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Evaluation of the Rate of Evolution in Natural Populations of Guppies (*Poecilia reticulata*)

David N. Reznick,* Frank H. Shaw, F. Helen Rodd, Ruth G. Shaw

Natural populations of guppies were subjected to an episode of directional selection that mimicked natural processes. The resulting rate of evolution of age and size at maturity was similar to rates typically obtained for traits subjected to artificial selection in laboratory settings and up to seven orders of magnitude greater than rates inferred from the paleontological record. Male traits evolved more rapidly than female traits largely because males had more genetic variation upon which natural selection could act. These results are considered in light of the ongoing debate about the importance of natural selection versus other processes in the paleontological record of evolution.

Natural populations of guppies in Trinidad are found in simple communities replicated in a series of drainages (1) that can be characterized as either high or low predation. In high-predation communities, guppies are found with the pike cichlid, *Crenicichla alta*, and other species of cichlids and characins. Some of these species prey preferentially on large, mature-size classes of guppies (1, 2). Predators are excluded from upstream portions of each drainage by rapids or waterfalls, yielding low-predation communities. Here, guppies co-occur with only the killifish *Rivulus hartii*. *Rivulus* is an omnivore that sometimes preys on guppies. Guppies from high-predation sites experience significantly higher mortality rates than those from low-predation sites (3).

When reared in uniform laboratory conditions, guppies from high-predation sites attain maturity at an earlier age and smaller size than their counterparts from low-predation communities. They also devote more resources to each litter, produce more, smaller offspring per litter, and produce litters more frequently than guppies from low-predation localities (4, 5). All of these differences have a genetic basis (5). The differences in mortality rates among high- and low-predation communities provide a potential mechanism driving life history evolution (3, 6).

We evaluated the role of predators in selecting for these patterns by creating two episodes of directional selection (7, 8). In two streams (tributaries to the El Cedro and Aripo rivers), we found waterfalls below which were guppies in a high-predation

community. Above the falls, there were no guppies and only the small guppy predator *R. hartii*. Guppies from below the barrier waterfall were introduced above the waterfall and hence moved from a high- to a low-predation site.

We evaluated the response to selection in the Aripo River study 11 years after the

introduction (8). The descendants of the transplanted guppies matured at a later age and larger size than the control population found below the barrier waterfall (Table 1). They also produced fewer, larger offspring per litter and devoted a smaller proportion of their consumed resources to reproduction early in life than did guppies from the control population. Their life histories had thus evolved to be similar to those of guppies naturally occurring in low-predation communities.

We evaluated the response to selection by fish in the El Cedro River 4 (7) and 7.5 years after the introduction. Males from the experimental site evolved delayed maturity and a larger size at maturity within 4 years (Table 1). No female life history trait showed a significant change by 4 years. By 7.5 years, females from the experimental site also matured at a later age and larger size than those from the control site (Table 1). No other aspect of the female life history had changed significantly by 7.5 years. Therefore, for this replicate, only some components of the life history have evolved thus far; all changes are in the same direc-

Table 1. Comparisons of guppies from low-predation (experimental) sites with those from high-predation (control) sites, made in controlled laboratory conditions on the second generation of laboratory-reared fish from each locality, following the methods of previous studies (7, 8). Differences between populations that are observed under such conditions are assumed to have a genetic basis and thus provide evidence that evolution has occurred. The means are least square means derived from the statistical comparisons of these values (22) and are adjusted for covariates and unequal sample sizes (7, 8). Sample sizes for the control in the Aripo River were 30 males and 29 females; for the experiment, 24 males and 22 females. In the El Cedro River 4-year assay, the control included 44 males and 43 females; the experimental included 42 males and 43 females. In the El Cedro River 7.5-year assay, the control included 36 males and 40 females; the experimental included 129 males and 110 females. "Response" is the estimated response to selection, or the difference between the control and experimental values. The statistical significance of the response is based on an analysis of variance (7, 8). Significance is based on one-tailed probabilities because the comparative studies provided a basis for predicting how the populations would evolve in response to the introduction. "Rate" is the estimated rate of change in darwins, calculated as $(\ln X_2 - \ln X_1)/\Delta t$, where X_1 and X_2 are the values of the trait at the beginning and end of the time interval, respectively, and Δt is the length of the interval in years. We estimated X_1 and X_2 using the values of the traits for the control and introduction populations, respectively. Gingerich (11) reports that artificial selection experiments attained rates of 12,000 to 200,000 darwins, with a geometric mean of 58,000 darwins. In contrast, the geometric mean rates for the fossil record range from 0.7 to 3.7 darwins, with the estimated rate being inversely proportional to the time interval over which it was evaluated; ns, not significant.

Measurement	1 σ means (standard error)		Response R	Rate (10 ³ darwins)
	Control	Experimental		
<i>Aripo River (11 years or 18.1 generations)</i>				
Male age (days)	48.6 (1.1)	58.2 (1.3)	9.6*	16.4
Male size (mg)	67.5 (1.6)	76.1 (1.9)	8.6*	10.9
Female age (days)	85.6 (2.2)	93.5 (2.6)	7.8*	8.0
Female size (mg)	162.3 (6.4)	189.2 (7.4)	26.8*	13.9
<i>El Cedro River (4 years or 6.9 generations)</i>				
Male age (days)	60.6 (1.8)	72.7 (1.8)	12.1*	45.0
Male size (mg)	56.0 (1.4)	62.4 (1.5)	6.4*	27.1
Female age (days)	94.1 (1.8)	95.5 (1.8)	1.4 (ns)	3.7
Female size (mg)	116.5 (3.7)	118.9 (3.7)	2.4 (ns)	5.1
<i>El Cedro River (7.5 years or 12.7 generations)</i>				
Male age (days)	47.3 (1.1)	52.5 (0.6)	4.9	13.9
Male size (mg)	71.5 (1.1)	74.4 (0.7)	2.9*	5.3
Female age (days)	75.8 (1.8)	80.4 (1.0)	4.6†	7.9
Female size (mg)	141.8 (5.1)	152.1 (3.2)	10.3*	9.3

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* $P < 0.05$. † $0.05 < P < 0.10$.

tion as in the Aripo experiment.

We analyzed the response to selection for just the age and size at maturity in males and the age and size at first parturition in females because these traits responded to the manipulation in both experiments. For each trait, we estimated the response to selection (R) as the difference between the mean of the trait for lab-reared guppies from the control and experimental populations (9) (Table 1). We then used R to quantify the relative rate of evolution in terms of darwins (10, 11). The estimated rates of phenotypic evolution range from 3700 to 45,000 darwins (Table 1). They are similar in magnitude to rates that have been obtained by artificial selection and four to seven orders of magnitude greater than those observed in the fossil record (11).

One notable result in the 4-year assay for

the El Cedro River is the 10-fold difference between the sexes for the rate of change in the age at maturity and a 3- to 5-fold difference for the size at maturity (Table 1). When the same comparisons are made in the 7.5-year assay, the relative rate of change ("Rate" in Table 1) for male age was still higher than that for female age, but the difference was far less pronounced; the absolute amount of change (R) was approximately equal. Males appear to have evolved to an end point within 4 years, so the 7.5-year result averages the same amount of change over a longer interval of time. On the other hand, females continued to evolve over the entire 7.5-year period. Differences in the estimated rates of change can also be attributed to changes in the experimental conditions between the 4- and 7.5-year assays (12).

The results for the Aripo River comparison are similar to those for the 7.5-year assay on the El Cedro; the relative rate of change in male age at maturity was twice the rate of female age, and the absolute rate of change was 23% greater (Table 1). The rate of change in size was slightly less for males than for females. If the pattern of evolution was similar to that in the El Cedro River, with males rapidly evolving to a new value then leveling off before the 11-year assay whereas the females continued to change, then this comparison would also tend to underestimate the rate of evolution in males relative to females.

Inferences about the strength of natural selection require estimates of generation time (13) and the genetic variance-covariance matrix (Table 2) (14), which quantifies the genetic variation available for a response to natural selection. The heritabilities of male age and size at maturity were high and significantly greater than zero in all four estimates (Table 2). These values are exceptionally high for life history traits: a more typical value is between 0.1 and 0.2 (15). The corresponding values for age and size at first parturition in females were lower, with the only significant value being for the age at first parturition in females from the El Cedro River (Table 2). One possible explanation for the difference in the heritabilities of male and female traits is that some of the genetic variation for these traits in males is associated with the Y chromosome, as reported for other species of Poeciliid fishes (16).

The genetic correlations between age and size at maturity (males) and age and size at first parturition (females) were very high for the Aripo River study and moderately high for the El Cedro River study. They were significant for both male comparisons (Table 2). The absence of significance for equally high correlations in the female data is attributable to the lower heritabilities for these traits in females. The genetic basis of age and size is thus broadly overlapping. Selection on one trait strongly influences the evolution of the other (9, 14).

The selection gradient β (Table 3) estimates the change in relative fitness as a function of the change in a trait as other traits are held constant. A positive coefficient implies that fitness increases as the value of the trait increases, whereas a negative coefficient implies that fitness increases as the value of the trait decreases (17). The bivariate β coefficients for males on the Aripo River indicate strong direct selection for increased age at maturity but weaker selection for decreased size at maturity (Table 3). The values for age and size increased together over time because of the positive genetic correlation between them

Table 2. Heritabilities and genetic variance-covariance matrices G . We used restricted maximum likelihood estimation (23) to estimate the genetic variance and covariance for the age and size at maturity. Data were derived from the same experiments that were used to evaluate the response to selection (Table 1). Heritabilities and genetic correlations are reported, with genetic variances and covariances in parentheses (24); ns, not significant.

	Males		Females	
	Age	Size	Age	Size
<i>Aripo River</i>				
Age	0.89* (0.0177)	0.91* (0.0169)	0.08 (ns) (0.0006)	1.68 (ns) (0.0040)
Size		0.88* (0.0178)		0.09 (ns) (0.0093)
<i>El Cedro River</i>				
Age	0.59* (0.0167)	0.52† (0.0077)	0.45† (0.0031)	0.55 (ns) (0.0018)
Size		0.998* (0.0135)		0.09 (ns) (0.0037)

* $P < 0.01$. † $P < 0.05$.

Table 3. Estimates of the linear selection gradients and standardized coefficients of selection for the two introduction experiments. The selection gradients $\beta = G^{-1}R$, where G^{-1} is the inverse of the additive genetic variance-covariance matrix and R is the vector of responses to selection (Table 1). These values were divided by the estimated number of generations during the experiment and multiplied by the phenotypic standard deviation estimated from the control population to yield the standardized coefficients reported here. Values are not reported for the El Cedro females at the end of the 4-year assay because there were no significant changes in the traits in question at that point. Coefficients of selection S were estimated as $S = P\beta$, where P is the phenotypic variance-covariance matrix. All computations were based on log-transformed data. Confidence intervals for β and S , given in parentheses, were calculated by parametric bootstrap. If the lower confidence limit is greater than zero, then the coefficient is significantly greater than zero (25).

Population	Parameter	β	S
<i>Aripo River</i>			
Males (univariate)	Age	0.068 (0.042, 0.180)	0.072 (0.050, 0.156)
	Size	0.065 (0.046, 0.111)	0.046 (0.037, 0.068)
Males (bivariate)	Age	0.193	0.201
	Size	-0.127	0.023
Females (univariate)	Age	0.290 (>0.045)	0.375 (>0.055)
	Size	0.013 (>0.003)	0.191 (>0.051)
<i>El Cedro River: 4-year assay</i>			
Males (bivariate)	Age	0.220 (0.019, 1.31)	0.310 (0.094, 1.91)
	Size	0.071 (-0.23, 0.369)	0.138 (-0.003, 0.282)
<i>El Cedro River: 7.5-year assay</i>			
Males (univariate)	Age	0.057 (-0.034, 0.883)	0.086 (-0.004, 0.618)
	Size	0.001 (-0.106, 0.103)	0.030 (-0.027, 0.086)
Females (univariate)	Age	0.172 (0.079, 0.659)	0.091 (0.043, 0.328)
	Size	0.281 (>0.032)	0.267 (>0.030)

(Table 2). The β coefficients for Aripo River females and El Cedro River males imply strong direct selection for increased age at maturity but weaker direct selection for increased size at maturity. Finally, the 7.5-year assay of El Cedro females indicates that direct selection on size was somewhat stronger than direct selection on age.

Coefficients of selection S are an alternative way of characterizing the process of natural selection (Table 3): S estimates the covariance between a trait and fitness. It incorporates the effects of direct selection on that character and the indirect effects of selection on correlated characters. When rates of evolution are compared, a given trait may evolve slowly because the intensity of selection on it is low, its heritability is low, or its rate of change is affected by selection on a correlated trait. We can use these coefficients, in combination with the other classes of information, to evaluate the causes of the apparent differences in the rate of evolution of males and females on the El Cedro River (Table 1). The rate of evolution tended to be higher in males (Table 1), possibly because males had higher heritabilities for these traits (Table 2). The most instructive comparison is between the 4-year values for males and the 7.5-year values for females because this avoids the bias caused by males reaching a plateau at 4 years. The coefficients of selection for age are higher for males than for females, but the magnitude of the difference (0.310 for males versus 0.091 for females) is smaller than the difference in the rate of evolution or the response (Table 3 versus Table 1). Similarly, in the Aripo River, the rate of change in male age at maturity is greater than that for female age, but the coefficient of selection is much higher for females. It thus appears that the differences in rate between the sexes are attributable to the differences in the heritabilities of the traits, with females evolving more slowly because of their lower genetic variance for age and size at maturity, rather than because of weaker selection on female traits.

If evolution can be so fast, why does it appear to be so slow in the fossil record? First, evolution is only sustained in response to a changing environment (18); when a new optimum is attained, no more evolution is expected [El Cedro River males in this study (19)]. Second, if environmental conditions vary erratically, so will patterns of evolution, as seen in Galápagos finches (20). Evaluating evolution with the fossil record averages across intervals of no change, intervals of rapid change, and possibly includes reversals in the direction of change, yielding an estimate of rate averaged over the entire interval (11). The net effect could well be no measurable change

in morphology, or "stasis." On the other hand, sustained directional selection can support far more rapid directional change than seen in the fossil record.

The evidence from studies of microevolution (19, 20) bears on the current debate over micro- versus macroevolution and the patterns of change recorded in the fossil record. In the fossil record, there is a well-established pattern of periods of little or no change (stasis) punctuated by brief intervals of rapid change associated with the origins of new taxa. Some have argued that selection among individuals within populations (natural selection) cannot account for these large-scale trends in evolution (21). Specifically, Gould and Eldredge argue for the necessity of bursts of speciation followed by species selection to sustain the rapid change associated with punctuations in the fossil record (21). Our work cannot address the efficacy of mechanisms other than natural selection, but it extends our understanding of what is attainable through this process. It is part of a growing body of evidence that the rate and patterns of change attainable through natural selection are sufficient to account for the patterns observed in the fossil record (18).

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- The 4-year assay was conducted at two levels of food availability. There was an interaction between food availability and population, such that the difference between the control and experimental sites was more pronounced at low levels of food availability (7). The 7.5-year assay was conducted at a single, high level of food availability, which resulted in compressed differences in male size and age, as one would predict from the interaction found in the 4-year assay. An assay run 9 years after the introduction with conditions similar to the 4-year assay produced results that were similar but without the interaction
- between locality and food availability. Therefore, we conclude that male size at maturity had plateaued 4 years after the introduction was made. The comparisons between the control and introduction populations in the 9-year assay were, respectively, as follows [mean(1 standard error)]: male age, 57.4(1.5) versus 67.6(1.1) days; male size, 62.4(1.5) versus 70.4(1.1) mg; female age, 84.0(1.5) versus 86.2(1.2) days; and female size, 130.9(3.7) versus 129.2(3.0) mg. The control and experimental populations differed significantly for male age and size at maturity. The probability that the female ages differed was marginally significant ($P = 0.08$), whereas the female sizes did not differ significantly.
- Guppies breed throughout the year and have overlapping generations (2). We estimated generation time T using the life table method [R. E. Ricklefs, *Ecology* (Chiron, Newton, MA, 1973)], which is based on estimates of birth and death schedules in natural populations. We estimated mortality rates from mark-recapture experiments on guppies from seven low-predation localities, including the Aripo and El Cedro experimental sites (3). Because the life table method requires age-specific survival, we estimated the ages of individuals in different size classes using growth rate equations derived for the same fish in the mark-recapture experiments (3) based on their sizes at the beginning and end of the recapture interval. We estimated size-specific fecundity with dissections and embryo counts from females collected from these same localities, plus from additional collections (4). We estimated the frequency of reproduction from laboratory studies, conducted at temperatures and food availabilities similar to those in the field (5). With this information, we estimated the mean generation time of fish in low-predation localities to be 210 days, or 1.74 generations per year.
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- Guppies from the experimental site on the El Cedro River (7.5-year assay) and the control site on the Aripo River (11-year assay) were reared in a three-generation, extended-pedigree, paternal half-sibling design. Paternal half-sibling matings were performed for the first and second generations of lab-reared descendants from each locality. The dependent variables (age and size at maturity in males and females) were quantified for the second and third laboratory-born generations. Sample sizes for the Aripo River were 16 wild-caught sires and 30 second-generation, laboratory-reared dams for the first generation cross, yielding data on 49 daughters and 57 sons. Of these daughters, 39 were used as dams for the next generation. They were mated to 23 wild-caught sires that were unrelated to individuals from the first two generations. These crosses yielded data for 115 daughters and 114 sons in the third generation. Sample sizes for the El Cedro River were 15 wild-caught sires and 40 lab-reared dams for the first generation, yielding data for 78 daughters and 97 sons. Twenty-eight daughters from this generation were mated to 11 unrelated, wild-caught males, yielding data for 73 daughters and 85 sons in the third generation. Sexes were analyzed separately because the genetic variance-co-

variance matrices indicated strong sex linkage for genetic variation in age and size at maturity.

25. The P and G matrices were sampled from a multivariate normal distribution using the estimates of P and G as means and their sampling variance-covariance matrix as variance. The values for R , the vector of responses to selection, were sampled from a multivariate normal distribution with means equal to R (Table 1) and a sampling variance-covariance matrix obtained from the multivariate analysis of variance that compared the control and experimental populations. Vectors of 1000 values of β and S were calculated, and the distributions of β and S were inferred from these. Some of the sampled G matrices were not positive definite and hence could not be inverted. In these cases, the implied estimates of β and S were either positive or negative infinity, depending on the associated value of R , and were retained as such in our set of 1000 β and S vectors. If more than 25 of the 1000 estimates were positive

infinity, the confidence interval was deemed to have no upper bound. Consequently, there are four different ways of reporting the results, depending on the nature of the G matrix and the results of the 1000 simulations: (i) One-sided confidence intervals are reported when singularity of more than 2.5% of the sampled G set the upper confidence limit at infinity. The one-sided value equals the 25th value of 1000 simulations (rank ordered from smallest to largest) and hence is equivalent to the lower bound of a 95% confidence interval. (ii) Confidence intervals with lower and upper bounds are reported when the simulations allowed us to set both an upper and lower limit to the distributions. The reported values are the 25th and 975th values of the 1000 simulations. (iii) Bivariate estimates of β and S were only possible for the males from both experiments. The only data set for which we could estimate the confidence limits for the bivariate analysis was the El Cedro males, for both the 4- and 7.5-year results. Here the limits mark the

two-dimensional range of the 950 simulated values closest (in Euclidean distance) to the estimates. (iv) Standard errors were not estimable for the bivariate estimates of β and S for males from the Aripo River because of the near singularity of the G matrix. In this case, the difference between β values for age and size at maturity are more pronounced in the bivariate analysis, which takes the high genetic correlation between them into account, than in the univariate analysis.

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Local Hormone Networks and Intestinal T Cell Homeostasis

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Neuroendocrine hormones of the hypothalamus-pituitary-thyroid axis can exert positive or negative immunoregulatory effects on intestinal lymphocytes. Small intestine epithelial cells were found to express receptors for thyrotropin-releasing hormone (TRH) and to be a primary source of intestine-derived thyroid-stimulating hormone (TSH). The gene for the TSH receptor (TSH-R) was expressed in intestinal T cells but not in epithelial cells, which suggested a hormone-mediated link between lymphoid and nonhematopoietic components of the intestine. Because mice with congenitally mutant TSH-R (*hyt/hyt* mice) have a selectively impaired intestinal T cell repertoire, TSH may be a key immunoregulatory mediator in the intestine.

The intestine constitutes an important host barrier to foreign antigen entry. This is reflected in the extensive complexity of the intestinal immune system, which is characterized by novel lymphocyte subsets (1) and by bidirectional intercellular communication between lymphocytes and epithelial cells (2). We recently demonstrated a role for neuroendocrine hormones in the development and regulation of intestinal T cells, in particular the TCR $\alpha\beta$, CD8 $\alpha\beta$ intraepithelial lymphocytes (IELs) (3, 4). Here, we describe a pathway of hormone synthesis and use mediated by thyrotropin (TSH), which links components within the small intestine and is used in local IEL immune regulation.

Freshly extracted small intestine cells (5) were characterized by flow cytometric analysis (6, 7). Populations of epithelial cells and lymphocytes were enriched to >97% purity, as verified by reactivity with monoclonal antibody (mAb) G8.8, a marker of murine epithelial cells, and a mAb to the CD45 leukocyte-common an-

tigen (LCA), a marker of nucleated hematopoietic cells (8–10) (Fig. 1). Purified IELs and epithelial cells were assayed for expression of the TSH β gene by reverse transcriptase-polymerase chain reaction (RT-PCR) (11). This yielded PCR products of the predicted size from both the IEL-enriched fraction and the epithelial cell-enriched fraction (Fig. 2), which were confirmed (12) by DNA sequence analyses (11). Because TSH production is controlled in part by TRH, purified IELs and epithelial cells were assayed for TRH receptor (TRH-R) gene expression by RT-PCR (11). A PCR product of the anticipated size was obtained from intestinal epithelial cells, whereas no PCR product was obtained from intestinal IELs (Fig. 2). The PCR product identified in epithelial cells was verified by reamplification using a nested upstream TRH-R primer located within the amplification region (11); this resulted in a single band of the anticipated 146-base pair size with sequence homology to murine TRH-R (11, 13).

Secretion of TSH by IELs and epithelial cells was measured by enzyme-linked immunosorbent assay (ELISA) (14) using supernatants of cells cultured with and without TRH according to published protocols (15).

Although TSH β was produced by both IELs and epithelial cells, epithelial cells produced considerably more TSH β than did an equivalent number of IELs (Fig. 3A), with maximal secretion occurring at 10^{-7} to 10^{-9} M TRH. This secretion pattern, including the high-dose prozone effect of TRH, is similar to previous reports of TRH-induced TSH secretion (15). TSH was detected in epithelial cell supernatants as early as 1 hour after stimulation (Fig. 3B), implying

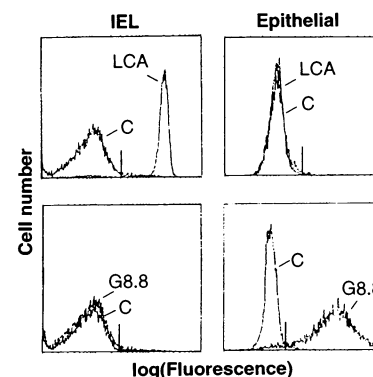


Fig. 1. One-color flow cytometric analyses, showing reactivities of LCA and epithelial cell antigen (G8.8) mAbs for IEL and epithelial cell populations. C, isotype-species-matched control mAb.

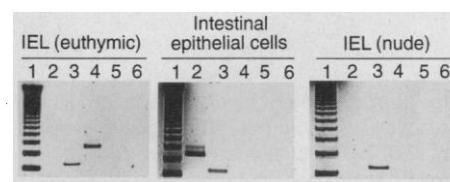


Fig. 2. RT-PCR analyses of gene expression in intestinal IELs and intestinal epithelial cells from euthymic mice and intestinal IELs from congenitally athymic nude mice. Lane 1 (in each panel), base pair standards; lanes 2 to 4, RT-PCR-amplified gene products for TRH-R, TSH β , and TSH-R, respectively; lane 5, primer controls in the absence of cDNA templates; and lane 6, controls for DNA contamination of RNA (that is, PCR analyses of RNA preparations after treatment with deoxyribonuclease but before cDNA construction).

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