can readily be adapted to the study of the genetic variability of isolated populations. If such a population has become rather uniform genetically, many of its members will possess a similar spectrum of histocompatibility alleles, and consequently the antigenic difference between them will be small. The more uniform the population, the longer will tissue transplants exchanged among its members survive, and the higher will be the percentage of accepted grafts. This method is most sensitive when transplants are made among the offspring of wild-caught individuals. This approach has been used to study 2 small populations of Xiphophorus (Cyprinodontiformes: Poecilicouchianus idae), the Monterrey platyfish, X. c. couchianus, restricted to the springs in the Huasteca Canyon near Monterrey, Mexico, and the northern platyfish, X. c. gordoni,2 found only in a few lagunas near Cuatro Ciénegas, Coahuila, Mexico.

MATERIALS AND METHODS

The Huasteca Canyon is about 1 mile long and 0.25 mile wide. Its floor is strewn with boulders and stones of all sizes and there is little vegetation. Until 10 or 15 years ago, the springs in this canyon drained into a small, central stream bed, the Rio Santa Catarina, but since then they have been diverted into man-made aqueducts and are now separated from each other. There are now 3 separate water systems, each of which is fed by 1 or more springs. On 3 occasions, these springs were thoroughly seined to determine the distribution and abundance of platyfish. Since the same seining technique was used throughout, the number of fish collected represents an index of the relative abundance of this species, but in some instances, the number of fish caught or observed may closely approach the total number of adult platyfish, because so few were present. X. couchianus keeps close to the banks and favors quiet waters with muddy bottoms, densely shaded by

² This platyfish has been described as a new species by Miller and Minckley (1963), but is given only subspecific rank by Rosen and Bailey (1963).

Taratian	Number of	Adult Platyfi	sh Observed		mber of Females Used				
Location	April 1961	June 1961	April 1962	f	for Analysis and Code				
Spring system I									
A-C	0	2	25	1	I-A				
В-С	0	0	0						
C-E	0	0	0						
D	4 pc	ol had evap	orated	2	I-D-1, I-D-2				
below E	not visite	d >200	0	1	I-E				
C-F	0	0	0						
M	0	0	0						
N	0	0	0						
Spring system II									
G-H	6	2	20	2	II-1, II-2				
below H	0	0	0						
Spring system III									
I–J	~30	~30	~30						
J	>150	not visited	>200	2	III-J-1, III-J-2				
J–L	0	0	0		•				
K	2	4	8	2	III-K-1, III-K-2				

TABLE 1. DISTRIBUTION OF Xiphophorus couchianus IN THE HUASTECA CANYON.

aquatic vegetation, as do the other species of platyfishes.

In the left branch of spring system I, platyfish were found in close proximity to the banks of a single deep spring pool (Table 1, Figs. 1A, 2), in a shallow pool of water near a dam where water had spilled over the banks (D), and in the ditch that runs for 2 miles through the village of Santa Catarina (below E). It should be noted that the dam (D) prevents platyfish from returning to the upper portions of this spring system. In spite of several hours of seining, no platyfish were ever found in the other branch (Fig. 1B-C) where the bottom is rocky and the current swift, in the manmade aqueduct (C-E) or in the shallow stagnant pools of the old stream bed (C-F). Similarly, no platyfish were found in 2 water holes and a spring 0.5-1 mile beyond the headwaters of spring system I (Fig. 1M, N).

In spring system II (Fig. 1G-H) which is only 1-2 ft deep and 3-4 ft wide, platy-fish were found in small numbers above the dam (H). Below the dam, 200 yards from its source, the water is channeled into a man-made ditch, an unsuitable habitat for platyfish.

The largest and possibly the most stable population of the Monterrey platyfish was encountered in spring system III which enters a rock pool 10 ft wide, 35 ft long, and

in some places 5 ft deep. This pool (Fig. 1J) is located on the north side of a cliff, and is shaded for most of the day. The platyfish were abundant in vegetation along the bank opposite the canyon wall and the adult population was estimated to consist of at least 200 fish. From the pool a shallow outlet (J-L), its sides and bottom reinforced by cement, leads to an underground aqueduct of the municipal water supply. Eighty feet from the pool, between the outlet ditch and the canyon wall, a small trickle of water is found in a depression in the ground (K). Except for a single hole, 1-2 ft deep underneath a boulder, the water is no more than 2 or 3 in. deep. A few X. couchianus were found here on each visit (Table 1).

The preceding description of the habitat of X. couchianus in the canyon holds only for the dry season. The rainy season near Monterrey is concentrated primarily during the late summer months and early fall when sudden heavy downpours and the steep denuded mountain slopes cause a rapid runoff, and silt-laden torrents race along the stream beds and across the canyon floor. Many fish undoubtedly are swept downstream and out of the canyon. The size of the population must be drastically reduced, to be eventually replenished by the surviving fish.

The other subspecies of X. couchianus, the northern platyfish, was collected in the small

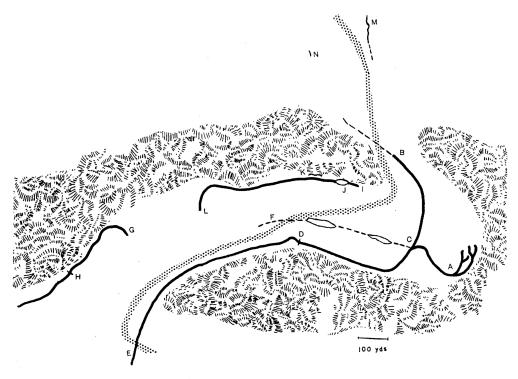


Fig. 1. The Huasteca Canyon; A, B, C, D, E, F—spring system I; G, H—spring system II; I, J, K, L—spring system III; D, H—dams; solid lines are watercourses; broken lines are intermittent streams; stippled area is dirt road.

Laguna Santa Tecla, which is about 100 ft across and 250 ft long and not more than 6 ft deep. In April 1961, a party headed by Dr. Robert R. Miller found the fish in 2 micropopulations close to the banks, I near the outlet and the other near an inlet in the southwest corner of the lake (Miller and Minckley 1963). Only a handful of fish were collected along the shore away from these 2 spots and none was seen or collected in the lake away from the bank. No platyfish were discovered in the outlet of the lake, which was explored for a distance of 1 mile. From the population near the outlet, Dr. Miller collected 195 fish, while 3 months later I caught, after repeated seinings, 62 fish from the other. During the winter of 1963, W. L. Minckley found this fish much more abundant at Santa Tecla and, in addition to being present in the 2 pockets, they were also seen all along the shoreline. The platyfish occurs also abundantly in 2 adjacent lagunas, Los Fresnos and Quintero.

All fish from the same collecting station were shipped together in plastic bags to the Genetics Laboratory. Each female was

then kept separately in an aquarium to which a male from the same locality was added. Most of the females from the Huasteca Canyon were gravid when collected and their progeny may have been derived from 1 or more males. The 4 platyfish from the Laguna Santa Tecla which were used in the experiment were immature and virgin. From each donor, 4 fin grafts were taken: anal, dorsal, and caudal fin, the latter being split into a dorsal and ventral half. The fins were transplanted according to the method described by Kallman and Gordon (1958). All operations were performed when both donor and host were between 8 and 30 days of age. No attention was paid to the sex of either host or donor. During the first 2 weeks the transplants were checked every other day, during the third and fourth postoperative week twice weekly, during the second month once weekly, and thereafter at 2-week or monthly intervals. A fin graft was scored as rejected when all its pigment cells had disintegrated. Histological examination of such grafts has shown that the destruction of pigment cells is closely correlated with the disintegration of the skin, bone, blood vessels, and soft membranous tissue between the fin rays. Water temperature for the duration of the experiment ranged from 24 to 29 C.

SUCCESS OF TRANSPLANTS

The original experiment which indicated that the Monterrey platyfish might be somewhat inbred in nature was performed with a fish collected by Dr. Gordon in 1958. The precise locality within the canyon from which the fish was collected is unknown. Four of 22 transplants exchanged among the offspring of this wild-caught female were accepted permanently (Table 2). These fish, which are designated as the P₁ generation, were then inbred in the laboratory for 5 generations, no attempt being made to mate females and males similar in antigenic makeup. Four lines of the third and 2 lines of the fifth inbred generations were tested. The percentage of accepted intrasib transplants increased with progressive inbreeding (Table 2).

In 1961, intrasib transplants were performed among the offspring of 10 females collected in 6 different localities in the Huasteca Canyon (Tables 1 and 3). The percentage of takes was highest from those sibs whose parents had lived in areas where the population density of platyfish was lowest. Often several broods per female were obtained, but with the exception of female II-1 results from different broods of the same female were similar and, therefore, the data have been combined. The percentage of takes among the first brood of II-1 was much higher than that of the second. Although from the first brood only



Fig. 2. Location A in the Huasteca Canyon.

5 hosts with their grafts intact survived for longer than 270 days, the actual number of takes was probably much higher. Ten fish died between the 120th and 210th day, but when checked on the 120th, 150th, and 180th day, degenerative changes could be detected in only 1 of them. In the other 9 fish, the grafts were in excellent condition at the time of death, and it seems very likely that most of these grafts would have survived permanently. This would have

TABLE 2. NUMBER OF SURVIVING FIN TRANSPLANTS EXCHANGED AMONG SIBS OF WILD-CAUGHT X. couchianus (1958) from the Huasteca Canyon and among Their Descendants (3rd and 5th INBRED GENERATION) .

CIL	Days after Transplantation												Percentage of		
Sib -	10	14	28	42	60	90	120	150	180	210	240	270+	Surviving Grafts		
Offspring of wild					,										
caught female	22^{1}	19	9	6	6	5	5	5	5	4	4	4	18		
F ₃ -1	9	8	7	7	6	5	4	4	4	4	4	4	44		
F ₃ -2	17	16	13	11	10	8	8	8	7	7	42	4	24 (41)		
F ₃ -3	20	18	8	6	5	5	5	5	5	5	5	5	25		
F ₃ -4	28	28	23	18	14	14	11	11	11	9	8	8	27		
F ₅ -1	12	12	12	12	11	7	5	5	5	5	5	5	42		
F ₅ -2	22	22	21	20	19	14	14	14	14	14	14	14	64		

Number of surviving fin transplants.
 Three fish died with their grafts in excellent condition.

TABLE 3. NUMBERS OF SURVIVING FIN GRAFTS AND SURVIVAL TIMES (DAYS) OF INTRASIB FIN TRANS-PLANTS AMONG THE PROGENY OF WILD-CAUGHT MONTERREY AND NORTHERN PLATYFISH.

	Days after Transplantation													Percentage of		
Sib	5	7	10	14	21	28	42	65	90	120	150	180	210	240	270	Surviving Grafts
Monterre	y platy	fish														
I-A	26	26	26	26	25	25	24	22	22	19	18	18	18	181		69
I-D-1	24	24	23	22	18	15	10	9	8	8	8	8	8	8	8	33
I-D-2	45	45	42	36	32	21	17	16	16	16	16	16	15^2	15	15	33
I-E	28	27	24	21	16	14	10	8	7	6	6	6	6	5	5	18
II-l	18	18	18	18	18	18	18	18	17	15	14^{2}	93	5 4	5	5	28 (72)
(brood 1))															
II-1	22	22	21	19	14	10	8	7	7	7	6	6	5	5	5	23
(brood 2))															
11-2	27	27	27	25	19	15	14	14	14	12	12	11^{2}	8^5	7^2	7	26 (41)
III-J-1	21	20	18	15	11	9	6	3	1	1	0					0
III-J-2	32	31	29	26	20	17	13	11	10	8	6	5	4	4	4	13
III-K-1	18	18	17	16	13	13	10	8	8	8	8	8	7	7	7	39
III-K-2	27	27	27	25	19	16	15	14	11	11	11	10	10	10	10	35
Northern	platy	fish														
1	74	74	71	62	38	33	20	16	14	12	11	11	11	11	11	15
2	42	41	33	27	18	13	10	8	7^2	7	6	6	6	5	5	12
3	9	8	7	5	5	2	2	2	2	2	2	1	1	1	1	11
4	18	15	12	8	4	1	0									0

¹ Hosts sacrificed on 240th day.

raised the incidence of takes to 72%. The different results obtained with the 2 broods of female II-1 may be the result of the second brood having been sired by a different male (or males) than the first brood. In nearly all grafts which survived for several weeks or months, the homograft reaction was a very slow and chronic process which extended over several weeks. Very few transplants were rejected after the 200th day, and in all of these degenerative changes could be seen long before.

The percentages of successful transplants among the offspring of 4 wild-caught northern platyfish were generally much lower (Table 3).

To determine how closely the populations of platyfish in the three isolated Huasteca spring systems are related to each other, tissue grafts were made from donors of one spring into hosts of another (Table 4). As with the intrasib transplants, all fish operated on were offspring of wild-caught females with the exception of fish from location I-E which represent the first inbred generation. Few of these transplants sur-

vived permanently and they were rejected faster than intrasib transplants, but they survived much longer than interstrain grafts of X. maculatus (Kallman and Gordon 1958). Although in some host-donor combinations relatively few grafts were exchanged, these results nevertheless indicate that fish from spring systems II and III were more closely related to each other than to the population of spring system I (Table 4).

Both the northern and the Monterrey platyfish react vigorously to foreign tissue transplants. Fins exchanged between members of these 2 distinct platyfish populations were destroyed within 2 weeks (Table 5).

DISCUSSION

The high percentage of surviving fin transplants exchanged among the progeny of wild-caught X. couchianus from the Huasteca Canyon provides a striking demonstration that a high degree of genetic homogeneity has been achieved in these isolated populations. Among the progenies of X. couchianus tested, the highest percentages of takes were obtained among those

² One host died with graft intact.

³ One graft rejected; 4 other hosts died with their grafts intact.

⁴ Four hosts died with their grafts intact.

⁵ One graft rejected; 2 other hosts died with their grafts intact.

TABLE 4. NUMBERS AND PERCENTAGES OF SURVIVING FIN TRANSPLANTS, AND SURVIVAL TIMES OF FIN GRAFTS IN Xiphophorus couchianus, Exchanged between Hosts and Donors Belonging to Different Springs.

D .	Locations From Which Hosts and Donors Were Taken:													
Days After Grafting	Donor- I-E Host- I-A	II I-A	III-J I-A	III-K I-A	I-E II	III-K II	II III-J	III-K III-J	II III-K	III-J III-K				
3	14	10	12	12	44	13	16	30	11	44				
	100	100	100	100	100	100	100	100	100	100				
7	14	10	10	12	44	13	14	30	10	44				
	100	100	83	100	100	100	88	100	91	100				
10	11	9	9	9	37	13	13	26	8	43				
	79	90	75	75	84	100	81	87	73	98				
14	10	8	8	7	35	13	11	22	7	35				
	71	80	67	58	80	100	69	73	64	80				
17	5	5	6	4	31	12	9	17	4	30				
	36	50	50	33	71	92	56	57	36	68				
21	3	3	3	2	21	11	5	7	2	24				
	21	30	25	17	48	85	31	23	18	55				
24	3	3	2	0	15	9	5	5	1	21				
	21	30	17		34	69	31	17	10	48				
28	1	2	0		1	6	3	4	0	17				
	7	20			3	47	19	13		39				
35	0	2			0	4	1	3		11				
00	•	20				31	6	10		25				
42		1				4	1	3		10				
		10				31	6	10		23				
49		0				4	0	3		7				
10		•				31		10		16				
60						4		2		3				
00						31		7		7				
90						4		1		2				
						31		3		5				
150						4		1		2				
100						31		3		5				
180						4		1		1				
100						31		3		3				
240						4		1		1				
						31		3		3				

sibs whose parents had been collected in the headwaters of spring systems I and II, where the population density is lowest. Conversely, there were few takes among broods of females collected in the rock pool where the Monterrey platyfish were most numerous and among the offspring of females of the Laguna Santa Tecla population, the size of which was considerably larger than that of the Monterrey platyfish in the rock pool. The percentage of takes among the fish derived from location K was significantly higher than among those from the rock pool only a few feet away. It is unlikely that in the small trickle of water at K a permanent platyfish population can be-

come established for any length of time. Very probably during periods of flood, platyfish from the rock pool are swept into it and trapped there. However, the extremely limited numbers of fish that can live in so small a body of water leads to strong inbreeding, and as shown by our experiments (Table 2), even 3 generations of brother-sister matings can lead to a decided rise in the percentage of accepted transplants. Since 3 to 4 generations of X. couchianus can be obtained per annum, the high percentage of takes among sibs of location K can be explained, even if their progenitors had arrived only a year before. Twenty to 30 years ago, X. couchianus was much more abundant in the

TABLE 5. Number of Surviving Fin Transplants, and Survival Times of Fin Transplants Exchanged between the Progeny of Wild-caught Xiphophorus couchianus from the Huasteca Canyon and from Cuatro Ciénegas. Temperature range 25–26 C.

	D	Days after Transplantation												
Host	Donor	4	5	6	7	8	9	10	11	12	13			
Monterrey platyfish (spring II)	Northern platyfish	14	14	10	9	8	5	4	2	1	0			
Northern platyfish	Monterrey platyfish (spring II)	13	11	10	7	6	5	3	1	0	0			

Huasteca Canyon than it is today, as shown by the late Myron Gordon (1953), who wrote about his 1930 visit to the canyon: "We had, it seemed, struck the ancestral home of all the X. couchianus in the neighborhood. They were swarming in that pool by the thousands. In one seine haul we collected more Monterrey platyfish than all the Museums of the world contained up to then. In twenty minutes we captured more than 500 specimens without causing any observable thinning of the platyfish population darting through the weedy jungle." Today, through man's diversion of the springs, these backwaters and pools are gone forever, together with their teeming platyfish population. During his 1958 visit, Gordon was able to collect only 8 fish and he feared that the entire species might soon be wiped out. How large the platyfish population was in 1930 and 1939 cannot be gathered from his accounts, but it was restricted to a single pool (Gordon 1943). Even at that time, however, a considerable degree of genetic homogeneity must have existed to account for the great similarity between the populations of the 3 springs today, presumably derived from the single spring pool and now occupying separated spring systems.

Kallman and Gordon (1958) showed that in the southern platyfish, X. maculatus, transplants between members of 2 related inbred strains (30 and 163) were rejected within 5 to 14 days. Kallman (1960) estimated that these strains differ at 3 or 4 histocompatibility loci. The rapid homograft rejection in X. maculatus contrasts sharply with the prolonged survival times of transplants exchanged between X. couchianus hosts and donors inhabiting different spring systems in the Huasteca Canyon. This indicates that the fish of all 3 springs are closely related and that many of them differ from each other by fewer histocompatibility genes

than does strain 30 from strain 163 of X. maculatus. The similarity of these populations must date back more than 15 years, when all springs drained into a central stream bed.

The survival of some transplants among the offspring of wild-caught northern platyfish appears surprising in view of the fact that these fish have been taken from a population apparently numbering thousands of fish. These results can perhaps be interpreted as indicating that these fish either do not possess as large a number of histocompatibility genes as other species or that for some reason the northern platyfish is less heterozygous for these genes than other species. Objections to the first possibility are that species belonging to the same genus are similar in their physiological and genetic makeup and, therefore, it seems unlikely that one species is characterized by a large number of histocompatibility loci while another species lacks most of them. The southern platyfish X. maculatus possesses at least 12 histocompatibility loci. The second possibility raises the question whether the effective population size of the platyfish in the Laguna Santa Tecla is really as large as it appears to be. All platyfish are relatively poor swimmers and are seldom encountered more than 1 or 2 ft away from the banks. They tend to aggregate in favorable pockets. I do not believe that platyfish in any appreciable numbers swim through small channels and streams from the Lagunas Los Fresnos and Quintero into the Laguna Santa Tecla. The gene flow between these locations is negligible. the effective breeding population of the fish in the Laguna Santa Tecla is much smaller than the number of platyfish in the Cuatro Ciénegas region. Also the fact that platyfish tend to aggregate in favorable pockets suggests that there is no free movement of fish from one end of the lake to the other. Fish at one end may mate primarily with fish that were born and matured in the immediate area. The effective population size may be quite small and the gene pool of the population at one end of the lake may be slightly different from that of the fish at the opposite end. The transplantation test may be a sensitive indicator of genetic differentiation on the micropopulation level.

Some problems raised by these experiments will eventually be clarified by similar studies on other species of Xiphophorus, some of which resemble the X. couchianus in their ecological requirements while others (swordtails) are more active swimmers that are not as restricted in their movements.

Dobzhansky (1951) and Wright (1938, 1940) have discussed in detail the relationship between population size and gene frequency. They emphasized the importance of the effective population size, which is the number of breeding males and females when the population is at its lowest ebb. There appears no doubt that the effective population size in these platyfish populations is much smaller than the actual number of fish observed. In the Huasteca Canyon, torrential rains in late summer and early fall produce floods that scour the stream bed and must wash many fish out of the canyon. New generations undoubtedly arise from individuals that survive at the bottom of deep pools or that remain hidden in dense vegetation (Gordon 1953). But not all surviving adults contribute equally to the next generation. Early maturing or more active males will inseminate a disproportionate number of females.

There was no evidence that graft rejection in these 2 platyfish populations was due to a strong histocompatibility gene. All results are consistent with the interpretation that the grafts were destroyed due to the additive effect of several genes. In the Monterrey platyfish, relatively few grafts were destroyed within the first 2 weeks after the operation and then primarily in those series in which either no takes or very few takes were obtained. The high percentage of successful fin transplants among the platyfish from Monterrey cannot be attributed to an immunological unresponsiveness, since they react strongly to grafts from the other subspecies, nor to any paucity of strong histocompatibility genes as has recently been suggested by Billingham et al. (1958, 1960) to explain the unexpectedly high percentage

of surviving skin transplants among Syrian hamsters.

Both subspecies of X. couchianus must have arisen from the same generalized Xiphophorus that invaded the Rio Grande drainage from the center of distribution of this genus farther south in the Rio Panuco system (Rosen 1960). Today, both forms have the typical distribution of relict species. They are restricted to 2 isolated pockets at the very periphery of the range of this genus. Within each of these 2 relatively small populations a certain degree of genetic homozygosity has been achieved and the antigenic difference between individuals of the same population is therefore small. This process has gone much further in the Huasteca Canyon than in the Cuatro Ciénegas region. However, different histocompatibility alleles have become fixed in the two populations of X. couchianus and this is the reason why each reacts vigorously against tissue transplants from the other.

SUMMARY

One subspecies of Xiphophorus couchianus, the Monterrey platyfish, is restricted to 3 isolated spring systems in the Huasteca Canyon near Monterrey, Mexico. The size of the population is extremely small, and in some springs fewer than 3 dozen adult individuals have been found. Until 10 or 15 years ago, all the springs ran into a central stream bed, but since then have been diverted into man-made aqueducts. Another subspecies, the northern platyfish, is found in 3 small lagunas near Cuatro Ciénegas, Mexico, about 150 miles to the northwest. This population is much larger and consists of several thousand adults.

Fin transplants were performed among the offspring of 11 wild-caught X. couchianus collected in 7 different spots in the canyon. The percentage of accepted intrasib grafts ranged from 0 to over 69. The highest incidence of takes was obtained among sibs whose parents had been collected in areas where the density of the population was low. The percentage of takes among the offspring of 4 Cuatro Ciénegas females ranged from 0 to 15. Many of the unsuccessful transplants survived for several weeks or months and exhibited the chronic type of graft rejection that is characteristic of cases in which host and donor differ by only a few histocompatibility genes.

Fin transplants exchanged among mem-

bers of populations from different spring systems in the Huasteca Canyon survived for 1 to 12 weeks and a few were permanently accepted. On the other hand, fin transplants from Monterrey platyfish into northern platyfish hosts and vice versa were all rejected in 12 days or less.

These 2 small platyfish populations have become homozygous for several loci. Members of the same population share a large number of histocompatibility genes and therefore the antigenic difference between individuals is small. Different histocompatibility genes have become fixed in the Monterrey and in the northern platyfish.

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GENETICS LABORATORY, NEW YORK AQUAR-IUM, NEW YORK ZOOLOGICAL SOCIETY, NEW YORK, N. Y.