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Evolution from transplants between genetically distinct populations of freshwater snails

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Abstract

In July, 1979, a series of 12 transplant experiments was begun using *Goniobasis proxima* (Say), a freshwater snail living in highly isolated populations in the southern Appalachians. At each site, 500 native snails were removed and replaced with 500 snails from a second population in which different alleles were fixed at one or two enzyme loci. The sites were resampled in 1983 to identify populations where the introduced genome had in fact become established. Only two of the twelve introductions were successful. Introduced genes have spread about 15–20 meters per year upstream and 5–10 meters per year downstream. Considering observed population sizes and age-specific survivorships, it appears that the observed frequencies of the introduced genomes at both sites are significantly greater than expected from simple diffusion. A selective advantage for the introduced snails or their F_1 progeny seems likely. This implies that the genetic differences between *G. proxima* populations may in some cases be due to random processes.

Introduction

Goniobasis proxima (Say) is a prosobranch gastropod widespread in small, softwater creeks in the piedmont and mountains from Virginia to Georgia. Population densities tend to decrease as stream size increases, so that each tributary has its own discrete population of snails. The ready availability of scores of conspecific populations, each of high density and easily sampled, has made *G. proxima* an unusually useful subject for evolutionary study (Dillon & Davis, 1980; Dillon, 1982, 1984a, 1984b).

Interpopulation divergence is very high in *G. proxima*, and heterozygosity unusually low. In a study of 25 populations from four adjacent drainages in Virginia, North Carolina, and Tennessee, Dillon (1982, 1984a) found different populations sharing no alleles at as many as seven of eight polymorphic loci examined. Evidence suggested that gene flow between even closely neighboring populations has been negligible for thousands of generations. Here I report the results of several transplant experiments involving pairs of *G. proxima* populations carrying 'marker' alleles at enzyme loci.

It is difficult to predict the genetic consequences of artificially introducing organisms from one isolated population into another. Certainly one possibility is that any introduced genes may simply diffuse throughout the recipient population, neither being selected for nor against. But if two populations have adapted to different environments over generations of isolation, for example, genomes transferred from one to the other may confer reduced fitness. Reduced fitness could also result from the disruption of 'coadapted gene complexes' in hybrids (Dobzhansky, 1950). So a second possibility is that introduced genes may be at a selective disadvantage and that their frequencies will decrease. A third possibility is that the frequencies of introduced genes may increase due to some selective advantage. If populations are indeed highly isolated, even truly superior alleles may be restricted to the

small area where they arise. The frequencies of such alleles may increase rapidly if artificially introduced into a second population. Foreign genomes may also be at a selective advantage if introduced into populations so isolated that inbreeding depression has become a problem.

There has been little research to suggest which of these three possibilities may pertain to natural populations. Selection has been commonly observed after artificial introductions into previously unoccupied areas or environments (reviews by Manly, 1985; Endler, 1986). Jones and Parkin (1977) and Greenwood and Parkin (1984) have estimated the relative survival rates of shell color morphs in transplanted populations of land snails using mark/recapture, although these experiments included only single generations and reproduction was not monitored. The transplant experiments of Dubinin and Tiniakov (1946) and Barker and East (1980) involving *Drosophila* were complicated by the lack of data regarding native population sizes.

Results obtained from several germane experiments have been diverse. Jain and Rai (1980) found that artificially introduced colonies of wild oats tended to have lower survivorships than native colonies. But the frequency of an introduced wing coloration gene increased initially, then stabilized, in a population of moths in England (Sheppard & Cook, 1962; Ford & Sheppard, 1969). Halkka *et al.* (1975) also introduced color polymorphisms into several natural populations of spittlebugs. When the recipient populations were large, they found that introduced genomes tended to be selected against, and gene frequencies tended to return to their pre-introduction values. But no marked selection in any direction was evident in one experiment where the recipient populations of spittlebugs was rather small.

Methods

Preliminary study

Four *G. proxima* populations from southern Virginia were examined in the preliminary study, designated HILL, IND, ROCK, and CRIP. As shown on the map of Dillon and Davis (1980), none of these populations is any closer than 30 km overland from any other. Table 1 shows that these populations were found to be fixed for distinct alleles at the octopine dehydrogenase locus (*odh*, EC 1.5.1.11) and/or the arylesterase 1 locus (*est*, EC 3.1.1.2). Dillon (1986) has established that inheritance is Mendelian at these two loci, and that the loci are unlinked. Octopine dehydrogenase isozymes were resolved using Poulik's (1957) discontinuous buffer system, and arylesterase was examined using a tris-EDTA-borate pH 8 buffer. A complete account of electrophoretic methods, together with recipes for buffers and stains, is given in Dillon (1982, 1985).

All possible reciprocal transplants between these four populations were made on July 11–13, 1979. Three separate creeks at each locality were selected as points of introduction. Then 500 snails at least one year old were collected at each point of introduction, and a like number of snails from one of the three other populations were released. Snails were transported in insulated containers, and in no case was any sample out of the creek for more than a few hours. Locality data for the 12 introduction areas are given in Dillon (1982).

In late May and early June of 1983, the 12 introduction areas were revisited. At least four samples of 30 one-year-old snails each were collected from each area. (In a sample of 30 diploid individuals there is a 95% chance of drawing at least one individual possessing an allele with a frequency of 0.05 or greater in the population at large). Samples were typically taken from the 10–20 meter stretch of stream around the point of release, 20 m downstream, 20 m upstream, and 40 m upstream. There was some variation in the number of sites, their size, and their placement, depending on snail densities and physical features of the stream. I then used

Table 1. Gene frequencies at two loci in the four preliminary transplant study populations.

Allele		HILL	IND	ROCK	CRIP
odh	109F		1.00		
	110				1.00
	112	1.00		1.00	
est	100			1.00	
	103	1.00	1.00		1.00

starch gel electrophoresis to determine whether any of the alleles introduced in 1979 were present in the one-year-old generation of 1983.

Only two of the 12 introductions were found to be successful. Snails from the IND population of Brush Creek (a tributary of the New River) near Independence, Virginia, were successfully introduced into two other populations, the HILL population of Little Snake Creek (also a tributary of the New River) near Hillsville, Virginia, and the ROCK population from Rock Castle Creek (a tributary of the Dan River) near Woolwine, Virginia. Table 1 shows that the HILL and IND populations are fixed for different alleles at the *odh* locus. The ROCK and IND populations are also fixed for different *odh* alleles, and are fixed for different *est* alleles as well. The ROCK/IND and HILL/IND populations, henceforth called 'RI' and 'HI', were subjects of further detailed study.

To determine the extent of the migration of introduced snails and their progeny, additional sample sites were established at the RI and HI populations in June, 1983. Thirty one-year-old snails were collected from several 20 m sites both upstream and downstream from the four initial sites. These were examined electrophoretically just as in the initial screenings. Only the stretch of stream inhabited by snails bearing introduced genes was considered in estimates of population size, survivorship, and gene frequency.

Population sizes

A separate mark-recapture study was performed at each site where snails with introduced genes were collected. Experimentation showed that snails could be marked with colored fingernail polish without apparent adverse effects. Then a large number of snails at least one year old were collected from each site, marked with a dab of color-coded nail polish, and returned to the stretch of stream whence they came. Marking was confined to the underside of the shell as a precaution against selective recapture. A second collection was made at each station three days later, and all marked and unmarked snails tabulated. Estimated population size and the standard error of that estimate were calculated using Bailey's (1951) modification of the Petersen method (Begon, 1979). The number of marks and recaptures were if at all possible over 100. Robson and Regier (1964) have shown that if N is approximately 1000, the number of individuals marked and the total recaptured need to be about 140 for a 95% probability of obtaining an estimate with a 0.5 accuracy.

Age structure

The life history of *Goniobasis* is fairly well known (Dazo, 1965; Stiven & Walton, 1967; Mancini, 1978; Dillon, 1982). Sexes are separate, with the number of females equal to or exceeding the number of males in the population. Females begin to lay eggs in the spring, sometimes as early as March, and typically egg-laying is over by July. Eggs develop directly into juvenile snails and hatch after about two weeks. The snails reach maturity in about a year, but females do not lay their first eggs until the spring two years after their birth. *Goniobasis proxima* occasionally lives 4–5 years.

One well-established method of estimating recruitment and survivorship in freshwater snail populations is the study of size classes (Calow, 1978; Jokinen, 1985). This technique is particularly useful in *G. proxima*, where the fairly restricted egg-laying season results in distinct annual cohorts. Thus a single site was chosen to represent the RI and HI populations, where visual collecting was easiest and underbrush and obstacles at a minimum. Then a slow, thorough search was conducted up the 20 meter stretch of stream, collecting all snails aged one year and older. Young-of-the-year snails, aged only a few months in June, were easily excluded from these collections by their very small shells. Shell height, considering the length of the last three whorls only (due to apical erosion) was measured using calipers (Dillon, 1984b). The largest snails were cracked open and examined for evidence of parasitic gigantism. Results were graphed and inspected to estimate the relative proportions of the year classes.

Expected gene frequencies

Dillon (1986) has shown that female *G. proxima*

mate at most once a year and store sperm for long periods. I shall assume here that females are inseminated only once in their lifetimes, prior to their second July, and will show later that this assumption is justified.

I also assume, for the present, that the number of snails in each study area, N, has remained constant since the summer of 1979. Then since 500 native snails one year old and older were removed and replaced with 500 introduced snails of comparable ages, the immediate allele frequencies would be:

$$p = 500/N, \; q = 1-500/N$$

where p is the initial frequency of the introduced allele or alleles and q is the frequency of the native alleles at the same loci.

Next I assume that population age structure in the spring and summer has remained constant from 1979 to 1983 and that fecundity does not vary with age. These would seem to be rather unrealistic assumptions, although Mancini (1978) found that annual fecundity in 53 *G. semicarinata* of varying ages ranged only from 29 young/female to 55 young/female.

Let the summer frequency of one-year-old snails be J_1, the frequency of two-year-olds be J_2, the frequency of three-year-olds be J_3 and the frequency of snails over three years old be J_4, such that $J_1 + J_2 + J_3 + J_4 = 1$. For notational convenience, let:

$$X = J_2/(1-J_1)$$
$$Y = J_3/(1-J_1)$$
$$Z = J_4/(1-J_1)$$

Thus X, Y and Z are the frequencies of the three reproducing age classes relative to all reproductives.

Consider first the population of egg-laying females in the spring of 1982, when the generation under study was born. The snails one year old and over in the summer of 1979, when the introductions were made, were in 1982 four years old and older. They laid a proportion Z of the eggs in 1982, of which p were homozygous for introduced alleles and q homozygous for native alleles (recall my assumptions regarding female mating). Three-year-old females laid a proportion Y of the eggs in 1982. These females were only two or three months old when the introductions were made in 1979, and were thus all homozygous native. No snails this small were introduced. In 1980 a proportion $p(1-J_1)$ of the females of this cohort were fertilized by introduced males and thus laid heterozygote eggs in 1982, while $J_1 + q(1-J_1)$ were fertilized by native males in 1980 and laid eggs homozygous for native alleles in 1982.

Finally, two-year-old females laid a proportion X of the eggs in 1982. They were born in 1980, the spring after the introduction, and thus a fraction p are homozygous introduced and q are homozygous native. In 1981, a fraction J_2 of these females mated with males from the homozygous native generation ahead of them. Thus J_2p laid heterozygous eggs and J_2q laid homozygous native eggs in 1982. The remaining fraction of the females born in 1980, $1-J_2$, mated in 1981 with a population of males of which p were homozygous introduced and q were homozygous native. Thus $p^2(1-J_2)$ laid homozygous introduced eggs in 1982, $2pq(1-J_2)$ laid heterozygous eggs, and $q^2(1-J_2)$ laid homozygous native eggs. Summing all possible outcomes:

$$E_{II} = Zp + Xp^2(1-J_2)$$
$$E_{IN} = Yp(1-J_1) + XJ_2p + X2pq(1-J_2)$$
$$E_{NN} = Zq + YJ_1 + Yq(1-J_1) + XJ_2q + Xq^2(1-J_2)$$

Where E_{II}, E_{IN} and E_{NN} are the expected frequencies of snails homozygous for all introduced alleles, heterozygous, and homozygous for native alleles respectively. Notice that $E_{II} + E_{IN} + E_{NN} = 1$. There is no opportunity for snails of any other genetic constitution (e.g., heterozygous at one locus, homozygous at a second) to occur in the one-year-old age class of 1983, even though the loci are unlinked.

Results

Gene flow

Results of the 1983 survey are shown in Figure 1. The introduced genes have spread remarkably long distances since 1979, considering the strong currents and the difficulty of the terrain. In the RI population, migration has averaged 15–20 m/y upstream and 5–10 m/yr downstream. The figures are similar in the HI population: 12–17 m/yr upstream and 2–7 m/yr downstream.

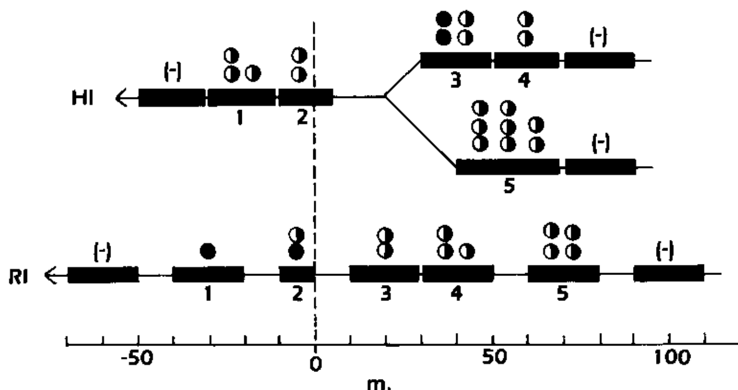

Fig. 1. Sampling results from the RI and HI populations. Sample sites are shown as darkened blocks on diagrammatic streams flowing from right to left. Of the 30 snails collected at each site, circles show the number that were heterozygous (half-darkened) and homozygous (fully-darkened) for introduced genes. The numbers below each site correspond to Tables 2 and 3.

Population sizes and observed genotype frequencies

Tables 2 and 3 show results from the mark – recapture studies performed on snails one year old and older in the RI and HI populations. No movement of marked individuals between sites was observed in either case over the three-day period.

Estimated snail densities at the five RI sites were comparable, ranging from 76 to 126 snails per linear meter of stream, so the results were pooled to obtain an estimate of population density over the entire 90 m sampled. The 90 m population size, 9538 ± 2274 (two standard errors), was extrapolated to 12720 ± 3032 for the 120 meters of stream inhabited by snails bearing the introduced genes. The observed frequencies of heterozygotes and homozygotes for introduced genes, O_{IN} and O_{II} respectively, were also estimated by pooling results from the five sites. Of 150 snails sampled, 10 were doubly heterozygous and 2 were doubly homozygous for the introduced alleles, so $O_{II} = 0.013$ and $O_{IN} = 0.067$ for the RI population.

In contrast, estimated snail densities were significantly heterogeneous in the five HI sites (Table 3). Thus results at the five sites could not be simply pooled to obtain an overall density or genotype frequencies. First the estimated mean and standard error for the number of snails inhabiting the Y-shaped

Table 2. Results of the mark-recapture study at the RI population.

Site	Length (m)	No. marked	Recap. per total	Est. N. (se)	Density (N/m)
1	20	130	11/214	2329 (628)	116
2	10	105	10/131	1260 (348)	126
3	20	120	15/264	1988 (467)	99
4	20	110	9/198	2189 (643)	109
5	20	130	19/234	1528 (319)	76
Pooled	90	595	64/1041	9538 (1137)	106

Table 3. Results of the mark-recapture study at the HI population.

Site	Length (m)	No. marked	Recap. per total	Est. N. (se)	Density (N/m)
1	20	100	11/267	2223 (605)	112
2	15	250	14/700	11683 (2889)	779
intersite	45	–	–	14400 (3645)[b]	320[a]
3	20	120	16/337	2386 (548)	119
4	20	100	9/189	1900 (558)	95
5	30	70	5/160	1878 (697)	63
Total				34480 (8942)	

[a] Average density of adjacent sites 2, 3, & 5.
[b] Back-calculated from density as estimated above.

'intersite' area were obtained by averaging values from the three adjacent sites. Then the population size in the area inhabited by snails bearing the introduced gene, 34480 ± 17884 (two s.e.) was obtained by totalling estimates from the six sites. The corrected overall frequencies of the two introduced genotypes were obtained as the sum of the j genotype frequencies weighted by population sizes at each site:

$$O_{IN}' \text{ or } O_{II}' = \sum_{j=1}^{5} (h_j)(n_j)/20070$$

where h_j was the frequency of the heterozygotes or introduced homozygotes in the jth site, 20070 was the estimated number of snails inhabiting the j sites, and n_j was the estimated number of snails inhabiting the jth site. (Correction for the intersite area was unnecessary). The resulting genotype frequencies for the HI population, $O_{IN}' = 0.089$ and $O_{II}' = 0.0079$, were lower than the uncorrected values, $O_{IN} = 17/150 = 0.113$ and $O_{II} = 2/150 = 0.013$. This is because introduced genomes tended to be more common in the sparsely-populated upstream sites.

Age structure

Figure 2 shows the distribution of shell lengths in snails one-year-old and over from the RI and HI populations. Three fairly distinct size classes are apparent in these data, corresponding to snails that are a few months over one, two, and three years of age. In addition, both populations contained a few very large snails. Since no evidence of parasitism was seen in these individuals, they are presumably aged four years and over. Values of J_1, J_2, J_3 and J_4 as applied to the calculation of expected gene frequencies are given in Table 4.

Expected genotype frequencies

Assuming the number of snails inhabiting the RI study area has remained constant, the substitution of 500 IND snails for 500 ROCK snails would, as a best estimate, produce an initial frequency of 0.039 for the introduced genes. Similarly, replacing 500 HILL snails with 500 IND snails would produce an immediate frequency of 0.014 for the introduced genes in the HI study area. Then if selection is absent

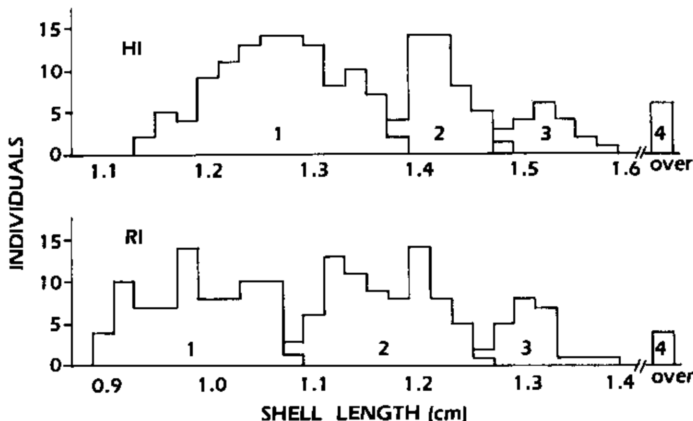

Fig. 2. Distribution of shell lengths (last three whorls) in snails from the HI and RI populations. Putative age in years is given for the size classes. Young-of-the-year snails have much smaller shells and were excluded.

Table 4. Age structure in sample populations HI and RI.

Age	HI		RI	
(years)	No.	Freq. (J)	No.	Freq. (J)
1	112	0.619	80	0.435
2	45	0.249	76	0.412
3	18	0.099	24	0.130
4+	6	0.033	4	0.023
Totals	181	1.000	184	1.000

and if spring and summer age structures and fecundities in the RI population have remained constant, the expected frequency of heterozygotes and homozygotes for introduced alleles in the one-year-old generation of 1983 would be 0.049 and 0.0023, respectively. At the HI population, similar assumptions would lead to expected frequencies of 0.0014 for introduced homozygotes and 0.018 for heterozygotes.

The observed frequencies of introduced genomes were generally much greater than these 'best estimates' of their expectation (Table 5). Binomial tests (Siegel, 1956:37) suggest that the excesses of homozygous introduced snails in both the HI and RI populations, and of heterozygotes in the HI population, are significant. The excess of introduced genomes in the HI population was sufficiently striking to prompt an examination of my assumptions regarding population size and age structure.

The best fit between observed and expected genotype frequencies obtainable by manipulating values of J would occur when $E_{II} + E_{IN}$ is maximized such that E_{II}/E_{IN} matches O_{II}/O_{IN}. A Monte Carlo program designed to accomplish this task provided, after approximately 10^5 iterations, $J_1 = 0.0$, $J_2 = 0.51$, $J_3 = 0.35$, and $J_4 = 0.15$, given the initial gene frequencies expected at HI, $p = 0.014$ and $q = 0.986$, and a ratio $E_{II}/E_{IN} = 0.0079/0.0891 = 0.0890$. But the frequencies of the introduced genomes observed at HI remain significantly greater than the resulting 'worst J' expected values (Table 5). Thus may results do not appear to be attributable to error estimating population age structure.

My initial values of p and q are dependent only upon my mark-recapture estimate of N, the population size. But the mark-recapture assumptions most likely violated in my application would lead me to underestimate true population size, and thus overestimate E_{II} and E_{IN}. The Petersen estimate assumes that all individuals are equally likely to be sampled and resampled. In my collections, however, it does seem possible that older and larger individuals were more likely to be both captured and recaptured, and on occasion some of my mark showed on the upper surface of the shell, perhaps making the bearer more conspicuous. These biases both detract from my hypothesis, and thus my findings that O_{II}' and O_{IN}' are greater than E_{II} and E_{IN} seem strengthened. But even if I have overestimated HI population size by two standard errors, giving $p = 0.030$, the resulting estimate of E_{IN} remains significantly lower than observation (Table 5). My finding that O_{IN}' is greater than E_{IN} in the HI population is so robust as to be unaltered by subtracting two standard errors from the population size and obtaining 'worst J' values based on the overestimate of p.

Discussion

All snails sampled from the RI population were either homozygous at both the *odh* and *est* loci, or they were doubly heterozygous. This confirms my assumption that females mate but once for life, perhaps immediately upon reaching maturity in the second spring but in all cases prior to the second July. Otherwise there would have been opportunity

Table 5. Comparison of observed frequency of introduced genotypes with frequencies expected under several assumptions.

		Heterozygotes	Homozygous Introduced
RI	observed	0.067	0.013
	best expectation	0.040	0.0023*
HI	observed (corrected)	0.089	0.0079
	best expectation	0.018**	0.0014*
	expected, worst *J*	0.025**	0.0022*
	expected, N-2 s.e.	0.036**	0.0030
	N-2 s.e. & worst *J*	0.050*	0.0044

* significant at the 0.05 level
** significant at the 0.01 level

for another generation of snails to pass between July, 1979, and the spring of 1982, and snails bearing only a single introduced allele should have been common.

The expected frequencies of the introduced genomes are rather low. But genetic drift is unlikely to play a significant role when 500 individuals are introduced into a native population as large as 12000 – 34000. Even assuming a population size two standard errors below that observed in the smaller population, the binomial standard error for gene frequency is only 0.0016. Compared to all other sources of variance, this is negligible.

The high frequencies of the introduced genes observed at both sites suggest that the introduced genome (that of the IND population in each case) is at a selective advantage. The data offer no evidence of heterosis. Although the frequency of the heterozygotes is high, the frequency of homozygous introduced snails is comparatively higher at both sites. This is consistent with the finding of Dillon (1984a) that gene frequencies in natural *G. proxima* populations may be more a function of random processes, such as mutation, migration, and genetic draft, than of selection.

These results from the wild are at variance with those expected from years of laboratory experimentation on 'coadapted gene complexes' (reviews by Endler, 1977; Wallace, 1981). But it is somewhat premature to judge the relative fitnesses of introduced genomes. Previous workers (Burton, 1987) have found that reduced hybrid fitness did not manifest itself until the production of an F_2 generation ('hybrid breakdown'). Future studies will address this possibility.

It should also be remembered that I found no evidence of the introduced alleles in ten of the twelve experiments attempted. Since a minimum of 120 individuals were sampled from each population, I had better than a 95% chance of seeing at least one individual carrying an allele with a frequency as low as 0.0125 in the populations at large. Setting aside the complication of overlapping generations, an introduced allele would have an expected frequency of 0.0125 if the recipient population were roughly 40000 individuals. Thus it would not be surprising if a marker allele were simply missed by experimental sampling error in one or two populations.

Experimental sampling error is probably insufficient to account for the absence of the marker allele from as many as ten populations, however. Given 1200 samples, there is better than a 95% chance of seeing at least one heterozygote carrying an allele with a frequency as low as 0.0013. It seems unlikely that population sizes at the ten sites could average the roughly 3.8×10^5 individuals needed to result in gene frequencies this low. Thus there is some evidence of selection against introduced genomes in this data set. The apparent success of introduced genomes in the RI and HI populations may therefore be exceptional.

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