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GENOTYPIC AND ENVIRONMENTAL COMPONENTS OF VARIATION IN GROWTH AND REPRODUCTION OF FISH HEMICLONES (*POECILIOPSIS*: POECILIIDAE)

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Abstract.—The frozen-niche-variation model was proposed to account for the coexistence of genetically related clones in naturally occurring unisexual populations. This model is based on two assumptions: 1) ecologically different clones have multiple independent origins from sexual ancestors; and 2) the population of sexual ancestors contains genetic variability for ecologically relevant traits. To test these assumptions, we produced 14 new "hemiclones" (nonrecombining haploid genotypes) of fish (*Poeciliopsis*: Poeciliidae). Our ability to synthesize many new hemiclones demonstrates the feasibility of multiple independent origins of nonrecombining genotypes. A substantial proportion (10–50%) of the phenotypic variation among hemiclones in size at birth, juvenile growth rate, and fecundity had a genetic basis. Thus, we conclude that multiple origins can give rise to an assemblage of genetically distinct hemiclones, each with a unique combination of life-history traits. Additionally, a comparative analysis of two natural hemiclones revealed that the synthetic strains represent a broad field of variation from which natural hemiclones can be selected.

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Coexisting plant and animal clones often exhibit considerable differences in their life-history characteristics and in their use of natural resources (for reviews see Bell [1982, 1985] and Bierzychudek [1985]). The frozen-niche-variation model was proposed to account for the coexistence of multiple clones in unisexual populations (Vrijenhoek, 1979*a*, 1984). Accordingly, distinct clones have multiple origins from genetically variable sexual ancestors. Selection then acts on the unisexual population to produce a local assemblage that exhibits minimal niche overlap among clones. A multiclonal assemblage is thus able to exploit more resources in a heterogeneous environment and, therefore, can displace its sexual ancestors from more of the available niches than can any single clone (Vrijenhoek, 1979*a*). Simulations of this scenario have revealed that, as long as the combined niche breadth of the clonal array does not completely eclipse that of the sexual ancestors, coexistence between sexual and asexual lineages is possible (Case and Taper, 1986). Bell's (1982) tangled-bank model leads to a similar prediction.

The frozen-niche-variation model is based on two critical assumptions. First, it assumes that ecologically different clones had independent origins from sexual ancestors. Clones are simply "frozen" genotypes that existed in the sexual gene pool. Electrophoretic data from numerous unisexual populations are generally consistent with the hypothesis that most clonal diversity arose via multiple origins (Lokki et al., 1975; Parker and Selander, 1976; Vrijenhoek et al., 1977, 1978; Moore, 1984; Harshman and Futuyma, 1985; Vrijenhoek, 1989). However, mutations have played a role in generating some clonal diversity (Saura et al., 1976; Lokki et al., 1976*a*, 1976*b*; Leslie and Vrijenhoek, 1980; Spinella and Vrijenhoek, 1982; Lynch and Gabriel, 1983; Lynch, 1985).

The second assumption of the frozen-niche-variation model, that the sexual ancestors of clones contain considerable genetic variability for ecologically relevant traits, has not been tested. Descriptions of phenotypic variation among naturally occurring clones do not provide a sufficient test of this hypothesis, because variation that was frozen among clones at the time of their origin cannot be distinguished from post-formational variation that accumulated due

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to subsequent mutation. Furthermore, extant clones may be a highly select subset of ancestral clones and, therefore, might not exhibit the range of phenotypic variation that can be frozen from the sexual gene pool.

The purpose of this study was to examine the second assumption of the frozen-niche-variation model by testing the hypothesis that newly formed clones can freeze heritable variation that is ecologically relevant. To accomplish this goal, we originally synthesized 33 new hybridogenetic strains of the unisexual fish *Poeciliopsis monacha-lucida* (Wetherington et al., 1987). These hybridogens transmit only a nonrecombinant, haploid *monacha* genome (i.e., hemiclone) between generations (see Materials and Methods, below). In this study, we were able to examine only 14 of these strains; the other 19 strains were extinct by the third generation because of low viability and reproductive output (Wetherington et al., 1987). We partitioned genotypic and environmental components of variance for four life-history traits of the 14 hemiclonal strains: length at birth, weight at birth, juvenile growth rate, and brood size. Our procedures for synthesizing these strains allowed us to examine genotypic variation that could be frozen at three levels: 1) from recombinant variation produced by a single sexual foundress; 2) from the differences among independent sexual foundresses; and 3) from differences between sexual foundresses from two allopatric populations.

Materials and Methods

Experimental Animal.—*Poeciliopsis* are small viviparous fishes that inhabit the springs, arroyos, and rivers of western Mexico. Multiple hybridization events between females of *P. monacha* and males of several related sexual species of *Poeciliopsis* (e.g., *P. lucida*) have produced a variety of diploid and triploid, all-female biotypes (Schultz, 1977). Allodiploid unisexual forms such as *P. monacha-lucida* (ML) are hybridogenetic (Schultz, 1969). During oogenesis, only the *monacha* chromosome set (M) is transmitted to the ova. The paternal *lucida* set (L) is expelled in a premeiotic cell division, thereby precluding synapsis and recombination (Cimino, 1972). In nature, the haploid M ova produced by ML females are fertilized by *lucida* sperm (L′) and a new ML′ hybrid expressing traits encoded by both parental genomes is produced (Schultz, 1966; Vrijenhoek, 1972). Only the maternal M genome of a hybridogenetic strain is transmitted clonally; the paternal L genome is substituted in each generation.

Synthesizing Unisexual Fish.—Initially, 33 new hybridogenetic strains of *P. monacha-lucida* were synthesized in the laboratory (for details of the crosses, see Wetherington et al. [1987]). Fourteen of these strains were examined in this study. These strains were produced by five sexually reproducing *P. monacha* foundresses. Each foundress was the first-laboratory-generation daughter of a wild-caught, naturally inseminated female. Foundresses A and B were collected in the Jaguari tributary of the Río Fuerte near the town of Agua Caliente, Sonora, Mexico (site AC; see Vrijenhoek, 1979*b* fig. 1), and foundresses C, D, and E were collected in the Río Mayo near the town of El Tabelo, Sonora (site TA). For comparative purposes, two natural hemiclones, ML/VII (strain S68-4 Cw) and ML/VIII (strain T70-3 Cw), were included. The natural hemiclones coexist with both *P. monacha* and *P. lucida* in the Río Fuerte at site AC (Vrijenhoek et al., 1978; Schenck and Vrijenhoek, 1986). All the hemiclonal strains were "standardized" by crossing them with an isogenic strain of *P. lucida* (S68-4 PC). This isogenic strain (which we designate L^i) has been maintained in the laboratory through sib matings for over 30 generations and should be homozygous at all loci (Angus and Schultz, 1983). Thus, the standardized ML^i strains were all genetically identical for their substitutable paternal genomes. Genetic differences among strains were directly attributable to the hemiclonal M genomes.

Experimental Design.—All fish were reared in 4-liter containers that were suspended in a 1,600-liter, filtered, circulating water bath (28° ± 1°C, 16L:8D photoperiod). Containers were perforated to allow solute exchange with the water bath. To minimize maternal effects, we isolated virgin females (ca. 120 days of age) in the water bath for 30 days prior to mating. This acclimation period allowed females to produce fertile eggs under controlled dietary,

temperature, and light conditions. Breeding females were fed high-protein commercial trout chow (Cosby-Hodges Milling Co.) ad libitum twice daily. The diet was supplemented daily with liver paste and frozen brine shrimp.

We partitioned genotypic and environmental components of variance for length at birth, weight at birth, juvenile growth rate, and brood size in the 14 synthetic strains. For analysis of birth size (lengths and weights), we examined offspring from 2–3 broods produced by two or three females of each strain. For analysis of growth rates and brood sizes, three offspring from each brood were randomly selected and reared individually. Offspring in some of the synthetic strains used in this study had birth defects (see Wetherington et al., 1987). These offspring were excluded from all analyses. Due to mortality, some broods were represented by only two individuals. The diet of the offspring consisted of measured quantities of trout chow fed twice daily. During the first week, each fish received 6.44 ± 0.18 mg per feeding. The rearing container was replaced weekly with a clean one, and the ration was increased according to a fixed schedule (Table 1). Fish of the same age received the same amount of food regardless of their size. Pilot studies revealed that this feeding regime provided each fish with excess food and minimized the accumulations of algae and uneaten food. Fish were preserved at 63 ± 3 days of age for brood-size assessment.

Measurement of Growth.—Standard length (distance from the tip of the snout to the end of the hypural plates) was determined from photographic negatives of the fish (Stearns, 1983). Fish were lightly anesthetized in tricaine methanesulfonate (MS222) (12 mg/100 ml of water), placed in a culture dish, and photographed using two transilluminating strobe lights. Standard length of offspring was determined on the day of birth and, subsequently, at weekly intervals for nine weeks.

Statistical Analyses.—Total phenotypic variance (V_P) among synthetic hemiclones was partitioned hierarchically into five components using an unbalanced nested analysis of variance (Fig. 1). To estimate within-population variance, hierarchical components were estimated separately for groups of hemiclones deriving from Río Fuerte versus Río Mayo foundresses. Genetic variance was partitioned into two hierarchical levels: 1) $V_{G(f)}$, the variance among independent *P. monacha* foundresses; and 2) $V_{G(h)}$, the variance among hemiclones synthesized from the same *P. monacha* foundress (hereafter referred to as sibling hemiclones). Because all offspring of a standardized hybridogenetic strain (i.e., ML^i) are genetically identical, all variance within a strain is due to environmental sources. Environmental variance was partitioned into three hierarchical levels: 1) $V_{E(m)}$, the variance among mothers within a strain; 2) $V_{E(b)}$, the variance among broods within mothers; and 3) $V_{E(o)}$, the variance among offspring within broods. Natural hemiclones ML/VII and ML/VIII were analyzed separately.

TABLE 1. Fixed feeding schedule for fish used in the analysis of growth rates. Mean ration (mg per feeding) and corresponding standard error for each of the nine successive weeks were estimated from ten replicates.

Age (weeks)	Mean ration (mg per feeding)	SE
1	6.44	0.18
2	8.84	0.15
3	16.31	0.22
4	27.74	0.22
5	33.91	0.25
6	38.63	0.26
7	43.44	0.41
8	49.70	0.44
9	56.31	0.48

For analysis of variance, the variation among offspring within a brood ($V_{E(o)}$) was used as the experimental error. Homogeneity of the experimental error was verified using the Kruskal-Wallis test (Conover, 1980 Ch. 5). Normality of residuals was verified by a graphical analysis using rankits (Sokal and Rohlf, 1981 Ch. 6). To guard against scaling effects (Bulmer, 1981 Ch. 4; Falconer, 1981 Ch. 17), independence of the variance and the mean was verified for each trait via regression, using all the broods within single females as observations. All analyses were performed using type-III sums of squares from the GLM procedure in SAS (SAS Institute, 1985). These are appropriate for an unbalanced design in which the effects are correlated, because each sum of

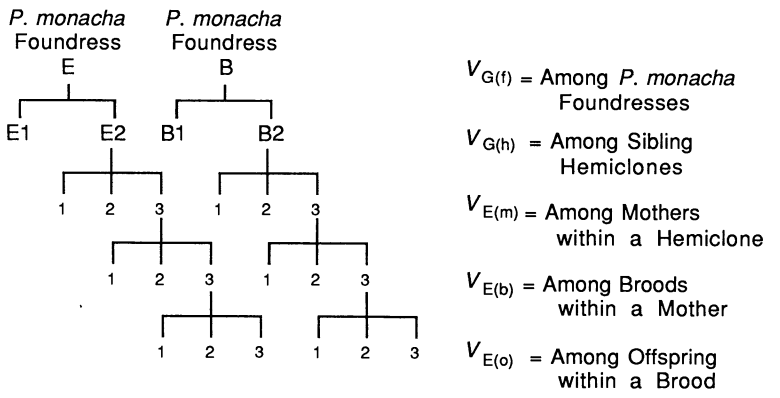

FIG. 1. Schematic representation of the experimental design using *P. monacha* foundresses E and B as examples. The total phenotypic variation in a particular trait was partitioned into genetic and environmental components. $V_{G(f)}$ and $V_{G(h)}$ include genetic sources of variation; $V_{E(m)}$, $V_{E(b)}$, and $V_{E(o)}$ include environmental sources of variation. Three mothers from each hemiclonal strain and three broods from each mother were used.

squares is calculated for a model that includes all other effects (Freund et al., 1986). Natural hemiclones ML/VII and ML/VIII were considered to be fixed effects. All other effects were considered to be random. Because the design was not balanced, F tests are approximate. Synthesis of mean squares via the Satterthwaite approximation (Sokal and Rohlf, 1981 Ch. 10) had a negligible effect on the F ratios when type-III sums of squares were used (less than a 5% change). In no comparison did the Satterthwaite method affect the significance level of the results. Therefore, the more straightforward approximate tests are presented. The components of variance were estimated by a

TABLE 2. Analyses of variance for hemiclonal genotypes derived from the Río Fuerte. Sources of variation: foundress = variation between founding *P. monacha* females; hemiclone = variation among hemiclones nested within a single foundress; mother = variation among mothers nested within a single hemiclonal genotype; brood = variation among broods nested within a single mother; offspring = variation among offspring nested within a single brood. Subscripts of mean squares (MS) in the F-ratio column correspond to levels (preceding column).

Source	*d.f.*	SS × 10^{-3}	Level	F ratio	F	P	Variance component × 10^{-3} (percentage of total)
A. Standard length at birth (R^2 = 0.56):							
Model	81	76.21	1	MS_1/MS_6	14.84	0.000	
Foundress ($V_{G(f)}$)	1	3.16	2	MS_2/MS_3	0.47	0.518	−0.006 (—)
Hemiclone ($V_{G(h)}$)	6	40.24	3	MS_3/MS_4	22.79	0.000	0.064 (41%)
Mother ($V_{E(m)}$)	22	6.47	4	MS_4/MS_5	0.70	0.814	−0.003 (—)
Brood ($V_{E(b)}$)	52	21.72	5	MS_5/MS_6	6.59	0.000	0.030 (19%)
Offspring ($V_{E(o)}$)	949	60.15	6				0.063 (40%)
B. Wet weight at birth (R^2 = 0.51):							
Model	82	837.64	1	MS_1/MS_6	12.83	0.000	
Foundress ($V_{G(f)}$)	1	2.36	2	MS_2/MS_3	0.04	0.854	−0.151 (—)
Hemiclone ($V_{G(h)}$)	6	380.19	3	MS_3/MS_4	8.61	0.000	0.527 (30%)
Mother ($V_{E(m)}$)	22	161.87	4	MS_4/MS_5	1.31	0.212	0.069 (4%)
Brood ($V_{E(b)}$)	53	298.61	5	MS_5/MS_6	7.08	0.000	0.387 (22%)
Offspring ($V_{E(o)}$)	1,011	804.64	6				0.796 (45%)
C. Juvenile growth rate (R^2 = 0.42):							
Model	74	71.21	1	MS_1/MS_6	1.48	0.023	
Foundress ($V_{G(f)}$)	1	4.03	2	MS_2/MS_3	1.54	0.261	0.015 (2%)
Hemiclone ($V_{G(h)}$)	6	15.72	3	MS_3/MS_4	2.69	0.048	0.059 (8%)
Mother ($V_{E(m)}$)	18	17.54	4	MS_4/MS_5	1.43	0.159	0.035 (4%)
Brood ($V_{E(b)}$)	49	33.34	5	MS_5/MS_6	1.05	0.409	0.010 (1%)
Offspring ($V_{E(o)}$)	148	96.33	6				0.651 (85%)

TABLE 3. Analyses of variance for hemiclonal genotypes derived from the Río Mayo. Sources of variation: foundress = variation between founding *P. monacha* females; hemiclone = variation among hemiclones nested within a single foundress; mother = variation among mothers nested within a single hemiclonal genotype; brood = variation among broods nested within a single mother; offspring = variation among offspring nested within a single brood. Subscripts of mean squares (MS) in the *F*-ratio column correspond to levels (preceding column).

Source	*d.f.*	SS × 10^{-3}	Level	*F* ratio	*F*	*P*	Variance component × 10^{-3} (percentage of total)
A. Standard length at birth (R^2 = 0.73):							
Model	49	65.46	1	MS_1/MS_6	20.77	0.000	
Foundress ($V_{G(f)}$)	2	26.09	2	MS_2/MS_3	11.73	0.038	0.114 (46%)
Hemiclone ($V_{G(h)}$)	3	3.34	3	MS_3/MS_4	1.76	0.205	0.010 (4%)
Mother ($V_{E(m)}$)	13	8.24	4	MS_4/MS_5	1.45	0.192	0.013 (5%)
Brood ($V_{E(b)}$)	31	13.54	5	MS_5/MS_6	6.79	0.000	0.047 (19%)
Offspring ($V_{E(o)}$)	376	24.19	6				0.064 (26%)
B. Wet weight at birth (R^2 = 0.66):							
Model	49	422.04	1	MS_1/MS_6	14.77	0.000	
Foundress ($V_{G(f)}$)	2	118.25	2	MS_2/MS_3	3.94	0.145	0.423 (26%)
Hemiclone ($V_{G(h)}$)	3	44.99	3	MS_3/MS_4	4.20	0.028	0.221 (14%)
Mother ($V_{E(m)}$)	13	46.39	4	MS_4/MS_5	1.05	0.430	0.027 (2%)
Brood ($V_{E(b)}$)	31	104.98	5	MS_5/MS_6	5.81	0.000	0.351 (22%)
Offspring ($V_{E(o)}$)	380	221.63	6				0.583 (36%)
C. Juvenile growth rate (R^2 = 0.52):							
Model	49	117.41	1	MS_1/MS_6	2.04	0.002	
Foundress ($V_{G(f)}$)	2	3.14	2	MS_2/MS_3	0.21	0.824	−0.130 (—)
Hemiclone ($V_{G(h)}$)	3	22.79	3	MS_3/MS_4	3.11	0.063	0.223 (13%)
Mother ($V_{E(m)}$)	13	31.76	4	MS_4/MS_5	1.40	0.216	0.095 (6%)
Brood ($V_{E(b)}$)	31	54.23	5	MS_5/MS_6	1.49	0.073	0.201 (12%)
Offspring ($V_{E(o)}$)	94	110.27	6				1.173 (69%)
D. Brood size (R^2 = 0.59):							
Model	49	117.41	1	MS_1/MS_6	2.04	0.002	
Foundress ($V_{G(f)}$)	2	3,039.62	2	MS_2/MS_3	15.77	0.026	38.890 (39%)
Hemiclone ($V_{G(h)}$)	3	289.10	3	MS_3/MS_4	1.58	0.246	1.351 (1%)
Mother ($V_{E(m)}$)	12	733.57	4	MS_4/MS_5	0.86	0.596	−0.673 (—)
Brood ($V_{E(b)}$)	25	1,781.93	5	MS_5/MS_6	1.29	0.196	5.898 (6%)
Offspring ($V_{E(o)}$)	120	4,303.00	6				54.679 (54%)

modification of Henderson's method (Searle, 1971 Ch. 11) in which estimates of the observed components were obtained by solving the system of linear equations prescribed by the type-III mean squares and their expectations (Milliken and Johnson, 1984 Ch. 19; Via, 1984). The procedures for analysis of variance resulted in the estimation of negative variance components (Searle, 1971 Ch. 10). Negative components are included in the estimate of total phenotypic variance. Estimates of variance components are only unbiased if the negative components are retained (Searle, 1971 Ch. 10).

RESULTS

Size at Birth.—Foundress effects ($V_{G(f)}$) on birth length and weight were not statistically significant for Río Fuerte *P. monacha* females (A and B) (Table 2, Fig. 2). Río Mayo foundresses (C, D, and E) had a significant effect on birth length, accounting for 46% of the total phenotypic variance, but they had no effect on birth weight (Table 3, Fig. 2). Hemiclonal effects ($V_{G(h)}$) were significant for Río Fuerte strains, accounting for 41% of the total phenotypic variance in length and 30% of the variance in weight. Río Mayo hemiclones had a significant effect on birth weight, accounting for 14% of the total phenotypic variance, but they had no significant effect on birth length. Nongenetic factors ($V_{E(m)} + V_{E(b)} + V_{E(o)}$) accounted for 59% of the total phenotypic variance in length and 71% of the variance in weight in Río Fuerte strains. Nongenetic factors accounted for 50% of the variance in length and 60% of the variance in weight in Río Mayo strains.

Juvenile Growth Rate.—A pilot study showed that growth rates tended to decel-

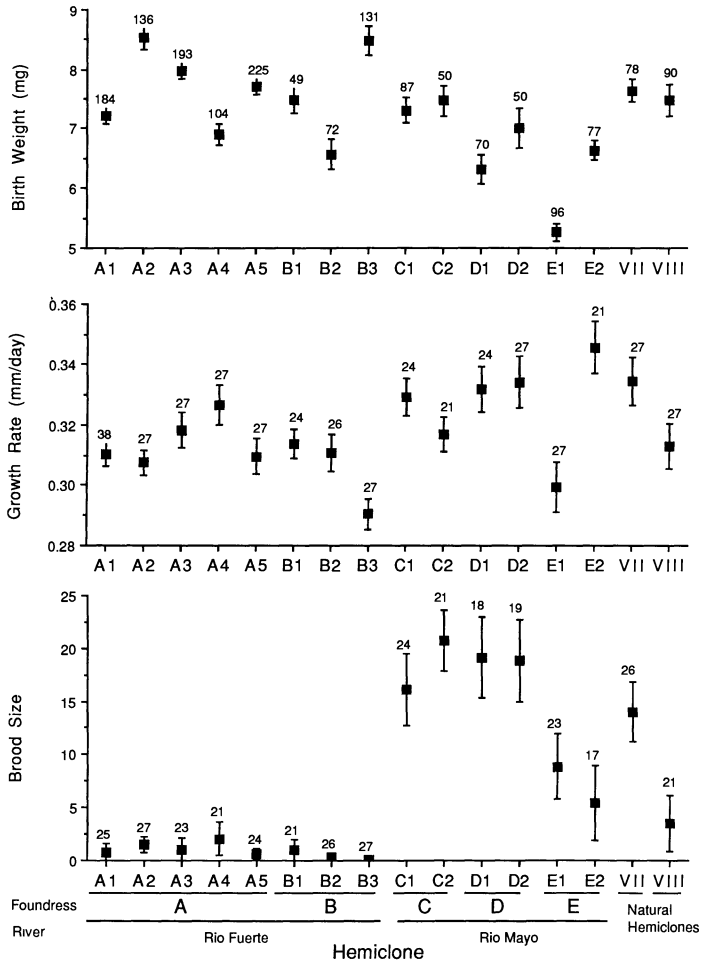

FIG. 2. Mean birth weight, mean juvenile growth rate, and mean brood size for 14 strains of synthetic hemiclones of *Poeciliopsis*. Natural hemiclones ML/VII and ML/VIII are shown for comparison. Mean birth weight and birth length exhibited virtually identical distributions; therefore, only birth weight is graphically represented. Vertical bars denote ±2 SE about the mean. Samples sizes are given above the bars for each strain.

erate after about 63 days, presumably as a consequence of resource allocation to egg production (Fig. 3). However, growth was approximately linear for the first 63 days. For the present study, we sacrificed the fish at nine weeks of age and estimated growth rates using the slope of the regression line for standard length on age (in days).

Foundress effects ($V_{G(f)}$) were not statistically significant either for Río Fuerte strains (Table 2, Fig. 2) or for Río Mayo strains (Table 3, Fig. 2). Hemiclonal effects ($V_{G(h)}$) were small but significant for Río Fuerte strains, accounting for 8% of the total variance (Table 2); although $V_{G(h)}$ accounted for 13% of the phenotypic variance in Río Mayo strains, the hemiclonal effects were not statistically significant ($P = 0.063$; Table 3). Nongenetic factors accounted for 90% of the total variance in Río Fuerte strains and 87% of the variance in Río Mayo strains.

Brood Size.—Ovaries were excised from the body cavity of 63-day-old preserved fish. The eggs were categorized into three groups: mature, immature, and atrophied. Mature and immature eggs were distinguished on the basis of size; all eggs smaller than 0.50 mm in diameter were classified as immature. Mature and immature eggs were always discrete categories within individual females. Atrophied eggs are decaying mature eggs. They are identified by the distri-

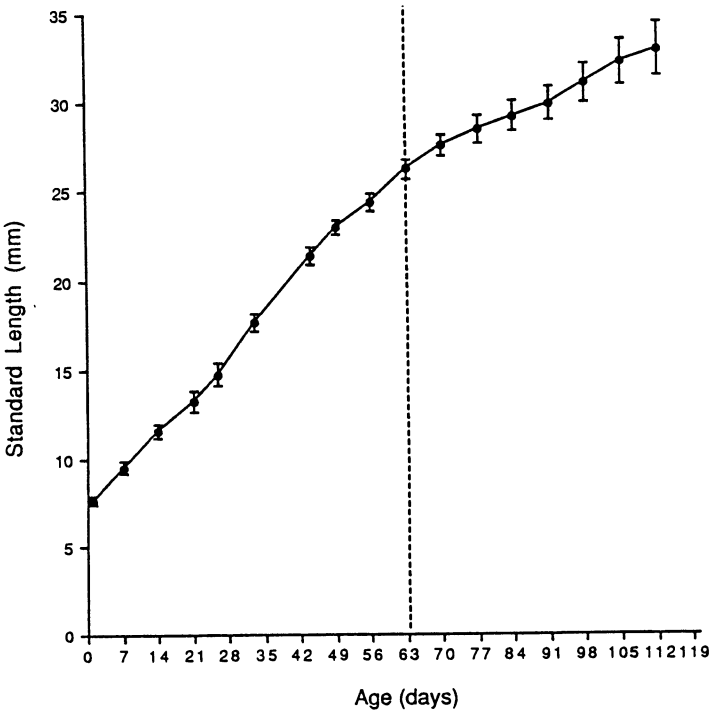

FIG. 3. Juvenile growth of a synthetic hemiclonal strain of *Poeciliopsis*. The dashed line marks 63 days of age, before which growth is approximately linear. Each point represents the mean standard length determined from 20 females. Vertical bars denote ±2 SE about the mean.

bution of oil droplets and a transparent yellow appearance. We used the number of mature plus atrophied eggs as an estimate of early reproductive investment. Since the fish used in this study were not inseminated, we assumed that all eggs produced during the study period were retained in the ovary at the time the female was sacrificed. We have no evidence to indicate that unfertilized eggs are resorbed or expelled from the body cavity (Meffe and Vrijenhoek, 1981).

Very few Río Fuerte hemiclonal females produced eggs before 63 days of age; thus, phenotypic variance in brood size could not be reliably estimated. Río Mayo foundresses had a statistically significant effect on brood size; $V_{G(f)}$ accounted for 39% of the total phenotypic variance (Table 3, Fig. 2). However, hemiclonal effects ($V_{G(h)}$) were not significant. Nongenetic factors accounted for 60% of the total phenotypic variance.

Natural Hemiclones.—Birth size, juvenile growth rate, and early reproductive investment of natural strains ML/VII and ML/VIII were contained within the range of expression of the total array of synthetic hemiclones. ML/VII fish grew significantly faster than ML/VIII fish ($F_{[1, 52]} = 3.99$, $P = 0.05$) and had larger brood sizes at 63 days of age ($F_{[1, 45]} = 26.39$, $P < 0.01$) (Fig. 2). Schultz and Fielding (1989) also reported that strain ML/VII grows faster than strain ML/VIII.

DISCUSSION

A substantial portion of the total phenotypic variance in the four life-history traits examined in this study had a genetic basis. Total genetic variance (V_G) for the Río Fuerte and Río Mayo populations were similar for the three jointly compared traits: 41% and 50% for birth length, 30% and 40% for birth weight, and 10% and 13% for juvenile growth rate, respectively. However, there were differences in the source of V_G between the two populations. For the Río Fuerte strains, V_G was mostly attributable to differences among sibling hemiclones ($V_{G(h)}$) derived from single *P. monacha* foundresses, but for Río Mayo strains, V_G was primarily attributable to differences among foundresses ($V_{G(f)}$). This difference

in partitioning of genetic variances might simply be an artifact of the small number of sexual foundresses (only five) or the small number of hemiclones per foundress (only 2–5) used in this study. Alternatively, genetic variance might be partitioned differently between additive, dominance, and epistatic components in the ancestral *P. monacha* populations. Furthermore, the *monacha* genomes frozen from these sexual populations might interact differently with the isogenic L^i genome used to create our standard-bred strains. Unfortunately, the present data do not allow us to discriminate among these alternatives.

The most striking phenotypic difference between Río Fuerte and Río Mayo synthetic hemiclones was in early reproductive investment. At 63 days of age, egg production in these fish was initiated by only a few hemiclonal females derived from Río Fuerte foundresses. In contrast, many mature eggs were found in Río Mayo synthetic hemiclones. Reproduction in poeciliid fishes is a complex function of size and age, which are themselves correlated (Kallman, 1984). The low reproductive investment of the Río Fuerte hemiclones may be a general characteristic of the *P. monacha* fish in this river, or alternatively, it may characterize only the Agua Caliente (AC) stocks we used, which were taken from the vicinity of a thermal spring.

Substantial genetic variation in birth size, brood size, and juvenile growth rate can be frozen during clonal synthesis. A previous study showed genetic variation in survival and fertility among 33 newly synthesized hemiclones (Wetherington et al., 1987). Because the substitutable paternal *lucida* genome was standardized in the synthetic *P. monacha-lucida* strains used in this study, genetic differences among strains were attributable only to the hemiclonal *monacha* genomes that were frozen from the *P. monacha* gene pool. To determine whether variation for other ecologically relevant traits can also be frozen, we are currently examining the morphology of feeding structures, foraging behavior, and predatory efficiency in both synthetic and natural *P. monacha-lucida* strains. Preliminary data suggest that these traits also exhibit significant interclonal variance.

New hemiclones can arise as siblings from a single sexual foundress or as nonsiblings from independent hybrid events. We found as much genetic diversity among sibling hemiclones as among independent hemiclones. This finding was surprising, because sibling hemiclones are more closely related than are independent hemiclones. In our experimental design, sibling hemiclones are identical by descent, on average, for 50% of their *monacha* alleles and 100% of their *lucida* alleles; thus the coefficient of relatedness (r) is 0.75. In contrast, hemiclonal strains synthesized from independent foundresses are not identical by descent for any *monacha* alleles but share the standardized paternal genome; therefore, $r = 0.50$. Thus, independent hybrid events might not be as critical for generating clonal diversity as previously thought. Single hybrid events can produce considerable clonal diversity among sibling hemiclones.

The present experiments clearly support the two critical assumptions of the frozen-niche-variation model. First, the ability to synthesize many hybridogenetic strains of *Poeciliopsis* demonstrates the feasibility of multiple independent origins of new hemiclonal lineages. Second, each time a new hemiclone arises, a unique genotype is frozen from the gene pool of the sexual ancestors. If the new hemiclone proves to be fertile and if it possesses a nonrecombinant mode of reproduction, it faithfully replicates the combination of life-history traits encoded by its clonal genotype. Multiple origins of new hemiclones from genetically variable sexual ancestors can generate a broad array of new nonrecombinant genotypes on which interclonal selection can act.

The two natural hemiclones studied herein (ML/VII and ML/VIII) differed in juvenile growth rate and early reproductive investment, but strain means for these traits were within the range of phenotypic variation exhibited by the synthetic hemiclones. Therefore, it is likely that the synthetic hemiclones represent the field of variation from which the natural hemiclones were selected. Studies with other clonal organisms have identified discrete life-history patterns that appear to be associated with the use of resources in spatially and seasonally heterogeneous environments (Solbrig and Simp-

son, 1974; Lynch, 1983; Harshman and Futuyma, 1985; Schenck and Vrijenhoek, 1986; Weider, 1987). However, life-history differences might not be necessary for coexistence of strains ML/VII and ML/VIII. Field and laboratory studies of these fishes revealed that strain ML/VIII is less patchy in its local distribution, more aggressive in its interactions, and more insectivorous in its diet (Keegan-Rogers and Schultz, 1984; Schenck and Vrijenhoek, 1986; R. E. Schenck, unpubl.).

Because natural hybridogens can substitute paternal alleles, the results obtained from the standardized ML^i strains might not be indicative of variation in natural populations. Hybridogenetic *Poeciliopsis* are capable of expressing all of the allozyme variation contained in the gene pool of the sexual host species (Vrijenhoek et al., 1977, 1978). Expression of paternal alleles has beneficial as well as detrimental aspects. If ML strains express the genotypes of the locally adapted sexual host species, paternal substitution may facilitate the spread of hybridogens into new habitats (Schultz, 1971). Apparently, *P. monacha-occidentalis* has spread to the northern rivers of Sonora by incorporating cold-tolerant *occidentalis* genomes (Bulger and Schultz, 1982). Alternatively, expression of paternal variation might be detrimental if resource partitioning is important for clonal coexistence. In this case, hemiclonal M genomes should be buffered against paternal sources of variation. If the L genome is not expressed, phenotypic differences encoded by the clonally inherited M genome should facilitate coexistence of clones with one another, as well as with their sexual relatives. The existence of spatial and dietary differences between strains ML/VII and ML/VIII in nature (Schenck and Vrijenhoek, 1986; R. E. Schenck, unpubl.) suggest that their hemiclonal M genomes buffer the phenotype from paternal sources of variance. We are currently examining the contribution of paternal substitution to the phenotypic variance of growth rate by varying the L genome of synthetic and natural ML strains.

Migration can also contribute to clonal diversity in unisexual fish populations. We compared synthetic hemiclones derived from only two sexual *P. monacha* populations: site TA in the Río Mayo and site AC in the Río Fuerte. On average, hemiclones from the Río Mayo had significantly lower birth weights and initiated reproduction at an earlier age than hemiclones from the Río Fuerte. Thus, some genetic differences among hemiclones can arise as a result of geographic variation in the *P. monacha* ancestors. Although we know of only one *P. monacha* population in the Río Mayo, at least five distinct populations of this species have given rise to endemic hemiclones in different tributaries of the Río Fuerte. However, the widespread distribution of some hemiclonal genotypes suggests that migration among tributaries has contributed to local diversity (Angus and Schultz, 1979).

According to the frozen-niche-variation model, the primary means by which a unisexual population can evolve is through recruitment of new clones from an evolving sexual ancestor (Vrijenhoek, 1979*a*, 1984). However, clonal diversity can also arise via mutation. Lynch and Gabriel (1983) showed that, under certain conditions, phenotypic evolution in unisexual populations can occur at a rate similar to that of a sexual population through accumulation of beneficial mutations at loci encoding polygenic characters. In a strictly parthenogenetic lineage of *Daphnia pulex*, two years of clonal divergence were sufficient to generate genetic variation for life-history traits on the order of 1–21% of the total phenotypic variance (Lynch, 1985). However, some believe that mutations are more likely to cause deterioration of clonal lineages, a phenomenon known as Muller's ratchet (Muller, 1964; Felsenstein, 1974; Maynard Smith, 1978; Pamilo et al., 1987; see also Kondrashov [1982, 1984]). The role of mutations in adaptive evolution of unisexual populations needs further investigation.

Although the present experiments demonstrate that *P. monacha* contains considerable genetic variation that can generate clonal diversity, these results cannot be used to estimate heritabilities of life-history traits for *P. monacha*. Dominance and epistatic components will be confounded with additive variation among hemiclones. Also, effects of hybridization might lead to overestimates of the genetic variation in the gene pool of the sexual ancestor. Incompatibili-

ties between different structural and regulatory genes in *monacha* and *lucida* genomes may alter developmental pathways in the hybrids (Ohno, 1969; Philipp et al., 1983; Parker et al., 1985), and thus unisexual hybrids might exhibit unusual phenotypes produced by novel gene interactions. The origin of unisexual hybrids could potentially release a burst of variability that cannot occur in either of the sexual ancestors. This would increase the opportunities for interclonal selection and thereby enhance the potential for ecological diversification of unisexual *Poeciliopsis* populations.

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