INFORMATION TO USERS

This reproduction was made from a copy of a document sent to us for microfilming. While the most advanced technology has been used to photograph and reproduce this document, the quality of the reproduction is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help clarify markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure complete continuity.

2. When an image on the film is obliterated with a round black mark, it is an indication of either blurred copy because of movement during exposure, duplicate copy, or copyrighted materials that should not have been filmed. For blurred pages, a good image of the page can be found in the adjacent frame. If copyrighted materials were deleted, a target note will appear listing the pages in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed, a definite method of "sectioning" the material has been followed. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For illustrations that cannot be satisfactorily reproduced by xerographic means, photographic prints can be purchased at additional cost and inserted into your xerographic copy. These prints are available upon request from the Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases the best available copy has been filmed.

University Microfilms International
300 N. Zeeb Road
Ann Arbor, MI 48106

Koepfer, Helen Roberta

SELECTION FOR INCREASED SEXUAL ISOLATION BETWEEN GEOGRAPHIC FORMS OF DROSOPHILA MOJAVENSIS

City University of New York PH.D. 1984

SELECTION FOR INCREASED SEXUAL ISOLATION BETWEEN GEOGRAPHIC FORMS OF *DROSOPHILA MOJAVENSIS*

by

HELEN ROBERTA KOEPFER

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1984

This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

Sept 7, 1984
Date

Chairman of the Examining Committee

Sept. 10, 1984
Date

Executive Officer

Lee Ehrman
Sept. 7, 1984

State University of New York
Institution

Robert J Calhoun
Sept 7, 1984

Queens College
Institution

Paul C Mundeugh
Sept 7, 1984

Queens College
Institution

Joseph Brookfield
Sept 7, 1984

city college.
Institution

Institution

Bruce Wallace

VPI and SU, Blacksburg, Va.
Institution

The City University of New York

Abstract

Selection for sexual isolation between two geographic forms of Drosophila mojavensis

Author: Helen Roberta Koepfer

Advisor: Marvin Wasserman

The aims of this study were to select for increased sexual isolation between peninsular and Sonoran forms of D. mojavensis baja, and to ascertain the effects of that selection on three types of interactions: 1) interactions between the geographic forms under selection; 2) interactions between the selected flies and unselected Sonoran D. arizonensis and Californian D. mojavensis; 3) interactions occurring within the selected peninsular and Sonoran forms, i.e., between males and females of the same form.

Extent of isolation was measured by the male choice method, and results of the study included four main findings: 1) There was an increase in isolation between selected peninsular males and Sonoran females which was primarily due to the altered behavior of the peninsular males; 2) Peninsular females and Sonoran males showed no

increase in isolation; 3) Selected peninsular males were also more isolated from Californian mojavensis females than were control line peninsular males. 4) Homogamic interactions between selected peninsular males and females, and between selected Sonoran males and females differed according to the origin of the heterogamic female with whom they were tested.

Acknowledgements

I once heard it said that failure is an orphan, but success has many fathers. I would like to change the words, but not the thought, and remember here the many parents of this success.

It has been my very good fortune to have Dr. Marvin Wasserman as a mentor and friend, ever generous with his knowledge, time and support. "Thank you" is a very small phrase, but there is really none large enough.

I would like to thank the members of my Advisory Committee, whose insightful questions and careful attention to my work were a collective gift I will long remember: Dr. Peter Chabora, who introduced me to ecology and thereby a new awareness of this world around us; Dr. Robert Calhoon, who showed me that numbers are not only a marvelous tool, but fun as well; Dr. Lee Ehrman, whose behavioral studies have taught me so much and whose enthusiam has always been a ready source of encouragement. To Drs. Joseph Grossfield, Paul Mundinger and Bruce Wallace, thanks for some comments and inquiries that set me to thinking all over again.

The members of the Queens College faculty and staff, as well as my fellow graduate students have provided valuable advice, assistance and support for which I am very grateful.

Finally: My mother, Mrs. Helen McLaughlin, was always there for us; My husband, Stephen Koepfer, who was willing not only to believe in my goals, but to give so much of himself to help me reach them; Our children Stephen and Diana - I am richer because they enter my world and in turn share theirs with me.

Table of Contents

List of Tables

List of Figures

Introduction

Overview of Investigation

Ethological isolation, in which behavioral differences prevent interspecific mating, is a widespread and effective type of reproductive isolation (Mayr 1966; Dobzhansky 1970). Muller (1939, 1942) and Dobzhansky (1940; 1970) were among the first to propose hypotheses for the evolution of ethological, or sexual, isolating mechanisms. The former suggests that they arise as by-products of the genetic divergence that occurs in allopatric populations as a result of adaptation to different environments, or as a consequence of genetic drift. Since many genes are pleiotropic, such genetic differences might sometimes have behavioral side effects which would decrease the incidence of interbreeding if the populations were to come into contact with each other. Dobzhansky's hypothesis is that any such behavioral differences would be at a selective advantage if genetically disparate allopatric populations become sympatric and produce hybrids of lowered fitness. Dobzhansky (1970) emphasized that the two ideas are not mutually exclusive, and

studies of the reproductive relationships between geographic strains of various Drosophila species support the idea that sexual isolation can develop in two stages (Ehrman, 1965; Manning, 1965; Anderson and Ehrman, 1967; Wasserman and Koepfer 1977; Kilias, Alahiotis and Pelecanos, 1980). During the first stage, geographically separate populations accumulate genetic differences while adapting to different environments. Selection for stronger isolation will occur if the populations meet, and if they are sufficiently disparate that interpopulation crosses produce hybrids that are inferior to nonhybrids in fertility and/or viability. Such selection would decrease both gamete wastage and the utilization of environmental resources by the less fit hybrids.

There is good experimental evidence that selection can change mating preferences, increasing or decreasing sexual isolation. This has been accomplished both within and between species. Thus Knight et al. (1956) and Crossley (1974) selected for assortative mating using the ebony and vestigial mutants of Drosophila melanogaster, and Ehrman (1971, 1973, 1979, 1983) observed the beginnings of sexual isolation between genetically marked D. melanogaster strains which were

being reared together, but which had been constructed in such a way that interstrain matings produced no viable offspring. Dobzhansky, Pavlovsky and Powell (1976) were able to increase isolation between strains of D. paulistorum which initially had exhibited only weak preferences for homogamic mating; selection raised the isolation level to that found between some of the naturally occurring paulistorum semispecies. Koopman (1950) and Kessler (1966) worked with the sibling species D. pseudoobscura and D. persimilis and Eoff (1975, 1977) selected for increased and decreased isolation between D. melanogaster and D. simulans, another pair of sibling species. Most work of this type has been concerned with testing the various speciation theories, and has been aimed at measuring any existing isolation and then attempting to change it. Little emphasis has been placed on behavioral analysis or the mode of action of the barriers to hybridization.

This investigation, therefore, was designed to explore several aspects of the development of sexual isolation. First, if two allopatric conspecific populations display a small amount of sexual isolation, can we put them into contact with each other and successfully select for an increase in isolation? If

yes, then we would wish to examine three facets of the isolation which developed. These are: 1) Variation in population responses. Have the populations responded equally, or similarly, to the selection procedure? 2) Specificity. How specific is the behavioral response which has evolved? That is, if isolation develops between the members of two populations which have been in contact, does this affect their relations with individuals of other populations, with whom they have had no contact? Thus, is there a specific recognition and response to one particular type, or a general recognition and response to any type which is foreign? 3) Variation in male and female responses. Successful mating depends upon a series of interchanges between male and female, beginning with recognition of a potential conspecific mate, and proceeding through various stages of courtship to copulation. Since this involves a continual exchange of appropriate stimuli between the partners, we might expect that the evolution of mate recognition and courtship behaviors would involve a process of coevolution, in which changes in one sex are accompanied by, or select for, complementary changes in the other. Therefore, if we have effected an increase in isolation between the males and females of

two populations, we would wish to determine which, if either, sex is more responsible for the changed interaction. That is, have selected males and females responded similarly, and/or to the same extent, to the pressure for increased discriminatory ability?

This study utilized Drosophila mojavensis and Drosophila arizonensis, two sibling species belonging to the mojavensis cluster of the repleta species group (Wasserman 1982 a,b). The species D. mojavensis comprises three geographical forms, which occur in California, U.S., the Baja Californian peninsula, Mexico, and mainland Mexico. A selection procedure was designed to increase isolation between the peninsular and mainland forms; the corresponding control line regimen assured random mating and maximum outbreeding. Isolation between the two forms was measured both before and after twelve generations of selective and control line procedures. Relationships of each form with D. arizonensis and Californian D. mojavensis were also examined.

The mojavensis Cluster

Distribution and host preferences

The members of the mojavensis cluster are three arid-adapted species which utilize a variety of cacti for food. The distributions of these species are shown in Figure 1. Drosophila arizonensis has the widest distribution, from southern Arizona and New Mexico into Guatemala. It has also been collected in low numbers in the Cape region of Baja California. Little cytological or morphological variability has been reported for this species. Its sibling species, D. mojavensis occurs in the Mojave and Sonoran Deserts of southern California, on Santa Catalina Island, U.S., and in Mexico on Baja California and along the west coast of Sonora and Sinoloa. It is also found on the islands in the Gulf of California. The Californian, peninsular and mainland forms differ from D. arizonensis and from each other in morphology, genetics, ecology and behavior. Mettler (1963) differentiated between the light colored Californian D. mojavensis mojavensis, which he called D. mojavensis Race A, and the Mexican D. mojavensis baja, which he called D. mojavensis Race B. The latter is smaller and darker, more closely resembling Drosophila

arizonensis. The subdivision was based on chromosomal inversion differences. Subsequently, Zouros (1973) divided D. mojavensis baja into subraces BI (mainland) and BII (peninsular) on the basis of electrophoretic allelic frequency differences at five polymorphic loci. D. navojoa has been collected from Navojoa, Sonora to the Isthmus of Tehuantepec. There is little information on this species, which is morphologically similar to D. mojavensis and D. arizonensis. Note that the three species are sympatric in southern Sonora and northern Sinoloa. For detailed collection information see Heed (1982).

In this paper, D. mojavensis mojavensis will be called Californian mojavensis or mojavensis (C); mainland D. mojavensis baja will be referred to as Sonoran mojavensis or mojavensis (S); Baja Californian D. mojavensis baja will be called peninsular mojavensis or mojavensis (P).

The habitat of these flies is one which is quite stressful and unfriendly to most Drosophila. They are able to survive by utilizing the necrotic tissue of various native Cactaceae. The rotting parts of these cacti serve as sources of nutrition and moisture, and as oviposition sites. Each form has a preferred host

cactus although each can utilize others as well (Fellows and Heed 1972; Heed 1978; Heed 1982; Kircher 1982); the major host plants are listed in Table 1. Californian mojavensis has been reared from Ferocactus acanthodes, a species of barrel cactus, in California, and from Opuntia demissa on Santa Catalina Island. D. navojoa utilizes Opuntia species, while D. arizonensis and the Mexican mojavensis utilize three highly specialized columnar cacti whose main distributions are also within the Sonoran Desert. D. arizonensis has cina cactus as its main host. The Mexican D. mojavensis forms utilize different host plants on opposite sides of the Gulf of California. Although both agria and organ pipe cactus are found in Baja California, peninsular mojavensis, (P), has been reared only from agria (Johnson, 1980). Agria is rare in Sonora, and a host plant shift has occurred to organ pipe; Sonoran mojavensis, (S), utilizes this giant cactus, except in the region of Desemboque del Rio San Ignacio, where agria is present and regularly used. This host plant shift is associated with changes in gene and chromosomal frequencies which will be discussed below.

Cytology of D. arizonensis and D. mojavensis

Salivary gland chromosomes have been particularly useful in elucidating the evolutionary relationships between these two species, and among the seventy - four other members of the Drosophila repleta species group (Wasserman 1982a,b). These are large, polytene chromosomes with characteristic banding patterns, puffs and constrictions which enable identification of individual chromosomes and of specific regions within chromosomes. Because of somatic pairing between homologues, heterozygosity for chromosomal mutations such as deletions, duplications and inversions is easily recognizable. Paracentric inversions are the most common type of chromosomal mutation within the repleta group. Two hundred thirty-five have been discovered to date, and have proven to be an extremely valuable aid in unraveling phylogenetic relationships between the member species. Sturtevant and Dobzhansky (1936) presented arguments to show that overlapping inversions yield chromosomal phylogenies. For example, given the gene orders #1: ABCDEFGHIJ and #3: ABFEHGCDIJ, one must propose an intermediate, hypothetical gene order #2: ABFEDCGHIJ, because it is impossible, by means of only two chromosomal break points, to convert either

#1 directly to #3, or the reverse. Possible phylogenies are then (A) 1 → 2 → 3; (B) 3 → 2 → 1; (C) 1 ← 2 → 3. (Wasserman (1963) argued that non-overlapping independent inversions can also be used as phylogenetic determinants if we assume that each inversion is a unique event. In this case, any two populations or species which have the same inversion are more closely related to each other than either is to a third population or species which lacks the inversion.

In the extensive investigations of Wasserman, the gene order found in D. repleta was considered the standard. The orders found in all other species were compared with that standard, with each inversion being given a binary name, the first part of which indicates the chromosome (X,2,3,4,5) and the second indicates the specific inversion (a,b,c,d,etc.). Many inversions were found, and it was necessary to go through the alphabet more than once, using superscripts, e.g. 2c, $2c^2$, $2c^3$, $2c^4$, etc. There is no relationship, therefore, between inversions having similar numbers and letters, but differing in their superscripts. Each species received a cytological formula, called its standard sequence, listing those inversions needed in order to convert its gene orders back to that which occurs in D repleta.

Thus the formula reveals those cytological changes which have occurred and survived during the history of the species.

The primitive sequence for D. arizonensis is Xabc; 2abcfgh; 3b; 4; 5, and that for D. mojavensis is Xabce; 2abcfghqr; 3abd; 4; 5. (Wasserman, per. comm.). As explained above, this informs us that the two species differ from each other by at least five inversions; i.e. D. arizonensis lacks Xe, 2q, 2r, 3a and 3d. It also tells us that no characteristic inversions occur in chromosomes 4 and 5. Flies carrying the primitive sequence of D. arizonensis were discovered only recently at one locality, Tomatlan; heretofore, Xabc; 2abcfghi; 3b; 4; 5 has been given as the D. arizonensis standard in all published work (Wasserman 1982b). Inversion 2i also occurs at Tomatlan and elsewhere. The primitive D. mojavensis sequence, which has never been collected, appears to have given rise to two cytological lines. One exhibits sequence SI, which differs from the primitive by three unique inversions in chromosome 2, and the other has the accepted D. mojavensis standard sequence: Xabce; 2abcfghqrs; 3abd; 4; 5. Sequence SI, which lacks inversion 2s, was found in a local population near San Ignacio, Baja California. This population, and all

others, also has chromosomes which differ from the primitive by at least one additional inversion, 2s (Wasserman, 1982b).

Californian mojavensis is cytologically monomorphic, all strains having the D. mojavensis standard sequence shown above. Mexican D. mojavensis is cytologically variable, however. It is polymorphic for a number of inversions that are restricted mainly to Baja California (Mettler 1963; Johnson 1980). The Sonoran mojavensis populations are essentially monomorphic for the second chromosome arrangement called La Paz and for the third chromosome standard. In contrast, five second chromosome arrangements, including La Paz, and two third chromosome arrangements can be found among the Baja Californian populations. Further, in some regions of the peninsula, the populations exhibit homozygosity for certain of these arrangements, while other areas exhibit great heterozygosity. It is of interest that all chromosomal heterozygosity of any consequence is associated with agria cactus, both in Baja California and in the unusual Sonoran population inhabiting the Desemboque region of Sonora, where there is agria.

D. arizonensis and D. mojavensis: reproductive relationships

Investigations concerning the reproductive relationships within and between these species were begun over forty years ago, and are continuing to yield an increasingly complex and complete picture of their interactions with each other and with their environments (Baker 1947; Crow 1941; Grant 1966; Johnson 1980; Markow 1981; Markow, Fogelman and Heed 1983; Mettler 1957, 1962; Mettler and Nagle 1966; Nagle 1965, 1969; Nagle and Mettler 1969; Patterson 1946; Wasserman and Koepfer 1977, 1980; Zouros 1981a,b,c; Zouros and d'Entremont 1974, 1980; Zouros and Vigneault 1983).

Gene exchanges between D. arizonensis and D. mojavensis are hindered by both pre- and postmating isolating mechanisms. The University of Arizona group under Dr. W. Heed has made extensive collections in Sonora, where the species are sympatric. These, plus the cytological investigations of Johnson (1980) have indicated that the two rarely, if ever, hybridize in nature.

This premating sexual isolation breaks down to varying degrees in the laboratory, depending upon the testing procedure and upon the form of D. mojavensis to

which D. arizonensis is exposed. Postmating barriers are present, however, which partially nullify this weakening of the primary barrier. For example, when Californian mojavensis females and D. arizonensis males were tested as single pairs, 73% of the females were inseminated but only 3% of those females produced offspring. In the reciprocal cross, D. arizonensis females with mojavensis (C) males, 66% of the females were inseminated and of these 74% produced progeny (Baker 1947), but the F_1 males were sterile. Such males produce immotile sperm and copulation results in a transfer of sperm-free semen (Crow 1941; Patterson 1946).

Using electrophoretic markers, Zouros and Vigneault (1983) have shown that male fertility depends on an interaction between the Y chromosome and two autosomes. Males with a D. arizonensis Y need at least one D. arizonensis chromosome 4 and one D. arizonensis 5 for sperm motility. Males with a D. mojavensis Y, however, must have two D. mojavensis homologues for chromosomes 4 and 5 in order to be fertile. This explains the sterility of males resulting from the cross of a D. arizonensis female and a D. mojavensis male (Zouros 1981a; Zouros and Vigneault 1983). As for hybrid inviability, Zouros (1981c) investigated chromosomal

effects on viability among progeny from various interspecific crosses and backcrosses. He found that only one chromosome (either 4 or 5) decreases viability when heterospecific, and this is mainly so when the rest of the genotype is mojavensis. He concluded that inviability of hybrids and backcross progeny is not a very potent postmating isolating mechanism for these two species.

In a series of population cage experiments begun in 1957, Dr. L. Mettler and the group at North Carolina State University studied the interactions of both Californian and Mexican D. mojavensis with D. arizonensis. They found that mojavensis (C) and D. arizonensis hybridized and produced fertile offspring; hybrid swarms were produced and chromosome replacement, heterosis, and genetic loads were investigated (Mettler 1957, 1962; Grant 1966; Nagle 1965, 1969; Nagle and Mettler 1969). With Mexican D. mojavensis, however, very little hybridization occurred, and D. mojavensis tended to replace D. arizonensis (Nagle 1965; Mettler and Nagle 1966). Since the distribution of Mexican D. mojavensis overlaps that of D. arizonensis, these results suggested that reproductive isolation might be stronger between the species where they are sympatric.

Further support for this idea was needed, however, since the strains used in these investigations came from peninsular populations of D. mojavensis, which were allopatric to D. arizonensis. This support came from the work of Wasserman and Koepfer (1977), and Markow (1981).

Wasserman and Koepfer used male choice tests to demonstrate that character displacement for sexual isolation does exist between mainland D .mojavensis and D. arizonensis in their region of sympatry in Sonora. Four populations of each species were tested: a) D. arizonensis from two localities allopatric to D. mojavensis: Tucson, Arizona and Venados, Hidalgo; b) D. arizonensis from two localities sympatric with D. mojavensis: Navojoa, Sonora and Caborca, Sonora; c) peninsular mojavensis, (P), from two localities allopatric to D. arizonensis: San Ignacio, Baja California Sur and La Presa, Baja California; and d) Sonoran mojavensis from the same two localities as the sympatric D. arizonensis strains: Navojoa, Sonora and Caborca, Sonora.

A summary of the data is given in Table 2. The conspecific tests yielded very low isolation indices (I). This is the index used by Malogolowkin-Cohen et al. (1965) and is equal to (the number of homogamic matings

minus the number of heterogamic matings) divided by (the total number of matings). The critical data are in the interspecific tests. When allopatric strains of the two species were paired, approximately 25% of the matings were interspecific, I is 0.497. In sympatric times sympatric tests, the interspecific matings drop to about 4%, I being 0.926. In tests involving one sympatric and one allopatric strain the frequency of interspecific crosses was about 15%, I being 0.699. The basis of the increased sexual isolation in sympatry is seen in Table 3, where the sexual isolation of the species is broken down according to regions. It can be seen that the higher isolation in the sympatric region is due entirely to the behavior of the sympatric mojavensis (S). It was found that the sympatric Sonoran mojavensis flies were more isolated from D. arizonensis, regardless of the site of origin of D. arizonensis, than were the allopatric Baja Californian mojavensis (P). There were no significant differences between sympatric and allopatric D. arizonensis males, or between sympatric and allopatric D. arizonensis females in their behavior toward D. mojavensis.

The behavioral interactions underlying this reinforcement of sympatric sexual isolation were

elucidated by Markow (1981) in a study of intra- and interspecific courtships involving allopatric and sympatric strains of D. mojavensis and D. arizonensis. In tests involving single pairs, the flies were observed for one hour and the pairs were scored for evidence of male courtship, female receptivity and eventual mating. In male choice tests, single males from either species were placed with two females, one from each species, and the type of female courted first was noted. The observed interactions indicated character displacement both for behaviors occurring during courtship and for male choices made prior to initiation of courtship. Thus, in pair tests involving D. arizonensis males and D. mojavensis females, courtships by allopatric and sympatric males did not differ significantly, but sympatric D. mojavensis females were much less receptive than allopatric females. Further, none of the interactions involving sympatric females resulted in mating. Likewise, in the reciprocal tests, D. mojavensis males with D. arizonensis females, origin of the D. mojavensis partner was again critical. A very low proportion of D. arizonensis females was receptive to the courtship of allopatric D. mojavensis males, and almost none were receptive to sympatric males; there

were no matings involving sympatric males. When the interspecific pair tests were repeated using hybrid F_1 D. mojavensis from reciprocal crosses between allopatric and sympatric regions, the SYM/ALLO hybrid males and females differed in behavior: hybrid females resembled allopatric D. mojavensis females in receptivity and mating, while hybrid males elicited responses typical for sympatric D. mojavensis males. It appears, then, that either male and female behaviors are controlled by separate genetic systems, or by the same system acting in a sex-influenced manner.

While Markow was carrying out the study described above, yet another "behavioral dissection" was occurring. In a series of elegant experiments utilizing chromosomal replacement and electrophoretic markers, Zouros (1981b) uncovered the chromosomal basis of the sexual isolation existing between D. mojavensis and D. arizonensis, and demonstrated that the genetic determination of sexual behavior is different in males and females of these species. The work involved replacing the chromosomes of D. mojavensis males with D. arizonensis homologues in order to see which chromosomes, or combinations of chromosomes, would change a male's behavior, increasing the probability of

his mating with a D. arizonensis female. Similarly, D. arizonensis females' chromosomes were replaced with those of D. mojavensis to discover which substitutions would make them more likely to accept mojavensis males. Female behavior was found to be affected by chromosome 2 and the chromosome marked with amylase, AMY, (5 or 4). Male mating behavior is determined mainly by two chromosomes, the Y and the one marked with phosphoglucose mutase, PGM, (i.e., either chromosome 4 or 5). It is interesting to note that these two chromosomes, Y and PGM-marked, are the same ones that are involved in male fertility.

The ethological barriers separating two populations are not always equally effective in reciprocal crosses. When this is so, we say that the sexual isolation is asymmetrical; that is, sexual isolation between females of one population (population A) and males of another population (population B) is greater than that found in the reciprocal cross (females of population B with males of population A). Such asymmetrical isolation exists both between D. arizonensis and mojavensis (P), and between mojavensis (S) and (P) (Zouros and d'Entremont 1974, 1980; Wasserman and Koepfer 1977, 1980; Markow, Fogelman and Heed 1983). In the interspecific

interaction, D. arizonensis females are more isolated from mojavensis (P) males than are (P) females from D. arizonensis males. In the intraspecific interaction between peninsular and Sonoran mojavensis, Sonoran, (S), females discriminate against peninsular, (P), males; peninsular females, however, do not discriminate against Sonoran males and in fact show a tendency toward heterogamic matings.

Evolution of D. mojavensis and D. arizonensis

The various studies summarized above have been of value not only in yielding an ever greater understanding of the current life of these species, but have also been critical in helping us to decipher events in their past history. Their cytological evolution as seen in the changes that have occurred in the salivary gland chromosomes indicates that D. mojavensis and D. arizonensis evolved from two contiguous cytological populations, differing from each other by the presence of 3a in the D. mojavensis precursor and its absence in the D. arizonensis precursor (Wasserman 1954, 1960, 1962, 1982a,b). After completing an exhaustive study of chromosomal polymorphism in D. mojavensis, Johnson (1980) suggested Baja California as the site of origin of D. mojavensis and Sonora as the site of origin of D. arizonensis; the Gulf of California, then, was the main barrier that had allowed the two populations to differentiate cytologically and ecologically. Wasserman and Koepfer (1977) proposed that a subsequent invasion of Sonora by D. mojavensis from Baja California led to a renewed contact between D. mojavensis and D. arizonensis, and reinforcement of sexual isolation in the sympatric area. This was accomplished by behavioral

changes in only one of the species. The smaller, migrant Sonoran mojavensis was able to change more quickly than D. arizonensis, whose distribution extended well beyond the region of sympatry. The behavioral modifications in the sympatric D. mojavensis were sufficient to perfect sexual isolation in Sonora and complete the speciation process.

This scenario not only explains the character displacement for sexual isolation between D. arizonensis and D. mojavensis, but is also consistent with the asymmetry in sexual isolation between D. arizonensis and peninsular mojavensis, and between the peninsular and Sonoran forms of D. mojavensis. Baja California is a stressful environment in which only D. mojavensis and three other, distantly related Drosophila species can survive. Very strong stabilizing selection has not been necessary, therefore, to assure reproductive isolation between these four species. On the mainland, however, D. arizonensis occurs with a number of closely related species with which it is at least potentially capable of crossing. Its courtship regimen must be quite precise and its discrimination level high to avoid heterogamic mating. We might expect, therefore, to find asymmetry in mating behavior, with mojavensis (P) females likely to

"err" and mate with D. arizonensis males, and D. arizonensis females quite consistently "correct" in accepting homogamic males as their partners (Wasserman and Koepfer 1980).

As for the asymmetry in mating preference between mojavensis (P) and (S), Zouros and d'Entremont (1980) suggest that it is a coincidental byproduct of selection for sexual isolation between mainland D. mojavensis and D. arizonensis. Due to the presence of the sibling species, mainland D. mojavensis females were selected to accept those males whose mating behavior did not overlap with that of D. arizonensis males. Thus the repertoire of acceptable and/or recognizable male conspecific courtship behavior was narrowed for the mainland form. Mainland females will reject those peninsular males whose behavior does not fall within this new norm, but peninsular females still recognize and accept mainland males. This hypothesis is consistent with the lack of any known postmating isolating mechanisms between the mainland and peninsular forms, and with the absence of closely related species on the peninsula. It has received additional support from the recent study of Markow, Fogelman and Heed (1983) on five cactophilic Drosophila, including peninsular and Sonoran mojavensis.

Three, including D. mojavensis, exhibit host plant shifts, utilizing different cacti on opposite sides of the Gulf of California. Only D. mojavensis encounters a sibling species on the mainland, however, and only mojavensis (P) and (S) exhibit sexual isolation from each other.

Artificial Selection for Increased Sexual Isolation in *Drosophilia*

As indicated in the Overview, Muller (1939, 1942) suggested that sexual isolation arises as an accidental byproduct of the genetic divergence that occurs while allopatric populations adjust to different environments. Because of pleiotropy, adaptive differences at certain loci may also result in some degree of ethological isolation (Wright 1955; Dobzhansky and Spassky 1962; Moodie 1982). This hypothesis is currently favored by Paterson (1978, 1981, 1982) and Robertson (Robertson and Paterson 1982). Dobzhansky (1940, 1970) stressed, however, that if the sexual isolation is not complete, matings between individuals of such genetically disparate populations are likely to produce ill-adapted hybrids. Natural selection, then, would act to strengthen the behavioral barriers to gene exchange, thereby minimizing or eliminating gamete wastage and resource use by such hybrids. Thus, the evolution of sexual isolation might also be a two-step process, with genetic differentiation occurring in allopatry and reinforcement of any existing ethological barriers in sympatry.

Evidence for the latter situation comes from a variety of organisms, including anurans (Littlejohn 1965; Littlejohn and Loftus-Hills 1968; Blair 1974; Fouquette, Jr. 1975), grasshoppers (Cohn and Cantrall 1974), damselflies (Waage 1975, 1979) and Drosophila (Ehrman 1965; Wasserman and Koepfer 1977). Littlejohn and Loftus-Hills studied two closely related Australian tree frogs, Hyla ewingi and Hyla verreauxi, in which the calls of allopatric populations are very similar and those of sympatric populations quite distinct. Sympatric females of both types showed completely homospecific preferences when exposed to the simultaneous calling of both types of sympatric male. Sympatric H. ewingi females responded equally to the calls of sympatric male H. ewingi and allopatric male H. verreauxi. Sympatric females of H. verreauxi responded completely to the calls of sympatric H. verreauxi males, but ignored the calls of allopatric conspecific males, a surprising result if allopatric and sympatric H. verreauxi are indeed conspecific. Unfortunately, this was not a complete test of the reinforcement hypothesis, as the responses of allopatric females were not examined at all, nor were sympatric H. verreauxi females given a choice between sympatric H. verreauxi males and

allopatric H. ewingi males. Further, only one intraspecific interaction was examined.

Waage (1975, 1979) investigated wing coloration in allopatric and sympatric populations of the damselflies Calopteryx maculata and C. aequabilis and found a significant divergence in coloration in the area of sympatry. Since this character is important for species recognition and premating isolation, and since the divergence is unique to the sympatric region, Waage presented it as a case of reproductive character displacement.

Drosophila paulistorum is a superspecies composed of six semispecies, or races. Each race inhabits a geographical area different from the others, but the distributions of some races overlap. Ehrman (1965) used Elens-Watteaux mating chambers to perform multiple choice experiments on pairs of semispecies which occur both allopatrically and sympatrically. She found that pairs which inhabit the same region are more sexually isolated than are allopatric pairs, which do not share an area. Her results are a clear example of character displacement for sexual isolation, as are the similar findings of Wasserman and Koepfer (1977) on D. mojavensis and D. arizonensis.

The conclusions drawn from these observational studies are further supported by the results of artificial selection experiments. A number of these have shown that selection can change mating preferences, increasing or decreasing sexual isolation both between and within species. Interspecific selection experiments have involved two pairs of sibling species, Drosophila pseudoobscura and D. persimilis (Koopman 1950; Kessler 1966) and D. melanogaster and D. simulans (Eoff 1975, 1977). Koopman set up three population cages containing D. pseudoobscura and D. persimilis. In each, the D. pseudoobscura were homozygous for the recessive eye mutant glass, and the D. persimilis were homozygous recessive for orange eye. Adult hybrids had wild eye color, and were discarded each generation. New populations were initiated each generation with nonhybrid progeny from intraspecific matings. For each cage, this selection regime resulted in nearly complete isolation between the species. The time required was short. In all cages, within seven generations, the percentage of hybrids had fallen to five percent or less: replicate 1: 5.2% hybrids at generation 6; replicate 2: 2.7% hybrids at generation 7; replicate 3: 3.3% hybrids at generation 3. These

percentages tended to remain low for the remainder of the experiment, twelve to twenty-two generations. Koopman, then, was the first to show that selection can strengthen the ethological isolation between a pair of species.

Kessler (1966) also worked with D. pseudoobscura and D. persimilis, but took a different approach. He selected both for and against sexual isolation, and measured male and female responses separately. The selection scheme involved reciprocal no choice situations in which selected flies of one sex were exposed to unselected, heterospecific individuals of the opposite sex. In the low isolation line, selected males and females which had mated heterospecifically in their respective tests, were bred to each other. In the high isolation line, selected males and females which had refused their heterospecific partners were mated to each other. Thus there was direct selection on the males and females in this work. Selection was effective in all but the high isolation D. persimilis males, and differential responsiveness was observed in male and female D. pseudoobscura, with females responding more rapidly than males. Female high and low lines of both species diverged by generation five, while high and low lines of

D. pseudoobscura males were not differentiated until generation nine. In addition, it was found that the selection procedure had altered the sexual activity levels of the flies. For D. pseudoobscura, high isolation females were less likely to mate than controls, and low isolation females were more likely to mate. In D. persimilis, mating activity was increased for both males and females in the low isolation lines. Further, at generation sixteen Kessler subjected selection and control line flies to multiple choice mating tests. Under these conditions, D. persimilis females rarely mated with D. pseudoobscura males, regardless of the direction in which they had been selected. For example, approximately 50% of low isolation D. persimilis females mated with D. pseudoobscura males in the no choice selection situation, but only 1.4% did so in the more natural conditions of the chamber. Kessler's results are valuable for a number of reasons. They support the contention that selection can change mating preferences, with the possibility of differential responses in males and females. They also serve as a caveat for other behavioral studies: when selecting, be aware that selection may affect more than one trait; when testing,

do not underestimate the importance of the testing regimen and the various responses possible with different testing schemes.

Intraspecific selection experiments involving D. melanogaster mutants were carried out by Knight, Robertson and Waddington (1956) and by Crossley (1974). The former authors selected for isolation between ebony and vestigial mutants of D. melanogaster. The procedure was similar to Koopman's in that wild type progeny from interstrain matings were destroyed, and the parentals of each new generation were mutants resulting from intrastrain matings. Clear results were achieved within eighteen generations, with control lines producing 66 percent hybrid progeny and selection lines 38 percent. Crossley (1974) repeated the experiment of Knight et. al. and included a behavioral analysis. Crossley concluded that changes in female discrimination were mainly responsible for the observed increase in isolation between the mutant strains.

Wallace (1954) also used a technique similar to Koopman's to select for isolation between the straw and sepia mutants of D. melanogaster. This, however, required seventy-three generations of anti-hybrid selection, and produced behavioral changes in sepia

females. Sepia females gave a 9:1 ratio of homogamic to heterogamic matings, while straw females mated equally with both sepia and straw males.

We can conclude, then, from these various experiments, that if incipient isolation exists between two types, it can be strengthened by selection. Further, divergence between the groups under selection may occur within a small number of generations. Lastly, one type or one sex may respond much more strongly than the other. The experiments to be described here illustrate these three points for Drosophila mojavensis.

Materials and Methods

Overview

Peninsular and mainland forms of D. mojavensis baja were subjected to selection for increased sexual isolation from each other, and also to a random mating control line regimen. Selected and control lines of the peninsular form were derived from flies collected in six Baja Californian localities, and will be referred to as mojavensis (P). Mainland flies were descended from four single pairs collected in Sonora; these will be called mojavensis (S). In addition to the two forms undergoing selection and control line procedures, unselected stocks of D. mojavensis mojavensis from southern California, called here mojavensis (C), and D. arizonensis from Sonora, mainland Mexico were also utilized. Table 4 summarizes the available collection information for all five types, and Figure 2 shows the collection localities. As indicated on the map, mojavensis (C) and mojavensis (P) are allopatric to each other and to the other two types, while D. arizonensis and mojavensis (S) are sympatric with one another.

In brief, the study included the following

procedures: 1) Preliminary tests measuring the sexual isolation between peninsular mojavensis, (P), and Sonoran mojavensis, (S). These were done before the establishment of selection and control lines; 2) Establishment of control and selection lines of (P) and (S); 3) Selection for increased isolation between selected lines of (P) and (S). Simultaneous maintenance of randomly mating (P) and (S) control lines; 4) Final tests measuring the extent of isolation existing between several combinations of peninsular and Sonoran flies: selected (P) x selected (S); control (P) x control (S); selected (P) x control (S); control (P) x selected (S); Final tests measuring isolation between Californian mojavensis, (C), and selected and control lines of (P) and (S), and between D. arizonensis and selected and control (P) and (S). Work was begun in July 1978 and was finished in October 1982.

Measurement of Sexual Isolation - The Male Choice Method

Male choice tests were used to examine interactions between the various types of fly, and also were the tool used for increasing isolation between mojavensis (P) and (S). In this method, two types of females are confined with one or more males of only one type. After a certain amount of time, the males are removed and either the females are dissected and their ventral receptacles examined for the presence of sperm or they are left alive and checked for production of progeny (Stalker 1942; Bateman 1949; Merrell 1960; Manning 1965).

In the choice situation utilized here, one male was exposed to four homogamic and four heterogamic females. Male and female virgins were collected, aged seven to ten days, reexamined immediately before testing to assure that they were in good physical condition, and then placed in a medium-containing shell vial measuring 95 mm. x 25 mm. They remained there for approximately forty hours, at 23^{o} C, under a cycle of twelve hours light and twelve hours of darkness. The females were then dissected and examined for the presence of sperm. Multiple replicas were run of each male/female

combination tested. For each combination, in half the replicas the left wings of the homogamic females were notched for identification purposes, and in the remainder the left wings of the heterogamic females were notched.

The data from such male choice tests can be converted into an index which measures the extent of sexual isolation existing between males and the heterogamic females to which they were exposed. Several such indices have been proposed, and compared, by various workers (Stalker 1942; Bateman 1949; Levene 1949; Merrell 1950, Malogolowkin-Cohen, Simmons and Levene 1965; Schaffer 1968; Koepfer 1970). The Charles-Stalker index (Stalker 1942) is equal to (the proportion of homogamic matings - the proportion of heterogamic matings) divided by (the sum of the proportions of heterogamic and homogamic matings). In 1965, Malogolowkin-Cohen, Simmons and Levene introduced an isolation index, I, which under the conditions in effect here, is numerically equal to the Charles-Stalker index, but has the advantage of a standard error. For this index, H_m = number of homogamic matings; H_t = number of heterogamic matings; N = total number of matings; SE = the standard error of I, and

$$I = (H_m - H_{t)} \ / \ N$$

$$SE = [(1-I^2) \ / \ N]^{1/2}$$

I varies from 1.0 (no heterogamic matings due to complete isolation) through 0.0 (random mating) to -1.0 (only heterogamic matings). Results of this study will be given in terms of I.

Experimental Procedures

Stocks used in testing and selection regimes

Since previous work has indicated the importance of initial genetic variability in artificial selection experiments (Bennett 1960; Thoday and Gibson 1970; Eoff 1977), the first step in the investigation was the synthesis of a genetically heterogeneous base stock of mojavensis (P). This was derived from flies collected in six Baja Californian localities in the following way. Mass matings of twenty to forty pairs were used to produce F_1 hybrids between flies of the following locality pairs:

Males	x	Females
Cuñaño	x	La Presa
La Presa	x	Loreto
Loreto	x	Mulege
Mulege	x	San Ignacio
San Ignacio	x	San Quitin
San Quitin	x	Cuñaño

215 virgin pairs of each hybrid type were then put into each of two population cages, and the resulting base stock was maintained in cages for two generations as illustrated in Figure 3. This system of cage maintenance

produced non-overlapping generations, with approximately 50% gene flow between the cages at each generation. Due to unusually high mortality, the flies were removed from the cages and maintained in vials by mass mating from generation two onward. This base stock was used in the preliminary tests and was also the stock from which selection and control lines of mojavensis (P) were derived.

A similar Sonoran base stock was not synthesized due to a change in the research plan. The original idea was to subject only (P) flies to selection for isolation from an (S) stock consisting of one isofemale line. However, after approximately two years of work with the (P) base stock, a decision was made to carry out selection on both the peninsular and Sonoran forms. Therefore all preliminary measurements involving mojavensis (S) were done on a single isofemale line, called the (S) base stock. The Sonoran selection and control lines were derived from that isofemale line and three others.

Final tests utilized Californian mojavensis, (C), descended from mass matings, and an isofemale line of D. arizonensis.

Testing before, during, and after selection: preliminary, selective and final male choice tests

Tests used in the study are classified as preliminary, final and selective. Preliminary tests were run on the base stocks before the start of selection and control line procedures; they involved only the peninsular and Sonoran forms and dealt with two situations: 1 (P) ♂ x 4 (P) ♀♀ + 4 (S) ♀♀ and 1 (S) ♂ x 4 (S) ♀♀ + 4 (P) ♀♀. Final tests were run on both control and selected lines of (P) and (S) after termination of selection and control line procedures. These examined the interactions of (P) and (S) with each other, and also with Californian mojavensis, (C), and with D. arizonensis. Table 5 shows the various final test situations which were analyzed. As indicated in column 1, six kinds of males were utilized in the final tests: control and selected peninsular, (P), males; control and selected Sonoran, (S), males; and males of mojavensis (C) and D. arizonensis. Each kind of male was exposed to four different choice situations, i.e. to four kinds of heterogamic females. Thus, there were twenty-four different final tests. There were sixteen replicates for each preliminary test and for each final test.

Selective tests were intrinsic to the selection regimen, involved only mojavensis (P) and (S), and will be described below.

Establishment and maintenance of control lines

There was one control line for mojavensis (P) and one for mojavensis (S); each was divided into four sublines. Each of the four (P) control sublines was derived from a single pair mating between flies of the peninsular base stock; these peninsular sublines were called Ca, Cb, Cc and Cd. For the (S) control line, the progenitors of each of the four sublines were one single pair from each of the Sonoran isofemale lines; these Sonoran sublines were called Cw, Cx, Cy and Cz.

The control lines were maintained by a random mating scheme which was chosen in order to maximize outbreeding, and which is shown in Table 6 for both peninsular and Sonoran forms. Each generation, there were four sets of parents per form, one set for each subline. The parents of a given subline consisted of one male from that subline and four females from a different subline. The male/female combinations changed each generation according to the revolving mating scheme shown in Table 7. This system was maintained for thirteen generations.

Establishment of selection lines; selection regimen

Sonoran and peninsular selection lines were established at the same time as the control lines and in an identical fashion. The mojavensis (P) selection sublines were called a, b, c and d. Sonoran, (S) selection sublines were w, x, y and z.

The selection procedure required the testing of sixteen males and sixty-four females of each type every generation. The procedure is outlined in Table 8, which shows the tests which might be made in one generation of selection. Each generation, sixteen male choice tests were run on (P) males with (S) females as the heterogamic type (i.e., sixteen tests, each involving one peninsular male with four peninsular females and four Sonoran females); simultaneously, sixteen tests were run on (S) males (i.e., one Sonoran male with 4 Sonoran females and four peninsular females). Four males from each (P) subline and sixteen females from each of the (P) and (S) sublines were represented in the (P) male choice tests. Likewise, four males from each (S) subline and sixteen females from each subline were represented in the Sonoran male choice tests. As shown in Table 8, all four males of a given subline were exposed to homogamic females from one other subline,

different from that of the males, chosen each generation according to the revolving mating scheme (Table 7), while each of the four males from that same subline was exposed to a different subline of heterogamic female. For example, in this generation, all c males were exposed to b subline homogamic females, but the four c males were exposed to four different kinds of heterogamic females; thus we see ♂c_1 x 4 b ♀♀ + 4 w ♀♀; ♂c_2 x 4 b ♀♀ + 4 x ♀♀, etc.

After dissection of the females, three results were noted for each set of tests. For the (P) male choice tests: 1) total result for the sixteen replicates; 2) performance of each individual (P) male; 3) performance of each (S) female subline as a whole. For the (S) male choice tests: 1) total result for the sixteen replicates; 2) performance of each individual (S) male; 3) performance of each (P) female subline as a whole.

The next generation of (P) and (S) selection line flies would be established as follows: 1) For the mojavensis (P) selection line, the individual male from each (P) subline which had shown the greatest isolation when tested with Sonoran, (S), females, was mated to four sisters and/or half-sisters of the females from the (P) subline which, in all tests, had shown the greatest

isolation from (S) males. The progeny resulting from this cross would be named for the male; i.e., subline names derive from the male parent. Thus, if peninsular females from subline d showed the greatest isolation from (S) males (w, x, y and z), then four d females would be mated to the one best male from each of the (P) sublines to produce the next generation. Similarly for the (S) selection line. There, the individual male from each Sonoran subline which had shown the greatest isolation from peninsular females, was mated to four sisters and/or half-sisters of the females from the (S) subline which, as a whole, had shown the greatest isolation from (P) males. Again, the subline name of the next generation comes from the male.

The data from generation O, the first set of tests utilized for selection purposes, will serve as an illustrative example (Tables 9 and 10). In tests involving (P) males (Table 9), there were 51 homogamic matings, (P) ♂ x (P) ♀, and 31 heterogamic matings, (P) ♂ x (S) ♀. The four best males were a_4, b_3, c_3 and d_3. These males were chosen as parents of their respective sublines for the next generation. To determine their female partners, consult Table 10, which shows tests involving (S) males with (P) females as the heterogamic

type. Here we see a tendency toward heterogamic mating, with 49 homogamic matings, (S) ♂ x (S) ♀, and 53 heterogamic matings, (S) ♂ x (P) This tendency was exhibited by females of sublines b, c and d, while the index for a subline females was positive, though of course, not significant. Therefore males a_4, b_3, c_3 and d_3 were each mated to four females from subline a. Their progeny were called sublines a, b, c and d respectively.

This same approach was used with the Sonoran selection line. Examining Table 9 by subline of heterogamic female, we see that the numbers of homogamic and heterogamic matings in tests involving w females were 14 and 9 (I = .22); in tests involving x females, 13 and 11 (I = .08); for y females, 10 and 3 (I = .54); for z females, 14 and 8 (I = .27). Sisters and half-sisters of the tested y subline females were therefore chosen as the female parentals of the next Sonoran selection line generation. Although the majority of (S) males tested showed either no isolation or an excess of heterogamic matings, males w_3, x_2, z_1 and z_3 did exhibit a slight excess of homogamic matings (Table 10). Therefore, males w_3, x_2 and z_1 served as male progenitors of their respective sublines for the next Sonoran selection generation, and each was mated to

four $\underline{y}$ subline females. Male y_1 and four $\underline{y}$ subline females were the parentals of the subsequent $\underline{y}$ subline. Since none of the $\underline{y}$ subline males had shown a preference for homogamic mating, male y_1 was chosen because he exhibited random mating rather than a tendency toward heterogamic mating and also because of the vigor which enabled him to mate with all eight females.

Selection was most stringent, following the above plan exactly, for only the first five generations. After that, selection was relaxed due to the ever-decreasing numbers of progeny in the selection lines. Peninsular and Sonoran selection lines were maintained by mass matings between sublines at generations five and six. After that, selection was as follows. (S) line: female selection at generation 9; selection on males and females at generation 10; (P) line: selection on males and females at generation 7; female selection at generation 10.

Results

Among the results of this investigation were three main findings, which will be treated in this chapter: 1) There was an increase in sexual isolation between selected lines of peninsular, (P), and Sonoran, (S), mojavensis. 2) There were also changes in the interactions between each of these and Californian mojavensis, (C). 3) The characteristics of these changes differed according to the male choice situation being examined.

The procedure which was utilized in attempting to increase isolation between mojavensis (P) and mojavensis (S) actually involved two different selection experiments which were being run simultaneously and which were dependent upon each other. Both used the (1,8) choice situation; that is, a single male and eight females. One dealt with the interaction occurring when a Sonoran male was confined with Sonoran and peninsular females: 1 (S) ♂ x 4 (S) ♀♀ + 4 (P) ♀♀. Selection here was on Sonoran males and peninsular females. The other involved the reciprocal choice situation, a peninsular male confined with peninsular and Sonoran females: 1 (P) ♂ x 4 (P) ♀♀ + 4 (S) ♀♀. Here,

peninsular males and Sonoran females were being selected. Results of this experiment will be discussed first.

I. Interactions involving peninsular, (P), males and/or Sonoran, (S), females. *

Ia. Increased isolation between selected peninsular males and selected Sonoran females. Selective test results: (P) ♂ x (P) ♀♀ + (S) ♀♀.

Table 11 lists the results of tests run on base stocks, and on selection lines, utilizing mojavensis (P) males with mojavensis (S) as the heterogamic female; i.e. 1 (P) ♂ x 4 (P) ♀♀ + 4 (S) ♀♀. Extent of isolation is indicated by the Isolation Index, I. The table includes data from preliminary tests run at generation 0, before the start of selection and control procedures; selective tests for generations 1 through 11; and final tests run at generations 12, 13 and 16 after selection had ceased completely. The selected sublines have been maintained by mass matings from generation 13 on. The response to selection as measured by I is also shown graphically in Figure 4.

* A summary of the interactions involving (P) males and/or (S) females can be found in Table 19.

Clearly, the selection regime led to an increased isolation between peninsular males and Sonoran females. Further, the response to selection was rapid and the effect was lasting. The first three generations of selection show an increase in I from 0.244 ± 0.107 to 0.870 ± 0.073, after which the level of isolation remains rather constant.

These results were analyzed by the chi square method of Brandt and Snedecor (Maxwell 1961) in order to ascertain the generations at which significant changes in isolation occurred. This method allows us to gauge the amount of heterogeneity within a set of samples, and then to determine the extent to which various subsets contribute to that heterogeneity. Since the eleven generations of selection involved five generations of stringent selection and six in which selection was weaker, the data were divided accordingly and each of the two sets was analyzed by the approach described above. Details of the method, plus an illustrative example, are given in the Appendix. This will be done for each statistical treatment utilized in this investigation.

Generations 0 to 5 were those in which the flies were subjected to stringent selection. I values for this

set of samples range from 0.244 ± 0.107 at the outset to 0.803 ± 0.076 at generation 5. There is significant heterogeneity within this set (X^2 = 30.72, 5 d.f., $p \leq .001$), and between the subsets composed of generations 0,1,2 and 3,4,5 (X^2 = 26.81, 1 d.f., $p \leq .001$). There are, however, no significant differences within either of those subsets (X^2 = 3.49, 2 d.f., $.20 > p > .10$; X^2 = 0.42, 2 d.f., $.90 > p > .80$).

Samples from generations 6 through 11 represent the time during which selection was sporadic due to decreasing numbers of progeny and the difficulty in obtaining sufficient flies for the required tests. These samples exhibit a significant heterogeneity (X^2 = 19.36, 5 d.f., $p \leq .01$), which is chiefly due to the drop in isolation at generations 9 and 10. The subsets represented by generations 9 and 10, and generations 6,7,8,11 differ significantly from each other (X^2 = 18.30, 1 d.f., $p \leq .001$), while there are no significant differences within either of these subsets (X^2 = 0.69, 1 d.f., $.50 > p > .30$; X^2 = 0.37, 3 d.f., $.95 > p > .90$).

It seems then, that there was a significant increase in isolation after just three generations of stringent selection, and that this effect was stable except for the unexplained temporary drop at generations

9 and 10.

Ib. Isolation found to be higher in selected lines than in control lines. Preliminary and final test results: (P) ♂ x (P) ♀♀ + (S) ♀♀.

The data discussed in section Ia) show an increase in isolation between selected mojavensis (P) males and selected mojavensis (S) females. However, it is of primary importance to compare the selection line isolation with that exhibited by the control line flies. The control lines were subjected to the same general regimen as were the selected lines, except, of course, there was no selection; parents of each succeeding control line generation were chosen at random. Three comparisons between preliminary and final test results were made for this purpose.

Recall that preliminary tests (generation 0) were run on the base stocks, before control and selection lines were established, and final tests were run on selection and control lines at the end of selection and control line procedures. Results of preliminary and final tests involving peninsular, (P), males with Sonoran, (S), females as the heterogamic type are given in Table 12. Numbers of homogamic and heterogamic matings, and the isolation index are given for each

test. There were sixteen replicates for each type of test. With one male and four females per replicate, a total of sixteen males and sixty-four females of each type were examined for each of the three kinds of test.

In order to determine whether or not there had been a change in the control line interaction between (P) males and (S) females, the following comparisons were made: preliminary results to final selection line results, preliminary to final control, and final selection to final control. This involved a two-step procedure utilizing two nonparametric tests: the Kruskal-Wallis one-way analysis of variance by ranks (Siegel, 1956; Gibbons, 1976) and a multiple comparison method attributed to Dunn (Dunn, 1964; Gibbons, 1976).

The Kruskal-Wallis procedure tests the null hypothesis that the samples under consideration come from the same continuous population, or from identical populations with respect to averages. If the probability value from the Kruskal-Wallis procedure indicates that the populations are not all the same, we may use a multiple comparison procedure to determine which pairs of populations differ significantly from each other.

Preliminary tests on base stock peninsular, (P),

males and Sonoran, (S), females yielded an isolation index I, of 0.244 ± 0.107, while final tests on selected flies gave an I value of 0.909 ± 0.063 at generation 12. Final tests on control line flies resulted in an I value of 0.514 ± 0.100 after thirteeen generations of control line regimen. The Kruskal-Wallis analysis indicated that preliminary, control and selection line responses were not identical with respect to isolation ($p \leq .001$). The Dunn procedure indicated that preliminary and control line responses did not differ significantly, and that the selection lines exhibited significantly more isolation than both the base stocks (i.e., preliminary tests) and the control lines ($p \leq .05$). We may conclude, therefore, that the control line random mating regimen did not affect isolation as measured by this method, and that the increased isolation between selected mojavensis (P) males and mojavensis (S) females is a result of the selection procedure.

Ic. Increased isolation in selection lines due to a change in preference, not sex drive. Final test results: (P) ♂ x (P) ♀♀ + D. arizonensis ♀♀ and (S) ♂ x (S) ♀♀ + D. arizonensis ♀♀.

The observed outcome of any "choice" test is not

only influenced by discrimination in mate choice, but also by the sex drives of the flies being tested. So when observing an isolation between types, we must try, if possible, to determine the relative contributions of type preferences and of mating activity levels. Since isolation between selected peninsular (P) males and Sonoran (S) females, as measured by I, has increased, it is necessary to inquire whether selection has actually changed the mating preferences of the flies, or has merely altered their sex drives. The answer to this lies in the male choice tests involving mojavensis (S) or (P) males with D. arizonensis as the heterogamic female: 1 (S) ♂ x 4 (S) ♀♀ + 4 D. arizonensis ♀♀ and 1 (P) ♂ x 4 (P) ♀♀ + 4 D. arizonensis ♀♀. Previous studies, discussed in the Introduction, have shown that isolation between D. mojavensis males and D. arizonensis females is quite high. In view of this, tests utilizing D. arizonensis as the heterogamic female were used to examine responses within the peninsular and mainland forms of D. mojavensis in what is almost a "no choice" situation for the male. That is, the behavior of control (S) males and females toward each other was compared with the behavior of selected (S) males and females when the male has little, if any, choice as to partner type.

The same comparison was made for (P) males and females. Contingency chi square tests were used for this, and the relevant data and X^2 values are shown in Tables 13 and 14.

Table 13 contains the data resulting from the final tests involving Sonoran, (S), males with D. arizonensis as the heterogamic female. Two comparisons were made: 1) numbers of (S) females mated in control line tests and in selection line tests; 2) numbers of D. arizonensis females mated in those same tests. Control tests and selection tests did not differ significantly for either homogamic or heterogamic matings. In tests involving control line (S) males and females plus D. arizonensis females, 46 (S) females were mated; tests using selected (S) flies plus D. arizonensis females yielded 48 homogamic matings ($X^2 = 0.04$, 1 d.f., $.90 > p > .80$). Similarly, there was no significant difference in numbers of heterogamic matings involving selected males and control males ($X^2 = 1.36$, 1 d.f., $.50 > p > .10$).

The same situation exists for peninsular mojavensis, (P), as is seen in Table 14. Here we see that 47 and 43 (P) females mated in control and selection line tests, respectively, while there were no matings at all involving D. arizonensis females.

Since control and selection lines of (P) and of (S) do not differ in number of observable homogamic matings per observation period, we may conclude that the sex drive, or attraction between members of each form, has not been drastically enhanced or diminished by the selection procedure. We can also note that the Sonoran and peninsular forms participate in about the same number of matings per observation period. Thus the increased isolation between selected (P) males and (S) females is due to a change in preference, and not to any change in mating activity levels.

Id. Responses of control and selected mojavensis (S) females and mojavensis (P) males to mojavensis (C), and to D. arizonensis. Final test results: tests involving either Californian mojavensis, (C), or D. arizonensis.

Final tests were also used to explore questions concerning specificity of response. That is, if selection has changed the interaction between Sonoran, (S), females and peninsular, (P), males, has it also altered the interaction of each with mojavensis (C) and/or with D. arizonensis? If yes, in which direction was the change - toward greater or lesser isolation?

The approach to this was by a comparison of final control and selection line responses within the following two sets of interactions: 1) Responses of control and selected (S) females to males of mojavensis (C) and D. arizonensis; 2) Responses of control and selected (P) males to mojavensis (C) females and to D. arizonensis females. Recall that Californian mojavensis, (C), and D. arizonensis had not been at all involved in the selection regime; there had been no contact between them and either selected or control lines of (P) and (S). Further, there had been no change in the procedures by which these two stocks were maintained.

Relevant data are found in Table 15. Responses of control and selected Sonoran, (S), females will be examined first. For this, we must compare the following two pairs of tests, whose results are shown on lines 1 and 2 of the table: 1) mojavensis (C) ♂ x mojavensis (C) ♀♀ + control mojavensis (S) ♀♀, (I = 0.165 ± 0.097); mojavensis (C) ♂ x mojavensis (C) ♀♀ + selected mojavensis (S) ♀♀, (I = 0.341 ± 0.102); 2) D. arizonensis ♂ x D. arizonensis ♀♀ + control mojavensis (S) ♀♀, (I = 0.964 ± 0.036); D. arizonensis ♂ x D. arizonensis ♀♀ + selected mojavensis (S) ♀♀, (I = 1.0).

The Kruskal-Wallis test was used to determine if these responses of control and selected (S) females differ significantly in either of the test situations; it indicated that they do not ($p = 0.18$). It appears, then, that selection has effected a change in the behavior of mojavensis (S) females which is specific to mojavensis (P) males. Recognition of and/or response to males of mojavensis (C) and D. arizonensis has not changed significantly.

A similar analysis was made for mojavensis (P) male behavior. Data from relevant test combinations are also found in Table 15: 1) control mojavensis (P) ♂ x control mojavensis (P) ♀♀ + mojavensis (C) ♀♀, ($I = 0.287 \pm 0.103$); selected mojavensis (P) ♂ x selected mojavensis (P) ♀♀ + mojavensis (C) ♀♀, ($I = 0.509 \pm 0.114$); 2) control mojavensis (P) ♂ x control mojavensis (P) ♀♀ + D. arizonensis ♀♀, ($I = 1.0$); selected mojavensis (P) ♂ x selected mojavensis (P) ♀♀ + D. arizonensis ♀♀ ($I = 1.0$). Clearly, there is no change in the relationship of mojavensis (P) males and D. arizonensis females which is measurable by the methods employed here. The interaction between (P) males and mojavensis (C) females has changed however. When tested by the Kruskal-Wallis procedure, selected (P) males are significantly more

isolated from Californian mojavensis, (C), females, than are control (P) males ($p \leq .05$). Thus, for peninsular males, selection has altered two interactions, that with Sonoran mojavensis females and that with Californian mojavensis females. In both cases, the change has been in the direction of an increased isolation.

Responses of control and selected (P) males and (S) females are presented graphically in Figures 5 and 6.

Ie. Mating patterns underlying the observed changes in isolation. Final test results: (P) ♂ x (P) ♀♀ + (S) ♀♀, and tests involving mojavensis (C).

The foregoing analyses indicated that selection had changed neither the relationships of mojavensis (P) and (S) with D. arizonensis, nor the mating activity levels of (P) and (S) when they are in the presence of D. arizonensis. There were significant increases in isolation between selected (S) females and selected (P) males, and between selected (P) males and mojavensis (C) females. In addition, there was an increase in isolation between selected (S) females and (C) males, but it was not significant. The following analysis was undertaken to further elucidate the behavioral changes underlying the observed increases in isolation.

Contingency chi square tests comparable to those discussed in section Ic) are shown in Tables 16 through 18. Table 16 shows a comparison of control and selected line interactions when (P) males were confined with (P) and (S) females. Selection lines exhibit a significant decrease in heterogamic matings ($p \leq .001$), and this is not surprising in view of the increased isolation as measured by I. What is, perhaps, unexpected is the accompanying decrease in homogamic matings ($p \leq .01$). Recall that there is no such decrease when selected (P) males are presented with (P) females and D. arizonensis females.

A similar situation exists for selected (P) males and females in the presence of mojavensis (C) females (Table 17). Again we see that two behavioral changes underly the increased isolation: significant decreases in both hetero- and homogamic matings, $p \leq .01$ and $p \leq .05$ respectively. Again, the decrease in heterogamic matings is more drastic than the decrease in homogamic pairings.

Now, if in fact, the sex drives of (P) males and females were not altered by selection (section Ic), and if the discriminatory ability of (P) males has sharpened regarding (S) and (C) females (sections Ib and Id), then what we are seeing is that the (P) male, at this point

in the selection process, is "paying a price" for his developing discriminatory ability. The selected (P) male makes fewer "mistakes" in mate choice than the control male, but he also mates with fewer homogamic females than does his unselected counterpart.

As for selected and control mojavensis (S) females with mojavensis (C) males, the overall picture is less clear. Although selected (S) females are more isolated from (C) males than are control (S) females, the Kruskal-Wallis procedure indicates that the difference in isolation is not significant. Contingency chi-square (Table 18), however, reveals a significant decrease in heterogamic matings among selected (S) females ($.02 > p > .01$). It may be that the selected females are also sharpening their ability to recognize (C) males, but the discrimination is not yet strong enough, or common enough in the selected group to effect a significant change in I value.

The various patterns discussed in this section are summarized in Table 19. Here we see that for selected peninsular males, increased isolation from both Sonoran and Californian females is associated with decreases in both hetero- and homogamic matings when the male is given a choice. Unfortunately, since all tests were done

by the male choice method, there is no analogous choice situation for selected Sonoran females, and we can only see that increased isolation from peninsular males and from Californian males is associated with decreased heterogamic pairings.

If. Increased isolation between selected (P) males and selected (S) females due mainly to (P) males. Final test results: (P) ♂ x (P) ♀♀ + (S) ♀♀; control flies x selected flies.

The tests discussed in the foregoing sections have clearly shown that selection changed the interaction between (P) males and (S) females, resulting in a heightened isolation between them. They do not tell us, however, whether the altered interaction is due to changes in one or both partners. In order to establish which, if either, partner contributed more to the isolation between selected flies, two additional types of final tests were run, and the following four situations were compared by the Brandt and Snedecor chi square method:

selected (P) ♂ x selected (P) + (S) ♀♀

selected (P) ♂ x control (P) + (S) ♀♀

control (P) ♂ x selected (P) + (S) ♀♀

control (P) ♂ x control (P) + (S) ♀♀

Results of these tests are given in Table 20.

The overall chi square indicates significant heterogeneity within the set of four (X^2 = 13.08, 3 d.f., $p \leq .01$). Comparisons between tests involving selected males and those utilizing control males indicate that these two subsets differ significantly (X^2 = 9.68, 1 d.f., $p \leq .01$). There are no significant differences between selected males (X^2 = 0.0008, 1 d.f., $.98 > p > .95$), or between control males (X^2 = 3.40, 1 d.f., $.10 > p > .05$). Control (P) males are more isolated from selected (S) females than they are from control (S) females, but the difference just misses a .05 level of significance (X^2 = 3.40, 1 d.f., $.10 > p > .05$).

When we compare tests involving selected females with those utilizing control females, the difference again shows a low, but not significant, probability (X^2 = 2.71, 1 d.f., p = .10). There are no significant differences between selected females, but tests utilizing control females do differ sharply in isolation (X^2 = 8.71, 1 d.f., $p \leq .01$). The difference is undoubtedly due to the presence (I = 0.905 ± 0.066) or absence (I = 0.514 ± 0.100) of a selected male.

The above considerations indicate that although both sexes have responded to selection for enhanced

discriminatory ability, it is the peninsular male which has been most strongly affected. This, of course, is what we have also seen regarding the relationships of the selected males and females with mojavensis (C).

II. Interactions involving Sonoran, (S), males and/or peninsular, (P), females. *

The results to be presented below issue from the tests involving Sonoran males with peninsular females as the heterogamic type. These data were analysed in essentially the same way as those discussed in section I), and will be presented in an analogous manner.

IIa. No overall change in isolation between selected Sonoran, (S), males and selected peninsular, (P), females. Selective test results: (S) ♂ x (S) ♀♀ + (P) ♀♀.

Table 21 lists the results of male choice tests utilizing mojavensis (S) males with mojavensis (P)

* Interactions involving (S) females and/or (P) males are summarized in Table 27.

as the heterogamic female; i.e. 1 (S) ♂ x 4 (S) ♀♀ + 4 (P) ♀♀. As in Table 11, extent of isolation is indicated by the isolation index, and the data shown are from preliminary tests (generation 0), selective tests (generations 1 through 11), and final tests (generations 12 and 13). The information is shown graphically in Figure 7.

Examination of the table and graph reveals that all coefficients save one are negative, and of the ten that are negative, one is significant. For this interaction, then, there is a tendency toward heterogamic mating, and this tendency was not reversed by the selection procedure. There was, in fact, a rather sharp drop in I value at generations 4 and 7. Unfortunately, no tests were run for this interaction at the intervening generations 5 and 6 due to drastically reduced numbers of progeny.

Since there appeared to be no overall change in isolation for the selected (S) males and (P) females, the data from generations 0 through 12 were treated as one set and analysed by the Brandt and Snedecor chi square method (Maxwell, 1961). Although the overall test does not reveal any heterogeneity between the eleven samples (X^2 = 7.29, 10 d.f., $.70 > p > .50$), it does

indicate that generations 4 and 7 are different from the rest (X^2 = 4.58, 1 d.f., $p \leq .05$). There are no significant differences between the other nine samples.

It appears, then, that selection did not effect any lasting change in the relationship between Sonoran, (S), males and peninsular, (P), females.

IIb. Isolation not found to differ in control and selection lines. Preliminary and final test results: (S) ♂ x (S) ♀♀ + (P) ♀♀.

Preliminary and final test results are shown in Table 22; they were compared by the Kruskal-Wallis procedure as were the results of the reciprocal interaction in section Ib). Preliminary tests on base stock Sonoran, (S), males and peninsular,(P), females yielded an isolation index, I, of - 0.039 ± 0.099, while final tests on selected flies gave an I value of - 0.116 ± 0.102. Final control line tests resulted in an I value of - 0.237 ± 0.111. These three samples do not differ significantly with respect to isolation ($.95 > p > .90$). We may conclude, therefore, that neither the control line regimen nor the selection procedure has significantly altered the interaction between mojavensis (S) males and mojavensis (P) females.

IIc. Responses of control and selected mojavensis (S) males and mojavensis (P) females to mojavensis (C), and to D. arizonensis. Final test results: tests involving either Californian mojavensis, (C), or D. arizonensis.

As before, final tests were used to look for secondary effects of the selection process. That is, although selection did not alter the (S) male/(P) female relationship, did it perhaps change the relationship of either or both with mojavensis (C) or with D. arizonensis? Again, final control and selection line responses were compared within two sets of interactions: 1) Responses of control and selected (P) females to males of mojavensis (C) and D. arizonensis; 2) Responses of control and selected (S) males to mojavensis (C) females and D. arizonensis females. The data and indices derived from them are presented in Table 23.

Responses of control and selected (P) females will be examined first. For this, we compare the following two pairs of tests, whose results are shown on lines 1 and 2 of the table : 1) mojavensis (C) ♂ x mojavensis (C) ♀♀ + control mojavensis (P) ♀♀, ($I = -0.162 \pm 0.096$) ; mojavensis (C) ♂ x mojavensis (C) ♀♀ + selected mojavensis (P) ♀♀, ($I = -0.048 \pm 0.109$); 2) D.

arizonensis ♂ x D. arizonensis ♀♀ + control (P) ♀♀, (I = 0.408 ± 0.108); D. arizonensis ♂ x D. arizonensis ♀♀ + selected (P) ♀♀, (I = 0.672 ± 0.091). In both these tests pairs, the selective I value is higher than the control I value, but the Kruskal-Wallis analyses indicate that the differences are not significant (p = .26 and p = .19, respectively).

Results of tests concerning control and selected (S) male behavior are found on lines 3 and 4 of Table 23: 1) control mojavensis (S) ♂ x control mojavensis (S) ♀♀ + mojavensis (C) ♀♀, (I = 0.241 ± 0.104); selected mojavensis (S) ♂ x selected mojavensis (S) ♀♀ + mojavensis (C) ♀♀ (I = - 0. 011 ± 0.107); 2) control mojavensis (S) ♂ x control mojavensis (S) ♀♀ + D. arizonensis ♀♀, (I = 0.878 ± 0.068); selected mojavensis (S) ♂ x selected mojavensis ♀♀ + D. arizonensis ♀♀, (I = 1.0). As in the case of selected (P) males, isolation from D. arizonensis females has remained at a very high level; no significant change can be detected. The isolation between selected (S) males and mojavensis (C) females has decreased, however, showing a significant ($p \leq .01$), and perhaps surprising, drop in I value from a small but significant 0.241 to a situation of essentially random mating. What we see, then, is that

selection has altered the relationships of both types of selected male with mojavensis (C) females, but the changes have been in opposite directions: increased isolation between (P) males and (C) females (section Id) and decreased isolation between (S) males and (C) females. Responses of control and selected (S) males and (P) females are presented graphically in Figures 8 and 9.

IId. Mating patterns in tests involving Sonoran males and peninsular females, with each other and with mojavensis (C).

Contingency chi square tests comparable to those discussed in section Ie) are shown in Tables 24 through 26. Table 24 contrasts control and selection line interactions when (S) males are confined with (S) females and (P) females. Selection lines exhibit a significant increase in homogamic matings, ($p \leq .05$), and no change in numbers of heterogamic pairings, ($.30 > p > .20$). This increase is not great enough to effect a significant change in I value, yet may indicate that selection is strengthening homogamic preferences in this interaction, albeit slowly.

The reverse situation occurs, however, in tests

involving (S) males with Californian mojavensis as the heterogamic female (Table 25). Here there is a significant decrease in homogamic matings, ($p \leq .05$), among selected flies, and an increase in heterogamic matings which is just short of a 0.05 significance level. These changes are the basis for the observed decrease in isolation between selected Sonoran males and Californian females.

The selected peninsular, (P), females are interesting in that they show no change in response to Sonoran, (S), males and yet exhibit a highly significant decrease in heterogamic matings, ($p \leq .001$), when paired with Californian, (C), males (Table 26). Again, the change is not reflected in a significantly increased isolation, but these results may indicate a heightened preference for mojavensis baja males, (P) and (S), over males of mojavensis mojavensis, (C). The patterns discussed in this section are summarized in Table 27.

IIe. Chi square analysis of tests involving control flies x selected flies. Final test results: (S) ♂ x (S) ♀♀ + (P) ♀♀.

The following types of situations were compared in the same manner as those discussed in section If;

selected (S) ♂ x selected (S) + (P) ♀♀

selected (S) ♂ x control (S) + (P) ♀♀

control (S) ♂ x selected (S) + (P) ♀♀

control (S) ♂ x control (S) + (P) ♀♀

Results are given in Table 28.

We have seen no increase in isolation between selected Sonoran males and peninsular females, and therefore it is not surprising to find a lack of significant heterogeneity among these four (X^2 = 1.09, 3 d.f., $.80 > p > .70$). There were also no significant differences between tests involving control and selected males (X^2 = 0.005, 1 d.f., $.975 > p > .900$), between selected males (X^2 = 0.23, 1 d.f., $.70 > p > .50$), or between control males (X^2 = 0.86, 1 d.f., $.50 > p > .30$). Comparisons between tests utilizing selected and control females yielded similar results (X^2 = 0.95, 1 d.f., $.50 > p > .30$), and a lack of significant differences between selected females (X^2 = 0.016, 1 d.f. p = 0.90) and between control females (X^2 = .12, 1 d.f.,

$.80 > p > .70$). These, then, simply substantiate the previous conclusion that neither mainland males nor peninsular females have moved away from original tendency toward heterogamic mating.

Discussion

The aims of this study were to select for increased sexual isolation between peninsular and Sonoran forms of D. mojavensis baja, and to ascertain the effects of that selection on three types of interaction: 1) interactions between the geographic forms under selection; 2) interactions between the selected flies and unselected Sonoran D. arizonensis and Californian mojavensis; 3) interactions occurring within the selected peninsular and Sonoran forms, i.e. between males and females of the same form.

Selection was successful in increasing isolation between mojavensis (P) and (S), in that the isolation between the selected Sonoran females and peninsular males increased from an initially low level (I = 0.244) to a level close to complete isolation (I = 0.909). This is comparable to the interspecific isolation level of I = 0.914 previously found between unselected mojavensis females and D. arizonensis males from Caborca, Sonora, the same locality as the selected D. mojavensis females of this experiment (Wasserman and Koepfer 1977). Evidence of this change in preference appeared rapidly, within three generations of selection, remained

relatively constant, and occurred without any concomitant change in the sex drive of either form. The latter is indicated by the equality of control and selection lines for total homogamic matings when D. arizonensis is the heterogamic female. The increase in isolation appears to be due primarily to the altered behavior of the selected peninsular males, as seen by the fact that these males are significantly more isolated from Sonoran females than are control males ($p \leq .01$, Table 20), and by their changed behavior when in the presence of their own and Sonoran females (Table 16). The selected peninsular male is also significantly more isolated from Californian mojavensis females than is his unselected counterpart (Table 15).

Peninsular females and Sonoran males showed no increase in isolation, and in fact maintained their initial tendency toward heterogamic mating. Selected Sonoran males are, however, significantly less isolated from Californian mojavensis females than are control (S) males (Table 23).

These results present us with a number of questions and areas for exploration which are relevant not only to this study, but to others as well. The response to selection was asymmetrical, as were the mating

preferences displayed by peninsular and Sonoran mojavensis in this and previous studies. We might ask, then, if this outcome was to be expected or is explicable in the light of current knowledge concerning the mating behavior of these flies. The response was also quite rapid, and this result invites comparison with other similar selection experiments, and speculation in view of the known genetics of D. mojavensis mating preferences. Finally, all significant changes in isolation, as measured by I, were due primarily to changes in the selected males. Alterations in female behavior, as indicated by contingency chi square tests, were less dramatic, but with one exception, were similar to those observed in the selected males. These observations offer certain clues as to the behavioral mechanisms underlying the observed changes in isolation, and emphasize again the intricate interplay of varying male and female responses which occur in both intra- and interspecific encounters.

As reviewed in the Introduction, and seen again in these results, peninsular and Sonoran mojavensis exhibit asymmetrical isolation in that Sonoran females prefer their own males, but peninsular females do not discriminate against Sonoran males, displaying instead

a tendency toward heterogamic mating. Zouros and d'Entremont (1980) consider this asymmetry to be a consequence of selection on the mainland for increased sexual isolation between Sonoran mojavensis and D. arizonensis; this began after the invasion of Sonora by D. mojavensis from the peninsula. They hypothesize that the range of behavior of the peninsular males overlaps that of the Sonoran D. arizonensis males. Therefore Sonoran mojavensis females, which are now sympatric with their sibling species, have been pressured to accept males whose behavior is within the range of D. mojavensis yet outside that of D. arizonensis; i.e., they have been selected to "tune their preference to a narrow area within the norm of the 'primitive' behavior" of the peninsular males (Zouros and d'Entremont). Females from the peninsula do not share their habitat with any closely related species, and therefore are not similarly pressured; they recognize and accept both (P) and (S) males as conspecific and are willing to mate with D. arizonensis males at a relatively high frequency.

This hypothesis gains additional support from the asymmetrical responses to selection observed in this study. When the experiment began, there was no isolation

between peninsular females and Sonoran males. This lack of isolation, actually a tendency toward heterogamic pairing, remained unchanged by selection, a result which, in hindsight, is not surprising. If we assume that (P) males offer a broad range of courtship cues, whether behavioral or chemical, and (S) males offer a narrow one, with both being equally acceptable to (P) females, there is simply little, if any, foundation upon which to build ethological isolation based on female choices. Similarly, the Sonoran males, although selected for precision in interspecific discrimination between D. mojavensis and D. arizonensis females, seem to be quite lacking in any mechanism for distinguishing between their own and peninsular females. They appear, then to reject and/or be rejected by, the sibling species and accept all conspecific females. Thus there was also no base upon which to erect an isolation by male choice. In fact, 149 out of 176 tested selected Sonoran males yielded data (tests in which a fly died were always discarded and sometimes not repeated; tests which did not result in any matings were also discounted); of these only 25 males displayed an excess of homogamic matings; 43 mated randomly and 81 exhibited an excess of heterogamic matings. As to peninsular

females, there are data for 44 sublines over the generations of selection. Of these, only 6 out of 44 sublines exhibited discrimination, however slight, against Sonoran males, i.e., mated more often with their own peninsular males than with Sonoran males. For the others, tests with Sonoran males resulted in random mating (6) or an excess of heterogamic pairings; i.e., in 32 sublines the peninsular females mated more with Sonoran males than with their own males.

In the reciprocal interaction, Sonoran females and peninsular males were placed in artificial sympatry and we have seen a refinement of their mating choices which seems to be due mainly to changes in the (P) males. (Tables 16, 19, 20). It is possible that by selecting for isolation, we have narrowed the behavioral range of peninsular males so that it falls outside the range acceptable to the Sonoran females. Since D. mojavensis males also exhibit both intraspecific and interspecific precourtship discrimination (Markow 1981, 1982; Markow and Richmond 1981), it is also possible that the discriminatory abilities of the peninsular males have been sharpened. The Sonoran females, whose discriminatory abilities were already finely honed when selection began, have not responded as dramatically as

have the peninsular males.

Viewing the data for this interaction by individual male and by female subline, we find that of 247 tested selected peninsular males, 219 yielded data. Only 9 of the 219 mated randomly; 7 of these 9 appeared in generations 0, 1 and 2, before any significant increase in isolation became evident. The remaining 210 males all showed positive preferences, usually strong, and 122 of the 210 mated only homogamically. Of the 52 Sonoran female sublines which were examined, 39 displayed complete isolation from peninsular males, and the rest showed positive, usually high, isolation indices. Thus, for this interaction, the tendency toward homogamic mating was already present, and the population was able to move rapidly to a new behavioral norm.

As mentioned in the Introduction, there have been a number of attempts to test the various speciation theories and to alter mating preferences both within and between species. Five of these utilized forms which are separated to some extent by pre- and/or postmating barriers. The remainder were attempts to erect an ethological barrier de novo, i.e. in the absence of any initial isolation. Robertson (1966) tested the two stage speciation model completely, that is, adaptation to

different environments followed by sympatry and/or selection for reproductive isolation. Soans, Pimentel and Soans (1974) as well as Hurd and Eisenberg (1975) tested both the allopatric and sympatric models of speciation by disruptive selection for negative and positive geotaxis in "allopatric" populations, and in "sympatric" populations between which there was some gene flow. Most other investigators have followed either of two basic plans. In one, members of a species are subjected to disruptive selection for a trait which is not directly related to mating behavior, after which the divergent populations are tested for sexual isolation. This has been done a number of times using sternopleural bristle number as the trait under selection (Thoday and Gibson 1962, 1970; Gibson and Thoday 1963; Scharloo, den Boer and Hoogmoed 1967; Chabora 1968; Barker and Cummins 1969 a,b; Spiess and Wilke 1984). Recently, Kilias et. al. (1980) tested the allopatric model by maintaining cage populations of D. melanogaster under different conditions of temperature and humidity for about five years and then testing for the presence of sexual isolation between the differentially adapted populations.

The second type of experiment, of which the present

study is an example, involves selection which is thought to affect mating behavior directly, such as selection against the hybrids or selection against individuals which participate in heterogamic matings (Koopman 1950; Wallace 1954; Knight, Robertson and Waddington 1956; Kessler 1966; Ehrman 1971, 1973, 1979, 1983; Crossley 1974; Eoff 1975, 1977; Dobzhansky, Pavlovsky and Powell 1976).

The following synopsis will show that results varied, both within and among experimental approaches, in success or failure in inducing ethological isolation. When isolation did appear, there was also variation in the selection time which was necessary and in the strength and stability of the response. The isolation index, I, will be used wherever possible in the comparisons to follow.

Robertson (1966) worked with two subpopulations of a D. melanogaster population, one of which had developed resistance to EDTA. Adapted and normal types were placed in paired cages, each type being supplied with its preferred medium. The cages were joined by glass tubing to allow migration between them. Although adapted/normal hybrids were less fit in either medium than the parental types, and in spite of extensive migration between

cages, no sexual isolation developed between EDTA-adapted and normal parental types after twenty-five generations of "sympatry". Flies from the original parental populations were also subjected to fourteen generations of selection for positive assortative mating in which parents of successive generations were chosen exclusively from matings between flies of the same population. This procedure also failed to effect any deviation from the original random mating between the parental types.

Soans et. al. (1974) and Hurd and Eisenberg (1975) did observe the development of significant sexual isolation between populations of Musca domestica subjected to intense (95%) selection for positive or negative geotaxis. The isolation was of the same magnitude between "allopatric" populations which had been kept separate, and between "sympatric" populations between which there was 30% (Soans et. al.) or 50% (Hurd and Eisenberg) gene flow. We cannot pinpoint exactly when the change in preference became evident since these workers did not test for isolation throughout the selection process. Soans et. al. ran mating tests after thirty-eight generations of selection, and Hurd and Eisenberg ran theirs after sixteen generations. Average

isolation indices were high, but varied according to the testing regimen being used: I = 0.50 for male choice tests in which only the first mating was noted, and I = 0.38 for multiple choice tests (Soans et. al.); I = 0.85 for male choice with first mating only and 0.54 for multiple choice (Hurd and Eisenberg). Asymmetrical mating preferences were observed in the male choice tests. Soans reported that (-) males and (+) females were more isolated then were (+) males and (-) females. Hurd, however, found the reverse situation, with (+) males and (-) females showing more isolation than the reciprocal pairing. It is interesting to note that when the first mating choices to be made in the male choice tests indicated isolation, the multiple choice tests did also, although there seemed to be more room for "error" in the latter. Further, these mating preferences developed de novo, in the absence of any observable pre- or postmating barriers.

In 1962 Thoday and Gibson reported that they had subjected D. melanogaster populations to disruptive selection for high and low sternopleural chaeta number for twelve generations. Each generation, individuals with high and low bristle numbers were chosen and allowed to mate at random. At generation twelve, there

was no overlap in chaeta number between high and low lines, and offspring of females with low numbers had fewer bristles than offspring of females with many bristles. Furthermore, forced hybridization between high and low lines gave rise to flies with intermediate numbers of bristles not seen in the selected flies. Thoday and Gibson suggested, therefore, that reproductive isolation had evolved de novo between high and low lines, but all subsequent attempts to repeat these experiments have failed to produce significant isolation. Possible reasons for this disparity in results were discussed by Thoday and Gibson (1970) and recently by Spiess and Wilke (1984).

Another recent test of the allopatric model did result in isolation between differentially selected D. melanogaster populations. Kilias et. al. (1980) did not choose a morphological trait, but instead kept various cage populations under different conditions of temperature and humidity, and then tested for isolation between them. They found that populations originating from different gene pools and maintained under similar environments exhibited random mating, while populations originating from a common gene pool but kept in different environments showed significant isolation

after three years. Isolation indices ranged from 0.229 to 0.339 and were stable over periods of time as long as thirty-one generations. This was so even when the flies were maintained in a common environment different from those to which the isolated populations had become adapted. The isolated populations were not initially separated by any post-mating barriers, although some pairs later displayed hybrid sterility. In this case, then, pre-mating barriers were not reinforcing a preexisting post-mating isolation, but had developed in its absence or concomitantly with it. This type of situation has been found in certain Hawaiian Drosophila (Craddock 1974; Kaneshiro 1980) and has been discussed by Powell (1978).

These differing laboratory results are what we would expect if mating preferences do arise as accidental byproducts of genetic divergence. In fact, they mirror the findings of Anderson and Ehrman (1967) in their review of the literature on mating behavior tests between allopatric strains of various Drosophila species. The survey revealed both random and non-random mating, with preferences for both homo- and heterogamic pairing, as well as asymmetrical preferences.

Selection experiments designed to directly affect

mating behavior have been more successful in altering mating preferences than those described above. These studies have involved either selection against hybrid progeny from matings between mutants of the same species or between members of different species, or the selection has been on flies whose mating choices have been tested. Most investigators have employed the former technique (Koopman 1950; Wallace 1954; Knight, Robertson and Waddington 1956; Ehrman 1971, 1973, 1979, 1983; Crossley 1974; Dobzhansky, Pavlovsky and Powell 1976). The latter approach was used in this study on D. mojavensis and in those of Kessler (1966) and Eoff (1975, 1977).

Only Koopman (1950) used anti-hybrid selection to increase isolation between two species. Since his work was reviewed in the Introduction, it will simply be emphasized here that these species are separated by ethological barriers and hybrid sterilty; that the required selection time was short, seven generations at most; and that the response was stable throughout the course of selection. Mass male choice tests (ten males with ten females of each type) on selected flies gave isolation indices between 0.50 and 0.60 at generations nine through fourteen.

Intraspecific experiments using anti-hybrid selection were done by a number of investigators. All were successful in increasing isolation between the chosen strains, but the time required was longer than that reported by Koopman, and the results were sometimes transitory. Those of Knight et. al. (1956) and Crossley (1974) are described in the Introduction, as are those of Wallace (1954). Knight et. al. and Crossley ran their selection regimes under a variety of conditions, but did observe a distinct decline in percentage of hybrids by generation eighteen at the earliest and thirty-one at the latest, with percentages remaining low for the remainder of the experiment, forty to fifty generations depending upon conditions. Unfortunately, no further tests were made after cessation of selection, so we do not know if the induced changes would have been stable in the absence of continuing selection. Wallace needed seventy-three generations of anti-hybrid selection on D. melanogaster to effect isolation between sepia females and straw males. Ehrman (1971, 1973, 1979, 1983) has been observing the development of sexual isolation between genetically marked, intersterile D. melanogaster strains which are being reared together. Extent of this isolation has fluctuated over the years. After two years

of "sympatry" it was 0.43 ± 0.08. At four years it was 0.64 ± 0.07, but later dropped to 0.26 ± 0.09. The most recent data to be published reveal that the isolation is again on the rise, with an index of 0.60 ± .07 after a total of 114 generations.

Dobzhansky, Pavlovsky and Powell (1976) described another intraspecific study. Here, selection was for isolation between the New Llanos and Orinocan semispecies of D. paulistorum. The case was of particular interest because New Llanos, originally interfertile with Orinocan, began to produce sterile hybrid males in such crosses after five years in the laboratory. In view of this new, post-mating barrier, Dobzhansky et. al. decided to select for ethological isolation between the two. Selection was partially successful in that the induced isolation was about as strong as the weakest isolation observed between semispecies of this group in nature. Having shown no isolation at the start, the selected semispecies exhibited an I of 0.52 ± 0.08 after twelve generations of selection, and one of 0.84 ± 0.05 after fifty. Thirty-one generations of subsequent selection did not bring the populations any closer to complete isolation.

Of the three studies utilizing selection on

individual flies, two achieved rapid responses to that selection. Kessler (1966), whose work was summarized in the Introduction, observed divergence between female high and low lines of D. pseudoobscura and of D. persimilis by generation five, and between high and low lines of D. pseudoobscura males by generation nine. As described in the Results of this report, there was a significant increase in isolation between peninsular, (P), males and Sonoran, (S), females after just three generation of selection. The isolation level remained fairly stable throughout the course of relaxed selection, and was still high at generation sixteen, after the sublines had been maintained in mass culture for three generations. Eoff (1975, 1977) attempted to increase isolation between D. melanogaster and D. simulans. He placed groups of five heterospecific pairs together for four days and used progeny production or lack thereof as evidence of mating or of refusal to mate heterogamically. Percentage of flies hybridizing was noted for each generation. Up and down lines for males and females of each species were followed for six to twenty-two generations, but interpretation of the data is difficult due to the large fluctuations in the percentage of hybridizing flies in the various

generations, and to the lack of analogous data for a control line. It is also questionable whether we can safely equate absence of progeny with lack of mating and/or with discrimination, since similar tests were not made on conspecific pairs to ascertain whether all females mate, and if all mated females produce offspring.

Although the development of sexual isolation lies at the heart of the speciation process, we have only just begun to unravel the complex web of conditions and interrelationships which is involved in the evolution of this isolating mechanism. The foregoing survey is not complete, yet it does highlight at least three factors to be considered when attempting to increase or induce behavioral isolation in the laboratory. First, and essentially, there must be sufficient genetic variability in male and female mating behaviors. Second, the selective procedure must be able to efficiently affect and mold this variability. Finally, the presence or absence of some initial isolation, as pre- or postmating barriers between the selected types seems to have some bearing on the probability, extent and rapidity of response to selection.

Consideration of the various experimental designs

described above leads to the conclusion that selection is most efficient if it is practiced on a trait which is directly related to mating behavior, and on individuals between which there is some initial isolation. Of the eleven cited experiments in which selection was on other than mating behavior, on types showing no apparent initial isolation, only four were successful in inducing some degree of behavioral isolation. Isolation indices, when measured, were relatively low, from about 0.23 to 0.54. Thoday and Gibson (1970) and Felsenstein (1981) have discussed the various factors influencing the outcome of such experiments. Chief among these are pleiotropy and linkage. That is, isolation is most likely to occur if the genes affecting the primary character have appropriate pleiotropic effects on mating behavior, or if there is close linkage between important loci affecting the primary trait and loci affecting isolation. It has been suggested that the results of Thoday and Gibson were the consequence of a special situation in which there was some linkage between the genes for bristle number and those for mate recognition (Scarloo et. al. 1967; Spiess and Wilke 1984). In fact, the computer simulations of Felsenstein indicated that no isolation will develop between subpopulations in the

absence of some initial linkage disequilibrium between the assortative mating locus (loci) and the loci under selection. Carson et. al. (1977), however, present evidence that selection for some unrelated trait may be accompanied by the chance fixation of a gene or gene combination which effects a strong behavioral isolation.

Of the ten experiments in which selection was directly on mating behavior, eight have been clearly successful in increasing isolation. Within these, those utilizing anti-hybrid selection have required greater lengths of time than the two in which selection was on previously tested individuals or their relatives. A rough ordering of the selection times gives us the following: Koepfer (3 generations) < Kessler (5 to 9 generations) < Koopman (9 to 14 generations) < Knight; Crossley (18 to 31 generations) < Dobzhansky et. al. (50 generations) < Wallace (73 generations) < Ehrman (114 generations). In addition to selective regime, we may also note that mojavensis (P) males and (S) females displayed some initial behavioral isolation, and D. pseudoobscura and D. persimilis are separated both ethologically and by hybrid sterility. It is probable that this preliminary isolation also contributed to the rapidity of response. Post-mating factors did not seem

to have as much influence, as Dobzhansky et. al. required fifty generations and Ehrman about one-hundred and fourteen generations of "sympatric" anti-hybrid selection by means of hybrid sterility. Drosophila mojavensis (S) males and (P) females were not separated by either pre- or postmating barriers, and here again we see a failure to induce isolation de novo.

In 1981 Sved proposed a polygenic model for the evolution of sexual isolation, and ran computer simulations of selection experiments utilizing no choice interstrain mass matings plus artificial elimination of the hybrids, i.e., design similar to that of Kessler. His results indicated that the evolution of pre-mating isolation would occur most efficiently if there were some initial or chance divergence in mating behavior in addition to the post-mating barrier between the selected forms. The experiments cited here seem to bear this out. He also found that a major factor in the selection response is the tendency for divergent males and females to reject mates from the heterogamic strain. However, there is also the tendency for these same individuals to reject members of their own strain. This, of course, makes the selection process inefficient and also leaves us with the ever-present question: Have we altered

mating preferences or merely produced a non-specific decline in mating ability in one or both selected strains? Sved suggests, therefore, that a more rapid and consistent progress toward isolation might be obtained by an experimental design which not only selects for rejection of heterogamic mates, but also simultaneously selects for acceptance of homogamic partners. The procedure utilized here for selection on mojavensis (P) and (S) may come close to fulfilling these requirements. In this regimen, a (P) male is exposed to four (P) and four (S) females. The male is being simultaneously selected for rejection of the foreign type and for mating ability with his own. It is hoped that the (S) females are being selected for rejection of the foreign male, and not merely for low mating ability. If this (P) male shows a high degree of isolation from the (S) females, and also mates with all or most of the available (P) females, he will subsequently be used as a progenitor of the next selection generation. For this he will be placed with four sibs and half-sibs of (P) females which have been selected for rejection of (S) males in the reciprocal test. In this final situation, both (P) males and (P) females are being selected for homogamic mating ability.

As stressed above, there will be no response to selection in the absence of sufficient genetic variability for mating behavior. A genetic analysis of the courtship behavior of the forms to be selected would, of course, give us a clue to the accessibility of that genetic system to selection, and an estimate of the ease with which a response might be obtained. Although there have been a number of such studies on closely related Drosophila species (Ehrman 1961; Grossfield 1966, 1975; Kilias and Alahiotis 1982; Tan 1946; Wood, Ringo and Johnson 1980), information concerning the genetic substructure of normal mating behavior and mate recognition is still scant.

Ehrman (1961) investigated the genetic basis of the sexual isolation existing between certain semispecies of Drosophila paulistorum, and concluded that the isolation is controlled by polygenes distributed throughout the species' three chromosomes. The effects of these polygenes are additive, and the isolation between the semispecies is nearly complete. Similarly, Kilias and Alahiotis (1982) found that the isolation which had developed between D. melanogaster strains while they were adapting to differing cage conditions also had a polygenic basis, and that the X, 2 and 3 chromosomes

were equally involved. In addition, they discovered that the cytoplasmic constitution of the strains was a major factor in determining the extent of isolation between them.

Wood, Ringo and Johnson (1980) examined the male mating behavior of D. melanogaster, D. simulans and of hybrids carrying a D. simulans X and a D. melanogaster Y. They analysed the frequencies of six transitions between sequential elements of male courtship, and found that D. melanogaster and D. simulans differ for four of the six. For two of these four, hybrid males are intermediate between the parental types; for one of the four, hybrids are the same as D. simulans; for the remaining transition, they behave like D. melanogaster. Their conclusions were that for the transitions in which hybrids are unlike D. simulans, at least some genes must be autosomal; for those in which hybrids resemble D. simulans, the genes may be X-linked or autosomal dominant over those of D. melanogaster.

The sibling species D. pseudoobscura and D. persimilis, were the subject of an early study by Tan (1946), who reported that the genes controlling female responsiveness to species-specific stimuli are located on the X and second chromosomes. Later it was found that

the male courtship songs of these two species differ in both qualitative and quantitative aspects (Waldron 1964; Ewing and Bennet-Clark 1968). Ewing (1969) demonstrated that the genes controlling the qualitiative aspects, i.e., the overall song pattern, are X-linked. Pulse interval, a quantitative trait, is controlled separately, either by polygenes or by one locus which confers an intermediate phenotype on the hybrid male.

Grossfield has given us extensive information regarding the influence of light on Drosophila mating behavior. Included here have been reports on the genetic control of light dependent mating behavior in two pairs of closely related species. In D. palustris mating is blocked by darkness, while in D. subpalustris mating is merely inhibited. Interspecific crosses between these two indicate that the ability to mate in darkness is controlled by an autosomal recessive, with genes on the X being involved in the degree of expression (Grossfield 1966). In another pair of siblings, males of D. triauraria cannot mate in darkness, while D. auraria males are light independent. The relevant genetic factors are located on chromosome 2. Since mating tests between the species, their F_1's and backcross progeny showed that only males which are homozygous for that

chromosome are unable to mate in the dark, the gene(s) appears to be recessive (Grossfield 1975).

For the D. arizonensis - D. mojavensis species pair, we are fortunate in knowing the locations of genes controlling both male and female behaviors. As reviewed in the Introduction, genetic determination of mating behavior differs in the two sexes (Markow 1981; Zouros 1981b). Female responses are controlled by genes on chromosome 2 and on the AMY-marked chromosome (4 or 5), while those of the male are determined by factors on the Y and on the PGM-marked chromosome (5 or 4). This sibling pair is one of the few studied in which the X is not at all implicated in mate recognition, and also is the only one in which the Y is clearly implicated. However, as Zouros points out, the chromosome bearing the PGM locus in D. arizonensis and D. mojavensis corresponds to the XR of D. pseudoobscura and D. persimilis. Thus, had he worked with the latter pair, Zouros would have reported that male behavior is determined by genes on the X and Y. We also know that hybrid F_1 mojavensis females from reciprocal (P) x (S) crosses resemble (P) females in receptivity and mating, while hybrid F_1 males elicit responses typical of (S) males. Thus, within D. mojavensis baja, (P) genes are

dominant in females and (S) genes are dominant in males.

The Y-linked genes affecting behavior may have been an important factor in the responses of D. mojavensis males to selection. First, any Y-linked trait in a male is immediately expressed phenotypically. Second, selection on the males was direct, so if any of the chosen males in a given generation were carrying Y-linked alleles which would alter their interactions with (P), (S), or (C) females, those alleles would, of course, be passed on to their sons. Selection on the females was indirect, on sibs and half-sibs, and presumably dealt with autosomal genes, whose effects would not be as readily expressed nor readily made homozygous. We might also note that the pair which never developed isolation consisted of (P) females and (S) males. If genes for peninsular female behavior and Sonoran male behavior are dominant, this was perhaps a system particularly intractable to selection.

Finally, now, we must look more closely at the specific interactions of the control and selected forms, both with each other and with unselected D. arizonensis and mojavensis (C). This will begin with a consideration of the effects of selection on (P) males and (S) females; these are summarized in Table 19. There have

been significant increases in isolation, as measured by I, between peninsular males and Sonoran females, and also between peninsular males and Californian females. The altered interaction is primarily attributable to the selected (P) males (Table 20), and is due to a drastic reduction in numbers of heterogamic matings, which is accompanied, however, by a decrease in homogamic matings as well. The same pattern occurs for the interaction between selected (P) male and (C) females. Although isolation, as measured by I, has not changed significantly between selected (S) females and (C) males, these females do exhibit a significant reduction in heterogamic matings when paired with (C) males; this change is in the same direction as that shown by the (P) males toward (C) females. There has been no observable change in isolation, and no significant change in numbers of homogamic and heterogamic matings when (P) males are confined with their own and D. arizonensis females. Similarly, there is no reduction in heterogamic matings when selected (S) females are confined with D. arizonensis males.

In analysing any increase in isolation due to selection we must attempt to decide among the possible underlying causes for the change. These include: 1) an

increased preference for homogamic matings; this might be expected to result in an increased number of homogamic pairings; 2) an increased recognition of and rejection of the heterogamic selected form, and perhaps of other foreign types as well; here we should see a decrease in heterogamic matings; 3) a combination of both these responses; 4) some change in mating ability or in drive, in one or both types, which is manifested as an apparent change in preference in either or both selected forms; here the direction of preference would be unpredictable.

If these are the possibilities, then there are at least two explanations for the observed changes in the interactions involving selected (P) males and (S) females. Either (P) males and (S) females have been selected for rejection of foreign types, or they have suffered some kind of decline in mating ability as evidenced by the overall decline in both homo- and heterogamic matings in tests confining the selected forms with each other, or with Californian mojavensis. There is no obvious evidence for increased homogamic preferences. There is a dilemma, however, in attempting to evaluate mating ability. If we measure mating ability as the total number of matings per observation period,

we then are confronted with conflicting evidence: No significant change for either (P) males or (S) females when D. arizonensis is the heterogamic type; decreased (P) male ability in the presence of (S) or (C) females and decreased (S) female ability in the presence of (C) males and females.

Considering that there seems to be no indicator of absolute mating ability here, and that all observed changes are in a direction consistent with that of selection, we might introduce the idea of relative mating efficiencies under different test combinations. We can then hypothesize that selection has caused a change in the mating preferences of (P) males and (S) females, and that the main observable effect is a rejection of any foreign D. mojavensis, whether the co-selected (P) or (S) geographic form, or the unselected Californian form. The initial isolation between the Mexican D. mojavensis forms and D. arizonensis was so high that it would be impossible to observe any increase by the methods used here.

Markow (1981; 1982) and Markow and Richmond (1981) have presented evidence that D. mojavensis males are capable of precourtship discrimination as indicated by the first female to be courted in a male choice

situation. These males preferentially court virgin females or females mated not less than forty-eight hours previously. Of more relevance here are the courtship interactions between D. mojavensis males and D. arizonensis females. Sympatric, mainland males have a high ability to distinguish between D. mojavensis and D. arizonensis females, while peninsular males lack this ability and court D. mojavensis first only 51% of the time. However, even when D. mojavensis males do initiate courtship with D. arizonensis, the courtship is very brief, three seconds or less. After this the male turns away abruptly and becomes immobile somewhere in the vial (Markow 1981). Clearly the D. arizonensis female sends an unambiguous and aversive signal to the sibling species male.

Since these two species look very much alike, and wing vibration doesn't begin until courtship has been initiated, Markow has suggested that precourtship discrimination is not based on visual or auditory cues, but rather on some form of chemical communication via tapping or air-borne signals. This is consistent with studies on early courtship behavior in Drosophila melanogaster. Shorey and Bartell (1970) have presented evidence for a volatile sex hormone produced by

D. melanogaster females which stimulates males to initiate courtship, and Jallon and Hotta (1970) have initiated an investigation into the "sex appeal focus" in D. melanogaster. "Sex appeal" is defined by Jallon and Hotta as a stimulus or set of stimuli which induces wing vibration; the stimulus appears to be originate somewhere within the abdomen, and is perhaps associated with the fat bodies.

In contrast to cues sent by a D. arizonensis female, the early signal(s) sent by a conspecific (S) or (C) female to a selected (P) male are probably quite similar to the cues of his own females. The fact that selected (P) males exhibit an increased isolation in tests involving these other forms of D. mojavensis, but also an overall decrease in total homogamic matings in such tests, may indicate that we are seeing an intermediate stage in the development of early courtship discrimination. At this stage of evolution, the male has a high probability of ultimately choosing the correct" mate, but suffers a loss in mating efficiency due to some residual difficulty in distinguishing quickly between the two conspecific forms with whom he is confined.

In 1965 Wallace and Felthousen introduced a test of

sexual isolation for Drosophila based on the idea of "mating interference". The technique utilized the elapsed time between the introduction of one or more conspecific pairs into a chamber and the occurrence of the first copulation. Elapsed times can be measured for a given conspecific pair and also for that pair in the presence of males and/or females of a second species. The conspecific pair can consist of allopatric or sympatric individuals, as can the introduced foreign type. If in such a situation, the male of the confined pair investigates the foreign flies, or if the foreign males investigate either the females or males of the confined pair, the elapsed time will be lengthened. Wallace and Felthousen predicted that if sexual isolation has been reinforced in areas of sympatry, then individuals of two different species will investigate each other less if they are of sympatric than of allopatric origin. Further, they suggested that a foreign species would interfere more in the mating if the male and female of the confined pair were of allopatric origin than if they originated in sympatry. Preliminary tests involved observation on geographic strains of D. melanogaster in various combinations with D. simulans, D. virilis and D.

nebulosa. Results agreed with the predictions.

The D. mojavensis - D. arizonensis results reported here may be showing another type of "mating interference," which also appears during the initial stages of sexual interaction, but which is observable in male choice tests. In this situation, the more different the foreign female, the less the interference. When a selected (P) male is confined with his own and D. arizonensis females, hetero- and homogamic signals are easily distinguished, and the male does not "waste" very much time in interspecific courtships. When confined with two conspecific females, one of his own geographic form and one of another, he is presented with much more similar signals, and spends more time trying to decipher them.

Preliminary observations made during the final tests involving (P) x (S) mojavensis may support this idea. The confined flies were observed for the first ten minutes after being placed in the vial, and it was simply noted whether or not the male initiated one or more courtships during that time. If he did, the choice of first female was also observed. No differences were found between control (P), control (S) and selected (S) males. The selected (P) males, however, initiated

significantly fewer courtships than the other three kinds of male. This was true both in the presence of control (S) females and selected (S) females. This may be evidence either for difficulties in early courtship discrimination and therefore a decrease in mating efficiency in this particular situation, or of course, for a general decline in mating ability.

Table 27 summarizes the results of the selection experiment utilizing peninsular females and Sonoran males. There has been no significant change in the isolation, as measured by I, for any interaction involving selected peninsular females, but the contingency chi square does indicate a significant decrease in matings between these females and D. mojavensis (C) males. Similarly, although the I value has not changed significantly for (P) females and (S) males, selected (S) males do show an increase in homogamic matings when confined with their own and (P) females. These same males show a decrease, however, in homogamic matings when the test involves their own and (C) females. As in the reciprocal experiment, there have been no significant changes in the relationship of either selected males or selected females with D. arizonensis.

These results also present us with the problem of adequately defining mating ability, for once again use of number of matings per observation period yields conflicting evidence for both selected males and females: No significant change for either (S) males or (P) females when D. arizonensis is the heterogamic type; increased ability for (S) males when in the presence of (S) and (P) females; decreased ability of (S) males when confined with their own and (C) females; decreased ability of (P) females when confined with (C) females and males.

As with selected (P) males, we have no indicator of absolute mating ability. However, in contrast to the (P) males, whose conduct was altered with both homo- and heterogamic females, selected (S) males exhibit changed responses only toward their own females, and these are in opposite directions in the two testing situations. The (P) females are similar to selected (S) females in that they now are more strongly rejecting of (C) males. Thus selected (P) males and females respond in similar ways to unselected mojavensis (C), while selected Sonoran males and females respond in opposite ways.

It has been suggested (Zouros and D'Entremont 1980) that the range of Sonoran male behavior is included

within the range of (P) male behavior, and that because of this (P) males and (S) males are equally acceptable to (P) females. This, of course, explains the lack of response to selection for isolation between peninsular females and Sonoran males. It is also possible that the courtship cues of Californian males (D. mojavensis mojavensis) differ from those of both the (P) and (S) Mexican forms (D. mojavensis baja) in some quantitative or qualitative way. The decrease in mating between selected (P) females and (C) males might then indicate an increased recognition and rejection of the other subspecies. The fact that both selected (P) males and (P) females exhibit the same response toward (C) indicates that this form may have had the potential to develop such a recognition.

The manner in which responses of selected Sonoran males vary according to the test situation is perhaps the most difficult observation to explain. The increased homogamic matings when in the presence of (S) and (P) females could indicate the beginnings of interform discrimination, but the decrease in homogamic mating without any concomitant change in heterogamic mating when (C) is the foreign female, is perhaps inexplicable without further testing.

Clearly, further testing is warranted for all of the observed interactions which have been reported here. The quantitative components of courtship and precourtship behaviors of the selected and control forms, both with each other and with the unselected mojavensis (C) and D. arizonensis, must be analysed in order to elucidate the behavioral elements which have been changed as a result of selection. Also necessary is a search for the chemical components of the observed interactions.

The research which has been reported here posed several basic questions regarding the evolution of sexual isolation. It has been rewarding in going far toward answering those questions, and in adding to our knowledge regarding behavioral interactions within the mojavensis cluster. Most stimulating, perhaps, is the wealth of new questions with which we are left, and new doors to inquiry which we are now free to open.

TABLES

Table 1 Major host plants of the mojavensis cluster species.

Species	Main Host
D. mojavensis (C)	Ferocactus acanthodes (barrel cactus)
D. mojavensis (S)	Stenocereus thurberi (organ pipe)
D. mojavensis (P)	Stenocereus gummosus (agria)
D. arizonensis	Stenocereus alamosensis (cina)
D. navojoa	Opuntia species

mojavensis (C) = mojavensis mojavensis (California)
mojavensis (P) = mojavensis baja (Baja California)
mojavensis (S) = mojavensis baja (Sonora)

Table 2 Summary of sexual isolation tests between D. arizonensis and D. mojavensis. Data show that sexual isolation between the species is highest in their region of sympatry (from Wasserman and Koepfer, 1977).

Type of Cross: Species	Region	Available females of each type	Percent Heterogamic Matings	Isolation Index ± S.E.
arizonensis x arizonensis	allopatric x allopatric	120	44.78	.104 ± .078
	allopatric x sympatric	480	47.20	.056 ± .038
	sympatric x sympatric	120	47.83	.043 ± .074
	Total	720	46.93	.061 ± .031
mojavensis x mojavensis	allopatric x allopatric	120	51.59	- .032 ± .080
	allopatric x sympatric	480	42.11	.158 ± .040
	sympatric x sympatric	120	43.45	.131 ± .082
	Total	720	43.95	.121 ± .033
Interspecific	allopatric x allopatric	480	25.16	.497 ± .040
	allopatric x sympatric	960	15.03	.699 ± .024
	sympatric x sympatric	480	3.71	.926 ± .019
	Total	1,920	15.33	.693 ± .017

Table 3 Isolation indices for interspecific tests between D. arizonensis and D. mojavensis. Data show that the enhanced isolation in the sympatric region is due to the behavior of the sympatric D. mojavensis (from Wasserman and Koepfer, 1977).

Species	Isolation Index ± S.E.	
	Allopatric	Sympatric
mojavensis females	.308 ± .040	.940 ± .017
mojavensis males	.779 ± .031	.922 ± .020
arizonensis females	.899 ± .022	.797 ± .030
arizonensis males	.538 ± .038	.598 ± .036

Table 4 Collection information for the Drosophila species used in this study.

Species		Stock Number	Collection Locality	Date	Stock Derived From	Collector
D. arizonensis		565.5E	Caborca,Sonora	1976	single pair	M. Wasserman
D. mojavensis mojavensis (C)		553.5 553.6	Anza-Borrego Dessert, California	1975	11 pairs	M. Wasserman
D. mojavensis baja						
	(S)	565.1C	Caborca, Sonora	1976	single pair	M. Wasserman
	(S)	565.1D	Caborca, Sonora	1976	single pair	M. Wasserman
	(S)	565.1E	Caborca, Sonora	1976	single pair	M. Wasserman
	(S)	565.1J	Caborca, Sonora	1976	single pair	M. Wasserman
	(P)	A202	Cunano, Baja California	1968	292	W. Heed
	(P)	360.1	La Presa, Baja California	1972		W. Johnson
	(P)	A199	Loreto, Baja California	1968	90	W. Heed
	(P)	A63.1	Mulege, Baja California	1962	single pair	J. Russell
	(P)	361.1 (A367)	San Ignacio, Baja California	1972		W. Johnson
	(P)	560.5	San Quitin, Baja California	1976	mass	M. Wasserman

Table 5 Strain of male and heterogamic female for each of the twenty-four final test combinations. For each test, one male was confined with four homogamic and four heterogamic females. There were sixteen replicates for each combination.

Strain of Male	Strain of Heterogamic Female
mojavensis (P) - control mojavensis (P) - selected	mojavensis (S) - control mojavensis (S) - selected mojavensis (C) arizonensis
mojavensis (S) - control mojavensis (S) - selected	mojavensis (P) - control mojavensis (P) - selected mojavensis (C) arizonensis
mojavensis (C)	mojavensis (P) - control mojavensis (P) - selected mojavensis (S) - control mojavensis (S) - selected
arizonensis	mojavensis (P) - control mojavensis (P) - selected mojavensis (S) - selected mojavensis (S) - selected

Table 6 Random mating system for maintenance of control lines. Each control line consists of four sublines. In every generation, the parents of a given subline are one male from that subline and four females from a different subline. The male/female combinations are repeated every third generation. Males and females from the same subline are never paired. The subline name comes from the male parent.

Parents of Generation	Matings to maintain peninsular, (P), control line — Sublines				Matings to maintain Sonoran, (S), control line — Sublines			
	C_a ♂ x ♀♀	C_b ♂ x ♀♀	C_c ♂ x ♀♀	C_d ♂ x ♀♀	C_w ♂ x ♀♀	C_x ♂ x ♀♀	C_y ♂ x ♀♀	C_z ♂ x ♀♀
n	a x d	b x a	c x b	d x c	w x z	x x w	y x x	z x y
n + 1	a x c	b x d	c x a	d x b	w x y	x x z	y x w	z x x
n + 2	a x b	b x c	c x d	d x a	w x x	x x y	y x z	z x w
n + 3	a x d	b x a	c x b	d x c	w x z	x x w	y x x	z x y

Table 7 Revolving mating scheme used in maintenance of the control line and in the male choice selective tests.

Generation	(P) ♂ x	(P) ♀♀	(S) ♂ x	(S) ♀♀
0, 3, 6, 9	a	d	w	z
	b	a	x	w
	c	b	y	x
	d	c	z	y
1, 4, 7, 10	a	c	w	y
	b	d	x	z
	c	a	y	w
	d	b	z	x
2, 3, 8, 11	a	b	w	x
	b	c	x	y
	c	d	y	z
	d	a	z	w

Table 8 Selection Procedure. Every generation, two sets of selective tests were run simultaneously: 16 utilizing peninsular, (P), males with Sonoran, (S), females as the heterogamic type, and 16 utilizing Sonoran males with peninsular females as the heterogamic type. Parentals of the next generation were the best male from each subline mated to four females from the subline which had shown the highest isolation in the reciprocal selective test.

Selective Tests For Peninsular Males and Sonoran Females

♂	♀♀		♂	♀♀		♂	♀♀		♂	♀♀	
(P)	(P)	+ (S)	(P)	(P)	+ (S)	(P)	(P)	+ (S)	(P)	(P)	+ (S)
a_1	d	w	b_1	a	w	c_1	b	w	d_1	c	w
a_2	d	$\underline{x}$	b_2	a	$\underline{x}$	c_2	b	$\underline{x}$	d_2	c	$\underline{x}$
a_3	d	$\underline{y}$	b_3	a	$\underline{y}$	c_3	b	$\underline{y}$	d_3	c	$\underline{y}$
a_4	d	z	b_4	a	z	c_4	b	z	d_4	c	z

Selective Tests For Sonoran Males and Peninsular Females

♂	♀♀		♂	♀♀		♂	♀♀		♂	♀♀	
(S)	(S)	+ (P)	(S)	(S)	+ (P)	(S)	(S)	+ (P)	(S)	(S)	+ (P)
w_1	z	a	x_1	w	a	y_1	$\underline{x}$	a	z_1	y	a
w_2	z	b	x_2	w	b	y_2	$\underline{x}$	b	z_2	y	b
w_3	z	c	x_3	w	c	y_2	$\underline{x}$	c	z_3	y	c
w_4	z	d	x_4	w	d	y_3	$\underline{x}$	d	z_4	y	d

a, b, c, d = peninsular sublines
w, x, y, z = Sonoran sublines

Table 9 Data from generation O, first set of selective tests utilizing peninsular, (P), males with Sonoran, (S) females.

(P) Males	Homogamic Matings to (P) Females		Heterogamic Matings to (S) Females		Isolation Index, I
a_1	3	d	2	w	0.20
a_2	3	d	3	x	0.00
a_3	0	d	0	y	0.00
* a_4	4	d	1	z	0.60
b_1	3	a	2	w	0.20
b_2	3	a	3	x	0.00
* b_3	4	a	1	y	0.60
b_4	4	a	2	z	0.33
c_1	4	b	3	w	0.14
c_2	3	b	3	x	0.00
* c_3	2	b	1	y	0.33
c_4	2	b	2	z	0.00
d_1	4	c	2	w	0.33
d_2	4	c	2	x	0.33
* d_3	4	c	1	y	0.60
d_4	4	c	3	z	0.14
	51		31		

I for the 16 tests = .244 ± 107

I for w ♀♀ ♂♂ = 0.22
I for x ♀♀ ♂♂ = 0.08
I for y ♀♀ ♂♂ = 0.54
I for z ♀♀ ♂♂ = 0.27

* best male of subline

Table 10 Data from generation 0, first set of selective tests utilizing Sonoran, (S), males with peninsular, (P) females.

(S) Males	Homogamic Matings to (S) Females		Heterogamic Matings to (P) Females		Isolation Index, I
w_1	4	z	4	a	0.00
w_2	3	z	4	b	- 0.14
* w_3	4	z	3	c	0.14
w_4	3	z	3	d	0.00
x_1	4	w	4	a	0.00
* x_2	4	w	3	b	0.14
x_3	2	w	4	c	- 0.33
x_4	2	w	4	d	- 0.33
* y_1	4	x	4	a	0.00
y_2	0	x	0	b	0.00
y_3	3	x	4	c	- 0.14
y_4	3	x	3	d	0.00
* z_1	4	y	3	a	0.14
z_2	2	y	3	b	- 0.20
z_3	4	y	3	c	0.14
z_4	3	y	4	d	- 0.14
	49		53		

I for the 16 tests = - .039 ± .099

I for a ♀♀ / ♂♂ = 0.03
I for b ♀♀ / ♂♂ = - 0.05
I for c ♀♀ / ♂♂ = - 0.04
I for d ♀♀ / ♂♂ = - 0.12

* best male of subline

Table 11 Summary of data for tests involving peninsular, (P), males and Sonoran, (S), females. Comparisons were made within generations 0 - 5 and 6 - 11 by the chi square method of Brandt and Snedecor. Subset membership is designated by vertical bars. Subsets a and b differ significantly ($p \leq 0.001$), as do subsets c and d ($p \leq 0.001$).

	Generation	Available Females of each type	Inseminated Females Homogamic	Heterogamic	Isolation Index	± S.E.
	0	64	51	31	*	.244 ± .107
a	1	56	55	22	***	.429 ± .103
	2	52	49	17	***	.485 ± .108
	3	52	43	3	***	.870 ± .073
b	4	52	25	1	***	.923 ± .075
	5	68	55	6	***	.803 ± .076
	6	216	173	21	***	.784 ± .045
c	7	64	50	5	***	.818 ± .078
	8	48	34	5	***	.744 ± .107
	9	64	58	22	***	.450 ± .100
d	10	64	61	18	***	.544 ± .094
c	11	64	51	5	***	.821 ± .076
	12	64	42	2	***	.909 ± .063
	13	64	48	2	***	.920 ± .055
	16	64	52	5	***	.825 ± .075

Interaction: 1 (P) ♂ x 4 (P) ♀♀ + 4 (S) ♀♀

Significance of Isolation Index: * $p \leq 0.05$
*** $p \leq 0.001$

Table 12 Comparison of preliminary and final tests utilizing peninsular, (P), males with Sonoran, (S), females as the heterogamic type. Arrows indicate significant differences in isolation ($p \leq .05$) between base stocks and selected lines, and between controls and selected lines.

Test Combination: 1 (P) ♂ x 4(P) ♀♀ + 4 (S) ♀♀

Type of Test	Available Females of Each Type	Inseminated Females Homogamic (P)	Inseminated Females Heterogamic (S)		Isolation Index ± S.E.
Preliminary - Base	64	51	31	*	0.244 ± .107
Final - Control	64	56	18	***	0.514 ± .100
Final - Selected	64	42	2	***	0.909 ± .063

* $p \leq .05$; ** $p \leq .01$; *** $p \leq .001$

Table 13 Results of final tests utilizing Sonoran, (S), males with *arizonensis* as the heterogamic female. Control and selection tests do not differ significantly in numbers of homo- and heterogamic matings.

	(S) ♀♀ (homogamic)			
	Mated	Unmated		
Control Test	46	18	64	
Selection Test	48	16	64	$X^2 = .04$
	94	34	128	$.90 > p > .80$

	arizonensis ♀♀ (heterogamic)		
	Mated	Unmated	
Control Test	3	61	64
Selection Test	0	64	64
	3	125	128

INTERACTION: (S) ♂ x (S) ♀♀ in presence of *arizonensis* ♀♀

COMPARISONS: Control (S) ♂♂ & ♀♀ vs. Selected (S) ♂♂ & ♀♀

Tests: 1 (S) ♂ (control) x 4 (S) ♀♀ (control) + 4 *ariz.* ♀♀

1 (S) ♂ (selected) x 4 (S) ♀♀ (selected) + 4 *ariz.* ♀♀

Table 14 Results of final tests utilizing peninsular, (P), males with *arizonensis* as the heterogamic female. Control and selection lines do not differ significantly in numbers of homo- and heterogamic matings.

(P) ♀♀ (homogamic)

	Mated	Unmated		
Control Test	47	17	64	
Selection Test	43	21	64	$X^2 = .34$
	90	38	128	$.70 > p > .50$

arizonensis ♀♀ (heterogamic)

	Mated	Unmated	
Control Test	0	64	64
Selection Test	0	64	64
	0	128	128

INTERACTION: (P) ♂ x (P) ♀♀ in presence of *arizonensis* ♀♀

COMPARISONS: Control (P) ♂♂ & ♀♀ vs. Selected (P) ♂♂ and ♀♀

Tests: 1 (P) ♂ (control) x 4 (P) ♀♀ (control) + 4 *ariz.* ♀♀

1 (P) ♂ (selected) x 4 (P) ♀♀ (selected) + 4 *ariz.* ♀♀

Table 15 Results of final tests performed on selected Sonoran, (S), females and peninsular, (P), males. Sixteen males and sixty-four females of each type were tested for each combination. Arrow indicates a significant difference in isolation ($p \leq .05$).

Form Under Selection; Sex	Tested With	Matings: Control Homo	Control Hetero	Selected Homo	Selected Hetero	Isolation Index ± S.E. Control	Selected
(S) ♀	(C) ♂	60	43	57	28	.165 ± .097	* .341 ± .102
	ariz. ♂	54	1	59	0	*** .964 ± .036	*** 1.000
(P) ♂	(C) ♀	56	31	43	14	* .287 ± .103 →	** .509 ± .114
	ariz. ♀	47	0	43	0	*** 1.000	*** 1.000

* $p \leq .05$; ** $p \leq .01$; *** $p \leq .001$

(C) = Californian mojavensis

Table 16 Results of final tests utilizing peninsular, (P), males with Sonoran, (S), as the heterogamic female. Selected (P) males participate in significantly fewer homo- and heterogamic matings than control (P) males.

	(P) ♀♀ (homogamic)			
	Mated	Unmated		
Control Test	56	8	64	
Selection Test	42	22	64	$X^2 = 7.36$ **
	98	30	128	$p \leq 0.01$

	(S) ♀♀ (heterogamic)			
	Mated	Unmated		
Control Test	18	46	64	
Selection Test	2	62	64	$X^2 = 13.33$ ***
	20	108	128	$p \leq 0.001$

INTERACTION: (P) ♂ x (P) ♀♀ + (S) ♀♀

COMPARISONS: Controls vs. Selection Lines of (P) & (S)

Tests: 1 (P) ♂ x 4 (P) ♀♀ + 4 (S) ♀♀ from control lines

1 (P) ♂ x 4 (P) ♀♀ + 4 (S) ♀♀ from selection lines

Table 17 Results of final tests utilizing peninsular, (P), males with Californian, (C), as the heterogamic female. Selected (P) males participate in significantly fewer homo- and heterogamic matings than control (P) males.

	(P) ♀♀ (homogamic)			
	Mated	Unmated		
Control Test	56	8	64	
Selection Test	43	21	64	$X^2 = 6.42$ *
	99	29	128	$p \leq .05$

	mojavensis (C) ♀♀ (heterogamic)			
	Mated	Unmated		
Control Test	31	33	64	
Selection Test	14	50	64	$X^2 = 8.77$ **
	45	83	128	$p \leq .01$

INTERACTION: (P) ♂ x (P) ♀♀ + (C) ♀♀

COMPARISONS: Control (P) oo vs. Selected (P) oo

Tests: 1 (P) ♂(control) x 4 (P) ♀♀ (control) + 4 (C) ♀♀

1 (P) ♂(selected) x 4 (P) ♀♀ (selected) + 4 (C) ♀♀

Table 18 Results of final tests utilizing Californian, (C), males with Sonoran, (S), as the heterogamic female. Selected (S) females participate in significantly fewer heterogamic matings than control (S) females.

	mojavensis (C) ♀♀ (homogamic)			
	Mated	Unmated		
Control Test	60	4	64	
Selection Test	57	7	64	$X^2 = .40$
	117	11	128	$.70 > p > .50$

	(S) ♀♀ (heterogamic)			
	Mated	Unmated		
Control Test	43	21	64	
Selection Test	28	36	64	$X^2 = 6.20$ *
	71	57	128	$.02 > p > .01$

INTERACTION: (C) ♂x (C) ♀♀ + (P) ♀♀

COMPARISONS: Control (P) ♀♀ vs. Selected (P) ♀♀

Tests: 1 (C) ♂x 4 (C) ♀♀ + 4 (P) ♀♀ (control): control test

1 (C) ♂x 4 (C) ♀♀ + 4 (P) ♀♀ (selected): selection test

Table 19 Summary of selection effects on Sonoran, (S), females and peninsular, (P), males. Selected Sonoran females are more isolated from peninsular males than are control females, and mate less often with both peninsular and Californian, (C), males than do control females. Selected peninsular males are more isolated from both Sonoran and Californian females than are control males, and mate less often with both their own and the foreign type of female in these interactions.

Interaction Under Selection: (P) ♂ x (P) ♀♀ + (S) ♀♀

Female	Foreign Male	Effect of Selection On Isolation I	p	Number of Matings with Foreign Male: X^2	
(S)	(P)	↑	*	↓	***
(S)	(C)	↑	n.s.	↓	**
(S)	ariz.	no change		no change	

Male	Foreign Female	Effect of Selection On Isolation I	p	Matings with Own Female: X^2		Number of Matings with Foreign Female: X^2	
(P)	(S)	↑	*	↓	*	↓	***
(P)	(C)	↑	*	↓	*	↓	**
(P)	ariz.	no change		no change		no change	

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Table 20 Comparison of final tests utilizing selected and control peninsular, (P), males with selected or control Sonoran, (S), females as the heterogamic type. The arrow indicates that selected (P) males are significantly more isolated ($p \leq .01$) from Sonoran females of either type than are control males.

Strain of Male	Heterogamic Female	Matings Homo	Matings Hetero	Isolation Index ± S.E.
(P) - selected	(S) - selected	42	2	*** .909 ± .063
	(S) - control	40	2	*** .905 ± .066
(P) - control	(S) - selected	52	8	*** .733 ± .088
	(S) - control	56	18	*** .514 ± .100

Significance of Isolation Index: *** $p \leq .001$

Table 21 Summary of data for tests involving Sonoran, (S), males and peninsular, (P), females. Comparisons were made within generations 0 - 12 by the chi square method of Brandt and Snedecor. Subset membership is designated by vertical bars. Subsets e and f differ significantly ($p \leq 0.05$).

	Generation	Available Females of each type	Inseminated Females Homogamic	Heterogamic	Isolation Index ± S.E.
	0	60	49	53	- .039 ± .099
	1	60	41	51	- .109 ± .104
e	2	40	33	30	.048 ± .126
	3	44	24	31	- .127 ± .134
f	4	60	20	43	* - .365 ± .117
	5	No tests			
	6	No tests			
f	7	32	19	30	- .224 ± .139
	8	64	43	50	- .075 ± .103
	9	64	46	54	- .080 ± .100
e	10	64	36	48	- .143 ± .108
	11	64	36	48	- .143 ± .108
	12	64	42	53	- .116 ± .102

Interaction: 1 (S) ♂ x 4 (S) ♀♀ + 4 (P) ♀♀

Significance of Isolation Index: * $p \leq .05$

Table 22 Comparison of preliminary and final tests utilizing Sonoran, (S), males with peninsular, (P), females as the heterogamic type. Base stocks, controls and selected do not differ in isolation.

Test Combination: 1 (S) ♂ x 4 (S) ♀♀ + 4 (P) ♀♀

Type of Test	Available Females of Each Type	Inseminated Females Homogamic (S)	Heterogamic (P)	Isolation Index ± S.E.
Preliminary - Base	64	49	53	- 0.039 ± .099
Final - Control	64	29	47	* - 0.237 ± .111
Final - Selected	64	42	53	- 0.116 ± .102

* $p \leq .05$

Table 23 Results of final tests performed on selected peninsular, (P), females and Sonoran, (S), males. Sixteen males and sixty-four females of each type were tested for each combination. Arrow indicates a significant difference in isolation ($p \leq .05$).

Form Under Selection; Sex	Tested With	Matings: Control Homo	Control Hetero	Selected Homo	Selected Hetero	Isolation Index ± S.E. Control	Selected
(P) ♀	(C) ♂	44	61	40	44	- .162 ± .096	- .048 ± .109
	ariz. ♂	50	21	56	11	** .408 ± .108	*** .672 ± .091
(S) ♂	(C) ♀	54	33	43	44	* .241 ± .104 ←	- .011 ± .107
	ariz. ♀	46	3	48	0	*** .878 ± .068	*** 1.000

* $p \leq .05$; ** $p \leq .01$; *** $p \leq .001$

(C) = Californian mojavensis

Table 24 Results of final tests utilizing Sonoran, (S), males with peninsular, (P), as the heterogamic female. Selected (S) males participate in significantly more homogamic matings than control (S) males.

	(S) ♀♀ (homogamic)			
	Mated	Unmated		
Control Test	29	35	64	
Selection Test	42	22	64	$X^2 = 4.55$ *
	71	57	128	$p \leq 0.05$
	(P) ♀♀ (heterogamic)			
	Mated	Unmated		
Control Test	47	17	64	
Selection Test	53	11	64	$X^2 = 1.14$
	100	28	128	$0.30 > p > 0.20$

INTERACTION: (S) ♂ x (S) ♀♀ + (P) ♀♀

COMPARISONS: Controls vs. Selection Lines of (S) and (P)

Tests: 1 (S) ♂ x 4 (S) ♀♀ + 4 (P) ♀♀ : from control lines

1 (S) ♂ x 4 (S) ♀♀ + 4 (P) ♀♀ : from selection lines

Table 25 Results of final tests utilizing Sonoran, (S), males with Californian, (C), as the heterogamic female. Selected (S) males participate in significantly fewer homogamic matings than control (S) males.

(S) ♀♀ (homogamic)

	Mated	Unmated		
Control Test	54	10	64	
Selection Test	43	21	64	$X^2 = 4.26$ *
	97	31	128	$p \leq .05$

mojavensis (C) ♀♀ (heterogamic)

	Mated	Unmated		
Control Test	33	31	64	
Selection Test	44	20	64	$X^2 = 3.26$
	77	51	128	$.10 > p > .05$

INTERACTION: (S) ♂ x (S) ♀♀ + (C) ♀♀

COMPARISONS: Control (S) ♂♂ vs. Selected (S) ♂♂

Tests: 1 (S) ♂ (control) x 4 (S) ♀♀ (control) + 4 (C) ♀♀

1 (S) ♂ (selected) x 4 (S) ♀♀ (selected) + 4 (C) ♀♀

Table 26 Results of final tests utilizing Californian, (C), males with peninsular, (P), as the heterogamic female. Selected (P) females participate in significantly fewer heterogamic matings than control (P) females.

	mojavensis (C) ♀♀ (homogamic)			
	Mated	Unmated		
Control Test	44	20	64	
Selection Test	40	24	64	$X^2 = .31$
	84	44	128	$.70 > p > .50$

	(P) ♀♀ (heterogamic)			
	Mated	Unmated		
Control Test	61	3	64	
Selection Test	44	20	64	$X^2 = 13.57$ ***
	105	23	128	$p \leq .001$

INTERACTION: (C) ♂ x (C) ♀♀ + (P) ♀♀

COMPARISONS: Control (P) ♀♀ vs. Selected (P) ♀♀

Tests: 1 (C) ♂ x 4 (C) ♀♀ + 4 (P) ♀♀ (control): control test

1 (C) ♂ x 4 (C) ♀♀ + 4 (P) ♀♀ (selected): selection test

Table 27 Summary of selection effects on peninsular, (P), females and Sonoran, (S), males. There is no significant change in isolation for any interaction involving peninsular females, although matings between selected peninsular females and Californian, (C), males have decreased significantly. Selected Sonoran males are less isolated from Californian females than are control males, and participate in fewer homogamic matings than control males when choosing between their own and Californian females. When the choice is between homogamic and peninsular females, however, selected Sonoran males mate more often with their own type of female than control males.

Interaction Under Selection: (S) ♂ x (S) ♀♀ + (P) ♀♀

Female	Foreign Male	Effect of Selection On Isolation I	p	Number of Matings with Foreign Male: X^2
(P)	(S)	↑	n.s.	no change
(P)	(C)	↑	n.s.	↓ ***
(P)	ariz.	↑	n.s.	↓ 0.10 p 0.05

Male	Foreign Female	Isolation I	p	Matings with Own Female: X^2		Effect of Selection On Number of Matings with Foreign Female: X^2
(S)	(P)	↑	n.s.	↑	*	no change
(S)	(C)	↓	**	↓	*	↑ 0.10 > p > 0.05
(S)	ariz.	no change		no change		no change

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Table 28 Comparison of final tests utilizing selected and control Sonoran, (S), males with selected or control peninsular, (P), females as the heterogamic type. There are no significant differences within this set of four test results.

Strain of Male	Heterogamic Female	Matings Homo	Matings Hetero	Isolation Index ± S.E.
(S) - selected	(P) - selected	42	53	- .116 ± .102
	(P) - control	40	58	- .184 ± .099
(S) - control	(P) - selected	46	56	- .098 ± .099
	(P) - control	29	47	* - .237 ± .111

Significance of Isolation Index: * $p \leq .05$

FIGURES

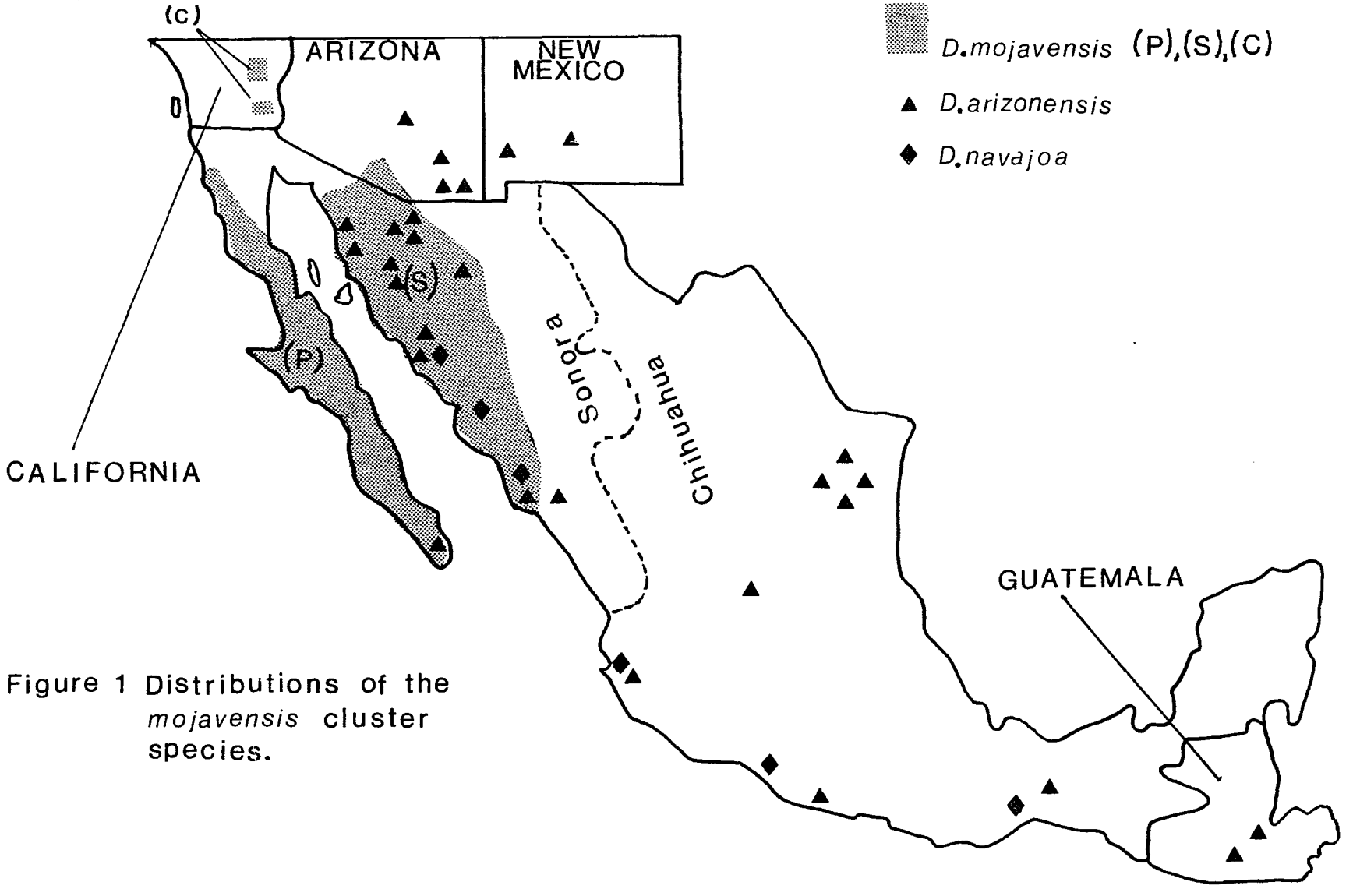

Figure 1 Distributions of the *mojavensis* cluster species.

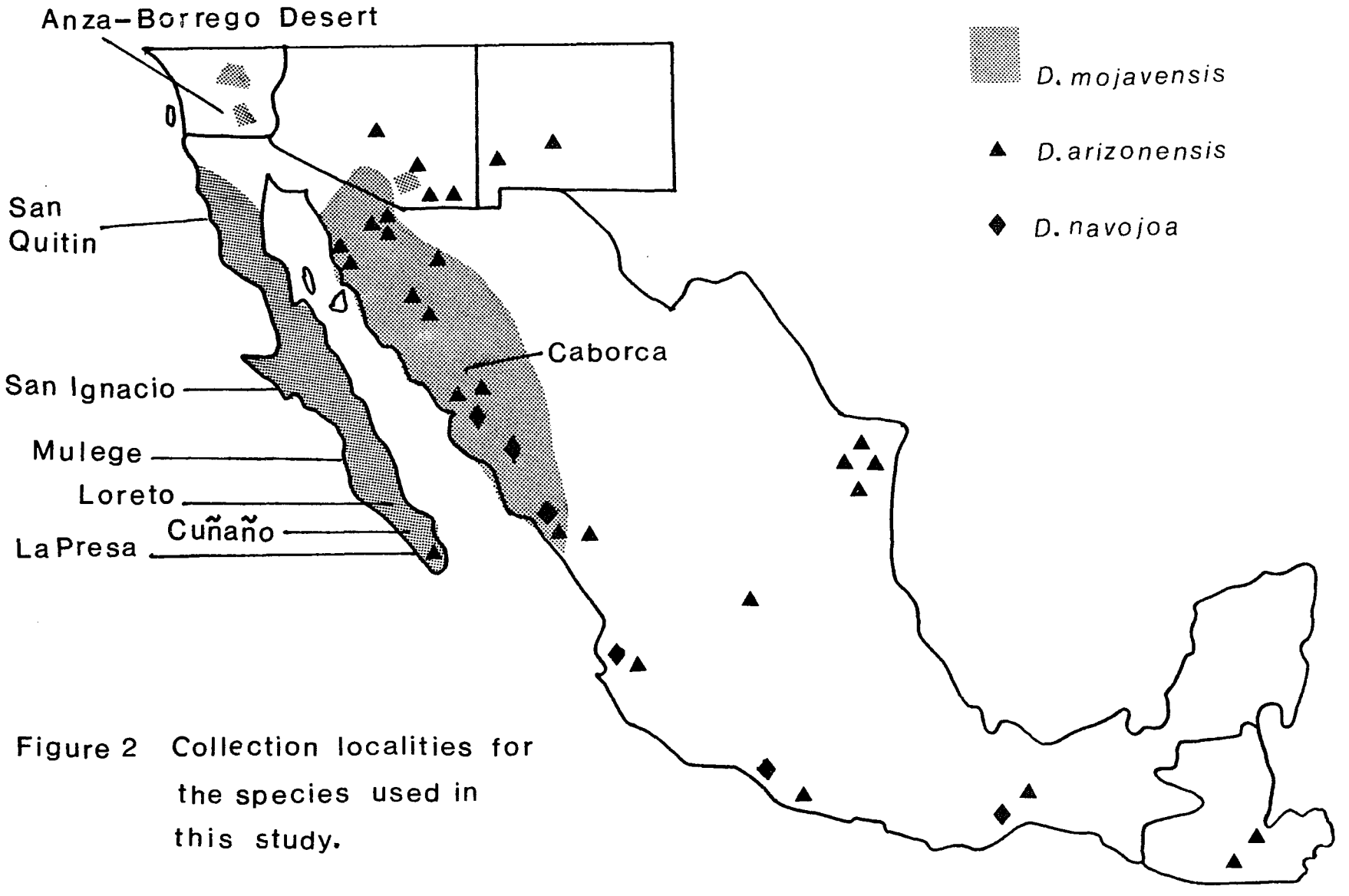

Figure 2 Collection localities for the species used in this study.

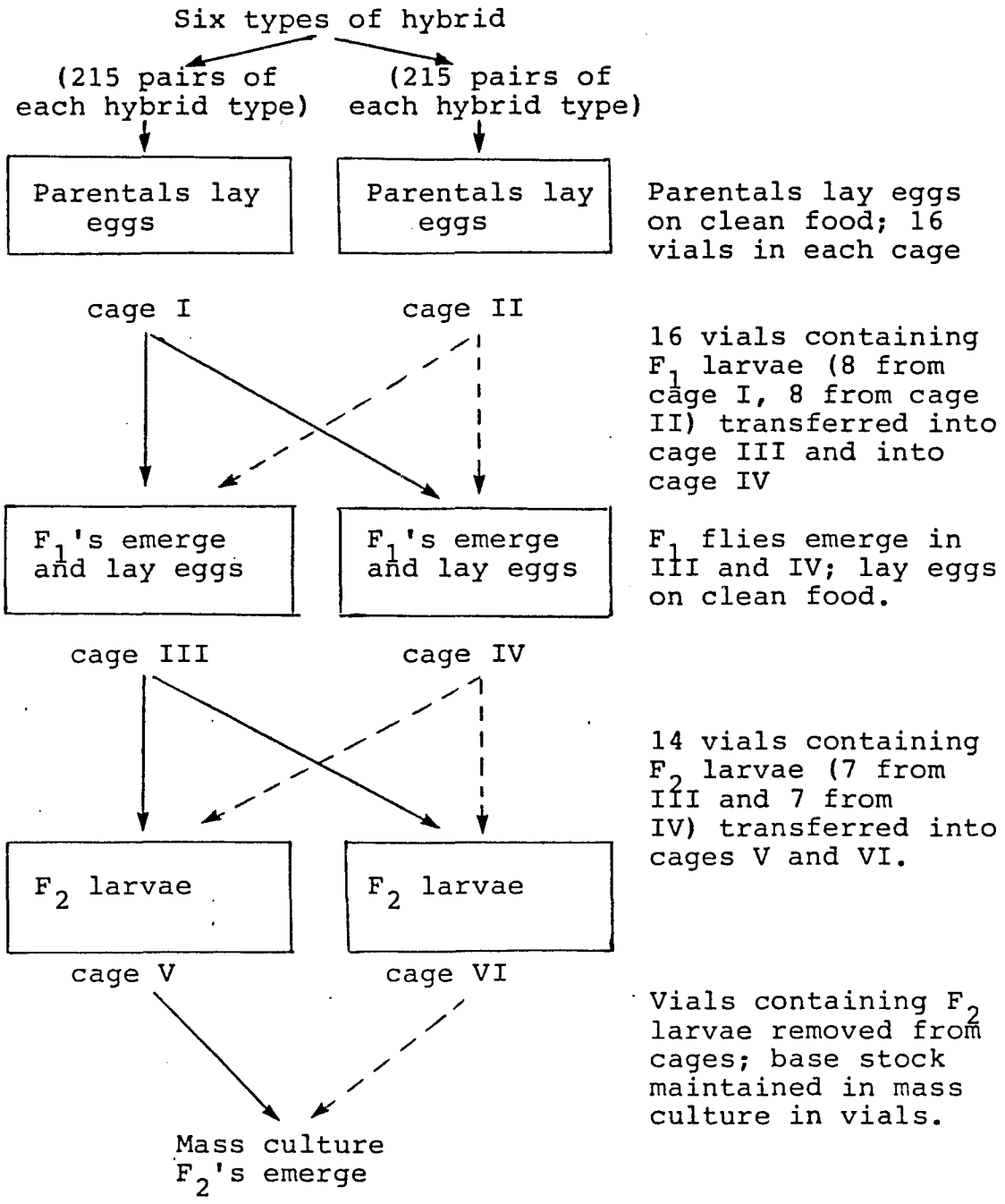

Figure 3 System of cage maintenance used to set up base stock of peninsular mojavensis, (P).

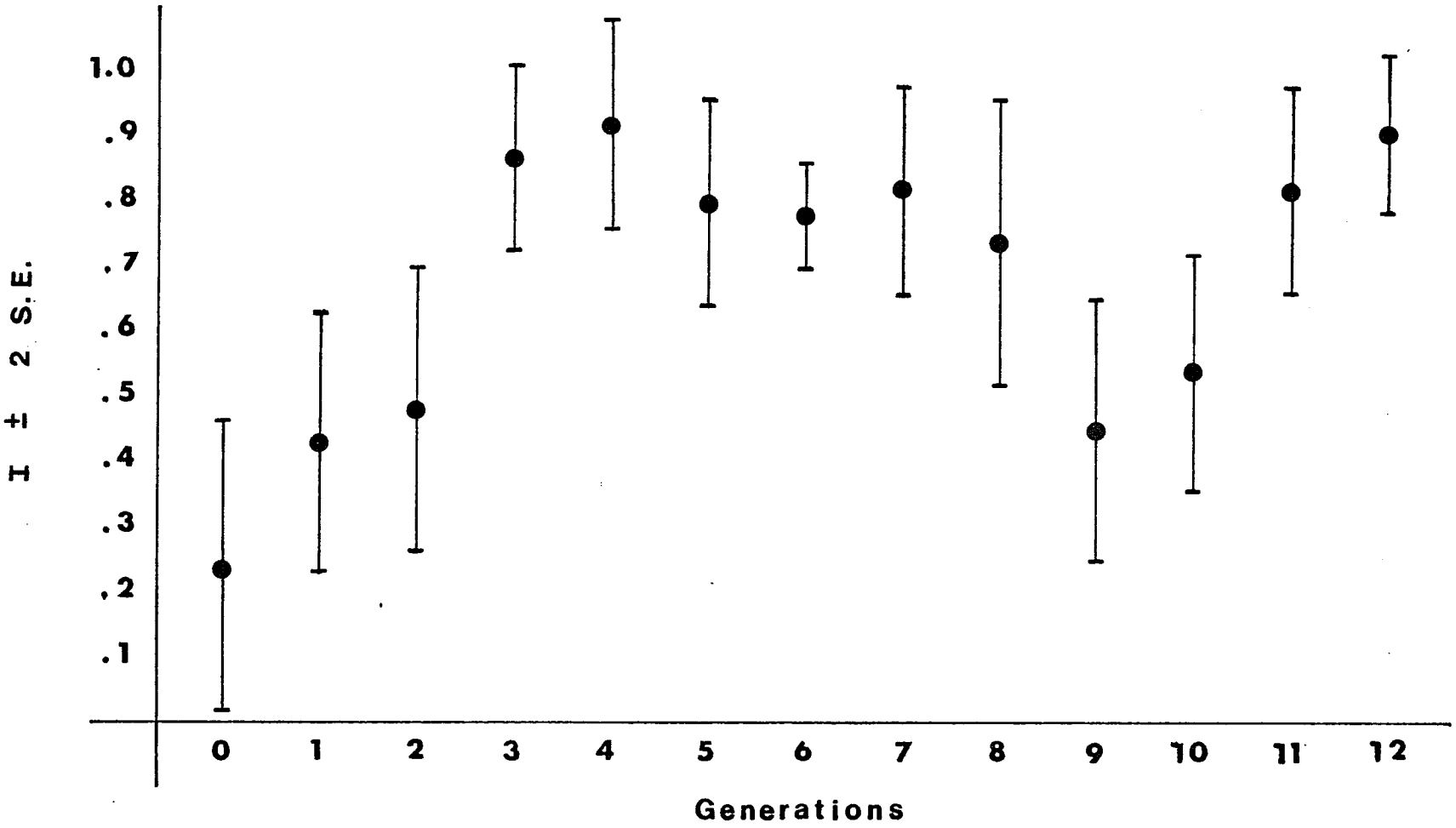

Figure 4 Response to selection for increased sexual isolation between peninsular males and Sonoran females.

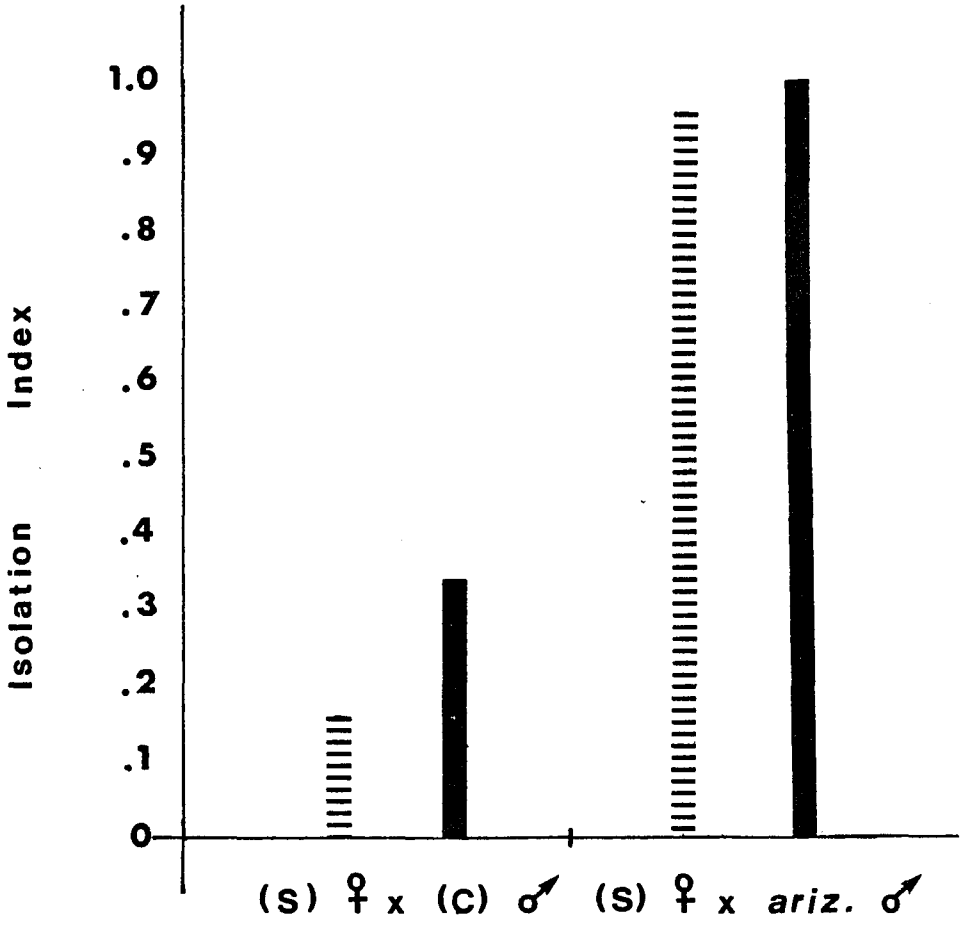

Figure 5 Responses of control and selected Sonoran females to foreign males.

control ♀♀

selected ♀♀

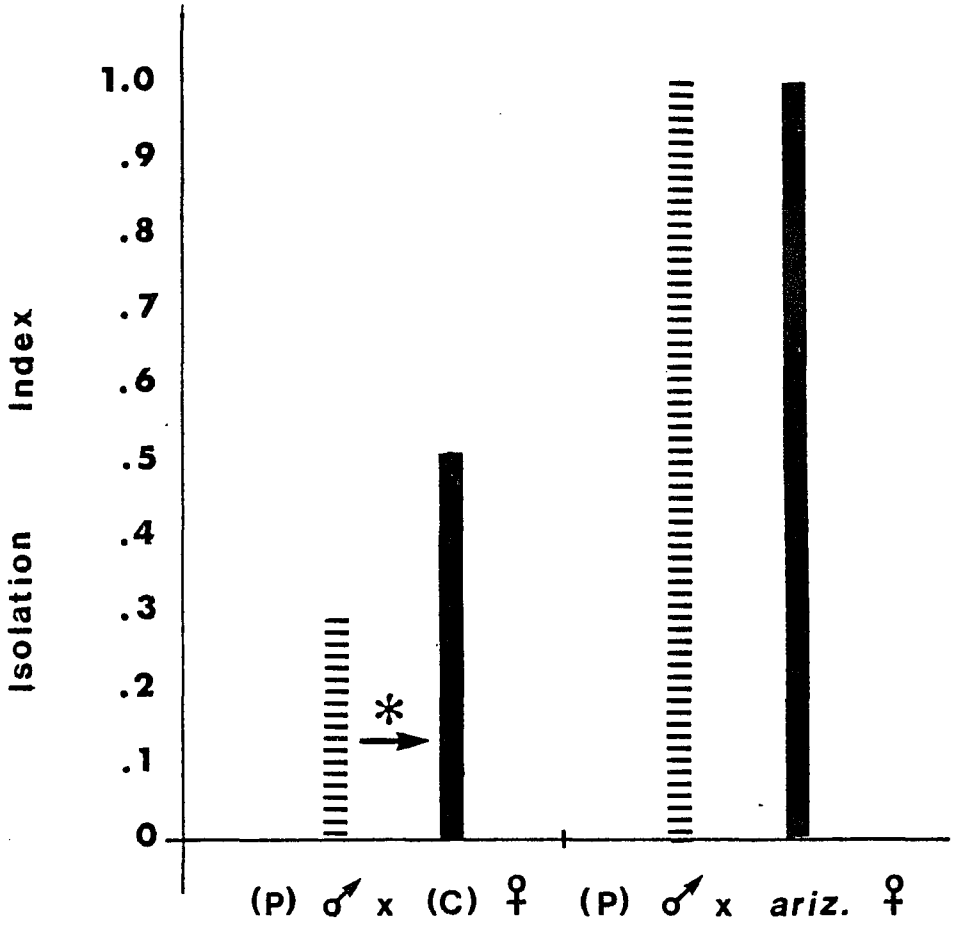

Figure 6 Responses of control and selected peninsular males to foreign females.

control ♂♂

selected ♂♂

* significant difference in isolation; $p \leq .05$

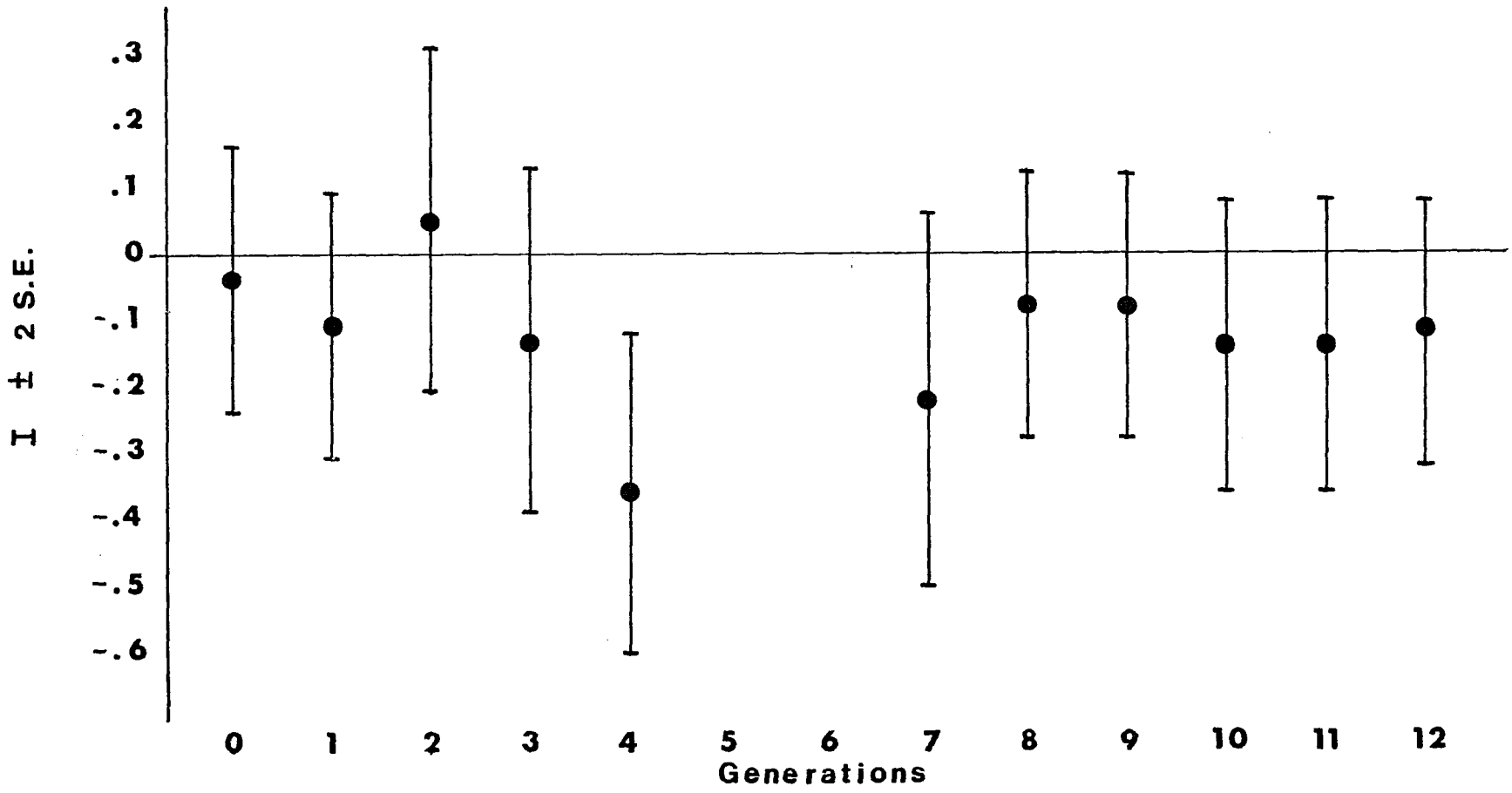

Figure 7 Isolation indices for selected Sonoran males and peninsular females.

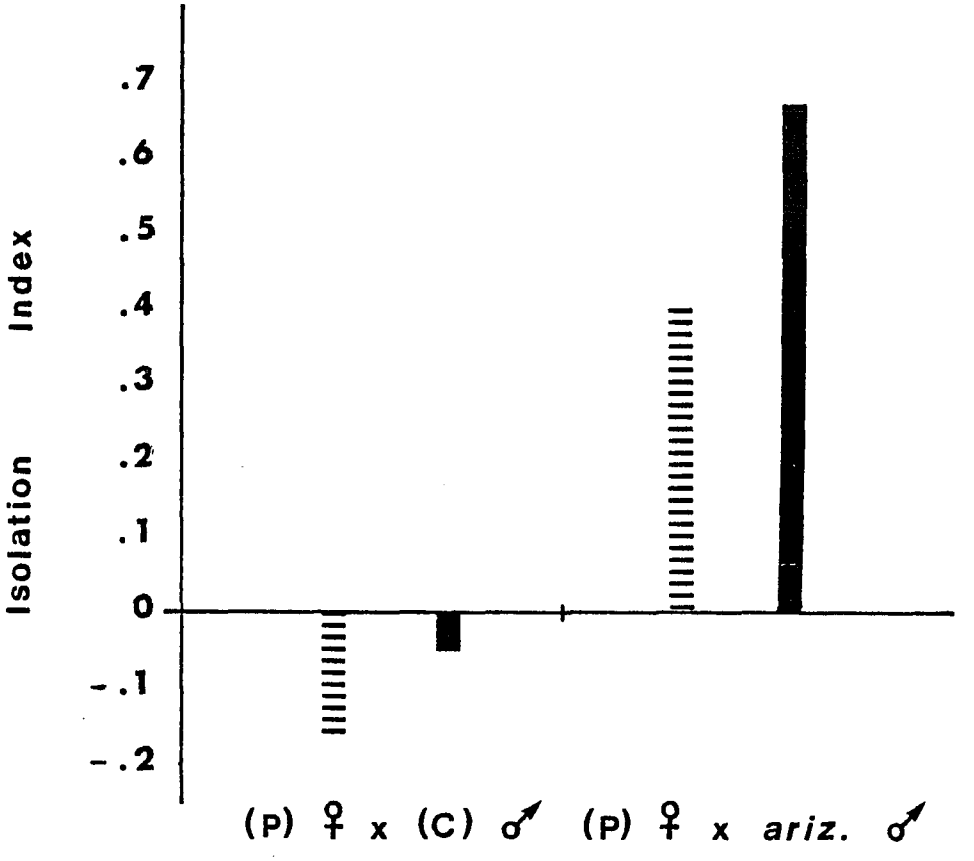

Figure 8 Responses of control and selected peninsular females to foreign males.

control ♀♀

selected ♀♀

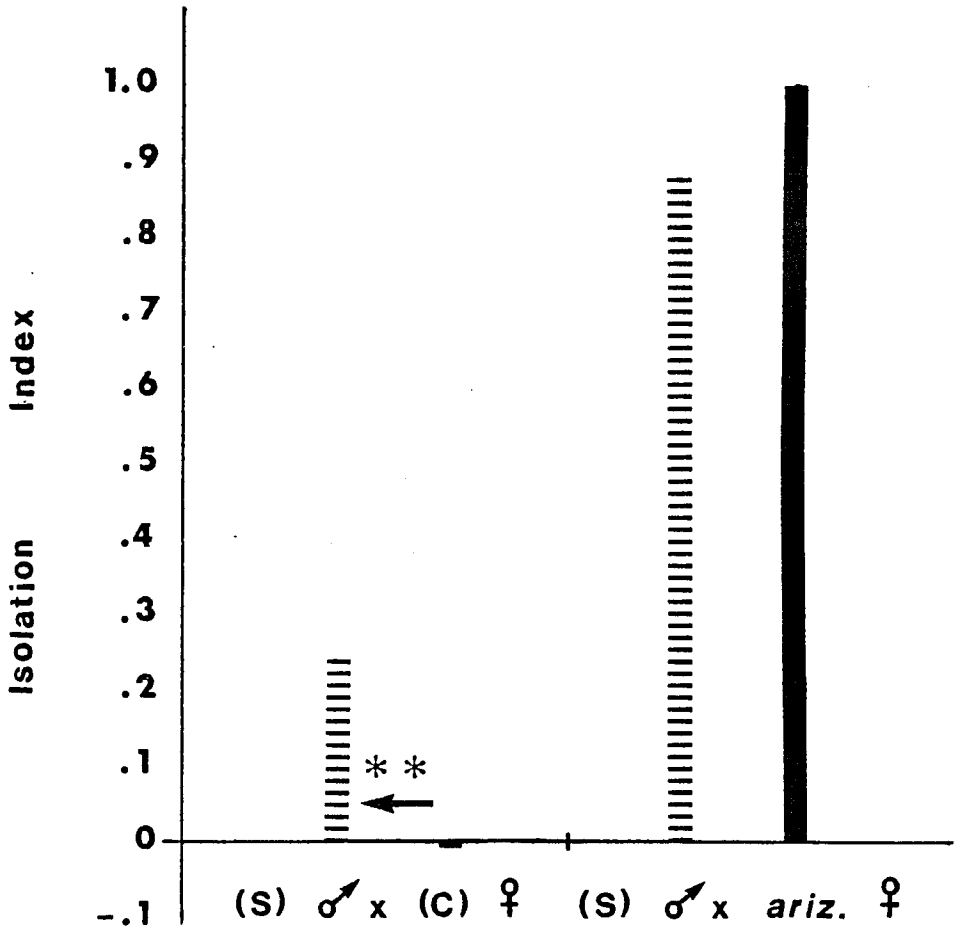

Figure 9 Responses of control and selected Sonoran males to foreign females.

control ♀♀

selected ♀♀

** significant difference in isolation; $p \leq .01$

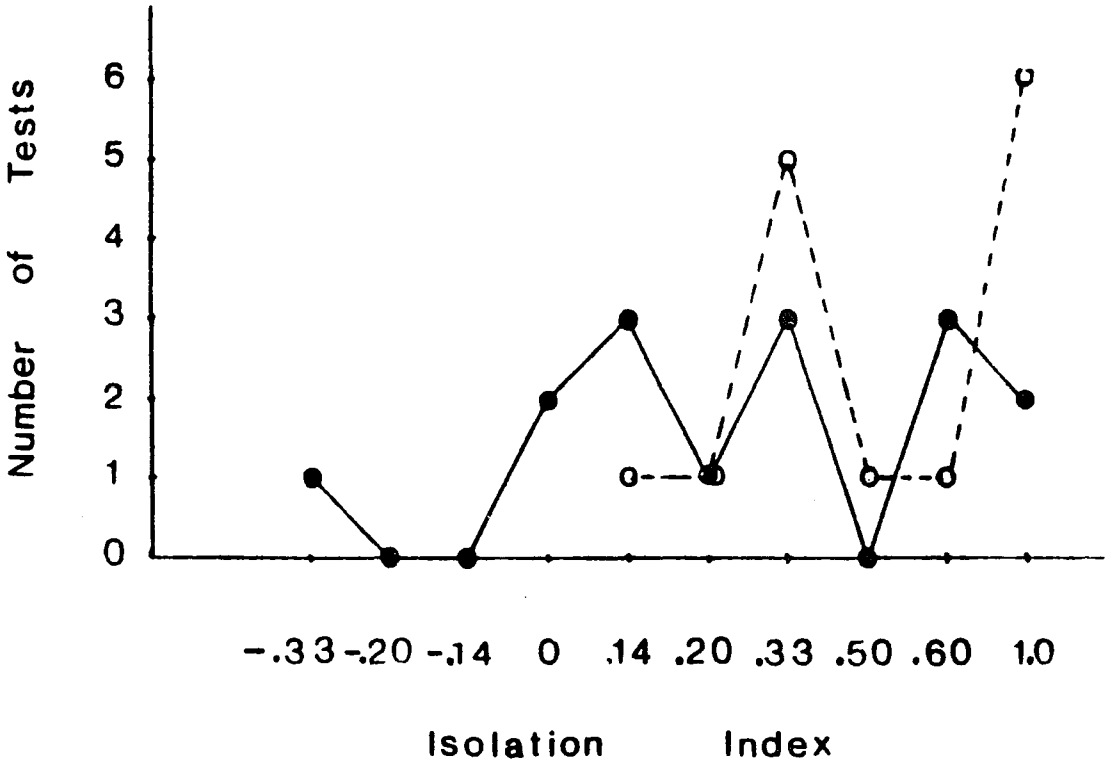

Figure 10 Frequency distribution of I

(P) ♂ x (P) ♀♀ + (C) ♀♀

●—● controls

o—o selected

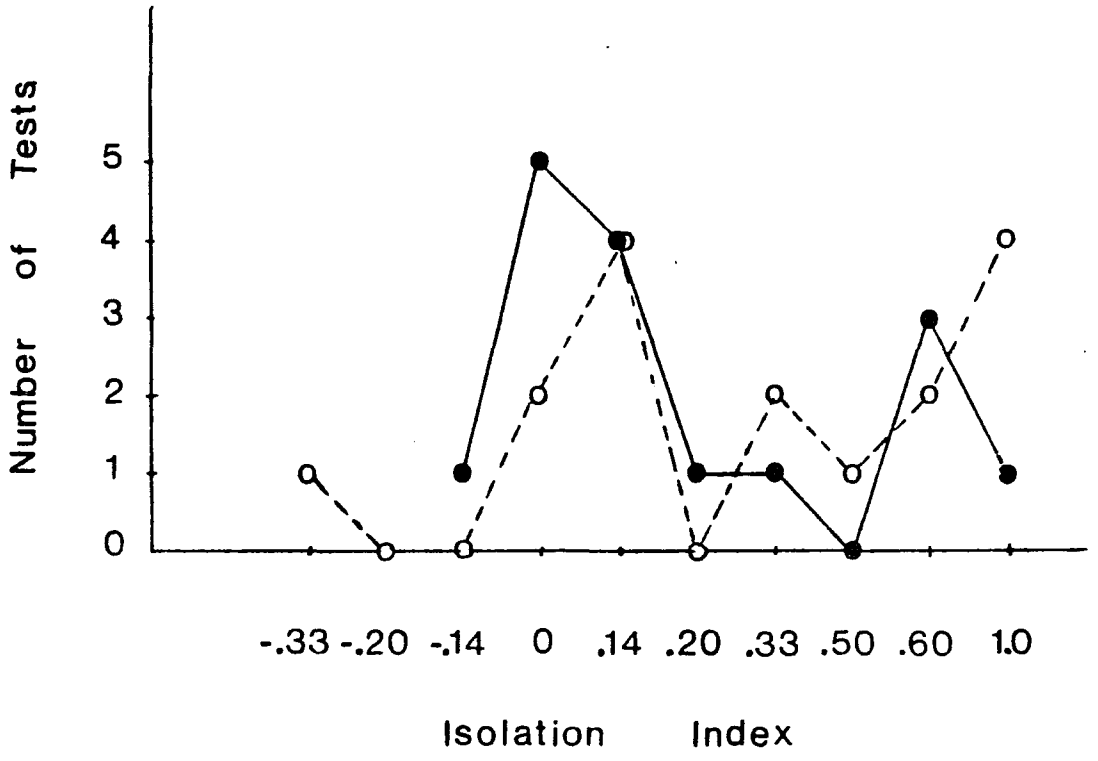

Figure 11 Frequency distribution of I

(C) ♂ x (C) ♀♀ + (S) ♀♀

●—● controls

○—○ selected

APPENDIX

Appendix

Chi Square Method of Brandt and Snedecor

This method utilizes an R x C contingency table to analyse data from a number of samples. It enables us to gauge the amount of heterogeneity within the set of samples, and then to determine the extent to which various subsets contribute to that heterogeneity. The procedure involves calculation of an overall chi square value for the entire set, followed by a subdivision of that overall value into additive components, each representative of a particular subset of the data. The general method is given in Maxwell (1961; pp. 52 - 62).

Illustrative example. Data from preliminary, selective and final tests for (P) male with (P) + (S) females are given in Table 11. The analysis for generations 0 to 5 will be shown below.

Step 1: Calculate Overall X^2 Value

Sample (Generation)	X_j Homogamic	$(n_j - x_j)$ Heterogamic	n_j Total	p_j (x_j/n_j)
0	51	31	82	.6219512
1	55	22	77	.7142857
2	49	17	66	.7424242
3	43	3	46	.9347826
4	25	1	26	.9615385
5	55	6	61	.9016393
$N = 6$	$T_x = 278$	$T - T_x = 80$	$T = 358$	

$\hat{p} = T_x/T = .7765363$; $\hat{q} = 1 - \hat{p} = .2234637$

$\hat{p}\hat{q} = .1735277$ $df = N - 1 = 5$

$$\text{Overall } X^2 = \frac{x_j p_j - \hat{p} T_x}{\hat{p}\hat{q}} = 30.722455^{***} \qquad p < .001$$

Since the overall X^2 value is highly significant, we conclude that there is heterogeneity among the samples from generations 0 to 5. The overall value is now partitioned into three components as shown below.

Step 2: Partitioning of Overall X^2

The overall value of X^2 based on five degrees of freedom is now partitioned into independent additive components as follows:

Components of X^2 due to	Degrees of freedom
(i) difference between generations 0,1,2 and 3,4,5	1
(ii) variation among generations 0,1,2	2
(iii) variation among generations 3,4,5	2
	5

(i) Component of overall X^2 due to difference between subsets composed of generations 0,1,2 and 3,4,5.

Sample (Generation)	X_j Homogamic	$(n_j - x_j)$ Heterogamic	n_j Total	p_j (x_j/n_j)
0,1,2	155	70	225	.6888889
3,4,5	123	10	133	.9248120
N = 2	$T_x = 278$	$T - T_x = 80$	$T = 358$	$\hat{p} = .7765363$
df = 1				

$$X^2 \text{ (0,1,2 vs. 3,4,5)} = \frac{x_j p_j - \hat{p} T_x}{.1735277} = 26.811642^{***}$$

This highly significant X^2 value indicates that these two subsets are different from each other in proportions of homogamic matings.

(ii) Component of overall X^2 due differences among generations 0, 1 and 2.

Sample (Generation)	X_j Homogamic	$(n_j - x_j)$ Heterogamic	n_j Total	p_j (x_j/n_j)
0	51	31	82	.6219512
1	55	22	77	.7142857
2	49	17	66	.7424242
N = 3	$T_x = 155$	$T - T_x = 70$	$T = 225$	$\hat{p} = .6888889$
df = 2				

$$X^2 \text{ (0 vs. 1 vs. 2)} = \frac{x_j p_j - \hat{p} T_x}{.1735277} = 3.493601 \quad .20 > p > .10$$

In view of this X^2 value, we conclude that the proportions of homogamic matings in samples from generations 0, 1 and 2 do not differ significantly.

(iii) Component of overall X^2 due to differences among generations 3, 4 and 5.

Sample (Generation)	X_j Homogamic	$(n_j - x_j)$ Heterogamic	n_j Total	p_j (x_j/n_j)
3	43	3	46	.9347826
4	25	1	26	.9615385
5	55	6	61	.9016393
$N = 3$ $df = 2$	$T_x = 123$	$T - T_x = 10$	$T = 133$	$\hat{p} = .9248120$

$$X^2 \text{ (3 vs.4 vs.5)} = \frac{x_j p_j - \hat{p} T_x}{.1735277} = .417212 \quad .90 > p > .80$$

This X^2 value is also non-significant. We conclude that the proportions of homogamic matings in the samples from generations 3, 4 and 5 do not differ significantly.

Step 3: Check the Calculations; Summarize Conclusions

Component of overall X^2 due to:	X^2	df	signif.
(i) differences between 0,1,2 and 3,4,5	26.811642	1	$p < .001$
(ii) differences within 0,1,2	3.493601	2	$p > .10$
(iii) differences within 3,4,5	.417212	2	$p > .80$
	30.722455	5	
calculated overall X^2	30.722455	5	$p < .001$

Conclusions: There is significant heterogeneity among the samples from generations 0 through 5. This heterogeneity is due to a significant

increase in isolation between (P) males and (S) females as manifested by a sharp decrease in the number of heterogamic matings. This decrease occurred after three generations of stringent selection.

The Kruskal-Wallis Test

The Kruskal-Wallis test is a nonparametric procedure which is used to determine whether two or more independent samples are from different populations. It tests the null hypothesis that the samples come from the same continuous population, or from identical populations with respect to location.

In the computation, all observations from the samples under consideration are ranked in a single series from smallest to largest, with ties being given average ranks. After this, the sum of the ranks in each sample is found. If the samples do not differ from each other, then rank sums will be approximately the same. The test determines whether the sums of ranks are so disparate that they are not likely to have come from the same population, or from identical populations.

The Kruskal-Wallis statistic, H, is defined below. If the null hypothesis is true, H is distributed approximately as chi square, with degrees of freedom equalling one less than the sample size.

$$H = \left[\frac{12}{N(N+1)} \sum_{i=1}^{a} \frac{(R_i)^2}{n_i} \right] - 3(N+1)$$

where a = number of samples
n_i = number of observations in i^{th} sample
N^i = sum of all observations
R_i = sum of ranks for the i^{th} sample
12 and 3 are constants

When ties are present, H is divided by a correction factor D, which is given below.

$$D = 1 - \frac{\sum u^3 - \sum u}{N(N^2 - 1)}$$

where u = number of ties per rank

Illustrative example. The Kruskal-Wallis test indicates that selected (P) males are more isolated from (C) females than are control (P) males (Table 15; Figure 6). The analysis of final tests involving selected and control (P) males with (C) as the heterogamic female will be shown below.

Of the sixteen tested control (P) males, fifteen participated in at least one mating; fifteen selected (P) males also mated at least once. Therefore, for this analysis we have two samples, control and selected (a = 2), and thirty observations to be ranked (N = 30).

To begin, an isolation index was calculated for each of the thirty tests. For example, four homogamic matings and three heterogamic matings gives an index of .14; four homogamic and two heterogamic gives I = .33, etc. These thirty indices were ranked, and then the sums of the ranks for control males and for selected males were found as follows:

Control (P) Males				Selected (P) Males			
I	(Rank	x	Number of Tests)	I	(Rank	x	Number of Tests)
- .33	1.0	x	1	- .33	1	x	0
0.00	2.5	x	2	0.00	2.5	x	0
.14	5.5	x	3	.14	5.5	x	1
.20	8.5	x	1	.20	8.5	x	1
.33	13.5	x	3	.33	13.5	x	5
.50	18.0	x	0	.50	18.0	x	1
.60	20.5	x	3	.60	20.5	x	1
1.00	26.5	x	2	1.00	26.5	x	6
	186.0		15		279.0		15

Sum of ranks for control males = 186.0
Sum of ranks for selected males = 279.0
df = 2 - 1 = 1

$$H = \left[\frac{12}{(30)(31)}\left[\frac{(186)^2}{15} + \frac{(279)^2}{15}\right]\right] - 3(31)$$

$$H = 3.72$$

H must now be corrected for ties:

I value	Ties (u)	u^3
- .33	0	0
0.00	2	8
.14	4	64
.20	2	8
.33	8	512
.50	0	0
.60	4	64
1.00	8	512
	28	1168

$$D = 1 - \frac{(1168 - 28)}{(30)(30^2 - 1)}$$

$$D = .9577308$$

$$H/D = 3.8841812^{*} \qquad .05 > p > .01$$

$$X^2_{(\alpha = .05)(1)} = 3.841$$

$$X^2_{(\alpha = .01)(1)} = 6.635$$

If the null hypothesis were true (i.e., if control and selected (P) males do not differ in isolation from (C) females), H would be distributed approximately as chi square with one degree of freedom. Since the calculated H is greater than 3.841, we may reject the null hypothesis and conclude that control and selected males differ in isolation from (C) females.

Since the Kruskal-Wallis procedure tests for differences in location, it is of interest to examine the mean and median I values for these two groups. The overall I value for the controls was .287 ± .103, while that for the selected males was .509 ± .114. For their fifteen individual I values, the controls had a median of .33, and a mean of .339. Selected males had a median I of .50, and a mean I of .606. Thus selection has effected a decided increase in the values of these location parameters. This can be seen clearly in Figure 10, which is a graph of the frequency distributions shown above.

For contrast, we can examine responses of control

and selected (S) females to (C) males. While the overall I values seem to be quite different, I for controls being .165 ± .097 and that for selected females equalling .341 ± .102, the Kruskal-Wallis statistic is not significant (H = 1.80; p = .1796). The frequency distributions given below and shown graphically in Figure 11 reveal the reason for this.

I	Number of Tests Control Females	Number of Tests Selected Females
- .33	0	1
- .20	0	0
- .14	1	0
0.00	5	2
.14	4	4
.20	1	0
.33	1	2
.50	0	1
.60	3	2
1.00	1	4

The median I value for the controls is .14, with mean equal to .234. The median I value for selected females is .33, with mean equal to .411. The distributions are largely overlapping, and neither mean nor median has changed drastically due to selection.

Multiple Comparison Procedure of Dunn

If a small probability value from the Kruskal-Wallis test indicates that three or more populations are not the same, we may then use an a posteriori multiple comparison test to determine which pairs of populations differ significantly from each other. The procedure used here is attributed to Dunn (1964) and explained in Gibbons (1976; pp. 181 -192).

In doing multiple comparisons, we obtain a joint probability statement concerning a set of comparisons, some of which may not be orthogonal. That is, we make simultaneous comparisons between some or all of the possible pairs of samples, and obtain an overall significance level α. This means that the probability that of all the statements being correct is $1 - \alpha$.

The method involves the following steps:

Observations are ranked as in the Kruskal-Wallis procedure, and the mean of the ranks of each sample is computed. The means are compared pairwise according to the following formula, which includes a correction for ties.

$$\left|R_i - R_j\right| \leq z\sqrt{\frac{[N(N^2 - 1) - (\Sigma u^3 - \Sigma u)][1/n_i + 1/n_j]}{12(N - 1)}}$$

where n_i = number of observations in the i^{th} sample

$R_i = R_i/n_i$ = mean of the ranks of the i^{th} sample

n_j = number of observations in the j^{th} sample

$R_j = R_j/n_j$

N = total number of observations in all samples

u = number of observations in all samples combined that are tied at any given rank

k = number of samples

$k(k - 1)/2$ = number of possible comparisons

z = critical z value. This is the quantile point of the standard normal distribution that corresponds to a right-tail probability of $\alpha/k(k - 1)$, or $\alpha/2p$ where p is the number of comparisons (see Table N, p. 232 in Gibbons)

After choosing the desired α level and appropriate z value, right and left sides of the expression are compared. The probability is at least $1 - \alpha$ that the above inequality holds for all pairs of means when the null hypothesis is true. All differences of means that are larger than the right hand side of the equation are considered significant at level .

Illustrative example. The Kruskal-Wallis test was used to evaluate the results of base stock (preliminary test), control and selection line responses for the male choice tests involving (P) males with (S) as the

heterogamic female. It indicated that the three are not the identical with respect to isolation ($p \leq .001$). The Dunn procedure was then applied in order to determine where the significant differences lay. This multiple comparison will be shown below.

Step 1: Compute Mean Ranks of Each Sample; Choose Significance Level

R_p = mean of ranks, preliminary test
$= 11.500000$
R_c = mean of ranks, controls
$= 22.266667$
R_s = mean of ranks, selected flies
$= 33.807692$

n_p = number of observations, preliminary test
$= 15$
n_c = number of observations, controls
$= 15$
n_s = number of observations, selected flies
$= 13$
$N = 43$

R_p = sum of ranks, preliminary test
$= 172.50$
R_c = sum of ranks, controls
$= 334.00$
R_s = sum of ranks, selected flies
$= 439.50$

$= .05$

$z = 2.394$

Step 2: Make the Comparisons

$$2.394\sqrt{\frac{[43(43^2 - 1) - (3434)][1/15 + 1/15]}{12(43 - 1)}} = 10.736698$$

$$\left|R_c - R_p\right| = 10.766667$$

$10.766667 > 10.736698$

$$2.394\sqrt{\frac{[43(43^2 - 1) - (3434)][1/15 + 1/13]}{12(43 - 1)}} = 11.141998$$

$$\left|R_s - R_p\right| = 22.307692$$

$22.307692 > 11.141998$

$$2.394\sqrt{\frac{[43(43^2 - 1) - (3434)][1/15 + 1/13]}{12(43 - 1)}} = 11.141998$$

$$\left|R_s - R_c\right| = 11.541025$$

$11.541025 > 11.141998$

Step 3: Summarize Test Results; Draw Conclusions

Means being Compared	Difference between Means	Comparison Figure
$R_c - R_p$	10.766667	10.736698
$R_s - R_p$	22.307692	11.141998
$R_s - R_c$	11.541025	11.141998

Since in each case the absolute value of the difference between the means is larger than the comparison figure, we conclude that base stocks, controls and selected flies exhibit significantly different degrees of isolation in these tests ($p < .05$). Thus, for (P) male with (P) + (S) females, the order of isolation is:

base stock (I = .244) < controls (I = .514) < selected (I = .909)

REFERENCES

References

Anderson, W. W. and L. Ehrman. 1967. Mating choice in crosses between geographic populations of Drosophila pseudoobscura. Am. Midland Nat. 81:47-53.

Baker, W. K. 1947. A study of the isolating mechanisms found in Drosophila arizonensis and Drosophila mojavensis. Univ. Texas. Publ. 4720:126-136.

Barker, J. S. F. and L. J. Cummins. 1969a. Disruptive selection for sternopleural bristle number in Drosophila melanogaster. Genetics 61:697-712.

Barker, J. S. F. and L. J. Cummins. 1969b. The effect of selection of sternopleural bristle number on mating behavior in Drosophila melanogaster. Genetics 61:713-719.

Bateman, A. J. 1949. Analysis of data on sexual isolation. Evolution 3:174-177.

Bennett, J. 1960. A comparison of selective methods and a test of the pre-adaptation hypothesis. Heredity 15:65-77.

Blair, W. F. 1974. Character displacement in frogs. Am. Zool. 14:1119-1125.

Carson, H. L., L. T. Teramoto and A. R. Templeton. 1977. Behavioral differences among isogenic strains of Drosophila mercatorum. Behav. Genet. 7:189-197.

Chabora, A. J. 1968. Disruptive selection for sternopleural chaeta number in various strains of Drosophila melanogaster. Am. Nat. 102:525-532.

Cohn, T. J. and I. J. Cantrall. 1974. Variation and speciation in the grasshoppers of the Conalcaeini (Orthoptera: Acrididae: Melanoplinae): the lowland forms of western Mexico, the genus Baytettix. San Diego Soc. Nat. Hist. Mem. 6:131 pp.

Craddock, E. M. 1974. Reproductive relationships between homosequential species of Hawaiian Drosophila. Evolution 28:593-606.

Crossley, S. A. 1974. Changes in mating behavior produced by selection for ethological isolation between ebony and vestigial mutants of Drosophila melanogaster. Evolution 28:631-647.

Crow, J. F. 1941. Studies in Drosophla speciation: I. The mulleri group. Genetics 26:146.

Dobzhansky, T. 1940. Speciation as a stage in genetic divergence. Am. Nat. 74:312-321.

Dobzhansky, T. 1970. Genetics of the Evolutionary Process. New York: Columbia University Press.

Dobzhansky, T., O. Pavlovsky and J. R. Powell. 1976. Partially successful attempt to enhance reproductive isolation between semispecies of Drosophila paulistorum. Evolution 30:201-212.

Dobzhansky, T. and B. Spassky. 1962. Genetic drift and natural selection in experimental populations of Drosophila pseudoobscura. PNAS 48:148-156.

Dunn, O. J. 1964. Multiple contrasts using rank sums. Technometrics 6:241-252.

Ehrman, L. 1961. The genetics of sexual isolation in Drosophila paulistorum. Genetics 46:1025-1038.

Ehrman, L. 1965. Direct observation of sexual isolation between allopatric and between sympatric strains of the different Drosophila paulistorum races. Evolution 19:459-464.

Ehrman, L. 1971. Natural selection for the origin of reproductive isolation. Am. Nat. 105:479-483.

Ehrman, L. 1973. More on natural selection for the origin of reproductive isolation. Am. Nat. 107:318-319.

Ehrman, L. 1979. Still more on natural selection for the origin of reproductive isolation. Am.Nat. 113:148-150.

Ehrman, L. 1983. Fourth report on natural selection for the origin of reproductive isolation. Am. Nat. 121: 290-293.

Eoff, M. 1975. Artificial selection in Drosophila melanogaster females for increased and decreased sexual isolation from D. simulans males. Am. Nat. 109:225-229.

Eoff, M. 1977. Artificial selection in Drosophila simulans males for increased and decreased sexual isolation from D. melanogaster females. Am. Nat. 111:259-266.

Ewing, A. W. 1969. The genetic basis of sound production in Drosophila pseudoobscura and D. persimilis. Anim. Behav. 17:555-560.

Ewing, A. W. and H. C. Bennet-Clark. 1968. The courtship songs of Drosophila. Behaviour 31:288-301.

Fellows, D. and W. B. Heed. 1972. Factors affecting host plant selection in desert-adapted cactiphilic Drosophila. Ecology 53:850-858.

Felsenstein, J. 1981. Skepticism towards Santa Rosalia, or why are there so few kinds of animals? Evolution 35:124-138.

Fouquette, M. J. Jr. 1975. Speciation in Chorus Frogs. I. Reproductive character displacement in the Pseudacris nigrita complex. Syst. Zool. 24:16-22.

Gibbons, J. D. 1976. Nonparametric Methods for Quantitative Analysis. Ohio: American Science Press, Inc.

Gibson, J. B. and J. M. Thoday. 1963. Effects of disruptive selection. VIII. Imposed quasi-random mating. Heredity 18:513-524.

Grant, B. S. 1966. Fitness component analysis of the interspecific hybrids of Drosophila arizonensis and Drosophila mojavensis. M. S. Thesis. North Carolina State University. Raleigh, North Carolina.

Grossfield, J. 1966. The influence of light on mating behavior in Drosophila. Univ. Texas Publ. 6615:147-176.

Grossfield, J. 1975. Genetic analysis of light dependent behavior in Drosophila. Genetics 80:37-38.

Heed, W. B. 1978. Ecology and genetics of desert Drosophila. In Ecological Genetics: The Interface. P. F. Brussard (ed.). New York:Springer-Verlag.

Heed, W. B. 1982. The origin of Drosophila in the Sonoran Desert. In Ecological Genetics and Evolution - The Cactus-Yeast-Drosophila Model System. J. S. F. Barker and W. T. Starmer (eds.). New York: Academic Press.

Hurd, L. E. and R. M. Eisenberg. 1975. Divergent selection for geotactic response and evolution of reproductive isolation in sympatric and allopatric populations of houseflies. Am. Nat. 109:353-358.

Jallon, J. and Y. Hotta. 1979. Genetic and behavioral studies of female sex appeal in Drosophila . Behav. Genet. 9:257-275.

Johnson, W. R. Jr. 1980. Chromosomal polymorphism in the desert-adapted species, Drosophila mojavensis. Ph.D. Dissertation. Univ. of Arizona.

Kaneshiro, K. 1980. Sexual isolation, speciation, and the direction of evolution. Evolution 34:437-444.

Kessler, S. 1966. Selection for and against ethological isolation between Drosophila pseudoobscura and Drosophila persimilis. Evolution 20:634-645.

Kilias, G. and S. N. Alahiotis. 1982. Genetic studies on sexual isolation and hybrid sterility in long-term cage populations of Drosophila melanogaster. Evolution 36:121-131.

Kilias, G., S. N. Alahiotis and M. Pelecanos. 1980. A multifactorial genetic investigation of speciation theory using Drosophila melanogaster. Evolution 34:730-737.

Kircher, H. W. 1982. Chemical composition of cacti and its relationship to Sonoran Desert Drosophila. In Ecological Genetics and Evolution. The Cactus-Yeast-Drosophila Model System. J. S. F. Barker and W. T. Starmer (eds.). New York: Academic Press.

Knight, G. R., A. Robertson and C. H. Waddington. 1956. Selection for sexual isolation within a species. Evolution 10:14-22.

Koepfer, H. R. 1970. Patterns of mating behavior in five geographic strains of Drosophila mercatorum. M. A. Thesis. Queens College of City University of New York.

Koopman, K. F. 1950. Natural selection for reproductive isolation between Drosophila pseudoobscura and Drosophila persimilis. Evolution 4:135-148.

Levene, H. 1949. A new measure of sexual isolation. Evolution. 3:315-321.

Littlejohn, M. J. 1965. Premating isolation in the Hyla ewingi complex (Anura: Hylidae). Evolution 19:234-243.

Littlejohn, M. J. and J. J. Loftus-Hills. 1968. An experimental evaluation of premating isolation in the Hyla ewingi complex. Evolution 22:659-663.

Malagolowkin-Cohen, C., A. S. Simmons and H. Levene. 1965. A study of sexual isolation between certain strains of Drosophila paulistorum. Evolution 19:95-103.

Manning, A. 1965. Drosophila and the evolution of behavior. In Viewpoints in Biology 4. J. D. Carthy and C. L. Duddington (eds.). London: Butterworths, pp. 125-129.

Markow, T. A. 1981. Courtship behavior and control of reproductive isolation between Drosophila mojavensis and Drosophila arizonensis. Evolution 35:1022-1026.

Markow, T. A. 1982. Mating systems in cactophilic Drosophila. In Ecological Genetica and Evolution: The Cactus-Yeast-Drosophila Model System. J. S. F. Barker and W. T. Starmer (eds.). New York:Academic Press.

Markow, T. A., J. C. Fogelman and W. B. Heed. 1983. Reproductive isolation in Sonoran Desert Drosophila. Evolution 37:649-652.

Markow, T. A. and R. C. Richmond. 1981. Drosophila males preferentially court virgin females. 11th Ann. Mtg., Behav. Genet. Assoc. (Abstract).

Maxwell, A. E. 1961. Analysing Qualitative Data. New York. Halsted Press, A division of John Wiley and Sons, Inc.

Mayr, E. 1966. Animal Species and Evolution. Cambridge, Mass.: Harvard University Press.

Merrell, D. J. 1950. Measurements of sexual isolation and selective mating. Evolution 4:326-331.

Merrell, D. J. 1960. Mating preferences in Drosophila. Evolution 14:525-526.

Mettler, L. E. 1957. Studies on experimental populations of Drosophila arizonensis and Drosophila mojavensis. Univ. Texas Publ. 5721:157-181.

Mettler, L. E. 1962. Fertility relationships of recombination-hybrid males from the cross of D. mojavensis and D. arizonensis. Dros. Inf. Ser. 36:89.

Mettler, L. E. 1963. D. mojavensis baja, a new form in the mulleri complex. Dros. Inf. Ser. 38:57-58.

Mettler, L. E. and J. J. Nagle. 1966. Corroboratory evidence for the concept of the sympatric origin of isolating mechanisms. Dros. Inf. Ser. 41:76.

Moodie, G. E. E. 1982. Why asymmetric mating preferences may not show the direction of evolution. Evolution 36:1096-1097.

Muller, H. J. 1939. Reversibility in evolution considered from the standpoint of genetics. Biol. Rev. 14:261-280.

Muller, H. J. 1942. Isolating mechanisms, evolution and temperature. Biol. Symp. 6:71-125.

Nagle, J. J. 1965. Studies on experimental sympatry between two sibling species. Dros. Inf. Ser. 40:82-83.

Nagle, J. J. 1969. Increase in genetic variability through interspecific hybridization between Drosophila mojavensis and Drosophila arizonenesis. Bull. New Jersey Acad. Sci. 14:23-28.

Nagle, J. J. and L. E. Mettler. 1969. Relative fitness of introgressed and parental populations of Drosophila mojavensis and D. arizonensis. Evolution 23:519-524.

Paterson, H. E. H. 1978. More evidence against speciation by reinforcement. S. Afr. J. Sci. 74. 369-371.

Paterson, H. E. H. 1980. A comment on "mate recognition systems. Evolution 34:330-331.

Paterson, H. E. H. 1981. The continuing search for the unknown and unknowable: a critique of contemporary ideas on speciation. S. Afr. J. Sci. 77:113-119.

Paterson, H. E. H. 1982. Perspective on speciation by reinforcement. S. Afr. J. Sci. 78:53-57.

Patterson, J. J. 1946. A new type of isolating mechanism in Drosophila. PNAS 32:202-208.

Powell, J. R. 1978. The founder-flush theory: An experimental approach. Evolution 32:465-474.

Robertson, F. W. 1966. A test of isolation in Drosophila. Genet. Res. 8:181-187.

Robertson, H. M., H. E. H Paterson. 1982. Mate recognition and mechanical isolation in Enallagma damselflies (Odonata:Coenagrionidae). Evolution 36:243-250.

Schaffer, H. E. 1968. A measure of discrimination in mating. Evolution 22: 125-129.

Scharloo, W., M. den Boer and M. S. Hoogmoed. 1967. Disruptive selection on sternopleural chaeta number. Genet. Res. 9:115-118.

Shorey, H. and R. J. Bartell. 1970. Role of a volatile female sex pheromone in stimulating male courtship behavior in Drosophila melanogaster. Anim. Behav. 18:159-164.

Spiess, E. B. and E. M. Wilke. 1984. Still another attempt to achieve assortative mating by disruptive selection in Drosophila. Evolution 38:505-515.

Siegel, S. 1956. Nonparametric Statistics for the Behavioral Sciences. New York: McGraw Hill.

Soans, A. B., D. Pimentel and J. S. Soans. 1974. Evolution of reproductive isolation in allopatric and sympatric populations. Am. Nat. 117-124.

Stalker, H. D. 1942. Sexual isolation studies in the species complex Drosophila virilis. Genetics 27:238-257.

Sturtevant, A. H. and T. Dobzhansky. 1936. Inversions in the third chromosome of wild races of Drosophila pseudoobscura and their use in the study of the history of the species. PNAS 22:448-450.

Sved, J. A. 1981a. A two-sex polygenic model for the evolution of premating isolation. I. Deterministic theory for natural populations. Genetics 97:197-215.

Sved, J. A. 1981b. A two-sex polygenic model for the evolution of premating isolation. II. Computer simulation of experimental selection procedures. Genetics 97:217-235.

Tan, C. C. 1946. Genetics of sexual isolation between Drosophila pseudoobscura and Drosophila persimilis. Genetics 31:558-573.

Thoday, J. M. and J. B. Gibson. 1962. Isolation by disruptive selection. Nature 193:1164-1166.

Thoday, J. M. and J. B. Gibson. 1970. The probability of isolation by disruptive selection. Am. Nat. 104:219-230.

Waage, J. K. 1975. Reproductive isolation and the potential for character displacement in the damselflies, Calopteryx maculata and C. aequabilis (Odonata: Calopterygidae). Syst. Zool. 24: 24-36.

Waage, J. K. 1979. Reproductive character displacement in Calopteryx (Odonata: Calopterygidae). Evolution 33:104-114.

Waldron, I. 1964. Courtship sound production in two sympatric sibling Drosophila species. Science 144:191-193.

Wallace, B. 1954. Genetice divergence of isolated populations of Drosophila melanogaster. Proc. 9th Int. Cong. Genet. 1:761-764.

Wallace, B. and N. Felthousen. 1965. Mating interference: A new test of sexual isolation. Evolution 19:552-555.

Wasserman, M. 1954. Cytological studies on the repleta group. Univ. Tex. Publ. 5422:130-152.

Wasserman, M. 1960. Cytological and phylogenetic relationships in the repleta group of the genus Drosophila. PNAS 46:842-859.

Wasserman, M. 1962. Cytological studies of the repleta group of the genus Drosophila. V. The mulleri subgroup. Univ. Tex. Publ. 6205:85-118.

Wasserman, M. 1963. Cytology and phylogeny of Drosophila. Am. Nat. 97:333-352.

Wasserman, M. 1982a. Evolution of the repleta group. In The Genetics and Biology of Drosophila. Vol. 3b. M. Ashburner, H. L. Carson and J. N. Thompson, Jr. (eds.). New York: Academic Press.

Wasserman, M. 1982b. Cytological evolution in the Drosophila repleta species group. In Ecological Genetics and Evolution - The Cactus-Yeast-Drosophila Model System. J. S. F. Barker and W. T. Starmer (eds.). New York: Academic Press.

Wasserman, M. and H. R. Koepfer. 1977. Character displacement for sexual isolation between Drosophila mojavensis and Drosophila arizonensis. Evolution 31:812-823.

Wasserman, M. and H. R. Koepfer. 1980. Does asymmetrical mating preference show the direction of evolution? Evolution 34:1116-1126.

Wood, D., J. M. Ringo and L. L. Johnson. 1980. Analysis of courtship sequences of the hybrids between Drosophila melanogaster and Drosophila simulans. Behav. Genet. 10:459-466.

Wright, S. 1955. Population genetics: the nature and causes of genetic variability in populatuions. Cold Spring Harbor Symp. Quant. Biol. 7:16-24.

Zouros, E. 1973. Genic differentiation associated with the early stages of speciation in the mulleri subgroup of Drosophila. Evolution 27:601-621.

Zouros, E. 1981a. An autosome - Y chromosome combination that causes sterility in D. mojavensis x D. arizonensis hybrids. Dros. Inf. Ser. 56:167-168.

Zouros, E. 1981b. The chromosomal basis of sexual isolation in two sibling species of Drosophila: D. arizonensis and D. mojavensis. Genetics 97: 703-718.

Zouros, E. 1981c. The chromosomal basis of viability in interspecific hybrids between Drosophila arizonensis and Drosophila mojavensis. Can. J. Genet. Cytol. 23:507-514.

Zouros, E. and C. J. D'Entremont. 1974. Sexual isolation among populations of D. mojavensis race B. Dros. Inf. Ser. 51: 112-113.

Zouros, E. and C. J. D'Entremont. 1980. Sexual isolation among populations of Drosophila mojavensis: Response to pressure from a related species. Evolution 43:421-430.

Zouros, E. and Vigneault. 1983. Speciation in Drosophila through hybrid sterility: the role of the Y chromosome. Genetics 104:s74.