FISHER INFINITESIMAL MODEL, ORR ADAPTIVE "WALK" MODEL AND BIOMETRICAL STUDIES OF CAROTENOID PATHWAY OF CARROTS[1]

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INTRODUCTION

Under the classic infinitesimal model, introduced by Fisher (1930), the character is determined by an infinite number of unlinked and nonepistatic loci, each with an infinitesimal effect, with the both assumption of normality and linearity. Most models of selection response are based on the either explicit or implicit assumption that the infinitesimal model adequately describes the underlying dynamics (Walsh, 2000). According to the model, genetic progress in an infinitely large population under constant selection intensity is predicted to be linear with time, with infinitesimal change in the allele frequencies and genetic variance. Although clearly incorrect as a description of biology, the infinitesimal model has provided reasonably accurate predictions of response to selection over moderate to long periods in large population (Gibson, 1999).

Orr (1998), based on QTL results and heuristic and approximated and mathematical derivation, suggested that the distribution of factors during adaptation assumes a pleasingly simple exponential form and that an exponential trend among factors fixed appears to be a general property of adaptation toward a fixed optimum. According to the author, the stepwise approach or an adaptive "walk" to the optimum involves several to many factors substitutions. So, after the first factor gets fixed the population moves closer to the optimum and thus the next factor fixed will, on average, have a smaller effect. The third factor in turn will typically be smaller than the second, and so on.

Carrot estimations for QTLs, minimum number of genes and heritabilities are presented in this study in order to present some highlights about these two models, to propose approaches to deal with this debate and also to make comments about practical implication related with engineering of quantitative traits.

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MATERIAL AND METHODS

Two unrelated F_2 populations were analyzed for crosses i) B493 (a dark orange inbred carrot with carotene content ranging from 180 to 210 ppm) x QAL (a white wild carrot, well distributed in temperate regions, with no carotene content): to study the factors that stimulate the pathway in presence of factors that block carotenoid production and ii) Brasilia (medium orange with carotene content around 150 ppm) x HCM (a very dark orange population with an average carotene content ranging from 460 to 499 ppm): to study the magnitude of factors contributing to carotenoid production when both parents have active pathways. The population sizes, to apply QTLs studies, were 180 and 160, respectively, for B493 x QAL and Brasilia x HCM. Major carotenes were quantified by high-performance liquid chromatography (HPLC), as described by Simon and Wolff (1987).

DNA extraction and AFLP reactions were conducted for phytoene, ?-carotene, ß-carotene, a-carotene and lycopene contents in both populations as presented by Santos and Simon (2002). Linkage and QTL analyses were conducted on individual coupling linkage maps for each parent. Estimates of the effective or minimum number of genes influencing all carotenoids products were determined by the methods of first moments or also called Sewall Wright's estimator by applying the generalization presented by Lande (1981). An estimate of heritability for all carotenoids contents was calculated according to equations presented by Burton (1951).

RESULTS AND DISCUSSION

Heritability values were around 50% for ?-carotene and ß-carotene, moderate values for lycopene, high values for phytoene and around 40% for a-carotene in the cross Brasilia x HCM. The heritability values were around or greater than 90% for all characters in the cross B493 x QAL (Table 1). The estimation of the number of loci showed that characters a-carotene and ß-carotene presented more genes than the other characters, in the Brasilia x HCM cross. In the B493 x QAL cross, except for a-carotene, all other characters showed a minimum number of genes around one (Table 1). The Brasilia x HCM heritabilities are somewhat less reliable than B493 x QAL since the number of F_1 and F_2 plants evaluated was smaller. All standard errors of minimum number of factors (Table 1) did not exceed the estimate of minimum of factors, indicating that inferences and conclusions were acceptable.

The low heritabilities values and larger number of genes estimated in orange x orange than in orange x white cross (Table 1) suggested continuous inheritance for α-carotene and β-carotene in the cross orange x orange and discrete inheritance for β-carotene in the orange x white cross,

supporting an adaptive evolutionary model suggested by H. Orr, in contrast to the R. Fisher infinitesimal model.

Table 1. Estimation of broad sense heritability and minimum number of genes or factors, with standard errors of estimates, controlling the inheritance of ?-carotene, a-carotene, ß-carotene, phytoene and lycopene in two F_2 populations of carrot.

Character	Brasília x HCM				B493 x QAL			
	Heritability		Number of loci and standard error		Heritability		Number of loci and standard error	
	1[1/]	2[2/]	1[1/]	2[2/]	1[1/]	2[2/]	1[1/]	2[2/]
ζ-carotene	0.46	0.48	0.17 ± 0.1	0.18 ± 0.1	0.92	0.98	1.13 ± 0.2	1.05 ± 0.2
α-carotene	0.32	0.36	4.00 ± 3.2	3.50 ± 3.0	0.83	0.95	3.93 ± 0.8	3.47 ± 0.7
β-carotene	0.28	0.42	1.67 ± 1.3	2.52 ± 2.3	0.97	0.99	1.12 ± 0.2	1.10 ± 0.2
Phytoene	0.53	0.89	0.22 ± 0.2	0.60 ± 0.4	0.97	0.99	1.12 ± 0.2	1.10 ± 0.2
Lycopene	0.44	0.66	0.36 ± 0.2	0.53 ± 0.4	0.51	0.88	1.83 ± 0.4	1.27 ± 0.3

Based on [1/] $\sigma^2_S = \sigma^2_{F_2} - \sigma^2_{F_1}$ and [2/] $\sigma^2_S = \sigma^2_{F_2} - [\frac{1}{2}\sigma^2_{F_1} + \frac{1}{4}\sigma^2_{P_1} + \frac{1}{4}\sigma^2_{P_2}]$

The locus R^2 explanation of the total variation range from 2.0% to 8.9% in the Brasilia x HCM population while in the B493 x QAL the range was from 0.1% to 19.3% (data not presented). For all carotenoid products the locus R^2 explanation was almost well distributed among loci in the Brasilia x HCM F_2 population while in the B493 x QAL a locus accounted for 72% (19.3/26.7) of the total model determination (data presented for two carotenoid products on Table 2).

Single marker analysis revealed locus explaining up to 13.8, 19.3 and 17.5% of total variation of ζ-carotene, β-carotene and phytoene, respectively in the cultivated x white wild carrot cross, while the largest single locus value in the cultivated x cultivated carrot cross was 8.4% (ζ-carotene).

Stepwise regression procedure reported R^2 values of 94 and 95% for the fitted models of phytoene, ζ-carotene and β-carotene, respectively, in the B493 x QAL cross, while in the Brasilia x HCM cross the initial stepwise model explained 48, 61 and 83% of the same carotenoid products, respectively. When the stepwise selected models were fitted with normal regression procedures, they explained much less than that explained by the initial stepwise procedure.

Based on the largest R^2 single locus explaining more of phenotypic variation in the orange x white background cross than observed with the orange x orange background it is

reasonable to assume that this study supports the adaptive evolutionary model suggested by Orr in contrast to the Fisher infinitesimal model.

Table 2. Group mean differences between absent and present alleles determined by stepwise regression, locus R^2 and linkage group of AFLP loci contributing to the phenotypic variation of ?-carotene and ß-carotene in the Brasilia x HCM and B493 x QAL F_2 populations.

Character	AFLP loci	Group mean differences	Locus R^2 (%)	Position	
				Linkage Group	cM
?-carotene	GAGCAC287-H	51.2	8.4	HCM-9	92.7
	ACCCTA290-B	33.5	3.3	Bsb-2	0.0
	AGGCAT462-H	-42.2	4.3	HCM-3	5.7
	ACACAA087-H	42.0	4.3	HCM-4	19.1
	AACCAA180-H	34.6	3.3	HCM-9	50.7
	GATCAT384/395	-	2.3	HCM-9	94.6
	AAGCTT134-H	27.0	2.0	HCM-9	40.4
Model R^2			28.3	-	-
ß-carotene	ACCCTA290-B	70.0	2.5	Bsb-2	0.0
	AACCAT202-4/B	90.1	3.3	Bsb-5	12.7
	GGTCTT207-B	91.0	3.3	Bsb-3	18.9
	GAGCAC080-H	90.5	4.0	HCM-4	44.7
	GAGCTT203-4/B	70.2	2.4	Bsb-8	19.2
	ACACAA334-B	81.1	3.1	Bsb-2	33.4
	GGACAA158-H	88.7	3.7	HCM-9	-
	GGGCTG082-B	-108.1	4.7	Bsb-5	-
Model R^2			40.4	-	-
?-carotene	AACCAT178-Q	-28.0	13.8	QAL-5	70.9
	GGACAG298-Q	-6.5	0.1	QAL-8	-
	GATCTC155-Q	-0.4	0.1	QAL-1	36.2
	ACACAA174-Q	-15.1	3.7	QAL-6	30.7
	ACCCTA401-Q	-11.9	2.7	QAL-5	-
	AGCCTT585-4	10.2	4.7	B493-4	-
	AAGCAG233-Q	-27.5	13.7	QAL-5	65.1
	GGACAG480-Q	-11.0	1.7	QAL-5	53.0
Model R^2			21.7	-	-
ß-carotene	GGTCAT322-Q	-30.0	6.4	QAL-8	52.1
	AAGCAG233-Q	-48.0	19.3	QAL-5	65.1
	GGACAG480-Q	-23.1	3.9	QAL-5	53.0
	GAGCTT364-Q	-24.2	4.8	QAL-5	55.8
Model R^2			26.7	-	-

One prominent application of carrot carotenoid pathway should be to study some long-standing problem in quantitative genetics and evolution of characters because there are many mutant lines with specific mutation or block in a step of the pathway. Experimental population should be careful designed, with appropriated population size and co-dominant molecular

markers, in order to study the evolution products accumulated in the pathway: from white carrots, without carotenoids products, to very dark carrots, with high contents of carotenoids.

Assuming the infinitesimal model as the correct one it is possible to point out that all efforts to identify, to clone and to engineer locus related with quantitative traits would be pointless, due to the small gain added with the introduced DNA. However, assuming that stepwise model is a realistic one to describe selection events, the marker assisted selection or the cloning and the engineering of those leading loci could be worthy and should be pursued for quantitative traits.

Conclusions

1. There are evidences for continuous inheritance of some carotenoid products in the cross orange x orange and discrete inheritance in the orange x white cross in carrots.
2. Estimations of QTLs, heritabilities and minimum number of genes in carrots support an adaptive evolutionary model suggested by H. Orr in contrast to the R. Fisher infinitesimal model.
3. Mutant lines of carotenoid pathway of carrots should be chosen as model to study some long-standing problem in quantitative genetics and evolution of characters.

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