LEARNED AND SELECTED FOOD OVIPOSITION PREFERENCE IN SINGLE AND MIXED SPECIES POPULATIONS OF *DROSOPHILA SIMULANS* AND *DROSOPHILA MELANOGASTER*

by

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BIOGRAPHY

The author was born in Niagara Falls, New York, on January 8, 1943. He received his elementary education in the Erie, Pennsylvania, Public Schools and his secondary education in the Roswell, New Mexico, and Mesa, Arizona, Public Schools. In 1961 he entered Arizona State University, graduating in 1968 with a Bachelor of Science degree in zoology and in 1970 with a Master of Science degree in zoology. He was awarded a fellowship by the Organization for Tropical Studies to participate in a tropical ecology course in Costa Rica and did so for two months in the summer of 1970. His doctoral program was initiated in the Department of Genetics at North Carolina State University in the fall of 1970.

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INTRODUCTION

One of the current dilemmas in ecology is concerned with explaining the origin of species diversity in temperate communities and the larger species diversity in the tropics. A related question is what happens when two species meet under natural conditions and compete for a limited resource. Does one usually eliminate the other, or drive it out, or is there some mechanism that allows coexistence? The approach to answer this question by previous workers has been to study competitive outcomes of two species in a closed environment where they compete for a limited resource - usually food. Consistent with Gauses' principle the outcome has usually been the elimination of the disadvantaged species. Occasionally a reversal of dominance occurred (*i.e.*, a reversal in numerical abundance) and in most of the exceptions this was found to be due to an increase in competitive ability through genetic change. This then has led to the question - How much additive genetic variability exists in natural populations for traits affecting competitive ability? - the hypothesis here being that it may be possible for two species to coexist by alternately undergoing genetic changes in competitive ability. Related studies have indicated there is little additive genetic variability and this had led to a conclusion that "The evolution of the species composition of a community may thus depend largely upon preadaptation of invading species to prevailing ecological conditions, including competitive pressures" (Futumya, 1970).

However, these previous studies may not be totally applicable to natural conditions, since they were conducted under a constant and uniform environment - an unusual condition in nature. In addition, the influence of behavior on the outcome of a competitive situation in a heterogeneous environment has not been adequately considered. Higher organisms have an advanced learned and instinctive behavior and either one may alleviate the type of "tooth and claw" competition of previous studies, and/or lead to a genetic change allowing coexistence.

The hypothesis of this research has been that in a heterogeneous environment, additive genetic variability exists in natural populations for traits that, although not directly associated with competitive ability, may lead to a more frequent coexistence of species under natural conditions than what would be expected from previous studies. The trait studied, food oviposition preference, is one the author feels to be ecologically important to *Drosophila*. Preference implies a choice between two or more foods and in this experiment they were a tomato and a potato based medium. One of the objectives was to determine if preference (defined as the fraction on tomato of the total eggs oviposited) is a trait that can be easily and effectively selected and, if so, whether the effects of selection would still be expressed in a mixed species population. The other objective was to determine how preference, as learned behavior, is modified by the presence of another species, and how it might influence the outcome of a competitive situation. The experimental organisms used to fulfill these objectives were *Drosophila simulans* and *D. melanogaster*.

REVIEW OF LITERATURE

Volterra (1926) was the first to theoretically consider interspecific competition using the Verhulst-Pearl logistic formulations for the growth of a single species in a limited environment. Later Gause (1934) redefined some of Volterra's parameters resulting in the following equations known as the Gause model of interspecific competition (Slobodkin, 1961):

$$\frac{dN_1}{dt} = r_1N_1\frac{K_1-N_1-aN_2}{K_1}$$

$$\frac{dN_2}{dt} = r_2N_2\frac{K_2-N_2-bN_1}{K_2}$$

The simultaneous growth of species 1 and 2 is given by dN_1/dt and dN_2/dt, respectively. N_1 and N_2 are the numbers of species 1 and 2 in the mixed population at time period t, K_1 and K_2 are the carrying capacities of each in the absence of the other species, and r_1 and r_2 are the intrinsic rates of increase, also in the absence of the other species. The inhibitory effect of species 2 on the growth rate of species 1 is quantified by coefficient a and, similarily, the inhibitory effect of species 1 on the growth rate of species 2 is given by b.

Based on this model four cases of competitive outcomes in a closed environment are possible.

Case 1. Both species completely overlap in necessary requirements and which survives depends on initial concentrations:

$$a > K_1/K_2 \quad \text{and} \quad b > K_2/K_1$$

Case 2. Species 1 is the sole survivor. The necessary requirements of species 2 overlaps completely within those of 1 which has a resource not available to species 2:

$$a < K_1/K_2 \quad \text{and} \quad b > K_2/K_1$$

Case 3. Species 2 is the sole survivor. The necessary requirements of species 1 overlaps completely within those of 2 which has a resource not available to species 1:

$$a > K_1/K_2 \quad \text{and} \quad b < K_2/K_1$$

Case 4. A stable coexistence is possible since each species inhibits its own population more than the other. The necessary requirements of each do not overlap completely, i.e., a resource is present for each that is not available to the other. This could be two food types in a heterogeneous environment, each of which is not mutually available to both species:

$$a < K_1/K_2 \quad \text{and} \quad b < K_2/K_1$$

Examples exist in the literature demonstrating the first three cases but these are not directly relevant to the thesis and will not be discussed. Gause (1935) demonstrated the fourth case with a mixed population of *Paramecium caudatum* and *P. bursaria* and a mixed population of *P. aurelia* and *P. bursaria*. In both mixed populations a coexistence resulted since *P. caudatum* and *P. aurelia* were more effective in consuming bacterial components of a mixed diet suspended in the upper layer of the liquid while *P. bursaria* preferred yeast cells sedimenting on the bottom.

Lerner and Ho (1961) demonstrated the importance of the genotype in competitive ability. Various inbred lines of *Tribolium castaneum* and *T. confusum* were competed with each other. Based on the outcomes they concluded that competitive ability, measured as percent survival, was genotypically determined.

Moore (1952), Pimental *et al*. (1965), and Ayala (1966) observed reversals in dominance of competing species and suggested genetic changes in competitive ability were responsible. *Drosophila melanogaster* was considered superior since it eliminated *D. simulans* in about 100 days. However, Moore found one case out of 20 in which *simulans* survived longer than usual. Competition experiments using *D. simulans* flies from the superior population demonstrated that a genetic change for increased competitive ability was responsible. He concluded that "... flies with improved competitive ability can be developed by selection and that this can be done in relatively short periods of time." Pimental *et al*. working with the house fly (*Musca domestica* L.) and the blowfly (*Phaenicia sericata* [Meig.]) observed an increase in competitive ability of the latter due to a genetic change which eventually resulted in the elimination of the house fly, initially the predominant species. They hypothesized that a coexistence of two species might be possible by an oscillation of genetic change in competitive ability with an eventual stabilization, but an oscillation of this type was not observed in their experiment.

Previous studies indicate that there is little additive genetic variability for competitive ability. Although Moore (1952) concluded that this ability can be selected in relatively short periods of time only one out of 20 competition populations was obtained in which a genetic change had occurred. Park and Lloyd (1955) were unable to detect any such changes in mixed *Tribolium* populations. Out of 28 experiments between *T. castaneum* and *T. confusum*, the latter survived in four but experiments using the *T. confusum* from these four populations demonstrated that it did not survive to any greater extent than previously. Futumya (1970) obtained inconclusive results in the following experiment. Equal numbers of *D. melanogaster* and *D. simulans* were placed in competition during oviposition. After oviposition the adults were discarded and the *D. melanogaster* progeny from this mixed larval population were used in the next generation of competition with stock *D. simulans*. *D. melanogaster* was selected in this manner for 10 generations. Based on the analysis of total population size, percent *D. melanogaster*, and total biomass, Futumya concluded "These results are consistent with the hypothesis that the genetic variance for traits relating to interspecific competition and resource utilization is highly nonadditive ..." Van Delden (1970) demonstrated a genetic increase in competitive ability of *D. melanogaster* resulting in a higher productivity (total number of flies), but this occurred only after more than 65 generations of competition with *D. simulans*. The selection procedure for competitive ability was similar to that used by Futumya, *i.e.*, a fixed number of each species was allowed to oviposit in the presence of each other for a specified time period at each generation.

Interspecific experiments have generally been unsuccessful in genetically changing competitive ability. However, Seaton and Antonovics (1967) and Bryant and Turner (1972) were successful in changing *intra*specific competitive ability. Seaton and Antonovics placed a mutant *dumpy* *D. melanogaster* stock in competition with a wild type stock during oviposition. The adult progeny from this mixed larval population were used in the next generation of competition. After three generations of this type of selection both the selected dumpy and selected wild type showed an increase in the total number of flies (i.e., productivity). In a mixed population the increased productivity of the selected stock did not result in a decreased population size of the other. They stated that "Selection has apparently caused dumpy to avoid wild type, not 'hit it harder' ...," and concluded that a divergence of requirements rather than an increase in direct competitive ability was a more likely outcome of selection in mixed species populations. However, it could be that what they observed was a repartitioning of a fixed amount of biomass to produce more individuals, but this possibility was not considered. A repartitioning of biomass occurred in the work of Bryant and Turner when they competed a green mutant strain with a wild type stock of the house fly (Musca domestica L.). Equal egg numbers of each stock type were manually placed on a food with green temporarily out of phase, relative to the wild type with respect to hatching time. The result was a selection in green for increased developmental rate. After five generations of selection the population size of green increased to approximately the same level as that of wild type but this increase was not accompanied by an increase in biomass.

Levins (1971) undertook a theoretical investigation of the Gause model to determine those parameters which could undergo a genetic change and lead to a stable coexistence of two species. He concluded that a genetic change in levels of saturation (K_1 and K_2) could result in a stable coexistence if the change was such that $a < K_1/K_2$ and $b < K_2/K_1$. He also stated that coexistence could occur if variability existed in the capacity to exploit particular elements of a heterogeneous resource supply. The elements could be considered to be the different food types of this research.

Clutterbuck and Beardmoore (1961) obtained indications that genetic variability exists in D. melanogaster for oviposition preference of foods adulterated with peppermint oil, juniper oil or lavender oil. They speculated on the possibility of such variability leading to polymorphic populations of these traits but did not consider the role they might have in interspecific competition. Moray and Connolly (1963) selected for food preference in D. melanogaster by retaining the adults that were attracted to media adulterated with peppermint oil and using these to give rise to progeny for the next generation of selection. As a result, it is not clear whether the selection is for food preference, oviposition preference, or both. In addition, some of the results were unexpected and contradictory.

Some examples in the literature of character displacement may be reflective of a genetic change that resulted from past competitive pressures. Brown (1956) describes character displacement as follows: "Two closely related species have overlapping ranges. In the parts of the ranges where one species occurs alone, the populations of that species are similar to the other species and may even be very difficult to distinguish from it. In the area of overlap, where the two species occur together, the populations are more divergent and easily distinguished, i.e., they 'displace' one another in one or more characters. The characters involved can be morphological, ecological, behavioral, or physiological; they are assumed to be genetically based."

PROCEDURE

Drosophila simulans and *D. melanogaster* were used as competitors since both have a nearly equal mean generation time, both were easily reared on the foods used, both have a high fecundity as well as dissimilar egg morphology, and since genetically variable base populations could be initiated with flies from natural populations. Other species preliminarily examined as possible competitors but found unsuitable were *D. hydei*, *D. buskii*, *D. annanasae*, *D. tripunctata*, *D. cardini*, *D. immigrans*, *D. robusta*, and *D. nigromelanica*.

The controls and base populations were maintained on a banana medium while the selected populations were maintained on the food type, either tomato or potato medium, for which they were selected to have increased preference. Other food types were investigated but the latter two were chosen since *simulans* and *melanogaster* have opposite natural preferences for them and each can support a large fly population.

The recipe for the banana medium is as follows:

water	900 ml
agar (Fishers laboratory grade)	10 gm
yeast ("Vita-Food" brand inactivated brewers yeast)	30 gm
banana (blended)	155 gm
Karo syrup	50 ml
mold inhibitor (tegosept)	25 ml

The same ingredients are used in making tomato and potato media except the banana and Karo syrup are left out and either 155 gm of Hunts tomato paste or 26 gm of potato flakes plus 155 ml of water are added instead.

Approximately 100 iso-female lines from collections around Miami, Florida were used to initiate the melanogaster base population on October 22, 1971. The simulans base population was initiated on February 13, 1972 with 40 iso-female lines originally collected from the vicinity of Jacksonville, Florida. Each was maintained in a Dobshansky-type population cage. After approximately 15 generations a tomato- and a potato-selected line (or population) was initiated for each species.

Selection occurred at the egg stage in a population cage containing eight cups of tomato and potato media. The media types were placed alternately in the cage to help cancel out irregularities in the physical environment. Oviposition was allowed to proceed (usually 24 hr) until a large progeny population (greater than 2,000 flies) was insured for the next generation. After oviposition one of the food types (with eggs) was discarded. In addition to preference, this procedure was also one for increasing fecundity in both the tomato and potato selection lines.

A number of methods were examined to determine preference for the selected and non-selected populations. Many were unsatisfactory because of the highly variable results relative to the large amount of time and work involved. The method adopted, shown in Figure 1, made it possible to perform a large number of preference determinations with a minimum of time and effort. Adults were placed into a glass vial 10 cm high and 3.3 cm in diameter. The two food types on the bottom were circular, each approximately 0.65 cm high and 1.2 cm in diameter, and were equidistant from each other and the side of the vial.

Since the preference determinations were staggered over time, this meant 19 separate preparations of the tomato and potato media. Special care was exercised during the cookings to be consistent from batch to batch for each food type. The estimated amount of yeast necessary for the 19 cookings of both food types was mixed thoroughly to homogenize possible differences in yeast species. The cooking followed a predetermined time schedule and was done in a double boiler to prevent extremes in temperature that might result in changes affecting preference. After cooking, additional water was added to compensate for unequal amounts of evaporation between preparations.

The media were poured into cups 6 cm high and 8 cm in diameter and refrigerated (down to approximately 7°C) at least six hours prior to use but were allowed to warm to room temperature before being removed from a cup and made into pellets. Pellets were made by slicing the medium into layers, placing these one on top each other five deep and then slicing through all five with a cork borer. Prepared in this manner, a pellet was slightly larger in diameter at the top than at the bottom. Before being lowered into a preference vial, with the tip of a spatula bent at a right angle, each pellet was turned over so that the larger diameter surface became the bottom. All pellets of one food type were placed into the vials before the other food was added. To prevent chemical contamination all tools and objects that came into contact with a food were washed with 95% ethanol and then rinsed with distilled water before coming into contact with the other type. The vials were stoppered with a white styrofoam plug and used for a preference determination within two hours after their preparation.

Preference was determined in groups of four or eight females (four of each species) depending on whether a single or mixed population was desired. Each species group of four females had a common genetic history (non-selected or one of the selected lines), a common larval food (tomato or potato) and a common adult food (tomato or potato). Obtaining females differing in the above characteristics meant collecting eggs from a control or selected population and then rearing the larvae and maintaining the adults on the appropriate food type. To collect eggs from a control population, flies were allowed to oviposit on banana medium but in a selected line they were given a choice between tomato and potato, as was done during selection. This procedure resulted in one more generation of selection and prevented a possible generation of reverse selection. Six food cups were obtained from the control and selected populations from which 150 eggs of melanogaster or 170 eggs of simulans were collected and put into a rearing vial of the appropriate food. Randomization in egg collection was attempted by obtaining 25 eggs from each cup for melanogaster (a total of 150), while for simulans this was modified to 30 eggs from each of four cups and 25 from the remaining two (a total of 170).

Larvae and adults were kept in a 21°C stockroom, except for a one to two day period prior to a preference determination at which time they were placed in a lab that varied between 25°C to 30°C. A total of 18 days for melanogaster and 16 days for simulans lapsed from the egg stage before determining preference. For this reason the simulans eggs were collected two days after melanogaster so that both of their preferences could be determined on the same day - a necessity for a mixed species test. Adults were lightly anesthetized with CO_2

approximately 24 hr prior to a preference determination, and sexed to separate out females which were allowed to recuperate in a vial containing fresh food of the appropriate type. Simulans was sexed two days after the first transfer but for melanogaster this was done two days after a second transfer. The 18 day as opposed to a 16 day period necessitated the second transfer, since larval activity of the first created a highly unfavorable adult environment in two days. Females were transferred to a preference vial with the aid of the device illustrated in Figure 2 and a paper funnel with a hole at the tip small enough to allow only one female to escape at a time. The device was placed over the hole and after extracting four females it was inserted between the side of the vial and the foam plug. A gentle outward blowing released the flies.

The preference tests will be referred to by number. Tables 1 and 2 enumerates the species composition, the genetic history, and the larval and adult food types of the females in a single and mixed species test, respectively. While examining both tables, recall that the controls were maintained on a banana medium except for the one generation needed to obtain flies for the single and mixed species tests.

An expanded experimental design was constructed for the single species tests of simulans (Tests 3 to 18, Table 1) since preference and fecundity in melanogaster was statistically more variable. The mixed species tests were of two types, those in which the females were obtained from the controls (Tests 23 to 26, Table 2), and those in which they were obtained from the selected lines (Tests 27 to 30, Table 2). In both types the females of a test were reared, i.e., spent

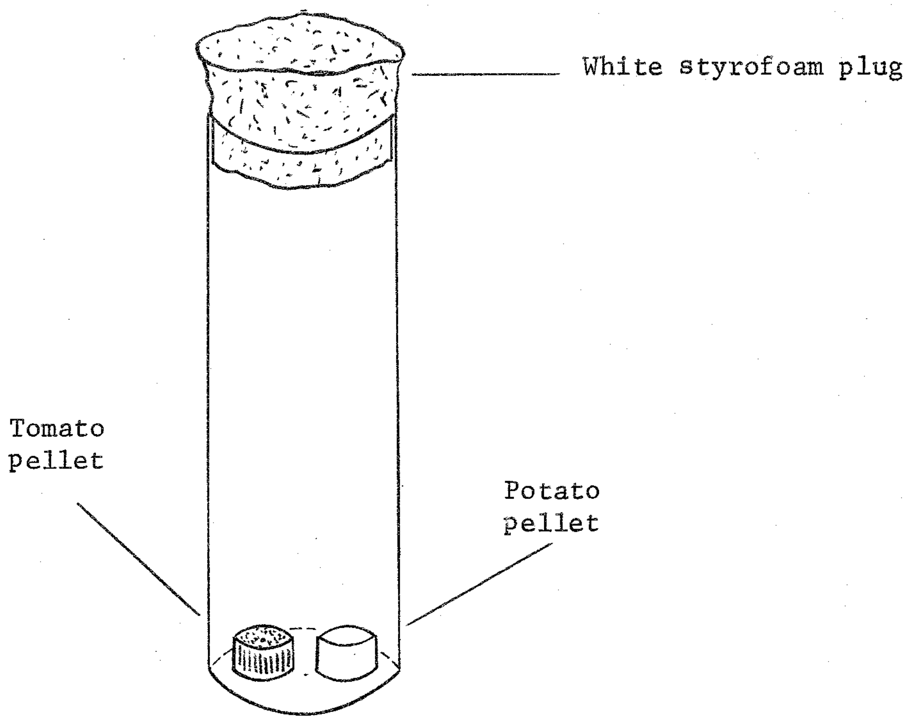


Figure 1. Preference vial. 3/4 actual size.

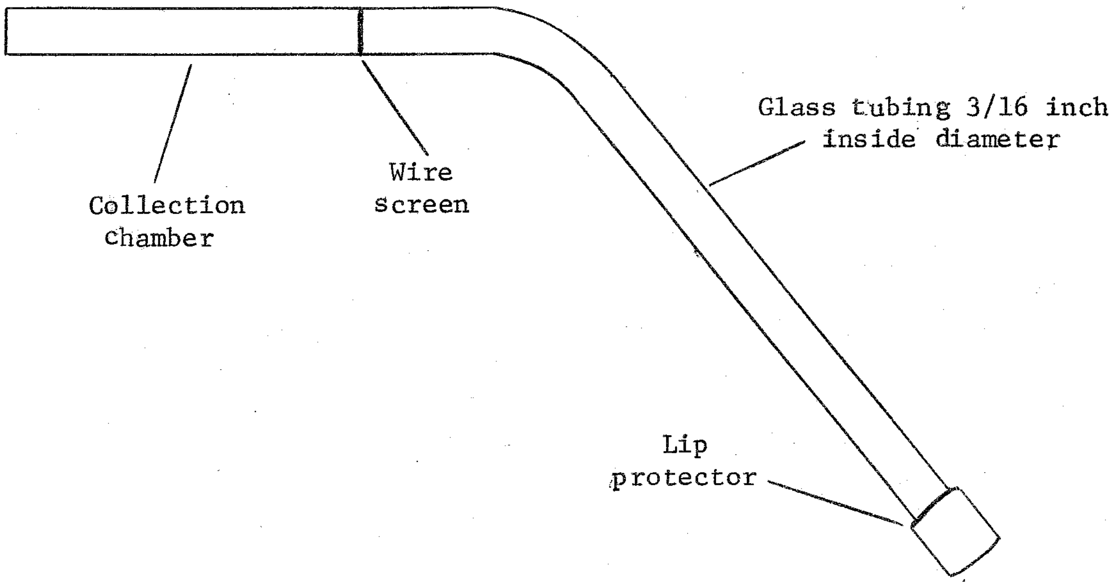


Figure 2. Instrument facilitating transfer of females to a preference vial. 3/4 actual size.

Table 1. Genetic history, larval food, and adult food of females used in single species tests.

	Test	Larval food	Adult food	Vials/Rep
Simulans control	3	tomato	tomato	8
	4	tomato	potato	6
	5	potato	tomato	6
	6	potato	potato	8
Simulans tomato line	9	tomato	tomato	8
	10	tomato	potato	6
	11	potato	tomato	6
	12	potato	potato	8
Simulans potato line	15	tomato	tomato	8
	16	tomato	potato	6
	17	potato	tomato	6
	18	potato	potato	8
Melanogaster controls	19	tomato	tomato	11
	20	potato	potato	11
Melanogaster tomato line	21	tomato	tomato	11
Melanogaster potato line	22	potato	potato	11

Table 2. Genetic history, larval food, and adult food of females used in mixed species tests.

	Test	Simulans rearing food	Melanogaster rearing food	Vials/Rep
Females from control lines	23	tomato	tomato	11
	24	tomato	potato	11
	25	potato	tomato	11
	26	potato	potato	11

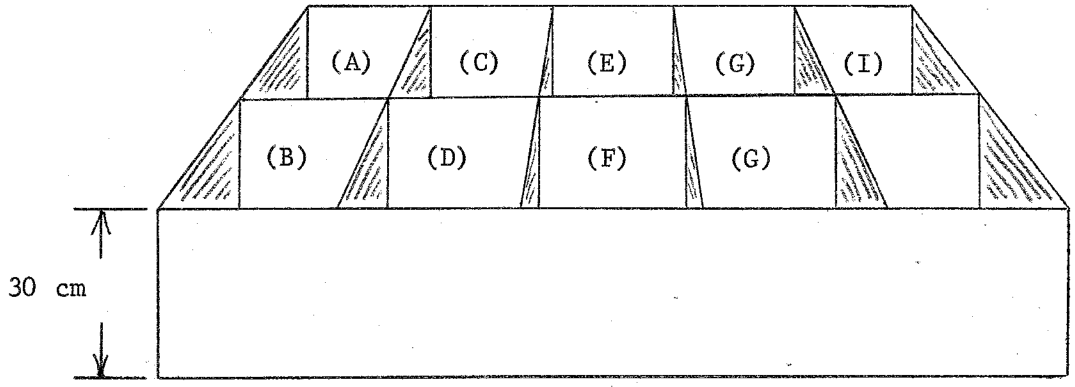
	Test	Simulans rearing food and selected line	Melanogaster rearing food and selected line	Vials/Rep
Females from selected lines	27	tomato	tomato	11
	28	tomato	potato	11
	29	potato	tomato	11
	30	potato	potato	11

their total life as larvae and adults, on one of the two foods. In the selection tests this food type was the same as the one for which they were selected to have increased preference.

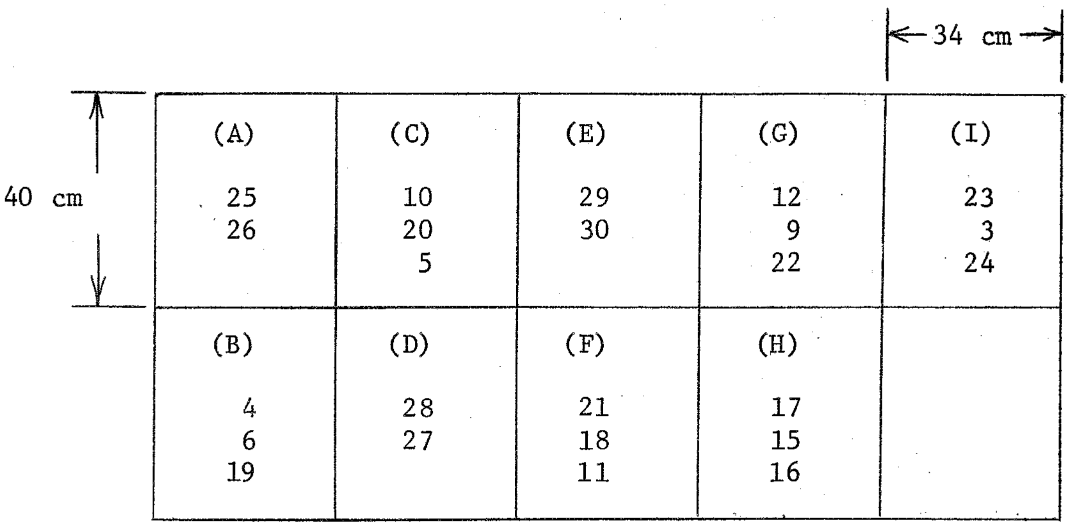
The experimental material was prepared so that all tests with the desired number of preference vials for each (given under the column heading "Vials/Rep" in both tables) could be set up in every replication. However, because of errors and other unforeseeable factors, a few replications are lacking some tests and/or have a greater or lesser than desired number of vials. Thirty-seven replications were spaced out over a three month period and this resulted in the control and selected lines undergoing approximately five more generations of maintenance and selection, respectively. Thus, the females from the selected lines in tests of the 37th replication were selected for approximately five more generations than those of the first.

All tests (single and mixed populations) were arbitrarily grouped into nine sets. The females of each set were allowed to oviposit 6 ± 1/2 hours, but (because of the time involved in preparation) a two to three hour time period separated the first and last. As a result, maintaining the six hour oviposition period meant removing some sets while others still had time to remain. To minimize disturbing other flies during this process, each set was placed in a white cardboard cubicle opened at the top and layered on the bottom with forest green colored blotting paper (Figure 3a). Vials were arranged in rows and the food types were alternated between vials in the manner shown in Figure 4. Since the fluorescent lights in the lab were parallel to these rows, it was felt this type of positioning would help cancel out unequal light intensities which might affect preference.

Figure 3b illustrates the manner in which tests were allocated to cubicles, each test combination of a cubicle representing a set. An attempt was made to cancel out effects of differential light intensity on preference both within and between cubicles by rotating sets and the tests within each set as follows. At each replication the set that went into cubicle A (Figure 3b) of the previous replication was placed in cubicle B, that of B was placed in C and so on until the set that went into cubicle I was placed in A. The tests of A set were also rotated in a similar manner within a cubicle.



a. Front view.



b. Overhead view.

Figure 3. Cubicle layout for determining preference. Cubicles are lettered from A to I. The numbers in the "Overhead view" (B) represents preference tests.

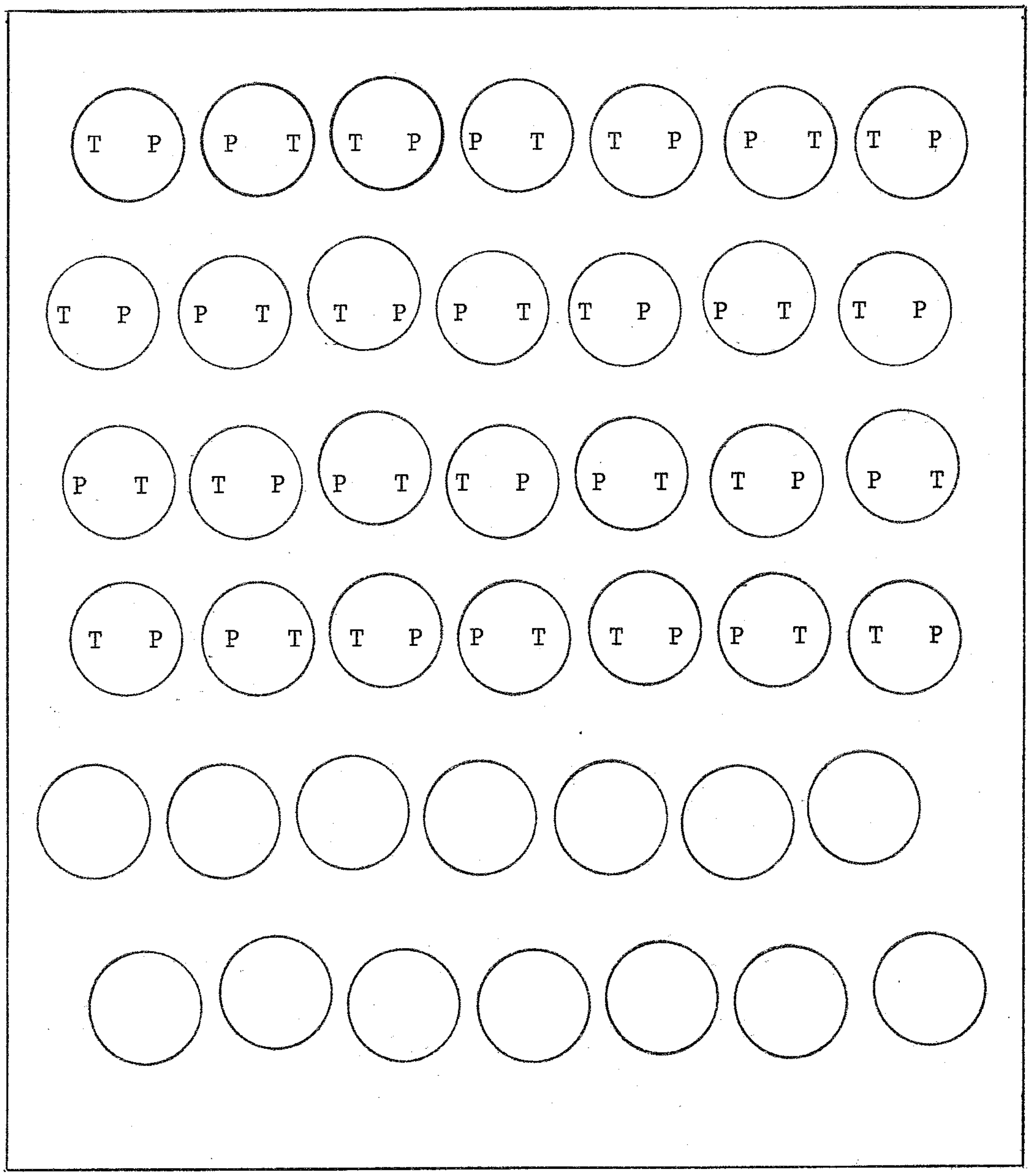


Figure 4. Preference vial and food pellet positioning within a cubicle. Preference vials are represented by circles, tomato pellets by the letter "T" and potato pellets by the letter "P."

After the six hour oviposition period, adults were discarded and the preference vials were stored at temperatures between 2°C and 8°C until a more convenient time for egg counting. The pellets could be stored as long as a week at these temperatures with few larval emergences; even then there was no difficulty in counting and distinguishing the species of an empty egg case.

Eggs were counted under a binocular dissecting microscope by rotating a food pellet resting on a 2x2 cm plastic square held between the two thumbs and forefingers. The square was covered with masking tape to keep the pellet from sliding, and a black line was drawn to mark the starting point of an egg count. For pellets from a mixed species vial, first the total eggs (both species combined) were counted, then the eggs of one of the two species, and finally, those for which a species determination could not be made. The number of eggs of the other species was obtained by subtraction.

ANALYSIS

Two basic measurements were obtained from each vial - preference and fecundity. If T = number of eggs on tomato and P = number of eggs on potato, then

$$\text{preference} = T/(T+P) \quad \text{and}$$

$$\text{fecundity} = T+P \quad .$$

Within a replication the mean preference and mean fecundity for a test was obtained by averaging over the number of vials that contributed to each measurement. There was no weighting for between replication differences in the number of vials contributing to a measurement mean. These means were the data points (*i.e.*, mean preference and mean fecundity) used in the analysis.

The analysis is divided into two sections, one utilizing the single species data (the "Single species analysis") and the other utilizing the mixed data (the "Mixed species analysis"). All analyses within each were of the factorial type except those involving the *melanogaster* single species data which consisted of difference between not more than two test means at a time.

Each data point is variable with respect to species composition (*i.e.*, *simulans* or *melanogaster* in a single species test or both species in a mixed test), genetic history of each species (non-selected or selected), larval food (tomato or potato), and adult food (tomato or potato). The factorials consisted of linear contrasts, *i.e.*, additions and subtractions, of these data points to determine the existence and magnitude of an effect. With appropriate construction any effects of the above variables cancel out except the one of interest. It would be arithmetically expressed as a linear contrast value. If that particular effect did not exist then the linear contrast value would equal zero, excluding random deviations. Within factorials linear contrasts were orthogonally independent to prevent confounding of effects and, thereby, making for an easier and more definitive interpretation of the analysis results.

The variance over replications for each contrast value was obtained in the usual manner, i.e.,

$$s^2 = \frac{\sum_{i=1}^{n} (X_i - \bar{X}^2}{n-1}$$

where n = number of replications, X_i = contrast value for replication i, and $\bar{X}$ = contrast value mean = $\frac{\sum_{1}^{n} X_i}{n}$.

If a replication was missing a test value needed in a contrast, then that contrast value was treated as missing and was omitted in calculating a mean and variance. As a result, there is not always the 36 degrees of freedom as expected from 37 replications.

The general null hypothesis for each single and mixed species analysis states that the contrast value equals zero, i.e., $H_0: u = 0$, and the alternate hypothesis is that the contrast value does not equal zero, i.e., $H_1: u \neq 0$. A t-test at the 0.05 level of significance was used.

Single Species Analysis

Although replication means are used in the analyses to follow, a summary of the preference and fecundity means, averaged over replications, for each test are given in Tables 3 and 4, respectively.

Table 3. Mean preferences averaged over replications in single species tests.

	Test	Preference	Degrees of freedom	Standard deviation
Simulans control	3	.5726	33	.0637
	4	.5474	34	.0775
	5	.5704	33	.0744
	6	.5047	34	.0725
Simulans tomato line	9	.6099	36	.0784
	10	.5858	36	.0668
	11	.5944	36	.0742
	12	.5448	36	.0671
Simulans potato line	15	.4940	36	.0612
	16	.4512	36	.0668
	17	.4528	36	.0642
	18	.4057	36	.0707
Melanogaster controls	19	.3833	36	.1121
	20	.4638	36	.1004
Melanogaster tomato line	21	.4219	36	.0997
Melanogaster potato line	22	.2776	35	.0735

Table 4. Mean fecundities averaged over replications in single species tests.

	Test	Fecundity	Degrees of freedom	Standard deviation
Simulans control	3	64.16	33	18.82
	4	78.83	34	24.84
	5	55.48	33	15.74
	6	78.70	34	24.43
Simulans tomato line	9	71.96	36	22.12
	10	82.41	36	29.02
	11	63.23	36	19.82
	12	89.52	36	22.09
Simulans potato line	15	69.28	36	19.56
	16	89.83	36	25.83
	17	61.99	36	17.11
	18	84.37	36	24.47
Melanogaster controls	19	46.32	36	20.00
	20	60.05	36	22.96
Melanogaster tomato line	21	36.93	36	15.70
Melanogaster potato line	22	65.15	35	26.71

The experimental design for simulans was essentially a 3x2x2 factorial - three genetic histories (a control, and a tomato-, and potato-selected line), two larval foods (tomato and potato), and two adult foods (tomato and potato) - and the data was analyzed accordingly. Orthogonal contrasts were constructed to give four meaningful main effects. The interactions that resulted were a consequence of the particular main effects that were chosen. The results of the analysis for preference are given in Table 5 and those for fecundity in Table 6.

For the preference analysis (Table 5), the "Selection effect" compares a tomato with a potato selected line. Since selection was in opposite directions this contrast detects selection response. The "Assymetrical selection effect" compares twice the control with the sum of the selected lines, and therefore, detects assymetry of selection response. The "Adult effect" and "Larval effect" determines the effect of different adult and larval foods, respectively, on preference.

Similarily for the fecundity analysis (Table 6), the "assymetrical selection effect" compares a tomato and a potato selected line but since selection was in the same direction, i.e., for increased fecundity, assymetry of response is detected. As with the preference analysis the "Selection effect" compares twice the control with the sum of the selected lines, but now this contrast detects a selection response for increased fecundity. The "Adult effect" and "Larval effect" determines the effect of different adult and larval foods, respectively, on fecundity.

Table 5. <u>Simulans</u> 3x2x2 factorial analysis results of preference.

Contrast	Test 3	Test 4	Test 5	Test 6	Test 9	Test 10	Test 11	Test 12	Test 15	Test 16	Test 17	Test 18	Preference contrast value[a]	d.f.	Standard deviation	Divisor
A. Selection effect	0	0	0	0	+	+	+	+	-	-	-	-	.0664***	36	.0218	8
B. Assymetrical selection effect	2	2	2	2	-	-	-	-	-	-	-	-	.0140***	32	.0169	16
C. Adult effect	+	-	+	-	+	-	+	-	+	-	+	-	.0216***	32	.0162	12
D. Larval effect	+	+	-	-	+	+	-	-	+	+	-	-	.0143***	32	.0151	12
E. A x C	0	0	0	0	+	-	+	-	-	+	-	+	-.0020	36	.0151	8
F. A x D	2	-2	2	-2	-	+	-	+	-	+	-	+	.0016	32	.0162	16
G. B x C	0	0	0	0	+	+	-	-	-	-	+	+	-.0038	36	.0149	8
H. B x D	2	2	-2	-2	-	-	+	+	-	-	+	+	-.0024	32	.0142	16
I. C x D	+	-	-	+	+	-	-	+	+	-	-	+	-.0047*	32	.0141	12
J. A x I	0	0	0	0	+	-	-	+	-	+	+	-	-.0027	36	.0148	8
K. B x I	2	-2	-2	2	-	+	+	-	-	+	+	-	-.0031	32	.0155	16

[a] On a per treatment mean basis, * .05 < P < .08, *** P < .001

Table 6. _Simulans_ 3x2x2 factorial analysis results of fecundity.

Contrast	Test 3	Test 4	Test 5	Test 6	Test 9	Test 10	Test 11	Test 12	Test 15	Test 16	Test 17	Test 18	Fecundity[a] contrast value	d.f.	Standard deviation	Divisor
A. Assymetrical selection effect	0	0	0	0	+	+	+	+	-	-	-	-	.21	36	5.29	8
B. Selection effect	2	2	2	2	-	-	-	-	-	-	-	-	-3.39***	32	4.39	16
C. Adult effect	+	-	+	-	+	-	+	-	+	-	+	-	-9.93***	32	5.87	12
D. Larval effect	+	+	-	-	+	+	-	-	+	+	-	-	2.46**	32	4.10	12
E. A x C	0	0	0	0	+	-	+	-	-	+	-	+	.77	36	4.29	8
F. A x D	2	-2	2	-2	-	+	-	+	-	+	-	+	.41*	32	3.46	16
G. B x C	0	0	0	0	+	+	-	-	-	-	+	+	-1.39	36	3.95	8
H. B x D	2	2	-2	-2	-	-	+	+	-	-	+	+	-.01	32	3.56	16
I. C x D	+	-	-	+	+	-	-	+	+	-	-	+	2.18***	32	3.24	12
J. A x I	0	0	0	0	+	-	-	+	-	+	+	-	1.75*	36	4.89	8
K. B x I	2	-2	-2	2	-	+	+	-	-	+	+	-	-.13	32	3.54	16

[a] On a per treatment mean basis, * P < .05, ** P < .01, *** P < .001

Since the experimental design of *melanogaster* was not as complex as that of *simulans*, only a few questions relating to preference and fecundity could be answered. The results of these analyses are given in Tables 7 and 8. The "Tomato selection effect" and "Potato selection effect" of Table 7 determines whether the selection procedure was effective in producing a genetic change in tomato and potato preference, respectively. For fecundity the "Tomato selection effect" and "Potato selection effect" of Table 8 detects genetic changes in the tomato and potato selected lines, respectively, and the "Rearing effect" compares the effect of the two foods on fecundity of females from the control line.

The results of the analysis for determining the existence of a correlation between preference and fecundity for the single species tests are given in Table 9. For any given test the analysis consisted of, first, calculating replication correlation coefficients. To perform these calculations, preference and fecundity values obtained from the same vial were treated as paired measurements. All paired values obtained from the vials representing a replication test were used in the standard correlation coefficient formula. A mean and variance of replication correlation coefficients was calculated and used in a t-test of the null hypothesis that a test correlation coefficient does not exist, *i.e.*, H_0:u = 0. The alternate hypothesis was that a test correlation coefficient does exist, *i.e.*, H_0:u = 0.

Mixed Species Analysis

From each vial the following measurements were obtained: *simulans* preference, *melanogaster* preference, combined preference, *simulans*

Table 7. *Melanogaster* analysis results of preference.

Contrast	Test 19	Test 20	Test 21	Test 22	Preference[a] contrast value	d.f.	Standard deviation	Divisor
A. Tomato selection effect	+	0	-	0	-.0193*	36	.0497	2
B. Potato selection effect	0	+	0	-	.0957***	35	.0496	2

[a] On a per treatment mean basis

* P < .05, *** P < .001

Table 8. *Melanogaster* analysis results of fecundity.

Contrast	Test 19	Test 20	Test 21	Test 22	Fecundity[a] contrast value	d.f.	Standard deviation	Divisor
A. Tomato selection effect	+	0	-	0	4.65***	34	6.08	2
B. Potato selection effect	0	+	0	-	-1.97	33	8.45	2
C. Rearing effect	+	-	0	0	-6.86***	36	7.86	2

[a] On a per treatment mean basis

*** P < .001

Table 9. Correlation analysis between preference and fecundity within single species tests.

Test	Correlation coefficient	d.f.	Standard deviation
3	.2081**	33	.3590
4	.1537	34	.4899
5	.0195	33	.4482
6	-.0708	34	.4117
9	.0219	35	.4122
10	-.0293	34	.5347
11	.1372	35	.4503
12	.0824	36	.4574
15	.0990	36	.3213
16	.0477	35	.3904
17	.1567**	36	.3387
18	.0452	36	.3436
19	.0395	36	.3178
20	-.0070	37	.3265
21	.0720	36	.3224
22	.0440	35	.3261

** $P < .01$

fecundity, *melanogaster* fecundity, and combined fecundity. If

A = the number of *simulans* eggs on tomato,
B = the number of *simulans* eggs on potato,
C = the number of *melanogaster* eggs on tomato,
D = the number of *melanogaster* eggs on potato,

then,

simulans preference = A/(A+B)
melanogaster preference = C/(C+D)
combined preference = (A+C)/(A+B+C+D)
simulans fecundity = A+B
melanogaster fecundity = C+D
combined fecundity = A+B+C+D .

Two possible sources of bias exist in the *simulans* and *melanogaster* data. It has already been mentioned that during the egg counting those eggs which could not be assigned to either species category were recorded as being indeterminate. This was not a frequent occurrence, about one in every 20 vials, and usually involved one to three eggs. In the single species vials it was observed that most of the eggs which fell into this category were usually those of *melanogaster*. Therefore, to simplify the mixed species analysis indeterminate eggs were added to the *melanogaster* totals. If an indeterminate egg occurred on a tomato pellet it was added to the total eggs of *melanogaster* on tomato ("C" was defined above) and similarly if it occurred on a potato pellet, it was added to the total of eggs on potato. Obviously, not all indeterminate eggs were laid by *melanogaster* and this, therefore, results in a possible bias. However, if the bias is consistent in direction and magnitude within and between replications, then the conclusions from the analysis should not be invalid as long as no direct comparisons are made between the single and mixed species data. In any case the bias should be quite small.

The second possible source of bias arises from the tendency of each species to produce a small number of eggs (approximately 1% in *melanogaster* and 5% in *simulans*) closely resembling those of the other species. However, if it is assumed that the author was consistent within and between replications in assigning these eggs to the *wrong* species category, then this bias should also not affect conclusions from the analysis for reasons already given.

Although replication test means are used in the analyses to follow, as in the single species data, the averages over replications for preference and fecundity are summarized in Table 10 and 11, respectively. The mixed data is complete in the sense that no test or measurement is missing in any of the 35 replications and subsequently all variances are based on 34 degrees of freedom.

Three types of a 2x2 factorial were used in the analyses. These are diagrammed below and designated in accordance with the type of tests contrasted within each (the number within the diagrams are the tests that were contrasted).

a) Control factorial

	melanogaster	
simulans	tomato	potato
tomato	23	24
potato	25	26

b) Selection factorial

simulans	*melanogaster*	
	tomato	potato
tomato	27	28
potato	29	30

c) Selection-Effect factorial

simulans	*melanogaster*	
	tomato	potato
tomato	27-23	28-24
potato	29-25	30-26

The Control factorial contrasts means of tests composed of females obtained from control lines while the Selection factorial contrasts means of tests composed of females obtained from the selected lines. The Selection-Effect factorial contrasts selection effects, *per se*, since all biological effects, besides those resulting from the selection procedure, should cancel out when the respective control mean is subtracted.

The results of the *simulans* and *melanogaster* preferences analyzed by the Control and Selection factorials are given in Tables 12, 13, 14, and 15. The "Rearing effect" of the Control factorials (Tables 12 and 13) determines whether learned behavior (*i.e.*, the effect due to rearing on different foods) is still expressed in the presence of the other species. The "Joint rearing and selection effect" of the Selection factorials (Tables 14 and 15) detects the existence of a joint effect between rearing and selection. Modification in preference due to the presence of the other species is detected by the "Other species effect" which, in Tables 12 and 13, is separated into two components,

Table 10. Mean preferences averaged over replications in mixed species tests.

	Tests	*Simulans* preference		*Melanogaster* preference		Combined preference	
		Preference	Standard deviation[a]	Preference	Standard deviation[a]	Preference	Standard deviation[a]
Control tests	23	.5637	.0791	.3747	.0777	.4852	.0710
	24	.5219	.0866	.4009	.0808	.4652	.0658
	25	.5184	.0713	.3116	.0973	.4433	.0636
	26	.4756	.0748	.3485	.0890	.4207	.0572
Selection tests	27	.5996	.0799	.4281	.1020	.5461	.0704
	28	.5867	.0932	.2580	.0714	.4446	.0514
	29	.3704	.0670	.4111	.1115	.3783	.0508
	30	.3718	.0787	.2283	.0626	.3147	.0592

[a] 34 degrees of freedom

Table 11. Mean fecundities averaged over replications in mixed species tests.

		Simulans fecundity		Melanogaster fecundity		Combined fecundity	
	Tests	Fecundity	Standard[a] deviation	Fecundity	Standard[a] deviation	Fecundity	Standard[a] deviation
Control tests	23	60.08	17.84	42.50	24.04	102.55	34.88
	24	59.04	17.69	54.31	25.78	114.34	38.34
	25	70.44	23.06	38.88	18.86	109.80	36.45
	26	72.71	25.01	57.01	24.48	131.31	47.49
Selection tests	27	65.16	19.80	32.62	14.63	98.24	29.50
	28	66.85	20.62	53.21	20.59	120.53	36.46
	29	82.00	25.17	34.97	14.75	117.20	33.38
	30	83.10	23.72	54.00	18.97	137.94	39.84

[a] 34 degrees of freedom

Table 12. Control factorial analysis results of *simulans* preference.

Contrast	Test 23	Test 24	Test 25	Test 26	Preference[a] contrast value	Standard[b] deviation
A. Rearing effect	+	+	-	-	.0229***	.0301
B. Other species effect	+	-	+	-	.0211***	.0254
C. Interaction	+	-	-	+	-.0002	.0188
D. Other species affect on tomato reared females	+	-	0	0	.0209***	.0294
E. Other species affect on potato reared females	0	0	+	-	.0214***	.0337

[a] On a per treatment mean basis, [b] 34 degrees of freedom,

*** P < .001

Table 13. Control factorial analysis results of *melanogaster* preference.

Contrast	Test 23	Test 24	Test 25	Test 26	Preference[a] contrast value	Standard[b] deviation
A. Rearing effect	+	-	+	-	-.0158*	.0441
B. Other species effect	+	+	-	-	.0289***	.0253
C. Interaction	+	-	-	+	.0027	.0369
D. Other species affect on tomato reared females	+	0	-	0	.0315***	.0450
E. Other species affect on potato reared females	0	+	0	-	.0262***	.0445

[a] On a per treatment mean basis, [b] 34 degrees of freedom,

* P < .05, *** P < .001

Table 14. Selection factorial analysis results of simulans preference.

Contrast	Test 27	Test 28	Test 29	Test 30	Preference[a] contrast value	Standard[b] deviation
A. Joint rearing and selection effect	+	+	-	-	.1110***	.0343
B. Other species effect	+	-	+	-	.0029	.0239
C. Interaction	+	-	-	+	.0036	.0168
D. Other species affect on tomato selected and reared females	+	-	0	0	.0065	.0284
E. Other species affect on potato selected and reared females	0	0	+	-	-.0007	.0300

[a] On a per treatment mean basis, [b] 34 degrees of freedom,

*** $P < .001$

the "Other species effect on tomato reared females" and the "Other species effect on potato reared females." In Tables 14 and 15 the two components are the "Other species effect on tomato selected and reared females" and the "Other species effect on potato selected and reared females." For example, the affect of melanogaster on simulans females reared on tomato media is given by the "Other species effect on tomato reared females" in Table 12.

Table 16 contains the results of the combined preferences analyzed by the Selection-Effect factorial. The "Simulans selection effect" determines whether the selected preference of simulans is still expressed in a mixed species population and likewise for melanogaster with respect to the "Melanogaster selection effect."

Table 15. Selection factorial analysis results of melanogaster preference.

Contrast	Test 27	Test 28	Test 29	Test 30	Preference[a] contrast value	Standard[b] deviation
A. Joint rearing and selection effect	+	-	+	-	.0882***	.0518
B. Other species effect	+	+	-	-	.0117	.0356
C. Interaction	+	-	-	+	-.0032	.0378
D. Other species affect on tomato selected and reared females	+	0	-	0	.0085	.0591
E. Other species affect on potato selected and reared females	0	+	0	-	.0148	.0437

[a] On a per treatment mean basis, [b] 34 degrees of freedom

*** $P < .001$

Table 16. Selection-Effect factorial analysis results of combined preference.

Contrast	Tests 27-23	Tests 28-24	Tests 29-25	Tests 30-26	Preference[a] contrast value	Standard[b] deviation
A. Simulans selection effect	+	+	-	-	.0264***	.0126
B. Melanogaster selection effect	+	-	+	-	.0153***	.0152
C. Interaction	+	-	-	+	.0051*	.0147

[a] On a per treatment mean basis, [b] 34 degrees of freedom,

* $P < .05$, *** $P < .001$

The results of the analyses for the measurement combined fecundity, analyzed by the Control and Selection factorials, are given in Tables 17 and 18. The "*Simulans* rearing effect" and the "*Melanogaster* rearing effect" (Table 17) determines whether fecundity differences, due to rearing on different foods, of *simulans* and *melanogaster*, respectively, are still expressed in the presence of the other species. The "*Simulans* joint effect" and the "*Melanogaster* joint effect" (Table 18) have the same interpretation except differences due to the joint effects of rearing and selection are detected.

The analyses for the measurements *simulans* fecundity (Tables 19 and 21, the Control and Selection factorial results, respectively) and *melanogaster* fecundity (Tables 20 and 22, the Control and Selection factorial results, respectively) give the same type of information as the above on combined fecundity. The advantage here, however, is that fecundity differences can specifically be traced to species differences. The "Rearing effect" (Tables 19 and 20), the "Joint rearing and selection effect" (Tables 21 and 22), and the "Other species effect" (all tables) have the same type of interpretations as other previous identically named effects, except here species specific differences in fecundity are detected.

Table 17. Control factorial analysis results of combined fecundity.

Contrast	Test 23	Test 24	Test 25	Test 26	Fecundity[a] contrast value	Standard[b] deviation
A. _Simulans_ rearing effect	+	+	-	-	-6.05**	11.89
B. _Melanogaster_ rearing effect	+	-	+	-	-8.33***	8.61
C. Interaction	+	-	-	+	2.43*	5.75

[a] On a per treatment mean basis, [b] 34 degrees of freedom,

* P < .05, ** P < .01, *** P < .001

Table 18. Selection factorial analysis results of combined fecundity.

Contrast	Test 27	Test 28	Test 29	Test 30	Fecundity[a] contrast value	Standard[b] deviation
A. _Simulans_ joint effect	+	+	-	-	-9.09***	12.13
B. _Melanogaster_ joint effect	+	-	+	-	-10.76***	9.18
C. Interaction	+	-	-	+	-.39	5.53

[a] On a per treatment mean basis, [b] 34 degrees of freedom,

*** P < .001

Table 19. Control factorial analysis results of _simulans_ fecundity.

Contrast	Test 23	Test 24	Test 25	Test 26	Fecundity[a] contrast value	Standard[b] deviation
A. Rearing effect	+	+	-	-	-6.01***	8.86
B. Other species effect	+	-	+	-	-.31	4.21
C. Interaction	+	-	-	+	.83	4.62

[a] On a per treatment mean basis, [b] 34 degrees of freedom

*** P < .001

Table 20. Control factorial analysis results of _melanogaster_ fecundity.

Contrast	Test 23	Test 24	Test 25	Test 26	Fecundity[a] contrast value	Standard[b] deviation
A. Rearing effect	+	-	+	-	-7.48***	9.02
B. Other species effect	+	+	-	-	.23	6.14
C. Interaction	+	-	-	+	1.58*	3.71

[a] On a per treatment mean basis, [b] 34 degrees of freedom

* P < .05, *** P < .001

Table 21. Selection factorial analysis results of *simulans* fecundity.

Contrast	Test 27	Test 28	Test 29	Test 30	Fecundity[a] contrast value	Standard[b] deviation
A. Joint rearing and selection effect	+	+	-	-	-8.27***	11.11
B. Other species effect	+	-	+	-	-.70	4.90
C. Interaction	+	-	-	+	-.15	5.09

[a] On a per treatment mean basis, [b] 34 degrees of freedom

*** P < .001

Table 22. Selection factorial analysis results of *melanogaster* fecundity.

Contrast	Test 27	Test 28	Test 29	Test 30	Fecundity[a] contrast value	Standard[b] deviation
A. Joint rearing and selection effect	+	-	+	-	-9.91***	6.76
B. Other species effect	+	+	-	-	-.78	3.33
C. Interaction	+	-	-	+	-.39	3.27

[a] On a per treatment mean basis, [b] 34 degrees of freedom

*** P < .001

DISCUSSION

One of the objectives of the research was to determine whether food oviposition preference could be changed by selection. In *simulans* and *melanogaster*, selection for both tomato and potato preference resulted in a statistically significant shift in the direction of selection (Table 5, contrasts A and B for *simulans* and Table 7, contrasts A and B for *melanogaster*). For *simulans* the mean preference of the control is .549 as compared to .584 and .451 for the tomato and potato line, respectively. (Table 3. The mean for the control is obtained by averaging over the four control tests, *i.e.*, test numbers 3, 4, 5, and 6. The means of the tomato and potato selected lines are obtained in a similar manner.) The magnitude of the shift, relative to the controls, is .035 for the tomato line and .098 for the potato line, both of which are highly significant ($P < .001$). Similarly, for *melanogaster* the magnitude of the shift is .039 for the tomato line and .186 for the potato line. (Table 3. The values are obtained by taking the differences of Tests 21 and 19 and Tests 20 and 22, respectively.)

In both species there was more progress in selecting for potato preference. This could reflect unequal selection intensities. During the selection procedure more eggs were observed to be consistently oviposited on the tomato media, and this may have resulted in a stronger selection intensity for potato preference.

Although the above observation (more eggs oviposited on tomato during selection) is consistent with the non-selected preference of *simulans*, .549 (the average of the control tests of Table 3), it is inconsistent with that of melanogaster, .424 (the average of Tests 19 and 20 of Table 3). Thus, while simulans prefers tomato more than 50% of the time, it is preferred by melanogaster less than 50% of the time. This inconsistency could be the result of either erroneous observation or a change in the control preference through time. However, it is felt that a more probable explanation is a confounding of adult feeding preference with oviposition preference. Support for this conjecture comes from the observation that, at least in some species, adult feeding and oviposition sites are separated (Carson and Stalker, 1951), but whether this can be extended to melanogaster and the food types used is uncertain. In addition, the overpowering smell of tomato medium (to the human sense of olfaction) may initially attract melanogaster (and simulans) females. Once on the food, the more confining nature of the cup (as opposed to the unrestricted movement of the females in the preference vials) may reduce the tendency of females to fly to another food for oviposition. An initial attraction to tomato could lead to a snowballing effect since an aggregative tendency between cups has been observed in population cages containing only one food type. The result is a clumping distribution of eggs, a phenomenon also noted by Del Solar et al. (1966).

These results show that, at least for simulans and melanogaster, additive genetic variability does exist in natural populations for preference - an ecologically important trait. These genetic shifts occurred in 15-20 generations of selection and it is also felt that for the reasons just discussed (adult aggregative tendencies and adult feeding preferences), the intensity of selection for either food may not be as severe as that which might occur under more natural conditions. These results are an exception to Futumya's (1970) conclusion that "...traits affecting interspecific competition or adaptation to a new environment may be largely non-additive..." The "new environment" may be (a) the result of migration, (b) the elimination of a food source (a changing environment), or (c) a previously non-existent competitive pressure from a closely related species. Others could be listed but, in short, any previously non-existent selective pressure could be considered as a new environment. Hutchinson's "Homage to Santa Rosalia or why are there so many kinds of animals?" calls attention to the problem of explaining the origin of the larger than expected species diversity, especially in the tropics. Dethier (1954) points out that, in general, phytophagous insect species are fairly restrictive in the food they prefer even though nutritionally another plant species may be adequate. He maintains that "...nutritionally unimportant token stimuli (attractants and repellants) are predominantly responsible for regulating the feeding preferences of phytophagous insects." Numerous feeding studies have shown that monophagous and oliphagous insects are capable of maintaining populations on other food plants usually not considered as part of their diet (see Brues, 1924; Painter, 1936; and Dethier, 1954 for a further discussion on this subject). Brown (1956) cites many examples of character divergence in natural communities and points out that some of the hybrid zones may actually be two species exhibiting character divergence rather than a hybridization along the zone of contact. Andrewartha and Birch (1954) maintain that the success of a species invading a new community is almost totally dependent upon the degree of preadaptation. However, the results of this study indicate that preference, an important ecological trait to phytophagous insects, is easy to select. Therefore, the success of a species may not necessarily depend upon the degree of preadaptation. The results also indicate that preference may be an exception to the rule that traits affecting fitness are to a large part non-additive (Robertson, 1955). Waddington's genetic assimilation work on salt tolerance (1959) supports these conclusions. One food with different salt concentrations could be considered as different types to which a species might adapt under the pressures of a natural environment. After 21 generations of severe selection, a salt tolerant strain was developed.

In addition to a preference shift in simulans, selection also increased fecundity but at about the same level in both selected lines. This is not unexpected since selection for fecundity was in the same direction for each (Table 6, contrasts A and B). However, the increased fecundity was detected by contrasting the selected lines with the control in the analysis. Females from the control, adapted to the banana medium, were not given banana as one of the choices during a preference test. Thus, the apparent increased fecundity may, instead, reflect a decrease in the control since banana was not one of the choices. This interpretation is the alternative to a real increase in the selected lines with or without the presence of banana medium.

In contrast to the simulans results, the melanogaster tomato line had a statistically significant reduced fecundity (Table 8, contrast A). There was no statistically significant difference between the potato line and the control (Table 8, contrast B), but the non-significant shift agrees with simulans by being in the direction of increased fecundity. These differences, relative to simulans, may be species specific but may also result from the higher variability of the melanogaster egg data, and possibly, a greater sensitivity to heterogeneities in the physical layout of the preference tests.

If the above results for simulans and melanogaster are real and not apparent (due to banana not being one of the choices in a preference test), then additive genetic variability is indicated for fecundity. This is inconsistent with the conclusion of Robertson (1957) that genetic variability for fecundity is non-additive. However, in his work the melanogaster females were already adapted to the media type - in this case cornmeal. It appears that with another dimension, namely adaptation to a new food, there is sufficient additive genetic variability to produce a selective response. Thus, the fecundity data further support the hypothesis that additive genetic variability exists in natural populations for traits that allow a species to respond to a new or changing environment. With respect to interspecific competition, the preference and fecundity results indicate that a species does not necessarily have to increase "tooth and claw" type competitive ability to avoid extinction but instead may easily shift to another niche (e.g., food type) not occupied by the other species.

The results of the simulans analysis also show that the type of food to which the larvae and adults were exposed influences food preference (Table 5, contrasts C and D). Whether these types of influence are the result of behavioral conditioning or habituation has still not been resolved in the literature and they will, therefore, be referred to as the "larval effect" and "adult effect" (see Thorpe, 1957, for a further discussion of conditioning and habituation). A nearly statistically significant (.05<P<.08) interaction between the larval and adult effects was present (Table 5, contrast I), but this may simply reflect the fact that in those tests where the larval and adult foods were different, a two to three day period elapsed after eclosion before the adults were transferred to the alternate food. To further support this interpretation, there was also a highly significant (P<.001) adult-larval interaction on fecundity (Table 6, contrast I). Thus, individuals that have spent both life stages on the same food type appear to have obtained an increased preference for potato and an increased fecundity.

Although the larval and adult effects for preference appear to differ in magnitude it has been shown by Hershberger and Smith (1967) that the magnitude of the larval effect (often referred to as "pre-imaginal olfactory conditioning") decreases with time. As a result, a statistically significant difference between the two would have little meaning since approximately seven days elapsed between eclosion and the determination of a larval effect whereas there was no time lapse in the determination of the adult effect. However, it should be noted that an adult effect on preference does exist. Although this has been shown in the ichneumonid parasite <u>Nemeritis canascens</u> by Thorpe (1938), only uncertain results have been obtained in <u>Drosophila</u> (Manning, 1967) to date.

A statistical breakdown into larval and adult effects was not possible for <u>melanogaster</u> and only the effect of rearing both life stages on each of the two food types was determined. Rearing on tomato medium resulted in a preference of .383 and rearing on potato medium resulted in a preference of .464 (Tests 19 and 20, respectively, of Table 3). When these values are compared to one another, one interpretation is that melanogaster acquires a reduced preference for the food type on which it was reared (recall that preference was defined as the fraction on tomato of the total eggs oviposited when comparing the two values). However, for simulans the opposite interpretation is reached. Instead of a reduction, it acquired an increased preference since tomato rearing versus potato rearing resulted in a preference of .573 versus .505 (Tests 3 and 6, respectively, of Table 3). Although the arithmetic difference between the tomato and potato reared preferences for both species (-.081 and .068 for melanogaster and simulans, respectively) are each highly significant ($P<.001$), this does not mean that for all possible foods, melanogaster will always prefer the food on which it was reared to a lesser extent and simulans will always prefer it to a greater extent. Clutterbuck and Beardmoore (1961) have already shown that when melanogaster is reared on a medium adulterated with peppermint or juniper oil, it prefers the adulterated medium to a greater extent than flies reared on normal media. However, when reared on media adulterated with lavender oil they are repelled to a greater extent than normal. That response is similar to the melanogaster results of this experiment, except lavender oil is known to be toxic to flies while tomato is evidently not since melanogaster is a pest of tomato fields (Stoner et al., 1972). It may be that melanogaster does respond in the same manner as simulans when reared on tomato but a false impression is obtained when the comparison is made with flies reared on potato. Presumably, according to this hypothesis, potato would be recognized as a less desirable food after spending one generation on it and, therefore, tomato would be preferred to a greater extent. Support for this interpretation comes from the fact that in simulans, fecundity was reduced (Table 6, contrast D) when larvae were reared on a potato as opposed to a tomato medium. It was also observed during the selection procedure that tomato was capable of producing a larger adult population from a given egg density and, therefore, the reduced fecundity may reflect a greater stress placed on larvae in the potato medium.

Directly opposed to this larval effect on fecundity is the result, also in simulans, that maintaining adults on potato instead of tomato, results in an increased fecundity ($P<.001$, Table 6, contrast C). The potato medium is softer and it may be that adults can more easily penetrate the solidified surface to obtain essential liquids and nutrients. Recall that the adults were maintained on fresh media 24 hours prior to a preference test and this is probably insufficient time for newly emerged larvae to break the surface to an extent that would significantly affect adult feeding.

The larval and adult effects are considered to be learned behavior (by workers in the field of behavior) and it is reasonable to expect them to have a role in the dynamics of natural populations. Drosophila are known to be generalized feeders and this, in part, is responsible for the world-wide distribution of melanogaster. The rearing effect on preference in one sense allows the population to "experiment" with new food sources. If the food is undesirable then females behaviorally tend to ignore it during oviposition, and also are able to more fully utilize an acceptable food resource. Thorpe (1939) and Cushing (1941) recognized the possible importance of the larval effect in terms of isolation and migration as they affect gene frequencies. In an ecological sense these behavioral traits may also influence the rate of dispersal or range expansion of a species. However, what has not been considered by previous workers is the possible influence that these traits may have on the outcome of a competitive situation.

In some of the preliminary work (before adopting the procedure used in this experiment), there appeared to be a positive correlation between preference and fecundity. If a correlation did exist then the type of analyses used and conclusions reached may be invalidated for both the single species tests just discussed and the mixed texts to follow. The results of testing for the existence of a correlation for each of the single species tests is given in Table 9. Only two out of the sixteen were statistically significant. There does not appear to be any pattern as to which test had a significant correlation and one significant result at the 5% level would be expected by chance alone. Although the results are consistent with a small amount of correlation, Two significant tests could have easily occurred by chance alone.

From the single species analyses it was found that larval, adult, and selection effects influence adult preference and fecundity, and the ecological implications to natural populations were discussed. How do these effects modify the response of a species to the presence of another, how do interacting species in turn modify these effects, and what are the implications to competitive situations in natural populations? An attempt will be made to answer these questions, but it has to be emphasized that the answers are inferred from interactions at the adult stage. Although the larval stage may be important in influencing the outcome of competition, Miller (1964a, 1964b) has concluded that *simulans* and *melanogaster* are ecologically equivalent at this stage. He suggested that competitive dominance is influenced by factors affecting adult fecundity and fertility.

To determine in what manner the presence of another species, during oviposition, modifies learned behavior (as acquired when reared on one food type versus another), it is necessary to look at the results of those mixed species tests in which females were obtained from the controls. These are Tests 23, 24, 25, and 26 (Table 2). Each species was reared, in the absence of the other, on either a tomato or a potato medium and has acquired a learned preference in accordance with the respective food type. Rearing on tomato versus potato for *simulans* resulted in a preference of .573 versus .505 (Test 3 versus 6, Table 3) and, likewise, for *melanogaster* a preference of .464 versus .383 resulted (Test 19 versus 20, Table 3). The interpretation, as stated in the "Single Species Analysis" section, is that *simulans* acquires a greater preference for the food type on which it was reared while *melanogaster* acquires a reduced preference. This is equivalent to stating that *simulans* prefers tomato when reared on tomato and prefers potato when reared on potato while *melanogaster* prefers potato when reared on tomato and prefers tomato when reared on potato. However, it has to be emphasized that these are *relative* preferences in the sense that they are not based on a single preference value (*i.e.*, a single species test value), but on a comparison of two values, one representing tomato reared females and the other potato reared females. Thus, although a species may prefer tomato greater than 50% of the time, if the (greater than .5) preferences are unequal when reared on a tomato versus a potato medium with a relativistic interpretation, one would be classified as tomato preferring and the other as potato preferring. To categorize _simulans_ and _melanogaster_ in this manner for each mixed test, the food type on which they were reared needs to be known. This is obtained from Table 2. For Test 23 _simulans_ was reared on tomato and, therefore, has a relative preference of tomato. _Melanogaster_ was also reared on tomato but has a relative preference of potato. The relative preferences of this and the other tests, obtained similarly, are listed as follows:

test number	_simulans_ relative preference	_melanogaster_ relative preference
23	tomato	potato
24	tomato	tomato
25	potato	potato
26	potato	tomato

The analysis results of the measurements _simulans_ preference and _melanogaster_ preference are given in Tables 12 and 13. The affect of _melanogaster_ on the learned preference of _simulans_ is obtained from Table 12. The "Other species affect on tomato reared females" is the arithmetic difference of Test 23 and Test 24 (_i.e._, Test 23 - Test 24). The contrast value obtained, .0209, is positive and highly significant (P<.001). This indicates that the preference of *simulans* is modified so that at the end of the oviposition period more eggs are on the food type which was preferred by *melanogaster* to a lesser extent. To see this, note that in Test 23 the *simulans* relative preference was tomato and the *melanogaster* relative preference was potato. According to the above interpretation, the preference of *simulans* for tomato should increase. In Test 24 both species have a relative preference for tomato and, therefore, the preference of *simulans* for tomato should decrease. Thus, Test 23 will have a higher preference value than Test 24 (since preference was defined as the fraction on tomato of the total eggs oviposited). The difference between the two (Test 23 - Test 24) should be positive, which is what was obtained. The highly significant and positive "Other species affect on potato reared females" (the arithmetic difference of Test 25 and Test 26) support this interpretation when the same methodology is followed.

The affect of *simulans* on the learned preference of *melanogaster* can be inferred from Table 3. The "Other species affect on tomato reared females" and the "Other species affect on potato reared females" are positive and highly significant. This indicates, following the same methodology for interpretation, that the preference of *melanogaster* is modified so that at the end of the oviposition period more eggs are on the food type which was preferred by *simulans* to a *greater* extent.

In addition to the above interspecific affects on learned preference, when the relative preference of both species are identical, an apparent reduction in fecundity results. This is inferred from the statistically significant and positive, "Interaction" effect of

Table 17. Since the effect is a contrast between those tests composed of species with different relative preference (Tests 23 and 26) and those composed of species with identical relative preferences (Tests 24 and 25), the above interpretation follows. However, the analysis was based on the measurement combined preference and, therefore, does not permit a further identification as to which species, simulans or melanogaster (or both), was responsible for the outcome. The results of the analyses to determine this, based on the measurements simulans fecundity and melanogaster fecundity, are in Tables 19 and 20. The non-significant "Interaction" effect of Table 19 (simulans fecundity) but significant "Interaction" effect of Table 20 (melanogaster fecundity) indicates that the cause is a reduction in melanogaster fecundity.

Previous competitive studies have usually involved placing two species in a closed environment containing only one food type as a limited resource. The outcome has depended, to a large extent, on the physiological and biological limitations for enduring crowded conditions and utilizing the resource to the fullest possible extent. A reversal in dominance, under the constant and uniform environments of these studies, depended on the degree of additive genetic variability for the traits involved. However, convincing evidence exists (Futumya, 1970; Mather and Cooke, 1962; McGill and Mather, 1971) that there is little additivity. This, and the outcome of the competitive studies, has led to a conclusion, typified by Futumya, that "The evolution of the species composition of a community may thus depend largely upon preadaptation of invading species to prevailing ecological conditions, including competitive pressures."

Thus, if an invading species cannot increase its competitive ability then it is "doomed" to extinction or is "driven" out. However, in none of the previous studies has an alternative to direct competition been incorporated into the experiment. For this reason, it is felt that the conclusion reached is not totally applicable under natural conditions since alternatives to direct competition probably do exist. In addition, previous workers have given little consideration to learned behavior as a factor influencing the outcome of competition. Learned and instinctive behavior is developed to a greater degree in higher organisms and it is on this level that two species may interact to avoid the "tooth and claw" competition of previous studies. In this research the heterogeneous environment was the two food types, and the learned behavior was the change in preference acquired by rearing on the one versus the other. The analysis results have shown that learned behavior can be modified when another species is present and in such a manner (as in simulans) that fewer eggs are oviposited on the food type preferred by the other species. This demonstrates that it may be possible for two species to coexit in a heterogeneous environment (of food), without either species having to undergo a genetic change. Under the constant and uniform conditions of previous studies, extinction of the disadvantaged species would be the most likely outcome. Even if coexistence was not possible, learned behavior may reduce the pressures placed upon a species, consequently reducing the probability of extinction until a genetic change did occur.

Selected behavior, in one sense, is instinctive behavior since it is not learned but has a genetic basis. To determine how the behavior of flies selected for preference is modified during interspecific competition and how this compares with non-selected flies (the control flies just discussed with respect to learned behavior), it is necessary to look at the results of those tests in which females were obtained from the selected lines. These are Tests 27, 28, 29, and 30 (Table 2). Recall that the females used in these tests were reared on the same food type for which they were selected for increased preference. The preferences, in the absence of the other species, of these types of females are the single species test means of Table 3. From this table, simulans tomato reared and selected females versus potato reared and selected females are found to have a preference of .610 versus .406 (Test 9 versus 18) and with the same comparison, melanogaster females are found to have a preference of .422 versus .278 (Test 21 versus 22). With a relative preference interpretation of these values, simulans is seen to prefer tomato when reared on and selected for tomato and prefer potato when reared on and selected for potato. These relative preferences are the same as those of the control. However, for melanogaster a reversal occurred. Previously, when reared on tomato it preferred potato and preferred tomato when reared on potato. Now, when reared on and selected for tomato it prefers tomato, and when reared on and selected for potato it prefers potato. To categorize simulans and melanogaster with respect to relative preference for each mixed species test, the food type on which they were reared and selected for preference needs to be known. This is obtained from Table 2. The following results:

test number	*simulans* relative preference	*melanogaster* relative preference
27	tomato	tomato
28	tomato	potato
29	potato	tomato
30	potato	potato

The analysis results of these tests are given in Tables 14 and 15 for *simulans* preference and *melanogaster* preference, respectively. In each table the interspecific effects that detect changes in preference due to the presence of the other species are the "Other species effect," the "Other species affect on tomato selected and reared females," and the "Other species affect on potato selected and reared females." None of these effects are statistically significant. Previously, it was found that learned behavior was modified by the presence of the other species. These results indicate, first, that selected behavior is exprssed under a competitive situation and, second, that the combination of learned and selected behavior (resulting from rearing on and having been selected for the same food type) is not modified, at least not to the extent as that of learned behavior alone, by the presence of the other species. This is equivalent to stating that the combination of learned and selected behavior has led to an increased independence from the presence of the other species with respect to food choice. The results could also be interpreted as an indication that instinctive behavior, *i.e.*, selected behavior, is less modifiable by interspecific interactions than learned behavior. However, since the effects of rearing and selection are confounded with each other in this analysis, it may be that the increased independence reflects a quantitative rather than a qualitative difference.

Previously from the analysis of learned behavior, there was found a reduction in combined fecundity for those tests in which both species had identical relative preferences. Indications were that a reduced fecundity of melanogaster was the most likely cause. Now, with the combination of learned and selected behavior, there is no longer a detectable interference on fecundity in either species, demonstrating again the increased independence during oviposition. This interpretation follows from the nonsignificant "Interaction" effect of Tables 18, 21, and 22 for combined fecundity, simulans fecundity, and melanogaster fecundity, respectively.

It has been shown that additive genetic variability exists in natural populations for preference. If this is to have any evolutionary significance for competing species, then the effects of selection have to be expressed under a competitive situation. This was inferred to have occurred in this research, but the inference was based on the joint effects of rearing and selection. The results of an analysis to detect the expression of selection, per se, are given in Table 16. The statistical method used should have cancelled out all effects except those attributable to selection. The "Simulans selection effect" and the "Melanogaster selection effect" are each highly significant, indicating that the effects of selection are expressed under a competitive situation for both species.

The results of this study support the hypothesis that additive genetic variability exists in natural populations for traits that, although not associated with direct competitive ability, could result in a coexistence of species through genetic change. The change could be a shift to a new niche. This could be considered as a type of character displacement, where instead of morphological characters, behavioral traits would exhibit a divergence along the zone of contact between two species populations.

SUMMARY AND CONCLUSIONS

From genetically variable base populations, a control, a tomato, and a potato selected line were initiated for *Drosophila simulans* and *Drosophila melanogaster*. Selection occurred at the egg stage by giving the adults a choice between the two foods and then discarding one of the food types with oviposited eggs. After approximately 20 generations of selection, learned behavior, selected behavior, and the joint effects of both were studied under a single and mixed species situation.

Definite shifts in learned and selected behavior were found. For *simulans* there was an increased preference for the food type of the larvae and of the adults. These effects were augmented when the larval and adult food types were identical. However, for *melanogaster* preference was negatively affected, *i.e.*, the other food, the one in which it was not reared, was preferred to a greater extent. In all selected lines a positive divergence occurred. Relative to the controls, selection for tomato in *simulans* and *melanogaster* resulted in an increased preference shift for tomato of magnitude 3.5% and 3.9%, respectively, and selection for potato resulted in an increased preference shift for potato of magnitude 9.8% and 18.6%, respectively.

Besides an effect on preference, it was found that food type also influenced fecundity. For *simulans* when the larval food was tomato, adults had greater fecundity than when it was potato. However, when adults were maintained on potato, their fecundity was greater than those maintained on tomato.

The selection procedure for preference was also one for increasing fecundity. In both the tomato and potato selected lines of *simulans*, a definite increase occurred. However, the tomato selected line of *melanogaster* decreased in fecundity and there was no statistically significant difference between the potato line and the control.

The interspecific conditions of a mixed species test resulted in changed learned behavior and fecundity. In the presence of *melanogaster*, the preference of *simulans* was modified so that more eggs were oviposited on the food type that was preferred to a lesser degree by *melanogaster* when not in the presence of *simulans*. The converse occurred with *melanogaster*. In the presence of *simulans*, it oviposited more eggs on the food that was preferred by *simulans* to a greater extent when not in the presence of *melanogaster*. Indications were that under these conditions *melanogaster* had a reduced fecundity.

Opposed to the above results, the joint effects of learned and selected behavior increased the independence of both species during oviposition. Thus, the presence of the other species no longer had an affect on preference and *melanogaster* no longer had a reduced fecundity.

One conclusion of this study was that learned behavior may alleviate direct "tooth and claw" type competition. The other conclusion was that additive genetic variability exists in natural populations of *simulans* and *melanogaster* for food oviposition preference, and this could result in a greater adaptability of the species to a new or changing environment, or a coexistence in a heterogeneous environment. The outcome of this study supports the hypothesis that a coexistence of species, in general, is possible through a genetic change of traits not directly affecting competitive ability.

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ABSTRACT

GORODENSKI, STANLEY ADRIAN. Learned and Selected Food Oviposition Preference in Single and Mixed Species Populations of *Drosophila simulans* and *Drosophila melanogaster*. (Under the direction of HENRY E. SCHAFFER).

Drosophila simulans and *D. melanogaster* were investigated to determine whether food oviposition preference (for a tomato and a potato base medium in this research) can be easily changed by selection, thus possibly allowing a species to readily adapt to a new or changing environment, or leading to a coexistence of species in a heterogeneous environment. Besides selected behavior, learned food oviposition preference, as acquired by rearing on different foods (the tomato and potato base mediums), was also studied to determine how learned behavior is modified by the presence of another species, and how it might influence the outcome of a competitive situation.

From genetically variable base populations, a control, a tomato and a potato selected line was initiated for each species. Selection occurred at the egg stage by giving the adults a choice between the two foods and then discarding one of the food types with oviposited eggs. After approximately 20 generations of selection, learned behavior, selected behavior, and the joint effects of both were studied under a single and a mixed species situation. This was done by introducing females - four of a species for a single species test and four of each species for a mixed test - into a vial containing a choice of both foods. Each species group of four females had a common genetic history (non-selected or one of the selected lines), a common larval food (tomato or potato), and a common adult food (tomato or potato).

Definite shifts in learned and selected behavior were found. For *D. simulans* there was an increased preference for the food type of the larvae and of the adults. These effects were augmented when the larval and adult food types were identical. However, in *D. melanogaster* preference was negatively affected, *i.e.*, the other food, the one on which it was not reared, was preferred to a greater extent. In all selected lines a positive divergence occurred. Relative to the controls, selection for tomato in *D. simulans* and *D. melanogaster* resulted in an increased preference shift for tomato of magnitude 3.5% and 3.9%, respectively, and selection for potato resulted in an increased preference shift for potato of magnitude 9.8% and 18.6%, respectively.

Besides an affect on preference it was found that food type also influenced fecundity. For *D. simulans*, when the larval food was tomato the adults had a greater fecundity than when it was potato. However, when adults were maintained on potato their fecundity was greater than those maintained on tomato.

The selection procedure for preference was also one for increasing fecundity. In both the tomato and potato selected lines of *D. simulans* a definite increase occurred. However, the tomato selected line of *D. melanogaster* decreased in fecundity and there was no statistically significant difference between the potato line and the control.

The interspecific conditions of a mixed species test resulted in changed learned behavior and fecundity. In the presence of *D. melanogaster* the preference of *D. simulans* was modified so that more eggs were oviposited on the food type that was preferred to a lesser degree by *D. melanogaster* when not in the presence of *D. simulans*. The converse occurred with *D. melanogaster*. In the presence of

D. simulans it oviposited more eggs on the food that was preferred by D. simulans to a greater extent when not in the presence of D. melanogaster. Indications were that under these conditions D. melanogaster had a reduced fecundity.

Opposed to the above results, the joint effects of learned and selected behavior increased the independence of both species during oviposition. Thus, the presence of the other species no longer had an affect on preference and D. melanogaster no longer had a reduced fecundity.

One conclusion of this study was that learned behavior may alleviate direct "tooth and claw" type competition. The other conclusion was that additive genetic variability exists in natural populations of D. melanogaster and D. simulans for food oviposition preference which could lead to a greater adaptability of the species in a new or changing environment, or a coexistence in a heterogeneous environment. The results support the hypothesis that additive genetic variability exists in natural populations for traits not directly associated with competitive ability.