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Heterosis Evaluation for Morphological Characters of Diallel Cross in Western Ethiopian Origin Coffee (C*offea arabica* L)

Dula Geneti[1], Bayetta Bellachew[2] and Ermias Habte[3]

[1]Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center, Jimma, Ethiopia
[2]Ethiopian Institute of Agricultural Research
[3]Research Officer International Livestock Research Institute · Feed and forages development, Addis Ababa, Ethiopia

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Correspondence:
Dula Geneti. Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center, Jimma, Ethiopia.

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Abstract

The choice of promising genotypes from diverse genetic base and subsequent utilization of hybrids is one of the breeding strategies to improve productivity. Hence, the present experiment was conducted with objective of to determine the level of heterosis of morphological traits in crosses among elite coffee materials from Western Ethiopia. The F_1's and parental lines were planted at *Mugi* Agricultural research testing site in RCBD design in three replications and investigated for their growth performance. The data were recorded for five stem-, four branch- and three leaf- characteristics. The analysis of variance exposed highly significant ($P<0.001$ and $P<0.01$) differences among 15 genotypes (5 parents and 10 F_1s) for all traits except for total number of nodes, leaf area and number of nodes per primary branch. The hybrids P_2 x P_5, P_2 x P_4, P_2 x P_3, P_3 x P_4 and P_1 x P_5 showed relatively high positive heterosis over (mid-parent)(MP) and (better parent) (BP) for most of the characters measured. On the other hand, the hybrid P_3 x P_5 consistently exhibited low or negative heterosis for all growth parameters.

Introduction

Coffee (Coffea *arabica* L.) belongs to the family Rubiaceae and the genus Coffea (Coste, 1992). The two important commercial species among 124 species in the genus Coffea (Davis *et al*., 2012) are *Arabica* coffee and Robusta coffee (Coffea canephora P.), in which the former is the only tetraploid species (2n = 4x = 44), while the latter is diploid (2n = 2x = 22) (Gichuru *et al*., 2008) both cover about 10 million hectares worldwide (Bunn, 2015). Coffee *arabica*, unlike many other coffee types is considered to be a 95percent self-fertile and only 5percent cross fertile species, meaning it can set fruit from its own pollen (Veddeler *et al*., 2008). In Ethiopia, the total land area coverage of *Arabica* coffee is estimated to be 700,474.69 ha with an annual average production of 469,091.1 tonnes, out of which over a half is consumed locally (CSA, 2016/2017).

Ethiopia is both the center of origin and diversification of C. *arabica* L. (Bayetta, 2001). The crop spreads widely in the country stretching from the river bank of Gambella plain (550m.a.s.l) to the central and Eastern highlands of the country with an altitude as high as 2600m (Bayetta, 1986). West Wollega is also endowed with the presence of high genetic variability of *Arabica* coffee. Ermias (2005) conducted studies on 75 West

Wellega coffee accessions and reported the presence of high genetic variability among the accessions for most of the traits studied. In spite of the presence such high genetic variability in west wollega/Ethiopia coffee, yield per hectare or productivity is low. Despite the existence of high genetic diversity in coffee population that provides immense opportunities for improvement program, shortage of improved varieties (pure line and hybrid varieties) is the major one (Bayetta, 2001; Mesfin, 1988; Babur, 2009). In any crop breeding program intended to address such problems like the ones mentioned above, heterosis studies is o of the basic breeding tools. Nevertheless, such studies on coffee are scanty at both national and international level.

Information on heterosis in C. *arabica* is relatively scanty compared to other crops since its hybridization studies had started quite recently. The perennial nature of the crop is another challenge as it requires several years to obtain meaningful results (Cilas*et al*., 1998). Consequently, research results on the effect of heterosis are limited.

Therefore, the present heterosis study was initiated to conduct systematic investigations by concentrating on crosses between variable parental lines originated from specific area, in this case western region of Ethiopia, and contribute towards improving productivity and quality in the long term.

Materials and Methods

Description of the Study Area

The study was conducted at Mugi research sub-stations of Jimma Agricultural Research Center (JARC), which is located in Kellem Wollega Zone. According to Anfilo District bureau of agriculture (BOA), Mugi is located 340 00’ to East and 8040’ to North and 610km from Jimma at an altitude of 1570 masl. The minimum and maximum temperature of the area is 11.6 and 26.3^{o}C, respectively with annual rainfall of 1655 mm/annum. Mugi is one of the major coffee producing areas in western Ethiopia which is characterized by wet humid sub-tropical climate.

Experimental Materials

Five pure line parents that were selected from national coffee collection program based on yield, disease and insect pest resistance, and canopy class was crossed in half diallel manner. The first parent, PX (P_1), was obtained from Southwestern national coffee collections trials but its specific accession number is unknown, hence designated as PX. The remaining four parental lines, W66/98(P_2), W78/84(P_3), W110/99(P_4) and W3/99(P_5) were screened from Western region’s national coffee collections established at Haru Sub-center. According to their canopy nature, P_1 is very open type, P_2 is medium compact, P_3 is medium open, P_4 is intermediate and P_5 is open canopy classes. Detailed description of these parental lines is given in Table 1. The breeding materials i.e. the five parental lines and 10 F_1 hybrids evolved from all possible crosses among the five parents were established in a breeding trial field at Haru Agricultural Research Sub-Center in 2015, in an attempt to develop hybrid coffee varieties for the area that can produce higher yield compared to the released pure lines.

Experimental Design and Field Management

A total of 15 genotypes were planted out in the trial field in August 2016. The trial was planted in RCBD design with three replications. The spacing between plants was 2m x 2m and the number plants per plot were six. Since the establishment of the trial, the field management practices were regularly conducted as per the recommendation of JARC and this standard

practices has continued throughout the experimental duration.

Data Collected

Data were collected for twelve morphological traits from the experimental plots during November 2017 to January 2018. Four very uniform coffee trees with no mechanical damage were carefully selected and tagged for each treatment. The marked trees were recorded for all the twelve characters considered as described below.

Stem Characters

Plant height (cm), Height up to first primary branch (cm), Total number of node (Counts), Inter-node length of the main stem (cm), Stem diameter (mm).

Branch Characters

Number of primary branches (Counts), length of primary branches (cm), Canopy diameter (cm), Number of node per-primary branch (Count).

Leaf Characters

Leaf length (cm), Leaf width (cm), Leaf area (cm2).

Data Analysis

Analyses of variance were computed for all the morphological characteristics considered in this study using XLSTAT, Computer program and SAS (SAS, 2004) version 9.0 software to test for genotypic and block differences. Least Significant Difference (LSD at P = 0.05 and P=0.01) was employed to test the significance of differences among the genotypes (five parents and ten hybrids). Further genetic analyses were carried out only for those characters that showed significant differences among the genotypes. Computation of heterosis was conducted using Microsoft Excel. The mathematical model or formulas applied are presented as follows:

The Analysis of variance was performed using mixed linear model as out lined to assess the differences among genotypes in their performance in morphological traits following the standard procedure suggested by Gomez and Gomez (1984) using SAS (9.0).

Thus the mathematical linear model for ij^{th} observation expressed as:

$$Y_{ij} = \mu + \tau_i + \beta_j + \epsilon_{ij}$$

Where;

Yij = the observation on the jth block and the ith treatment

μ = common mean effect

τ_i = effect of treatment i

β_j = effect of block j and

ϵ_{ij} = experiment error for treatments i in block j

Heterosis

The mid-parent heterosis (MPH) and better parent heterosis (BPH) in percent were calculated for the characters that showed significant differences for genotypes following the method suggested by Falconer and Mackay (1996):

$$MP\ (\%) = \frac{F1-MP}{MP}\ X\ 100$$

$$BP\ (\%) = \frac{F1-BP}{BP}\ X\ 100$$

Where, F_1 = Mean value of the cross

MP = Mean value of the two parents

BP = Mean value of the better parent

Test of significance for percent heterosis was made

using the t-test. The standard errors of the difference for heterosis and t-value were computed as follows suggested by Falconer and Mackay (1996):

$$SE\ for\ MP = \pm\sqrt{3me/2r}$$

$$SE\ for\ BP = \pm\sqrt{2me/2r}$$

$$t\ (mid - parent) = \frac{F1-MP}{SE\ (MP)}$$

$$t\ (better - parent) = \frac{F1-BP}{SE\ (BP)}$$

Where, SE (d) is standard error of the difference, Me is error mean square, r is number of replications and F_1, MP and BP were mean values of hybrids, mid and better parents, respectively. The computed t value was tested against the t-value at degree of freedom for error.

RESULTS

Analysis of Variance (ANOVA)

The analysis of variance revealed highly significant (P<0.01) differences between 15 genotypes (5 parents and 10 F_1s) for all traits measured except for total number of node, Leaf area and quantity of node per primary branch (Table 1).

Similarly, mean squares due to hybrids alone indicated highly significant difference for all characters, except total number of node, leaf area and number of nodes per primary branch.

Table 1: Mean Squares Due to Genotypes and Crosses for 12 Morphological Traits from Analysis of Variance (ANOVA)

Characters	Mean squares					
	Parents and Hybrids			Hybrids alone		
	Genotype (14)	Block (2)	Error (28)	Cross (9)	Block (2)	Error (28)
Stem Characters:						
Plant Height (cm)	612.33***	1707.25***	137.14	849.04**	1045.43**	174.98
Total number of node	2.39ns	6.098*	1.30	2.71ns	3.71ns	**1.70**
Stem Diameter	31.90***	58.49***	6.54	35.15**	31.01*	7.85
Height First Primary Branch	22.14**	1.40ns	14.25	16.89**	41.13*	6.46
Inter-node Length	1.82***	4.42***	0.45	27.11**	7.16ns	4.87
Branch Characters:						
Average Length Primary Branch	153.73**	85.78ns	36.39	181.2**	34.76*	47.88
Canopy Diameter	1001.65***	2408.09***	161.66	1236.31***	1396.84**	191.96
Number of Primary Branch	14.25***	50.29*	5.11	16.89**	41.13*	6.46
No. of node per primary branch	3.19ns	14.96***	1.69	3.90ns	13.62**	2.15
Leaf Characters:						
Leaf Length	2.41**	5.068**	0.68	3.46**	3.96*	0.71
Leaf Width	0.69***	0.43*	0.11	0.962***	0.370ns	0.122
Leaf area	72.36ns	194.27ns	48.22	92.45ns	168.84 ns	52.21

1***P < 0.001; **P > 0.001 and 0.01 *p > 0.01 and p 0.05; ns p > 0.05 (non-significant);df = degree of freedom ();

Block differences were highly significant (P<0.01) for characters plant height, stem diameter, leaf length and inter node length, while significant (P<0.05) for characters number of primary branch and leaf width.

Estimates of Heterosis

The level of heterosis expressed as percentage over the mid-parent (MPH) and over the better parent (BPH) was estimated in order to: (1) study the degree of heterosis that could be expressed in crosses among elite parental lines of different characteristics selected from West Wollega coffee population, and (2) be able to identify heterotic hybrids among the crosses involved in the present study for further test and commercial use. Details of these findings are discussed in the discussion part.

Stem Characters

Out of ten hybrids studied, seven hybrids exhibited positive mid- and better-parent heterosis for plant height (Table 2). Among these seven heterotic hybrids, P2 xP5 manifested the highest heterosis value of 21.1percent and 19.7percent over its mid-parent (MP) and better-parent (BP), respectively.

Table 2: Estimates of Heterosis as Percentage over the Mid-Parent (Mph) and Over the Better Parent (BPH) For Stem Characters

parents	Heterosis (percent) over							
	Plant height		Stem diameter		HFPB		Inter node length	
	MP	BP	MP	BP	MP	BP	MP	BP
P_1 x P_2	-2.1	-9.4	1.7	-15.7	2.8	-0.9	-7.4	-11.8
P_1 x P_3	14.2	7.2	11.4	-9.1	4.2	-3.4	3.3	1.8
P_1 x P_4	8.7	3.6	6.9	-9.4	-4.9	-11.6	7.6	1.5
P_1 x P_5	-7.0	-13.1	-4.3	-9.8	-26.2**	-28.0**	1.3	-5.3
P_2 x P_3	17.1	15.2	25.8	-8.2	16.1	11.4	5.9	2.3
P_2x P_4	15.8	12.2	20.0	-7.7	11.9	7.7	8.1	7.0
P_2 x P_5	21.1*	19.7*	25.8*	-7.4	5.5	-0.6	13.7	11.5
P_3 x P_4	10.9	9.2	6.5	-0.5	13.9	13.5	4.6	0.0
P_3 x P_5	-33.6**	-33.9**	-33.4**	-1.0**	-19.5	-27.1**	-28.6**	-32.3**
P_4 x P_5	12.8	10.5	15.3	-0.4	-17.5	-25.0**	12.0	10.9
Mean	5.79	2.12	7.57	-6.92	-1.37	-6.4	2.05	-1.44
SE (MPH)	8.28		1.80		2.66		0.47	
SE (BPH)	9.56		2.08		3.08		0.54	

*, ** significant at 0.05 and 0.01 prob. Level, .respectively, SE = Standard error, MP = mid parent, BP = better parent, HFPB = Height up to first primary branch

In contrast, the lowest and negatively significant mid- and better-parent heterosis values of -33.6percent and -33.9percent were observed with hybrid P_3 x P_5. As indicated earlier, all the rest hybrids exhibited positive but non-significant mid- and better- heterosis for the same character, except P_1 x P_2 and P_1 x P_5.

Better parent heterosis was lacking with all hybrids for stem diameter and only one hybrid, P_2 x P_5, manifested positive and significant mid parent heterosis with percentage value of 25.8percent. Additionally, seven hybrids showed positive but non-significant mid parent heterosis while two hybrids manifested negative mid-parent heterosis. The hybrid that exhibited negative non-significant mid parent heterosis value of -4.3percent was P_1 x P_5 while P_3 x P_5 showed negative and significant mid parent heterosis value of -33.4percent. In contrast, all hybrids revealed negative better parent heterosis for stem character.

Considering height up to first primary branch, four hybrids over mid-parent and seven hybrids over better-parent exhibited negative heterosis the remaining hybrids manifested positive heterosis.

The hybrid P_1 x P_5 exhibited significant negative heterosis values of -26.2percent and -28.0percent over

mid- and better- parent, respectively. This hybrid was the poorest performer among all hybrids for this particular trait. Out of the ten hybrids studied, six and three hybrids showed positive but non-significant mid- and better-parent heterosis, respectively. The highest better parent heterosis was manifested by the hybrid P_3 x P_4 followed by P_2 x P_3 with percentage values of 13.5percent and 11.4percent, respectively.

For inter-node length, none of the crosses exhibited positively significant mid- and better-parent heterosis. Among the hybrids, P_2 x P_5 and P_4 x P_5 showed high mid-parent heterosis value of 13.7percent and 12.0percent, respectively. These hybrids also exhibited the highest positive better parent heterosis value of 11.5percent and 10.9percent in that order. In the contrary, the hybrid P_3 x P_5 exhibited significantly negative mid- and better-parent heterosis value of -28.6percent and -32.3percent, respectively, and was the poorest performer for this particular trait.

Branch Characters

Most of the F_1's exhibited positive mid parent heterosis ranging from -44 to 25.3percent, and -42.1to 31.8 for canopy diameter and average length of primary branch, respectively (Table 3).

On the other hand, the magnitude of heterosis relative to better parents ranged from -48.5 to 28.0percent for canopy diameter and -48.8 to 28.5percent for Average length of primary branch. The highest mid parent heterosis values were manifested by the crosses P_2 x P_4 and P_2 x P_5 with percentage values of 25.3percent and 25.3percent, respectively for canopy diameter.

The highest mid parent heterosis was also observed in similar hybrids with percentage values of 31.1percent and 29.3percent, in that order for average length of primary branch. The cross P_2 x P_5 exhibited highest better parent heterosis values of 28.0percent for canopy diameter. The same hybrid, P_2 x P_5, also showed highest better parent heterosis values of 28.5percent for average length of primary branch. This result clearly showed that P_2 x P_5 was the best hybrid in expressing great improvement in canopy diameter and length of primary branches, characters which directly indicate improvement in the bearing areas of the hybrid and increase in its openness in terms of canopy nature. Therefore, this hybrid may require larger spacing while planting and need to be further studied for use as commercial hybrid.

On the other hand, certain hybrids exhibited the poorest performance for the above mentioned branch characters, The hybrid P_3 x P_5 manifested negatively significant mid parent heterosis value of -44.1percent while P_3 x P_4 also manifested negative but non-significant mid parent heterosis value of -0.7percent for canopy diameter. Similarly, P_3 x P_5 and P_3 x P_4 manifested negative mid parent heterosis values of -42.1percent and -4.0percent for average length of primary branch. The rest hybrids exhibited positive but non-significant heterosis over mid and better parent for these two important traits.

Table 3: Estimates of Heterosis as Percentage over the Mid-Parent (Mph) and Over the Better Parent (BPH) For Branch Characters

parents	Heterosis (%) over					
	Canopy diameter		Average length of primary branch		Number of primary branch	
	MP	BP	MP	BP	MP	BP
P_1 x P_2	1.5	-12.2	3.8	-9.7	6.3	2.2
P_1 x P_3	5.7	-0.2	-0.7	-3.2	17.0	12.6
P_1 x P_4	17.5	5.8	12.0	2.2	13.2	6.4
P_1 x P_5	9.2	-4.5	8.9	-5.7	22.4*	21.8*
P_2 x P_3	18.9	8.2	18.9	5.7	-6.7	-6.7
P_2 x P_4	25.3*	19.8	31.1*	24.3	1.5	-0.9
P_2 x P_5	25.3*	28.0*	29.3*	28.5*	3.5	-0.9
P_3 x P_4	-0.7	-5.7	-4.0	-10.3	8.1	5.6
P_3 x P_5	-44.1**	-48.5**	-42.1**	-48.8**	-24.6*	-27.8**
P_4 x P_5	22.1	18.2	27.3*	20.1	17.4	9.8
Mean	8.07	0.89	8.45	0.31	5.81	2.21
SE (MPH)	8.99		4.26		1.59	
SE (BPH)	10.38		4.92		1.84	

*, ** significant at 0.05 and 0.01 prob. Levels, .respectively, SE = Standard error, MP = Mid parent, BP = Better parent

Considering number of primary branches per tree which is the other important characters contributing to bearing area of a coffee tree, only P_1 x P_5 showed significant and positive heterosis percentage value 22.4percent and 21.8percent over mid- and better-parent, respectively. However, it was unfortunate that the three hybrids viz. P_2 x P_3, P_2 x P_4 and P_2 x P_5, which exhibited high heterotic values for canopy diameter and average length of primary branches, did not perform well for number of primary branches, a character which is another important component of potential bearing area. The hybrid P_4 x P_5, however, interestingly exhibited high and positive better parent heterosis consistently for all the three branch characters.

Generally, these hybrids namely, P_2 x P_3, P_2 x P_4, P_2 x P_5 and P_4 x P_5 appears very useful in the development of open type coffee hybrids and need to be further verified.

Leaf Characters

In this study, six hybrids for leaf length and seven for leaf width exhibited negative mid and better parent heterosis, while the remaining hybrids out of ten manifested positive heterosis for the respective character (Table 4).

Considering individual hybrids, P3 x P5 manifested the least leaf length and width. It expressed significantly negative mid- and better-parent heterosis values of -18.8percent and -21.1percent respectively for leaf length and -27.2percent and -27.3percent, respectively for leaf width. The magnitude of heterosis manifested for leaf characters appeared generally low.

Three hybrids viz. P_1 x P_4, P_2 x P_3 and P_2 x P_5 alone showed better parent heterosis values of 3.5percent, 3.0percent and 3.6percent for leaf length. For leaf width, the hybrids that exhibited positive better parent heterosis were P_2 x P_3, P_2 x P_4 and P_2 x P_5 and the magnitude of heterosis values were 3.0, 3.9 and 6.8, respectively. Earlier research findings indicated similar results of low heterosis percentage for leaf characters (Bayetta, 2001; Wassu, 2004; Ashanafi, 2013). In view of these findings, it may be difficult to improve leaf

characters through hybridization program.

Table 4: Estimates of Heterosis as Percentage over the Mid-Parent (Mph) and Over the Better Parent (BPH) For Leaf Characters

parents	Heterosis (%) over			
	Leaf length		Leaf width	
	MP	BP	MP	BP
P_1 x P_2	-1.4	-2.8	-0.2	-5.7
P_1 x P_3	-1.3	-2.1	-0.2	-3.4
P_1 x P_4	3.9	3.5	-0.2	-5.3
P_1 x P_5	-1.8	-5.2	-8.5	-11.6*
P_2 x P_3	3.6	3.0	5.5	3.0
P_2x P_4	-3.1	-4.2	4.3	3.9
P_2 x P_5	5.9	3.6	9.3	6.8
P_3 x P_4	-5.3	-5.8	-3.6	-5.6
P_3 x P_5	-18.8**	-21.1**	-27.2**	-27.3**
P_4 x P_5	3.0	-0.3	-1.8	-3.7
Mean	-1.53	-3.14	-2.26	-4.89
SE (MP)	0.5		0.23	
SE (BP)	0.67		0.27	

*, ** significant at 0.05 and 0.01 prob. Levels, .respectively, SE = Standard error, MP = mid parent heterosis, BP = better parent heterosis

Discussion

Analysis of Variance (ANOVA)

Mean squares due to genotypes both Parents and hybrids and hybrids alone indicated highly significant difference for all characters, except total number of node, leaf area and number of node per primary branch characters. Similar results were also reported by previous studies that showed significant differences among genotypes for morphological traits in different sets of crosses studied in coffee (Bayeta, 1991, 2001; Wassu, 2004 and Ayano *et al*, 2013).

Block differences were highly significant ($P<0.01$) and significant ($P<0.05$) for most of characters measured. This result was as expected since the experimental field was a gentle slope in nature and soil variation was expected within the field between top, middle and bottom parts.

Estimates of Heterosis

Considering heterosis analysis relative to the mid and better parent, positive mid- and better-parent heterosis were detected for all stem characters measured except positive better parent heterosis was lacking with all hybrids for stem diameter. In general, the magnitude of heterosis manifestation for this particular trait was very low. This result is not in agreement with the findings of the previous workers (Mesfin, 1982; Bayeta 2001; Wassu 2004), who reported higher magnitude of mid- and better-parent heterosis for thischaracter. Most probably, the deviations between the present and previous findings could be largely attributed to differences in parental lines involved and the environment under which the experiments were conducted.

Previously stated, canopy diameter and average length of primary branches are very useful traits in determining the potential bearing area of a coffee plant. Therefore, information about heterosis for these traits is imperative. In the present study, three hybrids viz. P_2 x P_3, P_2 x P_4 and P_2 x P_5 consistently exhibited high and positive better parent heterosis where that of the latter hybrid was significant for both canopy diameter and

average length of primary branches.

In contrary, the hybrids (e.g P_3 x P_5) that exhibited negatively high percentage heterosis may tend to develop in to compact type when fully matured and this could be a desirable character for those farmers who are interested to increase yield per unit area through high density planting. Similar result were reported by Bayetta *et al*. (2001) in *Arabica* coffee, who reported negatively high percentage heterosis could be develop in to compact type.

The same cross exhibited similarly high and negative heterosis for branch characters as discussed earlier also exhibited high and negative heterosis for leaf characters suggesting the unfavorable interaction of genes from the two parents for better branch growth and increase in leaf size. This conclusion appears consistent with other hybrid P1 x P3 which also exhibited negative mid- and better-parent heterosis for both leaf length and width. The magnitude of heterosis manifested for leaf characters appeared generally low. In view of these findings, it may be difficult to improve leaf characters through hybridization program.

Conclusion

The analysis of variance revealed highly significant ($P < 0.001$ and $P < 0.01$) differences among the 15 genotypes (5 parents and 10 F_1s) for all the traits considered except for total number of node, leaf area and number of nodes per primary branch. This clearly showed the presence of inherent variations among the genotypes for most of the characters studied. Considering individual hybrids, however, the level of heterosis was significant or highly significant over the mid-parent or better-parent for some morphological characters. In effect, the hybrids P_2 x P_5, P_2 x P_4, P_2 x P_3, P_3 x P_4 and P_1 x P_5 showed relatively high positive heterosis over MP and BP for most of the characters. On the other hand, the cross P_3 x P_5 consistently exhibited negative heterosis for all growth parameters which was significant or highly significant for most of the characters. This result might suggest that P_3 and P_5 to be genetically closely related to some heterotic group.

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