

PATTERNS OF HETEROSIS AND CROSSING BARRIERS
RESULTING FROM INCREASING GENETIC DISTANCE BETWEEN
POPULATIONS OF THE MIMULUS LUTEUS COMPLEX¹KAREN W. HUGHES² AND ROBERT K. VICKERY JR.³

Clausen (1962) noted that a common pattern of plant evolution is divergence resulting from the accumulation of genetic changes. In studies on the genera Layia and Madia he noted that increasing levels of divergence were associated with increasing inability to intercross. Grant and Grant (1960) in studies on the genus Gilia and Vickery (1966a, 1966b, 1967) in studies on Mimulus noted similar patterns, i. e., that increasingly divergent taxonomic levels were associated with increasing inability to intercross.

Heterosis or hybrid vigor is also associated with divergence in that divergent races or strains when crossed often show increased vigor and/or fertility. Elliott (1958) states that the degree of hybrid vigor increases as the genetic disparity of the parents increases. Whaley (1964) also notes that "there is at least a rough relationship between the amount of heterosis in a hybrid and the extent of the genetic differences between the parents." In natural populations heterosis may play a role in maintaining population heterozygosity. Co-adapted inversion complexes seem to be maintained by heterozygote advantage in natural populations of Drosophila pseudoobscura (Dobzhansky, 1949). Heterosis may also be induced by radiation in some strains of plants and animals. Mukai (1966) in studies on Drosophila melanogaster presents evidence that the amount of radiation inducible heterosis depends on the number of loci that are already heterozygous in the organism and argues that there is an optimum proportion of heterozygous loci. Tai and Vickery (1970) in studies on Mimulus indicate that the first indication of divergence may be heterosis in intrapopulation hybrids.

This paper will present evidence that populations, as they diverge, show first heterosis in their interpopulation hybrids then stronger and stronger interference with their ability to intercross. In order to test the hypothesis that diverging populations exhibit first heterosis then increasingly strong barriers to gene exchange, we selected populations of Monkey flowers from the Mimulus luteus complex of the snapdragon family for study. This complex exhibits many stages in the evolutionary process from minor genetic differences to complete crossing barriers

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Table 1. Populations of the *Mimulus luteus* complex

Culture Number	Collection Data
<u><i>Mimulus luteus</i> L.</u>	
5042*	Los Andes, Santiago Province, Chile. 1949. Altitude 1900 meters.
5043*	Valparaiso, Valparaiso Province, Chile. 1949. Altitude 600 meters.
6161	Auetrihue, Tucumen Province, Argentina. 1959.
6650*	Manzanar, Valparaiso Province, Chile. 1963.
6651	Salta de Laja, Bio Bio Province, Chile, 1964. Altitude 1100 meters.
6680*	Rio Blanco (Curacautin), Malleco Province, Chile. 1964. Altitude 1100 meters.
6685	Las Carbas, Cordillera de Los Andes, Nuble Province, Chile. 1963.
7514*	El Pangué, Coquimbo Province, Chile. 1966. Altitude 1700 meters.
7517	Valle del Rio Yesso Andes, Santiago Province, Chile. 1966. Altitude 1600 to 1800 meters.
7518*	San Jose de Maipo Andes, Santiago Province, Chile. 1966. Altitude 2200 meters.
7531*	Changue Pehuen, Neuquen Province, Argentina. 1965. Altitude 1300 meters.
9565*	Road between San Fernando and Vargas del Flaco, Colchagua Province, Chile. 1972. Altitude 700-900 meters.
<u><i>Mimulus tigrinus</i> Hort.</u>	
5016*	Commercial seed from Hallawell's seeds, listed as "Queen Prize". 1948.
5303*	Garden seeds from Hortus Botanicus, Coimbra, Portugal. 1949.
<u><i>Mimulus cupreus</i> Dom.</u>	
5684*	Uppsala Botanic Gardens, Uppsala, Sweden. 1950.
6606*	Commercial seed from Uppsala, Sweden. 1961.
7530	Mt. Chachit, Neuguen Province, Argentina. 1965.

* Populations for which complete crossing data were obtained.

between its populations (Vickery, 1969; Hughes, 1972). Polyploidy does not seem to be a complicating factor (Vickery et al., 1968).

Methods and Materials

Seventeen populations representative of the three main species of the Mimulus luteus complex of section Simiolus of the genus Mimulus (Scrophulariaceae) were chosen for investigation (Table 1). Most of the populations belong to the most abundant species of the complex, M. luteus L., and fewer populations to the rarer species M. cupreus Dom. and M. tigrinus Hort. The latter is a horticultural derivative of one or both of the other species (Grant, 1924). The wild species are widespread in the Andes, particularly in Chile and Argentina.

Cultures of each population were grown from seeds and established in the greenhouse. Quantitative data were gathered on 134 morphological traits from an average of eight plants in each culture. A wide variety of characteristics, e. g., number of stems and flowers; length of stems, internodes, leaves, pedicles, and seeds; pigmentation and texture of the plant parts; positioning of the flowers; etc. were used (Hughes, 1972).

The morphological similarities of the populations were determined by calculation of a shortest distance network developed by Prim (1957) and adapted to evolutionary studies by Edwards and Cavalli-Sforza (1964). In this method, the sum of the normalized character differences between two populations is the estimate of the distance between these populations on the Prim network. Populations with large numbers of differing traits are widely separated on the network and vice versa. The assumption implicit in the use of the Prim network for this study is that the sum of the character differences is proportional to the gene differences between the populations. It should be noted that Edwards and Cavalli-Sforza (1964) made assumptions of selective neutrality and independence for the traits studied that are not made here. It is highly unlikely that all 134 traits are either independent or selectively neutral. Such assumptions, however, are not necessary in this study as we are interested in an estimate of total genetic difference between populations regardless of the effects of selection on one independent trait or several co-adapted traits.

Plants from twelve populations were intercrossed in all combinations, i. e., in a complete diallel design. Partial crossing data was obtained for the remaining populations in the study (Hughes, 1972). The ability of the populations to intercross was examined by comparing interpopulation values with intra-population values for each of the following :

- A Total seeds from P_1 crosses. The total number of seeds per capsule, whether normal or abnormal, was counted for each cross.

- B Normal seeds from P_1 crosses. The proportion of normal plump seeds in each capsule as opposed to abnormal shrunken seeds was recorded for each cross.
- C Germination rate of seeds from P. crosses. The proportion of seeds which had germinated in a petri dish at 4 days was recorded. Normal germination spans a period of two weeks.
- D Total germination of seeds from P_1 crosses. The total proportion of seeds that had germinated in a petri dish at 50 days was recorded.
- E Presence of F_1 hybrid. The presence or absence of a viable F_1 hybrid plant was recorded for each cross.
- F Normal pollen produced by the F_1 hybrid. The percentage of normal as opposed to poorly stained or unstained pollen in iodine potassium iodide stain was recorded for each F_1 plant.
- G Total seeds produced by self pollinated F_1 hybrids. The total seeds per capsule, whether normal or abnormal, were recorded for each F_1 plant.
- H Normal seeds produced by self pollinated F_1 hybrids. The proportion of normal as opposed to shrunken abnormal seeds in each capsule was recorded for each cross.

These traits were selected to represent different stages in the interaction of genotypes from two different parents. If interpopulation values fell significantly below the corresponding intrapopulation values, a reduction in ability to cross or "partial barrier" to gene exchange was recorded for the cross for that trait. If interpopulation values were essentially zero but one or more hybrids of the next generation were obtained, a "severe barrier" was recorded for each such cross. If interpopulation values were zero and no hybrids of the next generation were obtained, a "complete barrier" was recorded for that trait for that cross. If interpopulation values were significantly higher than the corresponding intrapopulation values, "heterosis" was recorded. We chose to evaluate heterosis for the same traits used in determining inability of populations to intercross for consistency of analysis. Further, Whaley (1964) has presented evidence that early gene interactions may be primarily responsible for the later increase in height and vigor usually used as an indication of heterosis.

The relationship between morphological similarities (distance on the Prim network) and ability to cross was ascertained for each cross to determine if heterosis did occur between closely related populations and barriers between distantly related populations.

Table 2. Prim network distances and the presence of heterosis and/or barriers in crosses between population of the *Mimulus luteus* complex.

	Male Parent	5016T	5042L	5043L	5303T	5684C	6606C	6650L	6680L	7514L	7518L	7531L	9565L
1	5016T	313	340	99	-PB 675	555	479	H 383	414	227	P 314	H 395	
2	5042L	313	119	214	-PB 535	414	339	PP-H 243	101	86	174	B 82	
3	5043L	340	119	241	-PB 561	441	365	270	H 300	113	201	P 281	
4	5303T	99	241	241	B 576	456	370	H-P 275	H 315	128	206	296	
5	5684C	-PB 675	-PSS-SB 561	-PSS-SB 576	B 576	-PB 120	-PB 525	-PB 430	-PB 635	-PB 446	-PB 360	-PB 617	
6	6606C	555	441	456	-PB 120	405	405	310	515	328	241	497	
7	6650L	479	365	370	B 525	405	405	96	439	252	H 165	PP-P 421	
8	6680L	H 383	270	275	-PB 430	310	310	H 344	H 344	157	69	325	
9	7514L	414	300	315	-PB 635	515	439	H 344	H 344	187	275	183	
10	7518L	227	113	128	B 448	328	252	157	P 187	H-P 88	-P-H-P 88	B 168	
11	7531L	314	201	206	-PB 360	240	165	HH-P 69	275	88	H-P 88	H-PP 256	
12	9565L	395	281	296	B 617	497	421	P-P 325	P 183	168	PP-P 256	256	

Dashes represent crossing traits as follows: First dash, total seeds from F_1 crosses; Second dash, normal seeds from P_1 crosses; Third dash, germination rate of seeds from P_1 crosses; Fourth dash, total germination of seeds from P_1 crosses; Fifth dash, presence of F_1 hybrid; Sixth dash, normal pollen produced by the F_1 hybrid; Seventh dash, total seeds produced by self-pollinated F_1 hybrids; Eighth dash, normal seeds produced by self-pollinated F_1 hybrids. Where dashes are replaced by letters, heterosis (H), partial barriers (P), severe barriers (S), or complete barriers (B) were observed for that trait for that cross. The number under the dashes is the Prim network distance for that cross.

RESULTS

Complete barriers to gene exchange, severe barriers, partial barriers and heterosis were observed for either a single trait or for several traits in crosses between populations of the *M. luteus* complex (Table 2). Further, heterosis and/or barriers were observed for all crossing traits in the study. Correlation coefficients indicate that the crossing traits are independent, e. g., the percentage of abnormal seeds is not affected by the total seed production.

Population 5684C, one of the horticultural populations of *M. cupreus*, is isolated by complete barriers to gene exchange from all other populations of the *Mimulus luteus* complex. The other horticultural variety of *M. cupreus*, population 6606C does not show this pattern of barriers and can intercross freely and form hybrids with *M. luteus* and *M. tigrinus* populations. Crossing data (incomplete) for population 7530C, a natural *M. cupreus* population, indicate that this population also crosses freely and forms hybrids with the other two species (Hughes, 1972). Among the *M. luteus* cultures, population 9565L, an unusual violet flowered form, shows a reduction in ability to cross with other populations. Population 7531L also shows a reduced ability to cross with some populations. None of the *M. tigrinus* populations showed consistent crossing barriers.

Results of the Prim network analysis are given in Figure 1. Despite their crossing relationships, the two cultivated *M. cupreus* populations appear together on one branch of the network; however, the natural *M. cupreus* population 7530C is located at the opposite end of the network. The two *M. tigrinus* populations appear on a single branch. The *M. luteus* populations not only form the backbone of the network but form several side branches as well.

There is a significant correlation of the presence, number, and intensity of barriers with Prim network distance ($r = .58$, $P = .01$). Populations where hybrids show heterosis tend to be separated by low Prim network distances (Table 3).

Table 3. Means and Standard Deviations of Prim network distances for crosses showing combinations of heterosis and/or crossing barriers.

Crosses	Mean and Standard Deviation
Crosses exhibiting heterosis for one or more crossing traits	301 \pm 107
Crosses exhibiting both heterosis and partial barriers to gene exchange but for different crossing traits	261 \pm 120

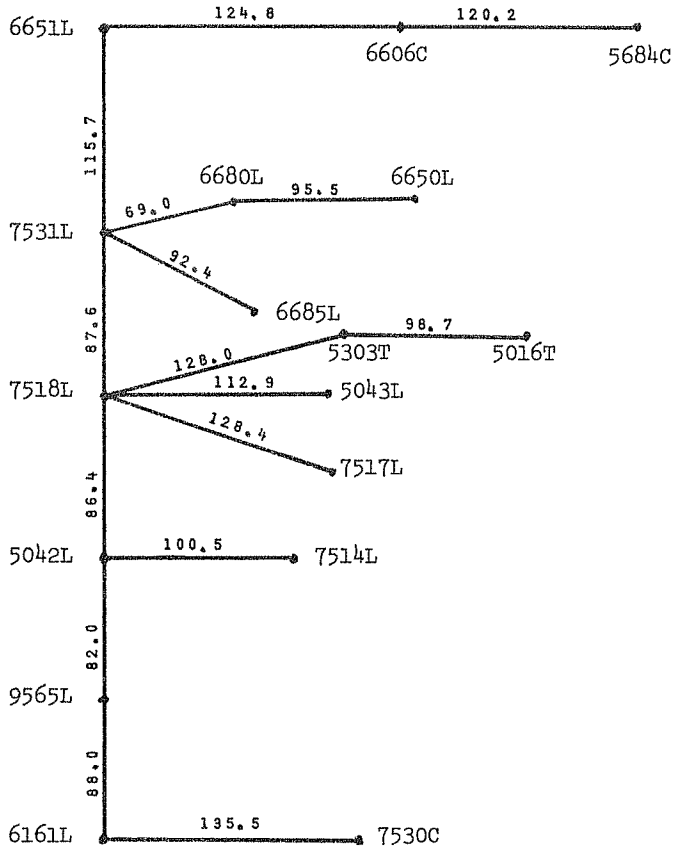


Fig. 1. Prim Network for seventeen populations of the *M. luteus* complex

Crosses exhibiting a single barrier to gene exchange only	286 ± 93
Crosses exhibiting two or more partial barriers	411 ± 64
Crosses exhibiting complete barriers	467 ± 180

DISCUSSION AND CONCLUSIONS

If populations, as they diverge, show first heterosis then increasingly strong barriers to gene exchange, low Prim network distances should be associated with the presence of heterosis while large Prim network distances should be associated with the presence of barriers to gene exchange. When several independent traits are involved with possibly differing rates of evolution, it is possible to obtain both heterosis and barriers to gene exchange in the same cross but for different aspects of development. Our data indicate that high Prim network distances are indeed correlated with increasingly strong barriers to gene exchange, that is, populations most widely separated on the network tend to show the strongest barriers while lesser distances are associated with lesser barriers. This agreement is not complete, however, and several population crosses either exhibit barriers to gene exchange while closely related on the Prim network or do not exhibit barriers while distantly related on the Prim network. Crosses between populations 7531L and 6680L between 7531L and 7518L show partial barriers to gene exchange yet are adjacent in the Prim network. Further, these populations are from widely separated sites. Such populations may represent examples of convergent evolution where similarities in the environment have created morphological similarities in otherwise genetically divergent populations. Conversely, the populations may be genetically similar but have evolved a single or very few critical gene differences which cause a reduction in the ability to cross. In either case, they do not follow the general pattern of correlation of morphological distance with the presence of barriers.

Another exception is found in the case of the three *M. cupreus* populations. Apparently the artificial selection exerted by horticulturalists on populations 5684C and 6606C has separated them from the wild population 7530C resulting in two morphologically distinct subgroups of *M. cupreus* that are widely separated on the network (Fig. 1). Population 5684C does not cross with any other population in the study and is separated from the other populations by very large Prim network distances. Population 6606C is also separated by very large Prim network distances yet shows no crossing barriers. Thus, intense phenotypic selection may or may not lead to corresponding barriers to gene exchange.

While the average Prim network distance for populations exhibiting heterosis is indeed lower than that for populations showing two or more

partial barriers and for populations showing complete barriers, it is slightly higher than that for populations showing only a single partial barrier (Table 3). To explain this we may hypothesize that the maximum heterozygosity that will result in heterosis (Mukai, 1966) does not differ significantly from heterozygosity which results in interference with the delicate physiological and metabolic processes of the hybrid and which, therefore, leads to partial crossing barriers. Thus Prim network distances showing heterosis and distances showing partial barriers may not be too different. This is supported by a group of crosses showing both heterosis and partial barriers but for different traits (Table 2). The problem is further complicated by the nature of the genes in heterozygous condition and by possibly differing rates of evolution for different genes.

Although the data are indicative that with increasing divergence we encounter first heterosis in the interpopulation hybrids, then interference with ability to intercross, they are not conclusive. The means representing average Prim network distances for crosses showing heterosis, heterosis and partial barriers, and partial barriers alone are very close and the standard deviations are large, however, the means represent evolutionary divergence between many different populations and for eight different traits. It is not surprising that there should be such variability, especially in the region between heterosis and partial barriers. It is clear, however, that heterosis is an initial result of a cross between two populations which have diverged to a moderate extent and that severe or complete barriers to gene exchange occur between populations that show a higher degree of evolutionary divergence. The evolutionary significance of heterosis is of interest. If a temporary isolating mechanism, e. g., geographical isolation between two populations is removed, then heterosis by favoring interpopulation hybrids might act in opposition to the developing evolutionary divergence and return the populations to a single gene pool.

SUMMARY

Ability of several populations of the *Mimulus luteus* complex to intercross was compared with an estimate of genetic divergence of the populations derived from a Prim network based on 134 traits. On the average we find that low levels of genetic differentiation result in heterosis or partial interference with ability to intercross and that with increasing genetic divergence, these are replaced with increasingly strong barriers to gene exchange.

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