GENETIC DISSECTION OF CLONALLY INHERITED GENOMES OF POECILIOPSIS. I. LINKAGE ANALYSIS AND PRELIMINARY ASSESSMENT OF DELETERIOUS GENE LOADS

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ABSTRACT

Theoretical considerations suggest that a high load of deleterious mutations should accumulate in asexual genomes. An ideal system for testing this hypothesis occurs in the hybrid all-female fish *Poeciliopsis monacha-lucida*. The hybrid genotype is retained between generations by an oogenetic process that transmits only a nonrecombinant haploid *monacha* genome to their ova. The hybrid genotype is re-established in nature by fertilization of these *monacha* eggs with sperm from a sexual species, *P. lucida*. The unique reproductive mechanism of these hybrids allows the genetic dissection of the clonal *monacha* genome by forced matings with males of *P. monacha*. The resultant F_1 hybrids and their backcross progeny were examined to determine the amount and kinds of genetic changes that might have occurred in two clonal *monacha* genomes.—Using six allozyme markers, four similar linkage groups were identified in each clonal genome. Segregation and assortment at these loci revealed no apparent differences between *monacha* genomes from sexually and clonally reproducing species. Mortality of F_1 and backcross progeny revealed differences between the two clonal genomes, suggesting that deleterious genes may accumulate in genomes sheltered from recombination.

RECENT discoveries of abundant genetic diversity in clonally reproducing populations have raised serious questions about the presumed evolutionary inflexibility of asexual reproduction (SUOMOLAINEN and SAURA 1973; PARKER and SELANDER 1976; LOKKI *et al.* 1975; VRIJENHOEK, ANGUS and SCHULTZ 1977, 1978; ATCHLEY 1977; PARKER *et al.* 1977; SOLBRIG 1971; VRIJENHOEK 1978). Nevertheless, the sources of clonal variation are only poorly understood. Asexual populations of eukaryotic organisms are secondarily derived from sexual ancestors. Thus, a major source of clonal variation is through continued recruitment of sexual genomes, which become "frozen" by a genetic mechanism that prevents recombination. Where clonal recruitment from sexual ancestors is no longer possible, variation still results from mutation at the genic and chromosomal levels. Many theoretical arguments over the rates of adaptive evolution in sexual and asexual lineages concern the relative significance of recurrent and novel mutations as sources of variability (CROW and KIMURA 1965, 1969; MAYNARD-SMITH 1968, 1971; FELSENSTEIN 1974). However, because most spontane-

ous mutations are deleterious, MULLER (1964) proposed a "ratchet mechanism" whereby an asexual population progressively accumulates a high load of deleterious genes that cannot be shed by the recombinational mechanism characteristic of sexual species. Unfortunately, the general lack of empirical information on mutational variability in asexual organisms has prevented a reasonable assessment of these arguments.

The present investigation attempts to determine whether mutations have accumulated in the clonal genomes of an all-female "species" of fish in the genus Poeciliopsis (Poeciliidae). The all-female fish arose by interspecific hybridization (SCHULTZ 1969, 1973a) and have retained high levels of genic heterozygosity owing to their clonal reproductive processes (VRIJENHOEK, ANGUS and SCHULTZ 1977, 1978). Natural selection would be ineffective in removing deleterious and lethal recessive mutations from clonal genomes, which are permanently "sheltered" in the heterozygous condition. Theoretical considerations led NEI (1970) to hypothesize that mutations should accumulate rapidly in "sheltered" nonrecombinant genomes, even if the carriers are at a slight disadvantage. However, an attempt to test NEI's hypothesis with *Oenothera biennis*, a plant lacking chromosomal recombination, did not reveal the predicted high level of genic heterozygosity (LEVIN 1975). Previous genetic studies of all-female Poeciliopsis, on the other hand, did uncover the presence of some novel forms of pigmentation and protein expression, such as silent alleles, that may have an adaptive basis as well (VRIJENHOEK and SCHULTZ 1974; VRIJENHOEK 1975; VRIJENHOEK, ANGUS and SCHULTZ 1977). In this series of studies, we take advantage of the mode of inheritance in diploid all-female Poeciliopsis to examine the following questions: (1) What is the extent to which deleterious and lethal genes have accumulated in various clones? (2) To what extent do such genes occur in the ancestral sexual species? (3) Have ancestral linkage arrangements been conserved in the clonal genomes? (4) Are ancestral cytogenetic features conserved in the various clones? We report here our first findings concerning questions 1 and 3.

Poeciliopsis monacha-lucida is a naturally occurring all-female "species" that arose by hybridization between the sexual species *P. monacha* and *P. lucida* in the rivers of northwestern Mexico (SCHULTZ 1969, 1973a). Although the hybrids express traits derived from both parental species, only the haploid *monacha* (M) genome is transmitted to the eggs (SCHULTZ 1966). The haploid *lucida* (L) genome is expelled from oogonia; therefore, recombination between the parental genomes does not occur (CIMINO 1972). The haploid *monacha* eggs produced by the all-female hybrids are fertilized in nature by sperm from *P. lucida* males; thus, the diploid M-L genotype is re-established in the progeny. This reproductive process, called hybridogenesis (SCHULTZ 1969), results in the M genome being transmitted clonally between generations, while the L genome is used only once and discarded. Based on electrophoretic and immunological studies, between two and ten genetically distinct clonal M genomes were found in every population of *P. monacha-lucida* examined (VRIJENHOEK, ANGUS and SCHULTZ 1978;

ANGUS and SCHULTZ, in press). Several of the clonal genotypes are widely dispersed geographically, suggesting that they may have persisted long enough to accumulate a considerable number of mutant genes.

The hybridogenetic reproductive mechanism persists as long as *P. monacha-lucida* females mate with *P. lucida* males. However, it can be disrupted in the laboratory by forced matings with males of *P. monacha* (SCHULTZ 1973b; VRIJENHOEK and SCHULTZ 1974). The progeny resulting from such crosses were used in backcrosses with both parental strains to study segregation and assortment of six gene loci encoding enzymes and to make a preliminary assessment of the load of deleterious genes in the clonal genomes.

MATERIALS AND METHODS

The two strains of *P. monacha-lucida*, Va-L and IIIb-L, used in this study are descendants of wild females collected in the Arroyo San Pedro of the Rio del Fuerte, Sinaloa, Mexico, in April 1973. They were maintained in the laboratory by matings with males from a highly inbred *P. lucida* strain (M61–31). Because progeny from the two strains were bred to have identical *lucida* genotypes, they were examined electrophoretically (VRIJENHOEK, ANGUS and SCHULTZ 1978) and immunologically (ANGUS and SCHULTZ, in press) to establish the differences due to their haploid *monacha* genotypes (haplotypes). Strain designations indicate differences in the clonal *monacha* genomes. For example, IIIb indicates this strain is one of several immunotypes (a, b, c, etc.) found among strains having the same electrophoretic haplotype, III. The *P. monacha* strain (S68–5) was collected in the Arroyo de los Platanos of the Rio del Fuerte in 1968 by R. J. SCHULTZ, and was maintained by brother-sister matings for nine generations prior to its use in this study. All matings were made with individual virgin females, which were isolated in ten-liter aquaria subsequent to insemination. The progeny were removed as soon as possible after birth to avoid cannibalism. Individual broods were reared in separate aquaria with approximately one fish per two liters.

The clonal genomes can be forced to recombine by placing them in a diploid *monacha* genetic background. Forced matings of the two *P. monacha-lucida* strains with males of the inbred *P. monacha* strain were accomplished using both natural and artificial inseminations (CLARK 1950). The results of these crosses can be represented as follows:

$$\text{Cross A: IIIb-L} \times \text{M/M} \rightarrow \text{IIIb/M}$$
$$\text{Cross B: Va-L} \times \text{M/M} \rightarrow \text{Va/M}$$

The dash between genomes represents hybridogenetic reproduction; the slash represents the presence of conventional Mendelian processes.

The genetic contents of the clonal genomes were examined with backcrosses of the F_1 hybrid progeny, Va/M and IIIb/M, to their respective parental strains (Table 1). The backcrosses to *P. monacha* are necessary to establish fertility and to examine segregation and assortment of the six marker genes in the F_1 progeny. Backcrosses to their respective *P. monacha-lucida* strains permitted an analysis of lethal and deleterious genes in the clonal genomes.

Patterns of inheritance for six gene loci encoding enzymes were examined in detail in this study. The electrophoretic techniques used in the analyses of the enzyme phenotypes were previously described (LESLIE and VRIJENHOEK 1977; VRIJENHOEK, ANGUS and SCHULTZ 1978). The six loci encoded an eye tissue lactate dehydrogenase (*Ldh-1*); a liver carboxylesterase (*Es-5*); a muscle isocitrate dehydrogenase (*Idh-2*); a glycyl-L-leucine peptidase (*Pep*); 6-phosphogluconate dehydrogenase (*Pgd*); and a mitochondrial form of aspartate aminotransferase (*Aat-3*). Previous genetic studies have established the Mendelian bases for the allelic variation at these loci (LESLIE and VRIJENHOEK 1977).

TABLE 1

Number, percent mortality and sex of offspring from crossing experiments

Cross	Female × Male	Number of matings	Mating success %	Number of offspring	Mortality† %	Mortality† χ^2	Sex of offspring‡ Females	Sex of offspring‡ Males	χ^2
Extraction with *P. monacha*									
A	IIIb-L × M/M	3	66.7	21	47.6	18.32***	0	11	9.09***
B	Va-L × M/M	6	66.7	95	22.1	3.16	0	74	72.01***
C§	Moc.-L × M/M	13	38.5	25	20.0	0.66	11	9	0.05
Backcrosses with *P. monacha*									
D	M/M × IIIb/M	7	100.0	117	20.5	3.34	30	44	2.28
E	M/M × Va/M	7	100.0	152	17.1	1.00	69	48	3.77
Backcrosses with *P. monacha-lucida*									
F	IIIb-L × IIIb/M	6	0.0	0	—	—	—	—	—
G	Va-L × Va/M	6	66.7	25	52.0	27.99***	0	12	10.08***
P. monacha × *P. monacha*									
H	wild M/M (outbred)	93	83.9	1223	14.2	—	199	231	2.38
I	S68–5 M/M (inbred)	3	100.0	90	13.3	0.05	19	58	18.75***

† H_0: Mortality in cross = mortality in outbred *P. monacha* (cross H).

‡ H_0: Ratio of males to females = 1:1. NOTE: Some progeny were sacrificed prior to sexual maturity.

§ Data from VRIJENHOEK and SCHULTZ 1974.

*** Significant at $p = 0.001$.

RESULTS

Extraction Matings: The first phase of this study involved placing clonal genomes into a homospecific genetic background. Crosses between Rio del Fuerte strains IIIb-L and Va-L and males of S68–5 *P. monacha* produced all male offspring (Table 1, crosses A and B). Upon gross examination, the F_1 hybrid offspring were not distinguishable from normal outbred *P. monacha* males. Previous electrophoretic studies of the all-female parental strains, IIIb-L and Va-L, revealed that they were both permanently heterozygous for distinct *monacha* and *lucida* alleles at 11 gene loci: *Mdh-2*; *Idh-1*; *Aat-1*; *Aat-2*; *Aat-3*; *Es-4*; *Es-5*; *Mp-1*; *Pep*; *Adh-2*; and *Gpd* (VRIJENHOEK, ANGUS and SCHULTZ 1978). Twenty-five F_1 male offspring, 18 from cross B and seven from cross A, were examined for their phenotypes at these 11 loci. Only *monacha* alleles were expressed in the 25 F_1 hybrids; as expected, the *lucida* alleles were not transmitted by the hybridogenetic females. The IIIb/M and Va/M hybrids were heterozygous at six loci owing to the distinct alleles transmitted by the S68–5 *P. monacha* strain. The haploid genotypes of the three genomes involved in these crosses are as follows:

IIIb = *Ldh-1*^{b}, *Pgd*^{a}, *Idh-2*^{a}, *Aat-3*^{a}, *Pep*^{a}, *Es-5*^{c}

Va = *Ldh-1*^{b}, *Pgd*^{a}, *Idh-2*^{a}, *Aat-3*^{a}, *Pep*^{a}, *Es-5*^{f}

S68–5 M = *Ldh-1*^{a}, *Pgd*^{c}, *Idh-2*^{b}, *Aat-3*^{b}, *Pep*^{b}, *Es-5*^{d}

Other strains of *P. monacha-lucida* from the Rio Mocorito produced both male and female progeny in extraction matings with S68–5 *P. monacha* (cross C, Table 1). There may be significant differences, therefore, among clonal genomes with regard to their sex-determining genetic complements. Just why these genomes produce only female progeny in the heterospecific *monacha-lucida* genetic background is not understood.

The percentage of mortality between birth and sexual maturity was examined in the F_1 hybrid progeny. Only the IIIb/M progeny from cross A exhibited significantly higher mortality than that of outbred *P. monacha* reared under the same laboratory conditions (Table 1). Mortality in these progeny was randomly distributed among broods, with both IIIb-L females contributing approximately equal numbers of offspring. A possible explanation for the high mortality in these progeny is suggested by the results obtained in the backcrosses.

Backcrosses with P. monacha: The IIIb/M and Va/M hybrid males were backcrossed with S68–5 *P. monacha* females to examine linkage arrangements of the six genetic markers in each strain (Table 1). All F_1 males involved in mating experiments were fertile, a result consistent with previous studies (SCHULTZ 1973b; VRIJENHOEK and SCHULTZ 1974). The mortality of offspring from backcrosses of Va/M and IIIb/M males was not significantly higher than that of outbred *P. monacha*. Both backcrosses produced male and female offspring in nearly equal numbers.

Genetic analysis of the backcross progeny indicated that meiosis occurred in the IIIb/M and Va/M males and, therefore, that the hybridogenetic mechanism had broken down. Alleles at the six marker loci segregated in a Mendelian fashion with only one exception, the *Aat-3* locus in Va/M (Table 2). Independent assortment was examined using a statistical test that takes into account unequal segregation at individual loci (BAILEY 1961, p. 32; MATHER 1957, p. 33). Significant associations among alleles at the *Ldh-1*, *Idh-2* and *Es-5* loci occurred in backcrosses with both strains (Tables 3 and 4). Previous work had suggested linkage between *Ldh-1* and *Idh-2* in *P. monacha* (LESLIE and VRIJENHOEK 1977;

TABLE 2

Segregation of alleles in backcrosses with P. monacha

	S68–5 M/M × Va/M			S68–5 M/M × IIIb/M		
	Allele‡			Allele‡		
Locus	*A*	*a*	χ^2†	*A*	*a*	χ^2†
Ldh-1	72	53	2.888	46	47	0.011
Pgd	61	64	0.072	38	55	3.107
Idh-2	69	56	1.352	52	40	1.565
Es-5	70	54	2.065	47	42	0.281
Aat-3	51	74	4.232*	48	45	0.096
Pep	47	51	0.163	43	36	0.620

† H_0: Ratio of $A:a = 1:1$.
‡ A = allele from S68–5 M genome; a = allele from clonal genome.
* Significant at $p = 0.05$.

TABLE 3

Linkage analysis of backcrosses of S68–5 P. monacha × *IIIb/M*

Locus A/Locus B		Parentals‡ *AB*	*ab*	Recombinants‡ *Ab*	*aB*	χ_L^2†	Recombination fraction	*S.E.*
Ldh-1	*Pgd*	18	27	28	20	0.097	0.52	0.05
Ldh-1	*Idh-2*	40	34	6	12	34.087***	0.20	0.04
Ldh-1	*Pep*	19	17	19	22	0.325	0.53	0.06
Ldh-1	*Aat-3*	20	19	26	28	2.419	0.58	0.05
Ldh-1	*Es-5*	42	39	3	7	55.396***	0.11	0.10
Pgd	*Idh-2*	21	24	16	31	0.043	0.51	0.05
Pgd	*Pep*	17	24	12	24	0.325	0.47	0.07
Pgd	*Aat-3*	19	26	19	29	0.097	0.52	0.05
Pgd	*Es-5*	20	25	17	29	0.011	0.51	0.05
Idh-2	*Pep*	21	16	20	20	0.117	0.52	0.06
Idh-2	*Aat-3*	24	17	28	23	1.087	0.55	0.05
Idh-2	*Es-5*	45	35	6	4	54.444***	0.11	0.03
Pep	*Aat-3*	22	19	19	17	0.325	0.47	0.07
Pep	*Es-5*	19	15	21	20	0.653	0.55	0.06
Aat-3	*Es-5*	23	17	25	26	1.330	0.56	0.05

† Calculation from BAILEY (1961, p. 32).
‡ Capital letters refer to S68–5 M alleles; small letters refer to clonal alleles for each locus pair.
*** Significant at $p = 0.001$.

TABLE 4

Linkage analysis of backcrosses of S68–5 P. monacha × *Va/M*

Locus A/Locus B		Parentals‡ *AB*	*ab*	Recombinants‡ *Ab*	*aB*	χ_L^2†	Recombination fraction	*S.E.*
Ldh-1	*Pgd*	36	28	36	25	0.072	0.49	0.04
Ldh-1	*Idh-2*	59	43	13	10	49.928***	0.18	0.03
Ldh-1	*Pep*	31	24	27	16	1.469	0.44	0.05
Ldh-1	*Aat-3*	25	27	47	26	3.528	0.58	0.04
Ldh-1	*Es-5*	61	43	11	9	56.903***	0.16	0.03
Pgd	*Idh-2*	36	31	25	33	0.648	0.46	0.04
Pgd	*Pep*	21	24	27	26	0.653	0.54	0.05
Pgd	*Aat-3*	28	41	33	23	1.352	0.45	0.04
Pgd	*Es-5*	36	29	25	34	0.290	0.48	0.04
Idh-2	*Pep*	23	21	30	24	1.020	0.55	0.05
Idh-2	*Aat-3*	26	31	43	25	0.968	0.54	0.04
Idh-2	*Es-5*	63	48	6	7	77.452***	0.11	0.03
Pep	*Aat-3*	21	33	26	18	1.020	0.45	0.07
Pep	*Es-5*	27	24	20	27	0.163	0.48	0.06
Aat-3	*Es-5*	28	32	22	42	0.129	0.51	0.05

† Calculation from BAILEY (1961, p. 32).
‡ Capital letters refer to S68–5 M alleles; small letters refer to clonal alleles for each locus pair.
*** Significant at $p = 0.001$.

VRIJENHOEK 1979). The present results add *Es-5* to this linkage group, at least in the clonal genomes. We are investigating whether this arrangement represents retention of the ancestral *P. monacha* linkage groups. The linkage maps for these loci in the IIIb and Va genomes and the number of progeny examined in the backcrosses (n) are as follows:

Va: *Ldh-1*—16.1—*Es-5*—10.5—*Idh-2*$n = 124$

IIIb: *Ldh-1*—11.1—*Es-5*—11.1—*Idh-2*$n = 90$

Based on a comparison of the standard errors of the recombination fractions (BAILEY 1961), these two maps are not significantly different.

Backcrosses with P. monacha-lucida: Since hybridogenetic females transmit only their clonal genome to their ova, one-half of the progeny resulting from a cross between a IIIb-L female and an IIIb/M male should be homozygous for the clonal allele at any particular locus. On the average, all the offspring will be homozygous for clonal alleles at 50% of their loci. Thus, this type of cross allows detection of deleterious gene combinations in the clonal genomes.

There were significant differences in the results of the backcrosses to the maternal strains. In the six backcrosses with Va-L (cross G), only 25 offspring were born to four females (7, 4, 2, and 12 progeny each) over a four-month period. Mortality was spread evenly among the four sibships (57.1, 75.0, 50.0 and 41.7%, respectively). All 12 of the survivors were males (Table 1), and no significant deficiencies of homozygous phenotypes for clonal allozymes encoded by six loci were observed (Table 5). In fact, one individual was homozygous for all six of the clonal alleles, indicating that no lethal genes are closely linked with these markers. The overall mortality in these sibships (52.0%) was significantly higher than that in the original extraction mating (cross B, 22.1%), as well as that in the backcross with *P. monacha* (cross E, 17.1%).

No viable progeny were obtained from six backcross matings involving IIIb-L (cross F). Fertilization had occurred in these matings because four of the six IIIb-L females contained an average of three embryos when sacrificed after four months. A fifth female had produced three still-born progeny at one time during

TABLE 5

Segregation of alleles in the backcross of Va-L × Va/M

Locus	Alleles†		χ^2‡
	A	*a*	
Ldh-1	7	5	0.083
Pgd	8	4	0.750
Idh-2	6	6	0
Pep	4	7	0.364
Es-5	7	5	0.083
Aat-3	5	7	0.083

† A = allele from S68–5 M. a = allele from Va genome.
‡ H_0: ratio $A{:}a = 1{:}1$.

the same period. Evidently, the mortality in these backcross embryos is considerably higher than that in those embryos resulting from the backcrosses involving Va-L.

DISCUSSION

Four linkage groups have been tentatively identified in each of the clonal genomes studied: Linkage Group I (*Ldh-1, Es-5, Idh-2*); LG II (*Pgd*); LG III (*Aat-3*); and LG IV (*Pep*). Since both clonal genomes have similar linkage maps, it is likely that this reflects a retention of the ancestral *monacha* arrangement. Previous studies on *P. monacha* are consistent with this interpretation (LESLIE and VRIJENHOEK 1977). Linkage Group I is of interest because the locus encoding an eye LDH have been found to be linked to two carboxylesterases in fishes of the genus Xiphophorus (MORIZOT, personal communication). Since *Es-5* encodes one of two carboxylesterases known in Poeciliopsis (PONTIER, personal communication) and since *Lhd-1* and *Es-5* are linked, there appears to be a conservation of this arrangement in the family Poeciliidae. Linkage tests on the other carboxylesterase in Poeciliopsis (*Es-4*) are in progress.

When matings between *P. monacha* and *P. lucida* give rise to a hybrid, a sexually reproducing *monacha* genome is essentially "frozen." Although the cytological details concerning the origin of the unisexual reproductive mechanisms are not known (CUELLAR 1974), all viable progeny from a *P. monacha* × *P. lucida* cross reproduce hybridogenetically (SCHULTZ 1973a). Since only 7% of such matings produce viable offspring, there may be a rare combination of genes, already in the *monacha* gene pool, that results in a predisposition toward hybridogenesis. If true, this predisposition should be detectable in the F_1 progeny, resulting from the genetic dissection of a unisexual strain. Mendelian segregation and assortment of clonal alleles, when placed in a homospecific *monacha* genetic background (Tables 2 to 4), indicate that there are no apparent differences between sexually and clonally inherited *monacha* genomes. The single exception to the Mendelian segregation ratios (*Aat-3* for Va/M) is probably due to chance.

VRIJENHOEK (1979) suggested that alleles in the clonal genomes may serve as a reservoir of variation for the sexual gene pool. These alleles would be accessible through the breakdown of unisexuals by accidental matings with *P. monacha* males. The fertility of the breakdown progeny found in this and previous studies (SCHULTZ 1973b; VRIJENHOEK and SCHULTZ 1974) supports the hypothesis that a potential exists for clonal genes to introgress into the sexual gene pool of *P. monacha*.

The crossing experiments permitted an examination of recessive genes in the clonal genomes of *P. monacha-lucida* strains IIIb-L and Va-L. Crosses between these strains and males of *P. monacha* (S68–5 M) produced all male F_1 hybrid offspring (IIIb/M and Va/M) that were morphologically and electrophoretically indistinguishable from normal *P. monacha* males. Backcrosses of these F_1 males with females of their respective hybridogenetic maternal strain are expected to produce offspring, which, on the average, are homozygous for the "clonal" alleles at 50% of their gene loci. Thus, the presence of deleterious recessive alleles in

the clonal genomes would be revealed by significant deficiencies of homozygous genotypes and reduced reproductive output.

A significant load of lethal equivalents might explain the failure to obtain more than a few offspring in the backcrosses (F and G; Table 1) with both all-female strains. This result is not due to infertility of the F_1 males, since they produced numerous offspring in backcrosses (D and E) with *P. monacha* females. It is more reasonable, however, to compare the former backcrosses, F and G, with matings A and B, both of which produced small numbers of F_1 hybrid offspring. Sperm from either S68–5 *P. monacha* or the F_1 hybrid *monacha* might have low competence in the reproductive tracts of the hybridogenetic females, accounting for this reduced productivity.

For the present, only rough comparisons of the lethal loads in strains IIIb and Va are possible. The backcross, Va-L × Va/M (cross G), produced about one-fifth as many viable offspring as the parental cross, Va-L × M/M (cross B), after adjustments were made for the number and length of mating experiments. If each lethal allele reduces the reproductive output of the backcross by 50%, it is estimated that the Va genome contains at least one embryonic lethal equivalent. A second lethal equivalent would account for the juvenile mortality, since only 12 out of the 25 progeny survived to sexual maturity. These deleterious genes are not closely linked to our six marker genes, since no significant deviations from Mendelian expectations were observed in backcross G (Table 5). In contrast, the IIIb-L × IIIb/M backcross (cross F) produced no viable offspring. Adjusting to the productivity of cross A, IIIb-L × M/M, at least 42 offspring were expected in backcross F. Thus, the IIIb genome probably contains a minimum of four to six lethal equivalents.

The deleterious genes revealed in the backcrosses with the IIIb-L unisexual strain may not be completely recessive in a diploid *monacha* genetic background. The F_1 hybrid IIIb/M males had significantly higher juvenile mortality than wild or inbred *P. monacha* (Table 1). Differential effects of the genetic background on the expression of lethal and deleterious genes have been previously reported (DOBZHANSKY and SPASSKY 1968; ANDERSON 1969).

Deleterious genes in the clonal genomes may have accumulated by mutation since the origin of these hybrid strains or may simply represent part of the normal variation in the *P. monacha* gene pool, which became "frozen" in the clonal genomes at the time of their origin. The population structure of *P. monacha* is one of small isolated colonies that frequently undergo severe bottlenecks in population size (VRIJENHOEK 1979). Thus, it is unlikely that there are many lethals in the gene pool of this species. Brother-sister matings within six independently derived strains of *P. monacha* revealed no evidence for deleterious genes in the first and second inbred generations. Prolonged inbreeding (12 generations) in one strain has led to aberrant sex ratios, but no decrease in survivorship (Table 1, cross I).

Additional evidence exists for the accumulation of potentially deleterious nonfunctional alleles in clonal genomes. A silent allele (*Ldh-1*^{0}) for lactate dehydrogenase is found in *P. monacha-lucida* inhabiting the Rio Sinaloa (unpublished),

and a silent allele (*Es-5*^{0}) for a carboxylesterase occurs in a related hybridogenetic fish *P. monacha-occidentalis* from the Rio Concepción (VRIJENHOEK, ANGUS and SCHULTZ 1977). Extensive electrophoretic surveys of natural populations of *P. monacha* have revealed no silent alleles at these loci (VRIJENHOEK 1979).

A progressive accumulation of nonfunctional mutations in clonal genomes would be expected to lead to decreased fitness in asexual populations (MULLER 1964). However, unisexual Poeciliposis are hybridogenetic; therefore, *de novo* mutations in the clonal *monacha* genomes are permanently sheltered in the heterozygous condition by the substitutable *lucida* genomes. Even if deleterious in the homozygous condition, nonfunctional or other mutations may improve the mating success and competitive abilities of *P. monacha-lucida*. The unisexuals must mate with males of *P. lucida*, which have a strong preference for conspecific females (MCKAY 1971). Sexual selection by *P. lucida* males would favor any mutations in *P. monacha-lucida* that would increase unisexual mimicry of *P. lucida* females. Mimicry of *lucida* genital pigmentation by some strains of *P. monacha-lucida* may have arisen by this process (VRIJENHOEK and SCHULTZ 1974; VRIJENHOEK 1975).

For traits not involved in mate selection, such as feeding structures and behavior, divergent evolution might be favored. Mutations in the clonal genomes might significantly alter niche requirements and life histories of individual unisexual strains and thereby decrease competition with the sexual host species and other clones (VRIJENHOEK 1978). The degree to which *de novo* mutation and "frozen" *monacha* variation contribute to clonal diversity is under investigation.

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