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BLASTODERM FATE MAP OF THE HONEY BEE (APIS MELLIFERA L.)

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Charles Potter Milne, Jr., B.A., M.S.

* * * * *

The Ohio State University
1977

Reading Committee: Approved By
Walter C. Rothenbuhler Adviser
Charles A. Triplehorn
Robert P. Holdsworth, Jr.

Department of Entomology
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And whatever you do in word or deed, do all in the name of the Lord Jesus, giving thanks through Him to God the Father.

Colossians 3:17
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Two people have been most helpful and encouraging on the long road of developing this dissertation research from a germ of an idea in June of 1973. My adviser, Walter Rothenbuhler, has assisted with advice and suggestions which have made the journey quicker and more certain. "The teaching of the wise is a fountain of life" (Prov. 13: 14a). I have enjoyed his friendship, encouragement and reproof and look forward to years of the same as we engage in similar avenues of research. Also, the artificial inseminations performed by him have proved indispensible in the course of this investigation.

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Finally, I wish to thank my Dad and Mother, Charles and Lottie Milne, for years of building and encouragement toward this goal. Also, I acknowledge and thank my Dad for several sessions of programming consultation in developing the computer program for data analysis on the IRCC computer.
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INTRODUCTION

I. Introduction of Dissertation Research

Many interesting single gene behavioral mutants have been isolated for the study of the genetic control of behavior in a wide variety of organisms (Benzer, 1971, 1973; Gould, 1974). An important step in the study of these mutants is the identification of the site of the primary tissue defect which results in the mutant behavior - the behavioral focus of the mutation (Hotta and Benzer, 1972). Since a behavioral mutation could affect the structure or function of any of the elements in the stimulus-response pathway, the identification of the behavioral focus is a challenging problem. This challenge is usually met by searching for a visible defect as well as one can.

The powerful technique of blastoderm fate mapping has been developed in insects for the identification of behavioral foci (Hotta and Benzer, 1972). In insects there is a correspondence between the location of a cell on the blastoderm and its fate in subsequent development. A blastoderm fate map is the demarcation on the surface of the blastoderm of prospective larval and/or adult structures. Since the fate mapping technique can identify the organ which may be defective, a considerable amount of time can be saved
(Hotta and Benzer, 1972). Also, mis-identification of the behavioral focus and investigation of a secondary effect of the mutation can be avoided by using fate mapping (Gould, 1974).

Two characteristics render fate mapping possible in insects. The first one is the unique insect blastoderm formation from a centrolecithal egg. Initial cleavage divisions are not accompanied by cell formation and the nuclei retain their relative positions in a syncytial cluster near the center of the egg. After several divisions the nuclei migrate to the periplasm at the surface of the egg and divide a few more times. A cellular blastoderm is then formed by the appearance of cell membranes around the nuclei. The second characteristic is the occurrence of appreciable numbers of gynandromorphs (sex mosaics) with their sharp lines of separation of male and female cells in many insect species.

The primary aim of the research reported in this dissertation is to develop a detailed blastoderm fate map for the honey bee (Apis mellifera L.) from gynandromorphs.

II. The Technique of Blastoderm Fate Mapping

Many of the principles used to develop a blastoderm fate map from gynandromorphs are similar to those used to develop a chromosome map from the progeny of appropriate crosses. Chromosome mapping from crossover frequencies was first proposed and accomplished by A. H. Sturtevant (1913) based on several ideas published earlier.
(Morgan, 1911). Sturtevant (1929) later presented the ideas basic to blastoderm fate mapping.¹

A fate map is developed from an analysis of the distribution of male and female tissue in a large number of gynandromorphs. Gynandromorphic adults develop from gynandromorphic or mosaic blastoderms. These mosaic blastoderms have large patches of male and female cells on the surface. Large patches of cells, rather than just a mixture of cells, arise because there is little mixing of nuclei either in the syncytial cluster or on the blastoderm surface (Garcia-Bellido and Merriam, 1969a). On the surface of the mosaic blastoderm there is a line of separation between the male and female cells. If two structures lie far apart on the blastoderm, this line of separation will frequently fall between them. On the other hand, if the structures lie close together, this line of separation will rarely fall between them. When the line of separation falls between the two structures they will be different in sex in the adult gynandromorph which develops. Otherwise, they will be identical in sex. Therefore, the frequency with which two structures differ in sex, in a population of gynandromorphs, is directly proportional to the distance on the blastoderm separating the areas which form these two structures.

¹Sturtevant (1929) did more than propose the ideas underlying contemporary blastoderm fate mapping. His tables 9 to 14 are tables of distances between pairs of structures in arbitrary units similar to sturts (Hotta and Benzer, 1972). This publication also contains the first blastoderm fate map (his figure 4) although it is not in the form usually presented today. He also reported that the gynandromorph data could indicate the relative size of each area on the blastoderm (Table 7) and the distance to the midline of the blastoderm (Table 8).
To determine the distance between two structures on the blastoderm, a population of gynandromorphs is classified as to whether the two structures are identical or different in sex. The frequency by which they differ in sex X 100 is the distance separating them. The units of distance (frequency X 100) on the fate map have been called "sturts" in memory of A. H. Sturtevant (Hotta and Benzer, 1972). Triangulation using the distances between structures is employed to construct a map showing the location of each structure (Garcia-Bellido and Merriam, 1969a; and others).

The relative size of the area giving rise to a structure is calculated from the frequency with which it appears mosaic. Each area is represented on the map by a circle with a diameter measured in sturts (frequency mosaic X 100) (Garcia-Bellido and Merriam, 1969a; and others). The distance from a structure to the midline of the blastoderm is equal to one-half the distance from that structure to the same structure on the other side of the blastoderm (Garcia-Bellido and Merriam, 1969a; and others). From a number of these a smooth outline of the map can be drawn.

III. Review of Literature

The vast majority of blastoderm fate mapping has been done in Drosophila on which the work started. It has been demonstrated in Drosophila that fate maps are consistent with classical embryological studies (Gelbart, 1974; Hall et al., 1974; Kankel and Hall, 1976). Fate maps have been developed for external and internal larval...
structures (Hotta and Benzer, 1973; Falk et al., 1973; Janning, 1974c) and internal adult structures (Hall and Kankel, 1973; Janning, 1974b; Kankel and Hall, 1976).

Three uses have been suggested or developed for fate maps. Kankel and Hall (1976) have suggested that the fate maps could be used to identify the primary effect (or focus) of developmental or hormonal mutations. Bryant and Zornetzer (1973) attempted to map the focus of several lethal mutations in Drosophila. But the greatest accomplishment of fate mapping and the most promising use for the future is for the identification of behavioral foci (Hotta and Benzer, 1972). Benzer and his colleagues (Benzer, 1971, 1973) have isolated many interesting single gene behavioral mutants in Drosophila. Each of these behavioral mutations is recessive and can be expressed in gynandromorphs by employing the appropriate crosses and techniques. The behavior of a gynandromorph can be classified as mutant or normal. In the same gynandromorph, several external cuticular structures can be classified male or female. Distances can then be calculated from the behavioral focus to each external structure in the same manner as between two external structures. From the location of the behavioral focus on the fate map, it is possible to infer the structure responsible for the behavior.

Hotta and Benzer (1972) have tested the ability of fate mapping to identify the behavioral focus of several recessive mutations whose foci were previously known. Five visual mutations, known to affect the photoreceptors of the eye (Hotta and Benzer, 1970), all mapped to the location of the compound eye on the fate map. The mutation
hyperkinetic causes a shaking of the legs under ether anesthesia and was known to affect the thoracic ganglia (Ikeda and Kaplan, 1970a,b). This mutation mapped on the fate map to a separate site for each leg in a region known to develop into the ventral nervous system.

Flies homozygous for the mutation drop-dead normally live for two days before dying at an exponential rate from a rapid deterioration of the brain (Benzer, 1971). Fate mapping placed this behavioral focus in the area which supposedly develops into the adult brain; but the full picture is more complex. The two foci for this mutation, one on either side of the blastoderm, are interacting. Two possible interactions can be envisioned for behavioral foci. In one case, mutant behavior is observed only if both foci are mutant. Alternately, a mutant behavior would be observed if either of the two foci were mutant. In these two cases the mutant focus is said to be submissive or domineering, respectively, to a normal focus. The analysis of these interacting foci has been extended by Merriam and Lange (1974). drop-dead was found to affect two bilaterally-submissive foci.

Another behavioral mutant examined by Hotta and Benzer was the muscle mutation wings-up, in which the wings permanently assume a vertical position shortly after emergence. The behavioral focus for this mutation is a bilaterally-domineering one located in an area known to produce mesoderm. Since muscles are of mesodermal origin, it was concluded that the fate map had correctly identified this behavioral focus.
Benzer (1973) has reported that the fate mapping technique has identified the behavioral focus of several other mutations, including a visual mutation known to affect the neurons of the lamina. Circadian rhythm mutations appear to map on the blastoderm in the region of the brain. The first step in courtship by a male involves orientation toward a female and vibration of the wings in response to impulses generated by the thoracic ganglion. Mapping of this courtship behavior on the fate map indicates that the brain is the behavioral focus and a male brain will produce a male behavior even though the thoracic ganglion may be completely female. Hall et al. (1973) reported the use of a temperature-sensitive, general locomotor mutant to map the control of leg movements to a site in the ventral nervous system region on the fate map, near that found for hyperkinetic.

Four different genetic procedures have been used to produce Drosophila gynandromorphs. The gene claret (ca) was used by Sturtevant (1929) to generate gynandromorphs both for his investigations and for Garcia-Bellido and Merriam (1969a) who constructed a fate map for D. simulans. An unstable, ring-X chromosome is the most popular technique for D. melanogaster (Garcia-Bellido and Merriam, 1969b; and others). The mutations mit (mitotic loss inducer; Gelbart, 1974) and pal (paternal loss; Baker, 1975) have also been used in D. melanogaster.

The study of these fate maps reveals that although different methods were used to produce the sex mosaics, the maps are all basically the same. One difference, however, is that the distances
in sturts between the structures on maps developed by some
investigators are different from those of others. As a result the
maps are different in overall size when placed on the same scale.
Several variations in gynandromorph formation, such as a change in
the number of patches on the blastoderm, a change in the relative
rates of division of the male and female nuclei, or a change in which
cleavage division the male nucleus is first generated, can affect the
distances between structures. In order to develop maps of comparable
size it was necessary to re-define the stunt, and rename it
"sturtoids" (Hall et al., 1974; Gelbart, 1974; Kankel and Hall, 1976).

It is also evident that even within the work of a single
investigator there is some variation in the precise location of
various structures and the overall shape of the blastoderm (Baker,
1975; Kankel and Hall, 1976). Kankel and Hall (1976) have shown
that the size of the blastoderm on the fate map is enlarged by adding
more structures to the map nearer the midline. There are some
ambiguities in Drosophila fate maps (Janning, 1974b) because circles
representing various structures often overlap, implying that cells
in a certain region develop into two different structures; almost
certainly this is not true (Garcia-Bellido and Merriam, 1969b).

With the exception of the genus Drosophila, little fate mapping
has been done, even though gynandromorphs are known for a wide
variety of insects, and blastoderm formation is similar in most
insects. Clark and Egen (1975) mention that a fate map is being
developed for Habrobracon juglandis. Milne (1976) has published the
only blastoderm fate map outside Drosophila as a preliminary map for
the honey bee. The honey bee is a promising organism in which to perform behavior-genetic studies, and gynandromorphs have been known since 1801 (Laubender). Male tissue in honey-bee gynandromorphs has been demonstrated, by genetic studies, to arise from accessory sperm development (Rothenbuhler et al., 1952). Rothenbuhler (reviewed in 1958) has selected a line of honey bees which can produce up to forty per cent gynandromorphs among the worker progeny.

The data for the preliminary honey-bee fate map was obtained from previously published data of the distribution of male and female tissue for nine structures in forty gynandromorphs produced by one queen (Drescher and Rothenbuhler, 1963). These gynandromorphs did not occur in the gynandromorph-producing line, but were produced by chilling eggs shortly after oviposition. The two recessive eye color mutants, snow and chartreuse, were used to aid the identification of male tissue.

Although it was not mentioned, a computer program was written to analyze the forty X eighteen array of data. The program calculated the distances between pairs of structures, which were used to hand construct a map. A midline could not be drawn for the map because the data were insufficient. The map appeared to be a good fit to the data when the distances between structures from the map were compared with the distances from the gynandromorphic analysis. As the distance between structures increased, it was apparent that the distance from the map was larger than the data indicated. The explanation of double crossing over was presented to account for this phenomenon (Hotta and Benzer, 1972).
The map constructed from the Drescher-Rothenbuhler data differs from the general arrangement of structures seen in the adult honey bee in the location of the sting and antenna. This indicates that, like Drosophila, rearrangements occur between the blastoderm and the adult. The maps for these two organisms differ only slightly. On the honey-bee fate map the proboscis appears much lower than on Drosophila fate maps. The fruit fly lacks mandibles and a sting, and these structures appear only on the honey-bee fate map. Only the honey-bee fate map (and Kankel and Hall, 1976, published while the honey-bee fate map was in press) indicates the relative size of the compound eye.

Another major difference from Drosophila maps was noted. Due to the nature of honey-bee gynandromorph formation by accessory sperm development near the cephalic pole of the egg, the map is somewhat distorted. Structures nearer the head were male more often than those in the abdomen. This distortion of the fate map is expected to make the head region abnormally large and the abdominal region abnormally small. Nevertheless, the relationships of the locations of the various structures should be retained.
MATERIALS AND METHODS

IV. Honey Bee Stocks

Three honey bee stocks were used for the research reported in this dissertation. The hairless stock contains a recessive, single-gene mutant (h) causing a loss of hair, primarily on the thorax (Mackensen, 1958). Queens of this stock were obtained from the University of California at Davis. Detailed information regarding these queens is presented in Table 1.

Two lines of queens produced the gynandromorphs used in this investigation. One line was obtained from the University of California at Davis and the other from the United States Department of Agriculture Bee Breeding and Stock Center in Baton Rouge. The USDA provided the line previously selected by Rothenbuhler (1958). Detailed information on the gynandromorph-producing queens is presented in Table 2.

V. Isolation of Gynandromorphs

Queens of the gynandromorph-producing line were introduced into queenless nucleus colonies with two or three combs of bees. Whenever gynandromorphs were to be collected from a queen, a frame of sealed brood was removed from the hive and placed in an emergence cage.
holding a single frame. Every twenty-four hours after placing the frame in an incubator (35°C and about 50% R.H.) the bees in the cage were individually inspected with the unaided eye. Those which appeared to be entirely worker were counted and discarded. Those bees which appeared to be gynandromorphs were counted and saved. Dead gynandromorphs found either on the bottom of the emergence cage, partially emerged, or unemerged were also counted and saved. The live gynandromorphs were either examined immediately, stored in a laboratory cage (Kulinčević and Rothenbuhler, 1973) and examined later, or frozen and examined later. Dead gynandromorphs were either examined immediately or frozen for later examination.

Gynandromorphs were isolated in this manner, rather than picking them live from the combs in the hive, for three reasons. The percent gynandromorphs produced by the queen was useful information and is known to vary with changes in environmental conditions (Drescher, 1965). All gynandromorphs developing to the adult stage were needed for examination. It is likely that some gynandromorphs may be killed, or die in the hive, soon after emergence (Witherell, 1971) and some may not even emerge. In the incubator, for example, it was noted that gynandromorphs with a male and a female mandible often did not emerge. Therefore, isolation of gynandromorphs in this manner avoided the complications of using a biased sample for examination. Finally, drifting could present a problem if bees were picked live from the combs because it was necessary to know the queen producing each gynandromorph. Exceptions were made in the case of queens producing low percentages of gynandromorphs. Live gynandromorphs
were taken off the combs for queen mating numbers 3616 (2), 3617 (3), 3618 (18), 3619 (20), 3626 (13), where the number in parentheses refers to the number of gynandromorphs taken and subsequently examined.

Some gynandromorphs isolated were not examined because they were either maternal gynandromorphs (polar body development to produce male tissue) or were not suitable for analysis because they had decomposed either in the comb before removal or in the freezer.

VI. Examination of Gynandromorphs

Examination of the gynandromorphs was performed under a stereo-dissecting microscope and consisted of recording the distribution of male and female tissue in each gynandromorph. Two genetic markers, ivory eye color (i) and hairless (h), aided in distinguishing the sex of several structures. Thirty-one structures and one behavior\(^1\) were examined in the gynandromorphs and the criteria used for classifying each is presented in Table 3. Not all thirty-two structures were examined in each gynandromorph. Dissections were performed to examine the hypophr angeal food gland, honey stomach and sting structures. A calibrated micrometer eyepiece was used to measure those structures for which the sex was distinguished by measurement. The criteria for classifying these structures were determined from the measurements of sixty workers and fifty drones reared in worker-sized cells.

\(^1\)The term structure is used loosely throughout this investigation, not only to refer to a morphological structure but to a behavioral focus as well.
An attempt was made to locate a behavioral focus on the fate map by examining the cell-cleaning behavior in several gynandromorphs. Shortly after emergence, workers are known to engage in cell-cleaning behavior (Rösch, 1925; Lindauer, 1952). This involves crawling head-first into a cell for an extended period of time. Drones do not engage in this activity. Some of the gynandromorphs were stored for several days in a laboratory cage containing a small section of worker comb affixed to the back. Several times the cage was quickly treated with carbon dioxide to immobilize the bees. The gynandromorphs immobilized head-first in the cells were separated from the rest and were said to exhibit the cell-cleaning behavior.

During the examination of a gynandromorph, each side was examined separately. The numerical data for the left and right sides were kept together for the data analysis. The numbers zero, one, two or three indicate that the sex for the structure was not determined, male, mosaic or female, respectively.

VII. Computer Analysis of Gynandromorphic Data

The FORTRAN IV program (GAP) written to analyze these numerical data is presented in Appendix A. The program is similar to the BASIC program written earlier for the preliminary honey-bee blastoderm fate map (Milne, 1976). The numerical data on the distribution of male and female tissue in each gynandromorph were key punched directly from the data sheets onto standard IBM cards. The FORTRAN IV program reads the data from these cards into two arrays. A larger array (0) contained the distribution of male and female tissue for each
gynandromorph. Each row represents a single gynandromorph, and each pair of columns represents the left and right side for each structure. The smaller array (N) is a parallel array containing the computer number of the queen (Table 4) which produced the gynandromorph.

The data were then analyzed nine times and each analysis is identified by a "match" number. All the data from the gynandromorphs were analyzed in the section identified as match = zero. Then those queens which produced a sizable number of gynandromorphs (computer queen numbers one to eight) had a separate data analysis performed on the gynandromorphs they produced (match number = computer queen number).

The first part of the data analysis by the FORTRAN IV program consisted of calculating the per cent male, per cent mosaic and per cent female for each structure. Each structure on the map for which no mosaic structures were found among the gynandromorphs was represented as a point. Those for which mosaic structures were found were represented by a circle with a diameter in sturts equal to the per cent mosaic structures found among the gynandromorphs. It is not expected that all of the blastodermal areas will have a circular shape, but this is the best approximation which can be given with the data at present. Detailed examination of the notum and wing of *Drosophila* reveals the noncircular nature of these foci (Garcia-Bellido and Merriam, 1969b; Ripoll, 1972). The result of this approximation in the honey bee will be a certain amount of overlap of the circles of closely located structures.
The second part of the data analysis by the FORTRAN IV program is the calculation of the distances in sturts between each structure and all the other structures on the same side of the blastoderm. In general, the distance between two structures is equal to the per cent of gynandromorph sides in which the two structures differ in sex. The exact method is somewhat complicated and is presented in Table 5. This is the same method used by the investigators on Drosophila, except that the values of P1, P2 and P5 are calculated for each pair of structures. Drosophila investigators usually assume that P1, P2 and P5 = 0.5, which is the maximum value. Since only a few large structures have been examined in Drosophila, this assumption creates only a small error in their work. The current investigation, however, is different because larger structures were examined. This refinement in the analysis of calculating P1, P2 and P5 has a considerable effect on the distances calculated, especially those between the structures with larger radii.

The final part of the analysis of the data performed by the FORTRAN IV program is to calculate the distance from each structure on one side of the blastoderm to the structures on the other side of the blastoderm. This is done in a manner similar to the distance calculations previously mentioned. The distance of major importance is the distance between a structure on one side of the blastoderm and the same structure on the other side. One-half of this distance is the distance in sturts between the structure and the midline of the blastoderm.
VIII. Computer Construction of Blastoderm Fate Map

Construction of the blastoderm fate map can begin after the analysis of the data from the gynandromorphs has produced the distances between pairs of structures, the radii of each structure, and the distance to the midline of the blastoderm. The goal of map construction is to develop a map which fits the data. Distances between structures as computed from the gynandromorphs should fit, or agree with, the distances between the structures on the map. Using a single set of data from gynandromorphs, it is possible, however, to construct several maps which differ slightly in the locations of structures and the fit to the data. The better a map is, the better it fits the data used to construct it.

All previously published fate maps have been constructed by hand. This is done by sequentially locating structures (points) by triangulation from the distances to other structures on a two-dimensional surface (a piece of paper). First, one point is located arbitrarily on the map. Next, a second structure is located arbitrarily on a circle surrounding the previously located point but at the appropriate distance from the point. A third one is added to the map at either of two places at the appropriate distance from the two already located. Then more points are located sequentially on the map at the given distances from the previously located points. The triangulation involves using a compass and ruler to make several arcs indicating the appropriate distance to each structure, and then choosing a location for the structure. Next, circles for each point
are drawn and the distances to the midline of the blastoderm marked. To complete the map, a smooth outline is drawn by hand to represent the midline.

There are three serious problems in constructing fate maps by hand. First, if a slight error is made early in the construction of the map, it is compounded as more points are added to the map. Second, it is difficult to locate a point on the map after several have already been located. With several arcs, not all intersecting at one point, choosing locations becomes somewhat subjective. Third, the map maker is hard pressed to take into account the considerations which affect the relative weights of the several distances to the previously located structures.

Researchers constructing fate maps by hand have attempted to deal with these problems by employing two refinements. First, instead of constructing one large map, submaps of the three body regions are constructed and then oriented together to provide an entire map. Also only the smaller distances are used to locate each point.

These refinements may have decreased the magnitude of these problems in map construction somewhat, but they have not eliminated them. In the construction of this fate map for the honey bee, a computer was used to eliminate each of these problems. The computer can avoid making slight errors early in the construction of the map. A properly programmed computer has no difficulty objectively locating a point even after many are already on the map. The
computer can easily take into account, numerically, the considerations which affect the relative weights of the several distances to the previously located points.

Construction of a fate map using a computer is mechanically similar to hand construction. First, three initial points are located by hand on a piece of graph paper. Additional points are located sequentially on the map by the computer. The computer accomplishes the triangulation and choosing of the location numerically. Finally, the circles for each structure and midline of the blastoderm are drawn by hand to complete the map.

After several points are located on a fate map, the general location of the next point can be determined. Its exact location can be found by identifying the point for which the distances on the map to the previously located points agree with or best fit the distances obtained from the analysis of the data from the gynandromorphs.

The fit of the map to the data can be examined in two ways. One involves placing the distances from the map side by side with the distances from the data and comparing them. It can also be done by plotting the distances from the data (x) and the distances from the map (y) on graph paper. If perfect fit were realized, all the points would fall on the x = y line. Less than perfect fit would be found at the locations for which some of the points were off the line x = y.

A computer cannot work subjectively with either columns of numbers or graphs, but the computer can numerically examine the fit
by calculating the distance from each point on the graph to the $x = y$ line. The sum of these distances, the fit value, decreases as the fit gets better.

The computer works with a map, not on paper, but consisting of the coordinates of the locations for each structure. To calculate the fit value for a tentative location the computer first calculates all the distances on the map to the other structures using these coordinates. Then the distances from each point to the $x = y$ line are calculated and summed.

The computer system used consisted of a Hewlett Packard 9830A computer (1760 words of memory, BASIC language) with a 9862A plotter, 9864A digitizer and 9866A thermal printer attached as peripheral units. Appendix B contains the BASIC program (BFMCP) written for this machine to construct fate maps.

The computer was programmed to use three or more nearby points to determine the location of another structure. Coordinates of a tentative location are sent from the operator-controlled cursor of the digitizer and the fit value calculated is displayed on the screen of the computer. If it is better than the previous best fit value (i.e. smaller) then this fit value, and the coordinates of that tentative location, are stored in memory until a better fit value is found. When the best fit value has been found, then the structure is assigned to that location on the fate map. The resolution of the fate map, limited by the resolution of the digitizer, was 0.25\text{sturts}. This represents a vast improvement over the poorer resolution possible in hand-constructed maps using a ruler, compass, pencil and paper.
In calculating fit values, several considerations must be taken into account. Not all previously located structures will be of equal weight in locating other structures. Each distance from each point to the \( x = y \) line on the plot of the distance from the data and the distance from the map is first multiplied by a relative weighting factor, between zero and one, before addition to arrive at the fit value. This overall weighting factor is a product of three separate weighting factors. If any of these weighting factors for a point is zero, then the overall weighting factor is zero and that point is not used in locating other structures. The maximum value for the weighting factor is one, and points for which the value is one are of maximal value in locating other structures.

The first weighting factor has a value of zero or one. If there was any uncertainty regarding the validity of using the data for a structure in locating others, this weighting factor was set equal to zero. The structures for which the first weighting factor was zero are listed in Table 6.

As the number of gynandromorph sides used to determine a specific distance decreases, the variance will increase. In the construction of blastoderm fate maps, distances based on larger samples are therefore relatively more important in locating a structure than those based on smaller samples. The second weighting factor was set equal to \( \frac{n-1}{N-1} \). As the sample size \( n \) approaches zero, this weighting factor approaches zero, and as the sample size approaches \( N \) (the largest sample size used), it approaches one.
The third weighting factor involves a phenomenon observed in mapping of loci on chromosomes. As the distance between two loci increases, double crossovers decrease the apparent, calculated distances from data. This also has a similar effect in mapping structures on blastoderm fate maps (Hotta and Benzer, 1972; Milne, 1976). Haldane (1919) has expressed this mathematically for gene mapping in a mapping function which can be expressed for blastoderm fate mapping in sturts (Figure 1). This figure also demonstrates that the maximum distance between any two structures, from gynandromorph data, is fifty sturts (Hall et al., 1974). Therefore, in locating a structure with respect to those previously located, smaller distances are more accurate and are of relatively more value. Also, the fact that the map is being constructed on a flat sheet of paper while the data represents arc distances on a curved blastoderm surface infers that smaller distances are less in error (Garcia-Bellido and Merriam, 1969a). To calculate this third weighting factor, the operator, prior to the location of the point and upon inspection of the various distances to the other points, selects a value for the cutoff value (C). Distances larger than C sturts have the weighting factor set to zero. For distances up to C, the weighting factor is \( 1 - \frac{D}{C} \). As D, the distance, approaches C or zero, the weighting factor approaches zero or one, respectively.

Since the computer is not programmed to use long distances, an artifact can appear when the computer finds the best fit location for a structure. This occurs when the data indicates a best fit location near a point which should be far away. Then the operator must
override the computer's attempt to do this and look for the next best location. The location finally selected is a good fit, but the fit is not as good as the best fit point. The structures subsequently located by the computer are a better fit using the selected point than the best fit point.

After all the structures had been located on the map, the computer was programmed to make an overall fit plot by plotting the distances from the map \((y)\) and distances from the data \((x)\). This allowed subjective appraisal of the map. Another BASIC program was designed to take the coordinates of the structures and draw a map, with circles around the structures at the appropriate diameters, if desired. Finally, hand triangulation was used to locate the midline of the blastoderm and a smooth outline drawn.
RESULTS AND ANALYSES

IX. Results

Combs of brood from twenty queens of the gynandromorph-producing lines were emerged in an incubator and 78,192 bees were inspected during this investigation. Sixteen of these queens produced the 1870 gynandromorphs isolated. 1574 gynandromorphs were examined and the distribution of male and female tissue recorded. These data are recorded in Appendix C in addition to the computer queen number of the queen producing each gynandromorph. Table 4 contains the percent gynandromorphs produced by each queen during the period gynandromorphs were collected and the number of gynandromorphs examined.

X. Analyses of Distances between Structures

It is possible that, unlike Drosophila, there could be large differences in the maps or distances between structures based on the analysis of gynandromorphs from different females. This was investigated by comparing the distances between pairs of structures from different queens. Figures 2 - 8 contain the graphs of distances for queen 3549 (42.39% gynandromorphs) plotted against those for each of the other queens. The least squares line, correlation coefficient
and F-statistic were calculated for each graph. In each case the F-statistic was extremely large, implying a significant relationship between the queens for the distances. This indicates there is a high probability that the maps for the queens compared will be similar.

If the maps of different queens were identical, the slope of the least squares line would be one and the y-intercept would be zero. But this is not the case as the slopes and y-intercepts were not equal to the expected values (Figures 2-8). This indicates that in developing a set of distances to use in constructing a map from all the gynandromorphs, the gynandromorphs could not simply be pooled as though they came from one queen. Instead, each queen's distance data was transformed to conform to the data for queen 3549 (42.39% gynandromorphs) by taking each individual distance and subtracting the appropriate y-intercept and dividing by the slope. These transformed distances, when plotted against the distances for queen 3549 (42.39% gynandromorphs), gives a slope of one and a y-intercept of zero. All the transformed distances for each queen could then be combined to form composite distances which were then used for constructing a map. Table 7 contains the composite distances between pairs of structures calculated by this method.

The factors in gynandromorph formation causing the deviations in slope and y-intercept from the expected values are unknown at this time. It is known, however, that the slope and y-intercept are independent of the per cent gynandromorphs produced (Table 8).
It is possible that the distance between two structures may vary as the per cent gynandromorphs produced varies. If, for example, the average number of male patches increased along with the per cent gynandromorphs produced, then the distance between two structures would increase. To investigate this possibility, values for twenty-seven different distances were compared in six queens with a range in gynandromorph production of almost two hundred-fold. In no case was the correlation coefficient so large that a significant F-statistic was generated (Table 9). Therefore, the distance between structures on the blastoderm is independent of the per cent gynandromorphs produced.

XI. The Blastoderm Fate Map

The composite distances of Table 7 were used to construct the fate map in Figure 9. It indicates the location of each of the thirty-two structures examined as placed by the computer. No circles around structures or midline are drawn in order that the locations may be clearly seen. Figure 10 is the graph of the distances from the map and the distances from the data showing the fit of the map to the data used to construct it. The expected fit would be one similar to that of Figure 1, where adherence to the $x = y$ line is seen for small distances and an asymptotic approach to a maximum value of fifty sturts for larger distances. To provide for a visual comparison of this with the fit observed in <i>Drosophila</i> hand-constructed maps, Figure 11 has been prepared from Kankel and Hall's (1976) Figure 9 and Table 2. Even without the consideration that several of the
points on the honey-bee map are uncertain for various reasons, the computer-generated honey-bee map is a better fit to the data. Points considerably below the \( x = y \) line at short distances on the map are indicative of a poor fit to the data.

If the points which are uncertain, for various reasons, are omitted from the map, a truly remarkable fit of the map to the data is achieved (Figure 12). Those which were omitted had the first weighting factor equal to zero and were denoted prior to map construction (Table 6). When the fit of the uncertain structures was examined, it is clear that the computer was able to place these with a fit nearly as good as for hand-constructed maps (Figure 13).

Four structures were examined two different ways in two separate groups of gynandromorphs. The sex of the antennae, glossae, labial palpus and galea were distinguished both by the eye and by measuring with a calibrated, micrometer eyepiece. Inspection of Figure 9 demonstrates that they map in essentially identical locations regardless of the method used to distinguish the sex of the structure.

Nine of the thirty-two structures had the first weighting factor set to zero and their inclusion on the map should be examined. Since it was difficult to distinguish the sex of the gena and frons in the gynandromorphs, their locations on the map will be tentative.

The compound eye was listed as an uncertain structure because of its large size on the blastoderm and the ease of detection of mosaic eyes. While there may not be a great deal of mixing in the syncytial cluster before blastoderm formation, there probably are stray nuclei which do end up in patches of otherwise uniformly sexed nuclei. This
will cause the structure to be mosaic. If mosaic structures are easy to detect, then the distances to other structures will decrease. The position of the compound eye is probably farther away from the remainder of the points on the map and the exact location on the map is therefore tentative.

The center ocellus of the honey bee proved to be a unique structure in its sex determination (position). It was noticed upon inspection of the gynandromorph data that a female center ocellus was never found if either side ocellus were male. Also a male center ocellus was never found with two female side ocelli. This is the male bilaterally-domineering type of focus and it is located at the position of the side ocellus. Therefore it will be omitted from the map. My results with respect to the center ocellus agree well with the drawings and descriptions of gynandromorphic honey bees (Eckert, 1934) and *Hypodynerus tuberculentris* (Spin.) (Cooper, 1959). However, Sakagami and Takahashi (1956) and Clark et al. (1971) present reports for the honey bee and *Habrobracon juglandis*, respectively, which conflict with my findings.

Janning (1972, 1974a) and Kankel and Hall (1976) have shown that digestive system structures can be mosaic. It was not possible by the techniques employed to detect a mosaic honey stomach. The honey stomach is probably a structure composed of two non-interacting foci on either side of the blastoderm. It was not possible, therefore, to locate it from the data and will be omitted from the map.

Work on behaviors in *Drosophila* by Hotta and Benzer (1972) has indicated that most behavioral foci appear to be interacting.
Therefore the cell-cleaning behavior examined in the honey bee may be such a focus. Since only the cell cleaners were isolated, it was not possible to locate the focus if interacting, and the cell-cleaning behavior will be omitted from the map.

The sex distribution found in the wings was unusual. Only a rare fore wing and an occasional hind wing were male, the rest being female or mosaic. This is consistent with results of Drescher (1975) that the shape of the wing may indicate one sex and bristle distribution on the surface of the wing may indicate the other sex. The cause of this difficulty in sex determination is unknown. Even though this is an indication that something is unusual and the data should not be used, they map in the approximate locations expected from the Drosophila work (Garcia-Bellido and Merriam, 1969a). The location of the wings on the map is, therefore, tentative.

The sex distribution of the food gland was also unusual. Less than twenty per cent of the food glands in the gynandromorphs examined were male. This could indicate that there is an interaction between the foci (left and right sides). If there is no interaction between the foci during the development of gynandromorphs, the sex distribution (both sides male, bilateral mosaics, or both sides female) should be binomial. Table 10 contains the analysis of the sex distribution to determine if it is binomial. Since a significant $\chi^2$ was obtained, something is affecting the sex distribution of the food gland. Therefore, this structure should be omitted from the fate map.
The data indicate that there are fewer bilateral mosaics for
the food gland than expected. Two plausible explanations can be
envisioned for this result. The first is that female is partially
domineering. But, the data do not support this explanation. Table
10 shows that there are fewer bilateral mosaics than expected. The
number lost from this class (about sixteen) is offset by equal gains
(of about eight) in the two classes with both sides male or both
sides female. The second explanation is that some of the bilateral
sex mosaics for the food gland, or a structure nearby on the
blastoderm, die before forming the adult.

The honey-bee blastoderm fate map presented in Figure 14 has
the previously mentioned changes taken into account. A circle for
each structure is drawn with the appropriate diameter and some
overlap of nearby structures is noted. This is not expected on the
actual blastoderm. Several distances to the midline of the
blastoderm are marked and a smooth outline has been indicated.

XII. Polarization of the Honey Bee Blastoderm

It is possible that the honey-bee gynandromorph and/or
gynandromorphic blastoderm is longitudinally polarized (Sakagami and
Takahashi, 1956; Drescher, 1975; Milne, 1976). A polarized
blastoderm occurs when one area is predominately one sex in a
population of mosaics. Honey-bee gynandromorph formation by
accessory sperm development near the point of penetration (cephalic
pole) of the egg could lead to the head being predominately male and
the abdomen predominately female.
This was examined in the data collected in this investigation in a manner similar to Drescher (1975). Table II contains the data and the analysis of these data to discern if either the adult or the fate map is polarized by examining the correlation between the longitudinal location in the adult or on the map and the per cent male. The average of the per cent male for all the structures classified in each body region was similar. As Drescher (1975) has reported, there is no significant relationship between the longitudinal location in the adult and the per cent male. This is not in agreement with observations by Sakagami and Takahashi (1956) on about forty gynandromorphs nor results reported in Milne's (1976) preliminary investigation.

There is a highly significant correlation (0.925) between the location in the adult and the location on the blastoderm fate map (Table II). There are, however, some necessary longitudinal rearrangements which must occur before the adult is formed. No significant correlation was detected between the location of a structure on the fate map and the per cent male with which it occurred in the gynandromorphs. This indicates that there is no significant longitudinal polarization of the blastoderm as a whole. There is also no significant polarization in the thoracic region of the blastoderm. But there is a significant negative correlation between the location of the structures within the region of the blastoderm fate map which will produce the head and the per cent male. The sign of the correlation coefficient indicates that on the fate map the anterior portion of the head region (toward the
cephalic pole and including the compound eye, ocelli, clypeus and labrum) is predominately male, while the posterior portion of the head region (mouthparts) is predominately female.

This phenomenon will be unique to the honey bee since different methods of gynandromorph formation are used in *Drosophila*. A polarization of the blastoderm in the head region will have an effect on the map. It will cause the head region to be abnormally lengthened in respect to the rest of the map. Even though this may occur, the relative locations of the structures should be retained.
DISCUSSION

The goal of the research reported in this dissertation was to produce a detailed blastoderm fate map for the honey bee from an analysis of gynandromorphs. This goal has been achieved. In addition, several features of gynandromorph formation in the honey bee have been explored, and the technique of fate mapping improved.

In this research, over 100,000 bits of information were collected and used in making the fate map. This is two to five times the amount of data used for any of the Drosophila maps. Analysis of these data was greatly simplified by the use of a computer, a procedure which was introduced only recently (Baker, 1975; Kankel and Hall, 1976; Milne, 1976).

Kankel and Hall (1976) briefly describe their analysis program and some of the differences between it and the one used here are trivial. One major difference between the two programs lies in the values of P1, P2 and P5. Previous researchers on Drosophila have assumed values of 0.5 for these variables. The program utilized for this honey-bee map uses the radii of the two structures and an estimate of the distance separating them to calculate the precise value, which is often less than 0.5. Therefore, the program developed here for the analysis of data from gynandromorphs for fate maps is much more accurate, especially for the honey bee. It would
also be more accurate in the analysis of data in *Drosophila*, whenever the structures appear mosaic. If all the structures are single bristles, there will be no difference between the data analyses provided by either program. When structures are mosaic, such as the eye, antenna or wing in *Drosophila*, a large improvement in the data analysis would be provided by the program developed for the honey bee. It is also expected that a certain fraction of the variation between the locations of structures on the maps would be eliminated (Baker, 1975; Kankel and Hall, 1976).

After the analysis of the data, in all the earlier studies, the actual construction of the map has been done by hand. Already discussed was the fact that hand construction has three serious problems which lessen the accuracy of the map. The expected resolution and accuracy of hand-constructed maps is about two or three sturts. Use of a computer to construct the map, done for the first time in this investigation, eliminates these three problems and increases the accuracy. The resolution and accuracy of the computer-constructed maps is 0.254 sturts, about a ten-fold improvement.

The longitudinal location of structures on the honey-bee fate map (Figure 14) is highly correlated with the longitudinal location of structures in the adult. Structures forming each body region are grouped on the map. There are, however, rearrangements which must occur between the blastoderm stage and the adult. This is identical to the situation described in *Drosophila* (Hotta and Benzer, 1972). As in *Drosophila*, there is no clear grouping of the structures into
clusters which will form the imaginal discs (Garcia-Bellido and Merriam, 1969a). Scattering of these areas which will form the adult structures appears to be the rule on the blastoderm.

The preliminary honey-bee fate map (Milne, 1976) agrees well with the locations determined in this investigation. Only forty gynandromorphs, produced by chilling the eggs after oviposition, were used to develop that map compared to the 1574 for this more detailed map. The head structures (compound eye, antenna, mandible and proboscis) are in approximately the same position on both maps. All of the structures are somewhat larger in this more detailed map. The thoracic structures located on the preliminary map are also in approximately the same location on both maps. A major difference, however, is the location of the sting. In choosing the location for the sting on the preliminary map, two locations seemed possible from the data. The one chosen appeared to be a slightly better fit and was therefore selected. The other position, however, appears now to have been the correct one because this investigation, based on more gynandromorphs, places it in a similar location. Therefore, in general, the preliminary honey-bee fate map and the detailed map developed here are similar, even though different methods were used to produce the gynandromorphs.

Seven entire blastoderm fate maps have been published for Drosophila, one for D. simulans and six for D. melanogaster. Comparison of the map of Garcia-Bellido and Merriam (1969a) for D. simulans with the D. melanogaster maps reveals no discrepancies larger than between any two D. melanogaster maps. Therefore, it is
possible to compare the fate map developed here for the honey bee with maps developed for both species of *Drosophila*. Inspection of the maps for overall body layout shows a remarkable similarity for locations of the head, legs, thorax and abdomen.

Closer inspection of the head region reveals some similarities and differences in the location of the structures on the blastoderm. There are four head structures which are located in relatively similar locations on all maps with respect to each other. The honey-bee antenna (flagellum + scape) and the antenna of the fly (or a part of the antenna) are located in the same location on the maps for the two insects. Both maps show the compound eye located in approximately the same location. The ocellar bristles of *Drosophila* and the ocelli of the honey bee map in similar locations. The palpus of the fly and the galea of the honey-bee are maxillary structures and map in similar locations on the respective maps.

By contrast, three other head structures appear in different locations. *Drosophila*’s proboscis and the glossa + labial palpus of the honey bee are both labial structures but it appears consistently higher on the *Drosophila* maps. The clypeus of the honey bee and the oral vibrissae of *Drosophila*, located in similar locations in both adults, appear at different places on most maps. Only one *Drosophila* map (Gelbart, 1974) indicates a location for the vibrissae similar to the honey-bee clypeus location, while five maps indicate a location which is above and slightly to the rear of the antennae (Garcia-Bellido and Merriam, 1969a; Hotta and Benzer, 1972; Janning, 1974b; Baker, 1975; Kankel and Hall, 1976).
This honey-bee map shows the frons and gena located below the antennae. The *Drosophila* orbital bristles, located on a similar portion of the head, appear in a different location on the maps. One *Drosophila* map (Baker, 1975) places it between the antennae and the ocellar bristles, whereas five maps locate the orbital bristles near the vibrissae, above the antennae (Garcia-Bellido and Merriam, 1969a; Hotta and Benzer, 1972; Gelbart, 1974; Janning, 1974b; Kankel and Hall, 1976).

There is close agreement on the maps between all the thoracic structures examined in the honey bee and similar structures in *Drosophila*. The sting and preliminary map location of the abdomen are similar in location to the tergites and sternites located on *Drosophila* maps.

Several structures such as the mandible, sting and labrum, not found in the fruit fly, appear on the honey-bee map. Other structures, such as the metathoracic pleuron and the haltere (hind wing), have not been mapped in *Drosophila*. There are also many structures which appear on some of the *Drosophila* maps which are not located on this honey-bee map.
SUMMARY

Blastoderm fate mapping is the identification of areas on the blastoderm which will give rise to various larval and/or adult structures. The map is developed from an analysis of the distribution of male and female tissue in a large number of gynandromorphs. The technique of blastoderm fate mapping has been developed in insects for the identification of the anatomical structure (behavioral focus) affected by a behavioral mutation which is responsible for the mutant behavior (Hotta and Benzer, 1972).

The aim of the research reported in this dissertation was to develop a detailed honey-bee fate map for morphological structures. No detailed map has been published outside the genus Drosophila. Milne (1976) has published a preliminary honey-bee map of nine structures based on the analysis of forty gynandromorphs.

Two lines of gynandromorph-producing queens produced the gynandromorphs isolated. For each queen, single frames of sealed brood were allowed to emerge in an incubator and the gynandromorphs were collected and examined. Thirty-one structures and one behavior were classified as to whether they were male, mosaic or female. Occasionally, a structure was not classified. A FORTRAN IV program (Appendix A) was written to calculate the per cent male, mosaic and female for each structure, the distance between pairs of
structures, and the distance between structures located on opposite sides of the blastoderm. A BASIC computer program (Appendix B) was written to construct a map from the values calculated by the FORTRAN IV program. In some respects the method the computer employs is similar to hand construction of maps by triangulation. Three points are located by hand and the computer then locates the remainder sequentially at the location of best fit. The location of best fit is that location for which the distances from that point to the rest of the structures agree well with the distances computed by the FORTRAN IV analysis of the gynandromorphs. Then the areas for the various structures are indicated by drawing a circle and a smooth outline for the map is drawn indicating the midline of the blastoderm.

Gynandromorphs were isolated and examined from sixteen queens (Table 4). The distribution of male and female tissue for the thirty-two structures in 1574 gynandromorphs is recorded in Appendix C. Distances between structures were independent of the per cent gynandromorphs produced by a queen (Table 9). Furthermore, distances were approximately the same when they were determined from progeny of different queens (Figures 2-8). This indicated that the maps were probably similar.

Table 7 contains the distance data calculated from the gynandromorphs which were produced by the six queens producing most of the gynandromorphs. The BASIC program was used to construct the fate map (Figure 9) which was a good fit to the data (Figure 10). Four structures were classified by two different methods to distinguish the sex in two separate groups of gynandromorphs, and were found to map in similar locations.
Nine of the thirty-two structures classified had some doubt raised regarding their validity in locating other structures (Table 6). The center ocellus is a complex, interacting focus which maps at the position of the side ocellus. With respect to a center ocellus, a male side ocellus is bilaterally domineering. A large structure, such as the compound eye, in which small patches of male or female tissue can be easily detected, will be located too close to the remainder of the structures on the map. The position of the gena, frons and wings is tentative. Both the honey stomach and cell-cleaning behavior are probably complex foci which cannot be located by the techniques employed. The sex distribution of the food gland was unusual, indicating that it should be omitted from the map. With these changes the honey-bee fate map is presented in Figure 14.

The possibility of a greater amount of male tissue at one or the other end of a gynandromorphic blastoderm or adult was examined. No such longitudinal polarization was found for the adult or the blastoderm as a whole. But there was a significant polarization within the region of the blastoderm which produces the head. The cephalic pole region of the head is predominately male, whereas the posterior region is predominately female. Presumably this kind of polarization results because sperm enter the egg at the cephalic pole and male tissue of gynandromorphs develops from accessory sperm. This polarization will effect a lengthening of the head region of the map with respect to the rest of the map.
The FORTRAN IV program employed in the development of this detailed honey-bee map is more sophisticated than those used previously for *Drosophila*. This is especially true when analyzing structures which are mosaic in some gynandromorphs.

Previous fate maps for *Drosophila* and the honey bee were constructed by hand. The BASIC program developed and employed in this investigation eliminates the problems inherent in this method and increases the resolution and accuracy of fate maps an estimated ten-fold.

The longitudinal position of structures on the detailed honey-bee map and the position in the adult are highly correlated (Table II). This detailed map also agrees well with the preliminary honey-bee fate map (Milne, 1976). The general layout of the honey-bee map is identical to that found in *Drosophila*. Four head structures (flagellum + scape, compound eye, side ocellus, and galea) are located in approximately the same locations on maps of both species. On the other hand, three head structures (glossae + labial palpus, clypeus, and frons + gena) are located in different areas. All the thoracic structures and the abdomen are found to be in the same locations on both the *Drosophila* maps and this honey-bee map. Several structures (mandible, sting structures, labrum, metapleuron and metathoracic wing) located on this honey-bee map do not appear on any *Drosophila* map.
Table 1. Queens of hairless stock from which drones were obtained for inseminating queens of gynandromorph-producing stock

<table>
<thead>
<tr>
<th>OSU mating number</th>
<th>Inseminated at¹</th>
<th>OSU mating number of mother of</th>
<th>Genotype of</th>
</tr>
</thead>
<tbody>
<tr>
<td>3558</td>
<td>Davis (161-32)</td>
<td>-</td>
<td>+/+</td>
</tr>
<tr>
<td>3613</td>
<td>OSU</td>
<td>3558</td>
<td>+/h</td>
</tr>
<tr>
<td>3614</td>
<td>OSU</td>
<td>3558</td>
<td>+/h</td>
</tr>
<tr>
<td>3615</td>
<td>OSU</td>
<td>3558</td>
<td>+/h</td>
</tr>
</tbody>
</table>

¹Davis = University of California at Davis  
OSU = The Ohio State University  
²op = open mated to free flying, non-hairless drones
<table>
<thead>
<tr>
<th>OSU mating number&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Inseminated at&lt;sup&gt;2&lt;/sup&gt;</th>
<th>OSU mating number of mother of Queen</th>
<th>Drones</th>
<th>Genotype&lt;sup&gt;4&lt;/sup&gt; of Queen</th>
<th>Drones</th>
</tr>
</thead>
<tbody>
<tr>
<td>3536</td>
<td>Davis (179-734)</td>
<td>3537</td>
<td>op</td>
<td>gyn</td>
<td>+</td>
</tr>
<tr>
<td>3537</td>
<td>Davis (189-732)</td>
<td>op</td>
<td>gyn/+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3537 op</td>
<td>-</td>
<td>3537</td>
<td>op</td>
<td>gyn</td>
<td>+</td>
</tr>
<tr>
<td>3538 op</td>
<td>-</td>
<td>3537</td>
<td>op</td>
<td>gyn</td>
<td>+</td>
</tr>
<tr>
<td>3548 USDA (44-3)</td>
<td>3326</td>
<td>3326</td>
<td>i/+</td>
<td>gyn i</td>
<td>gyn</td>
</tr>
<tr>
<td>3549 USDA (47-4)</td>
<td>3326</td>
<td>3326</td>
<td>i/+</td>
<td>gyn i</td>
<td>gyn</td>
</tr>
<tr>
<td>3550 Davis (344-13)</td>
<td>-</td>
<td>-</td>
<td>gyn</td>
<td>gyn</td>
<td>gyn</td>
</tr>
<tr>
<td>3551 Davis (344-15)</td>
<td>-</td>
<td>-</td>
<td>gyn</td>
<td>gyn</td>
<td>gyn</td>
</tr>
<tr>
<td>3616 OSU</td>
<td>-</td>
<td>3613&lt;sup&gt;3&lt;/sup&gt;</td>
<td>i/i,</td>
<td>gyn h</td>
<td></td>
</tr>
<tr>
<td>3617 OSU</td>
<td>-</td>
<td>3613&lt;sup&gt;3&lt;/sup&gt;</td>
<td>i/i,</td>
<td>gyn h</td>
<td></td>
</tr>
<tr>
<td>3618 OSU</td>
<td>-</td>
<td>3613&lt;sup&gt;3&lt;/sup&gt;</td>
<td>i/i,</td>
<td>gyn h</td>
<td></td>
</tr>
<tr>
<td>3619 OSU</td>
<td>-</td>
<td>3613&lt;sup&gt;3&lt;/sup&gt;</td>
<td>i/i,</td>
<td>gyn h</td>
<td></td>
</tr>
<tr>
<td>3620 OSU</td>
<td>-</td>
<td>3613&lt;sup&gt;3&lt;/sup&gt;</td>
<td>i/i,</td>
<td>gyn h</td>
<td></td>
</tr>
<tr>
<td>3621 OSU</td>
<td>-</td>
<td>3613&lt;sup&gt;3&lt;/sup&gt;</td>
<td>i/i,</td>
<td>gyn h</td>
<td></td>
</tr>
<tr>
<td>3622 OSU</td>
<td>-</td>
<td>3613&lt;sup&gt;3&lt;/sup&gt;</td>
<td>i/i,</td>
<td>gyn h</td>
<td></td>
</tr>
<tr>
<td>3624 OSU</td>
<td>-</td>
<td>3613&lt;sup&gt;3&lt;/sup&gt;</td>
<td>i/i,</td>
<td>gyn h</td>
<td></td>
</tr>
<tr>
<td>3625 OSU</td>
<td>-</td>
<td>3613&lt;sup&gt;3&lt;/sup&gt;</td>
<td>i/i,</td>
<td>gyn h</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>SS = supersedure queen

<sup>2</sup>op = open mated to free-flying drones

Davis = University of California at Davis

USDA = United States Department of Agriculture Bee Breeding and Stock Center, Baton Rouge

OSU = The Ohio State University

<sup>3</sup>drones from hive headed by queen 3613 could have been from 3614 or 3615

<sup>4</sup>gyn = gynandromorph-producing line

<i> = ivory eye color

<sup>h</sup> = hairless
Table 3. Structures in gynandromorphic honey bees classified as male, mosaic, female or not determined

<table>
<thead>
<tr>
<th>Structure (abbreviation)¹</th>
<th>Number for computer analysis</th>
<th>Criteria for distinguishing sex in a gynandromorph</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound eye (E)</td>
<td>1</td>
<td>male facet larger than female facet, ivory eye color in some males</td>
</tr>
<tr>
<td>Side ocellus (SO)</td>
<td>3</td>
<td>male ocellus located in front of head, female ocellus located on top of head</td>
</tr>
<tr>
<td>Center ocellus (CO)</td>
<td>5</td>
<td>male ocellus located in front of head, female ocellus located on top of head</td>
</tr>
<tr>
<td>Frons (Fr)</td>
<td>7</td>
<td>somewhat sunken, hairy &amp; dark in male; somewhat bulging, hairless &amp; light in female</td>
</tr>
<tr>
<td>Gena (Ge)</td>
<td>9</td>
<td>somewhat sunken, hairy &amp; dark in male; somewhat bulging, hairless &amp; light in female</td>
</tr>
<tr>
<td>Antenna (Ant)</td>
<td>11</td>
<td>length estimated by eye; male longer than female; mosaic intermediate</td>
</tr>
<tr>
<td>Clypeus (Clp)</td>
<td>13</td>
<td>male hairy and lighter in color than female</td>
</tr>
<tr>
<td>Labrum (Lm)</td>
<td>15</td>
<td>male hairy and lighter in color than female</td>
</tr>
<tr>
<td>Mandible (Md)</td>
<td>17</td>
<td>male smaller and pointed; female larger and cup-shaped</td>
</tr>
<tr>
<td>Galea (Ga)</td>
<td>19</td>
<td>length estimated by eye; female longer than male; mixed is intermediate</td>
</tr>
<tr>
<td>Labial palpus (LbPlp)</td>
<td>21</td>
<td>length estimated by eye; female longer than male; mixed is intermediate</td>
</tr>
<tr>
<td>Glossa (GIs)</td>
<td>23</td>
<td>length estimated by eye; if curved, curve is toward male side; female longer than male, mosaic intermediate</td>
</tr>
<tr>
<td>Food gland (FG1d)</td>
<td>25</td>
<td>absent in male; present in female</td>
</tr>
<tr>
<td>Basitarsus of prothoracic leg (Btar1)</td>
<td>27</td>
<td>female is stouter, darker &amp; has fewer but longer hairs than male; mosaic has both female and male areas</td>
</tr>
<tr>
<td>Structure (abbreviation)</td>
<td>Number for computer analysis</td>
<td>Criteria for distinguishing sex in a gynandromorph</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Basitarsus of mesothoracic leg (Btar&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>29</td>
<td>female is darker and wider; mosaic is intermediate in width</td>
</tr>
<tr>
<td>Tibia of metathoracic leg (Tb&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>31</td>
<td>presence of pollen basket, concave and shiny in female; hairy and convex in male</td>
</tr>
<tr>
<td>Flagellum of antenna (Fl)</td>
<td>33</td>
<td>length measured - (&lt;3.00 \text{ mm} = \text{female} ; &gt;3.50 \text{ mm} = \text{male} , \text{intermediate} = \text{mixed} )</td>
</tr>
<tr>
<td>Scape of antenna (Scp)</td>
<td>35</td>
<td>length measured - (&gt;1.15 \text{ mm} = \text{female} ; &lt;1.10 \text{ mm} = \text{male} , \text{intermediate} = \text{mixed} )</td>
</tr>
<tr>
<td>Fore wing (W&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>37</td>
<td>length measured - (&lt;9.25 \text{ mm} = \text{female} ; &gt;10.1 \text{ mm} = \text{male} , \text{intermediate} = \text{mixed} )</td>
</tr>
<tr>
<td>Hind wing (W&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>39</td>
<td>width measured - (&lt;2.00 \text{ mm} = \text{female} ; &gt;2.45 \text{ mm} = \text{male} , \text{intermediate} = \text{mixed} )</td>
</tr>
<tr>
<td>Mesopleuron (Pl&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>41</td>
<td>hairless gene - hairless = male; hairy = female</td>
</tr>
<tr>
<td>Mesonotum (N&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>43</td>
<td>hairless gene - hairless = male; hairy = female</td>
</tr>
<tr>
<td>Mesosternum (S&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>45</td>
<td>hairless gene - hairless = male; hairy = female</td>
</tr>
<tr>
<td>Styllet of sting (Stl)</td>
<td>47</td>
<td>present in female; absent in male</td>
</tr>
<tr>
<td>Lancet of sting (Lct)</td>
<td>49</td>
<td>present in female; absent in male</td>
</tr>
<tr>
<td>Sheath lobe of sting (Sh)</td>
<td>51</td>
<td>present in female; absent in male</td>
</tr>
<tr>
<td>Honey stomach (crop) (HS)</td>
<td>53</td>
<td>larger in female than male</td>
</tr>
<tr>
<td>Metapleuron (Pl&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>55</td>
<td>hairless gene - hairless = male; hairy = female</td>
</tr>
<tr>
<td>Galea (Ga)</td>
<td>57</td>
<td>length measured - (&gt;3.60 \text{ mm} = \text{female} ; &lt;3.00 \text{ mm} = \text{male} , \text{intermediate} = \text{mixed} )</td>
</tr>
<tr>
<td>Labial palpus (LbPlp)</td>
<td>59</td>
<td>length measured - (&gt;2.50 \text{ mm} = \text{female} ; &lt;2.00 \text{ mm} = \text{male} , \text{intermediate} = \text{mixed} )</td>
</tr>
<tr>
<td>Glossa (Gls)</td>
<td>61</td>
<td>length measured - (&gt;2.60 \text{ mm} = \text{female} ; &lt;2.25 \text{ mm} = \text{male} ; \text{if curved, curved toward male side} )</td>
</tr>
<tr>
<td>Cell cleaning (CC)</td>
<td>63</td>
<td>if seen cleaning cells = female; see text for full explanation</td>
</tr>
</tbody>
</table>

<sup>1</sup>Abbreviations from R.E. Snodgrass (1963), and D. J. Borror and D. M. DeLong (1971)
<table>
<thead>
<tr>
<th>Queen mating number</th>
<th>Computer queen number</th>
<th>% Gynandromorphs in queen's progeny</th>
<th>Number of gynandromorphs classified</th>
</tr>
</thead>
<tbody>
<tr>
<td>3537</td>
<td>1</td>
<td>18.53</td>
<td>.559</td>
</tr>
<tr>
<td>3549</td>
<td>2</td>
<td>42.39</td>
<td>490</td>
</tr>
<tr>
<td>3619</td>
<td>3</td>
<td>2.55</td>
<td>146</td>
</tr>
<tr>
<td>3549</td>
<td>4</td>
<td>26.00</td>
<td>115</td>
</tr>
<tr>
<td>3537</td>
<td>5</td>
<td>12.21</td>
<td>90</td>
</tr>
<tr>
<td>3548</td>
<td>6</td>
<td>8.962</td>
<td>80</td>
</tr>
<tr>
<td>3626</td>
<td>7</td>
<td>0.711</td>
<td>38</td>
</tr>
<tr>
<td>3618</td>
<td>8</td>
<td>0.259</td>
<td>37</td>
</tr>
<tr>
<td>3536</td>
<td>9</td>
<td>0.12</td>
<td>4</td>
</tr>
<tr>
<td>3617</td>
<td>10</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>3616</td>
<td>11</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>SS 3537</td>
<td>12</td>
<td>0.06</td>
<td>2</td>
</tr>
<tr>
<td>3620</td>
<td>13</td>
<td>0.056</td>
<td>2</td>
</tr>
<tr>
<td>3621</td>
<td>14</td>
<td>0.031</td>
<td>1</td>
</tr>
<tr>
<td>3623</td>
<td>15</td>
<td>0.023</td>
<td>1</td>
</tr>
<tr>
<td>3624</td>
<td>16</td>
<td>0.026</td>
<td>1</td>
</tr>
<tr>
<td>3551</td>
<td>17</td>
<td>0.046</td>
<td>1</td>
</tr>
<tr>
<td>3550</td>
<td>18</td>
<td>1.30</td>
<td>1</td>
</tr>
<tr>
<td>SS 3537</td>
<td>19</td>
<td>0.06</td>
<td>1</td>
</tr>
</tbody>
</table>

1SS = supersedeure

2unsue of exact percentage because two laying queens were present in hive
Table 5. Method for computation of distance between two structures on the blastoderm fate map

Distance between structures A & B = \( \frac{\Sigma x}{\Sigma n} \times 100 \),

where \( \Sigma x \) = number of times A & B differed in sex in the gynandromorph sides examined

\( \Sigma n \) = number of gynandromorph sides examined

<table>
<thead>
<tr>
<th>Sex of structure A</th>
<th>Sex of structure B</th>
<th>( x^2 )</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 3</td>
<td>0, 1, 2, 3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1, 3</td>
<td>P1</td>
<td>1</td>
</tr>
<tr>
<td>1, 3</td>
<td>2</td>
<td>P2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>P5</td>
<td>1</td>
</tr>
</tbody>
</table>

1 sex of structure: 0 = not determined, 1 = male, 2 = mosaic, 3 = female

2 P1 = chance of the line separating male and female cells on the blastoderm intersecting the line connecting the centers of the two structures, given structure A is mosaic

P2 = chance of the line separating male and female cells on the blastoderm intersecting the line connecting the centers of the two structures, given structure B is mosaic

P5 = chance of the line separating male and female cells on the blastoderm intersecting the line connecting the centers of the two structures, given structures A and B are mosaic

P1 and P2 were calculated from the radii of structures A and B and an estimate of the distance separating them by subroutine CASE1 (see Appendix A).

P5 was calculated from the radii of structures A and B and an estimate of the distance between them by subroutine CASE2 (see Appendix A).
Table 6. Structures classified in gynandromorphs for which the first weighting factor is zero and which are not used in locating any other structure

<table>
<thead>
<tr>
<th>Structure</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound eye</td>
<td>Some eyes which should be male or female are changed into mosaics by random (stray) nuclei which then decreases distances to other structures</td>
</tr>
<tr>
<td>Center ocellus</td>
<td>Inspection of gynandromorph data indicates it is located at the same position as the side ocellus and that male is bilaterally-dominating. The computer program is unable to locate this type of structure or use it to locate others</td>
</tr>
<tr>
<td>Frons and gena</td>
<td>was rather difficult to distinguish sex</td>
</tr>
<tr>
<td>Fore wing, hind wing, and food gland</td>
<td>sex distribution was unusual, too many female structures</td>
</tr>
<tr>
<td>Honey stomach</td>
<td>hard to distinguish sex, mosaic structures could not be detected</td>
</tr>
<tr>
<td>Cell cleaning</td>
<td>uncertain as to whether this really measures a behavior and whether it is a complex focus</td>
</tr>
<tr>
<td>SO</td>
<td>CD</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>25.48</td>
<td>28.25</td>
</tr>
<tr>
<td>19.95</td>
<td>27.61</td>
</tr>
<tr>
<td>45.63</td>
<td>41.66</td>
</tr>
<tr>
<td>9.39</td>
<td>17.61</td>
</tr>
<tr>
<td>13.00</td>
<td>27.09</td>
</tr>
<tr>
<td>27.93</td>
<td>28.40</td>
</tr>
<tr>
<td>3.92</td>
<td>30.47</td>
</tr>
<tr>
<td>30.77</td>
<td>33.70</td>
</tr>
<tr>
<td>15.37</td>
<td>22.44</td>
</tr>
<tr>
<td>11.69</td>
<td>15.81</td>
</tr>
<tr>
<td>8.53</td>
<td>32.50</td>
</tr>
<tr>
<td>36.87</td>
<td></td>
</tr>
</tbody>
</table>

*sex distinguished by using unaided eye
1sex distinguished using calibrated micrometer eyepiece
2see Table 3 for abbreviations of structures
Table 7

es between structures for construction of the honey-bee blastoderm fate map

<table>
<thead>
<tr>
<th>b2</th>
<th>Fl2</th>
<th>Scp2</th>
<th>V2</th>
<th>V3</th>
<th>Pl2</th>
<th>N2</th>
<th>S2</th>
<th>Stl</th>
<th>Lct</th>
<th>Sh</th>
<th>HS</th>
<th>Pl1</th>
<th>Ga</th>
<th>LbPl0</th>
<th>Gls2</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>.51</td>
<td>19.88</td>
<td>28.62</td>
<td>39.64</td>
<td>32.78</td>
<td>38.93</td>
<td>28.30</td>
<td>33.34</td>
<td>60.86</td>
<td>59.03</td>
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Table 8. Relationship between the per cent gynandromorphs produced and the slope and y-intercept of the least squares line when the distances between structures are examined for two queens.

<table>
<thead>
<tr>
<th>Queen mating number</th>
<th>Least squares line</th>
<th>% Gynandromorphs produced</th>
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<tr>
<td></td>
<td>Slope</td>
<td>y-intercept</td>
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<tr>
<td>3537</td>
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<td>0</td>
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<td>1.045</td>
<td>-0.683</td>
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<tr>
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<td>1.031</td>
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<td>3548</td>
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<td>4.910</td>
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<tr>
<td>3618</td>
<td>1.289</td>
<td>-5.908</td>
</tr>
</tbody>
</table>

Relationship investigated | Correlation Coefficient | F-statistic^2 |
--------------------------|-------------------------|---------------|
Slope and % gynandromorphs produced | -0.356 | 0.872 |
\% gynandromorphs produced and y-intercept | - | 0.508 |

^1 from Figures 2 - 8 comparison to queen 3549 (42.39\% gynandromorphs)

^2 significance at <0.05 would be found for a F-statistic ≥5.99, degrees of freedom = 1,6

^3 unsure of exact percentage because two laying queens were present in hive
Table 9. Correlation between per cent gynandromorphs produced and the distance between two structures for six queens

<table>
<thead>
<tr>
<th>Distance</th>
<th>Correlation Coefficient</th>
<th>F-Statistic</th>
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<tbody>
<tr>
<td>From</td>
<td>To</td>
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<td>SO</td>
<td>Clp</td>
<td>-0.365</td>
</tr>
<tr>
<td>SO</td>
<td>Lm</td>
<td>-0.243</td>
</tr>
<tr>
<td>SO</td>
<td>Md</td>
<td>-0.347</td>
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<tr>
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<td>Btar(_1)</td>
<td>-0.231</td>
</tr>
<tr>
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<td>Btar(_2)</td>
<td>-0.243</td>
</tr>
<tr>
<td>SO</td>
<td>Tb(_3)</td>
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<td>Lm</td>
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<td>Md</td>
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</tr>
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<td>Btar(_2)</td>
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<td>Md</td>
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<td>Btar(_1)</td>
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<tr>
<td>Btar(_2)</td>
<td>Tb(_3)</td>
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</tbody>
</table>

1 Queens with computer numbers one to eight (Table 4)

2 See Table 3 for abbreviations of structures

3 Significant (≤0.05) F-statistic is >5.99, degrees of freedom = 1, 6
Table 10. Analysis of the sex distribution of the food gland among gynandromorphs

<table>
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<th>sex distribution</th>
<th>number of gynandromorphs</th>
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<td>completely male</td>
<td>13</td>
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<tr>
<td>bilateral mosaic</td>
<td>25</td>
</tr>
<tr>
<td>completely female</td>
<td>90</td>
</tr>
</tbody>
</table>

\[
\text{Fraction of female sides} = \frac{180 + 25}{2 \times 128} = 0.8008
\]

\[
\text{Fraction of male sides} = \frac{26 + 25}{2 \times 128} = 0.1992
\]

<table>
<thead>
<tr>
<th>sex distribution</th>
<th>number expected for a binomial distribution</th>
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<td>completely male</td>
<td>((0.1992)^2 \times 128 = 5.08)</td>
</tr>
<tr>
<td>bilateral mosaic</td>
<td>(2 \times (0.1992) \times (0.8008) \times 128 = 40.84)</td>
</tr>
<tr>
<td>completely female</td>
<td>((0.8008)^2 \times 128 = 32.08)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>sex distribution</th>
<th>completely male</th>
<th>bilateral mosaic</th>
<th>completely female</th>
<th>total</th>
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<tr>
<td>observed</td>
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<td>25</td>
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<tr>
<td>expected</td>
<td>5.08</td>
<td>40.84</td>
<td>82.08</td>
<td>128</td>
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</tbody>
</table>

\[
\frac{(\text{observed}-\text{expected})^2}{\text{expected}} = 12.35, 6.14, 0.76 \quad \chi^2 = 19.25
\]

Critical value (level of significance \(\leq0.001\), degrees of freedom = 2) \(\leq13.82\)
Table 11. Analysis for longitudinal polarization of the blastoderm or adult in gynandromorphs

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<th>Location2</th>
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<td>51</td>
<td>154</td>
<td>33.77</td>
<td>thorax</td>
</tr>
<tr>
<td>Fl_3</td>
<td>7</td>
<td>82</td>
<td>29.33</td>
<td>head</td>
</tr>
<tr>
<td>Scp_5</td>
<td>7</td>
<td>84</td>
<td>44.47</td>
<td>head</td>
</tr>
<tr>
<td>Pl_2</td>
<td>36</td>
<td>144</td>
<td>50.12</td>
<td>thorax</td>
</tr>
<tr>
<td>N_2</td>
<td>29</td>
<td>137</td>
<td>53.00</td>
<td>thorax</td>
</tr>
<tr>
<td>S_2</td>
<td>39</td>
<td>140</td>
<td>34.91</td>
<td>thorax</td>
</tr>
<tr>
<td>St_1</td>
<td>98</td>
<td>213</td>
<td>43.37</td>
<td>abdomen</td>
</tr>
<tr>
<td>Lct</td>
<td>98</td>
<td>205</td>
<td>38.88</td>
<td>abdomen</td>
</tr>
<tr>
<td>Sh_1</td>
<td>98</td>
<td>213</td>
<td>44.15</td>
<td>abdomen</td>
</tr>
<tr>
<td>Pl_3</td>
<td>42</td>
<td>156</td>
<td>51.42</td>
<td>thorax</td>
</tr>
<tr>
<td>Ga_3</td>
<td>16</td>
<td>116</td>
<td>33.16</td>
<td>head</td>
</tr>
<tr>
<td>LbPlp_5</td>
<td>17</td>
<td>115</td>
<td>24.37</td>
<td>head</td>
</tr>
<tr>
<td>Gls_5</td>
<td>18</td>
<td>122</td>
<td>25.26</td>
<td>head</td>
</tr>
</tbody>
</table>

Table: Correlation coefficients and F-statistics

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Correlation coefficient</th>
<th>F-statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult with blastoderm</td>
<td>0.925</td>
<td>141.510</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Adult with % male</td>
<td>0.090</td>
<td>0.196</td>
<td>NS</td>
</tr>
<tr>
<td>Adult with % male - head</td>
<td>-0.272</td>
<td>1.120</td>
<td>NS</td>
</tr>
<tr>
<td>Adult with % male - thorax</td>
<td>-0.440</td>
<td>1.197</td>
<td>NS</td>
</tr>
<tr>
<td>Blastoderm with % male</td>
<td>-0.326</td>
<td>2.862</td>
<td>NS</td>
</tr>
<tr>
<td>Blastoderm with % male - head</td>
<td>-0.822</td>
<td>29.098</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Blastoderm with % male - thorax</td>
<td>-0.465</td>
<td>1.377</td>
<td>NS</td>
</tr>
</tbody>
</table>
Table 11 (continued)

<table>
<thead>
<tr>
<th>Body region</th>
<th>Number of structures</th>
<th>% male</th>
<th>$X$</th>
<th>$s^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>head</td>
<td>16</td>
<td>43.18</td>
<td>192.60</td>
<td></td>
</tr>
<tr>
<td>thorax</td>
<td>7</td>
<td>41.91</td>
<td>82.04</td>
<td></td>
</tr>
<tr>
<td>abdomen</td>
<td>3</td>
<td>42.13</td>
<td>8.09</td>
<td></td>
</tr>
</tbody>
</table>

$^1$see Table 3 for abbreviations of structures

$^2$number, in arbitrary units, equal to longitudinal position in the adult or on the blastoderm fate map. The fate map in Figure 9 was used for the blastoderm and the drawing of the honey bee on p. 147 by Snodgrass (1963) was used for the adult. To obtain the values for the longitudinal positions an arbitrary starting point to the left of the figure was chosen. Then a metric ruler was placed longitudinally across the figure with zero at the starting point. The value was obtained by noting the value on the ruler at which the structure (or base of the structure) occurred.

$^3$% male = % male/(% female + % male) from the analysis of all 1574 gynandromorphs

$^4$sex distinguished using unaided eye

$^5$sex distinguished using calibrated micrometer eyepiece
HALDANE'S MAPPING FUNCTION

\[ P = \frac{1}{2} (1 - e^{-2D}) \]

- \( P \) = distance from map
- \( D \) = distance from data
- \( e \) = base of natural logarithms

(Haldane, 1919)

Figure 1. Haldane's gene mapping function expressed for blastoderm fate mapping
Figure 2. Relationship of distances between structures from two queens (#3549 and #3537) producing the percentage of gynandromorphs indicated on the axes.
Figure 3. Relationship of distances between structures from two queens (#3549 and #3619) producing the percentage of gynandromorphs indicated on the axes.

NUMBER OF POINTS = 36

SLOPE = 0.8801
Y-INTERCEPT = 1.5331

CORREL. COEFF = 0.8400

F-STATISTIC = 81.4962

$H_0 : R=0$
$H_1 : R \neq 0$

$P = 1.5 \times 10^{-10}$
Figure 4. Relationship of distances between structures from two queens (#3549 and #3549) producing the percentage of gynandromorphs indicated on the axes.
Figure 5. Relationship of distances between structures from two queens (#3549 and #3537) producing the percentage of gynandromorphs indicated on the axes.

NUMBER OF POINTS = 120

SLOPE = 1.0309
Y-INTERCEPT = 4.3245

CORREL. COEFF = 0.8372

F-STATISTIC = 276.4430

$H_0 : R = 0$
$H_1 : R \neq 0$

$P = 1.0 \times 10^{-32}$
Figure 6. Relationship of distances between structures from two queens (#3549 and #3548) producing the percentage of gynandromorphs indicated on the axes.

Number of points = 120

Slope = 0.3424

Y-intercept = 4.9104

Correlation coefficient = 0.8734

F-statistic = 379.6457

H₀: R = 0
H₁: R ≠ 0

p = 1.1 × 10⁻³⁸
Figure 7. Relationship of distances between structures from two queens (#3549 and #3626) producing the percentage of gynandromorphs indicated on the axes.

NUMBER OF POINTS = 36

SLOPE = 1.3672
Y-INTERCEPT = -7.4197

CORREL. COEFF = 0.8589

F-STATISTIC = 93.9245

$H_0 : R = 0$
$H_1 : R \neq 0$

$P = 2.6 \times 10^{-11}$
Figure 8. Relationship of distances between structures from two queens (#3549 and #3618) producing the percentage of gynandromorphs indicated on the axes.
sex determined by the unaided eye

sex determined by calibrated micrometer eyepiece

see Table 3 for abbreviations of structure

Figure 9. Computer constructed blastoderm fate map of the honey bee for all thirty-two structures
Figure 10. Fit of computer constructed blastoderm fate map (Figure 9) to the data (Table 7) for all thirty-two structures.
Figure 11. Fit of Drosophila blastoderm fate map to the distance data; From: Kankel and Hall (1976)
Figure 12. Fit of honey-bee blastoderm fate map (Figure 9) to data (Table 7) for twenty-three structures with first weighting factor equal to one (Table 6)

Number of points = 214
Figure 13. Fit of honey-bee blastoderm fate map (Figure 9) to distance data (Table 7) for nine structures with first weighting factor equal to zero (Table 6).
Figure 14. The honey-bee blastoderm fate map.
APPENDIX A

FORTRAN IV computer program (GAP) used to analyze data from gynandromorphs.
118 CONTINUE
NC=NC+1*V3(2)+V3(3)
IF(NC.F0.0) GO TO 120
XP=FLAT(E1S(1))/FLOAT(NC)*100.
XM1=FLAT(E1S(2))/FLOAT(NC)*100.
1.X=FLAT(E1S(3))/FLOAT(NC)*100.
S20((M+1)/2)*XP1S7.
C 119 1 CONTINUE
FORM(2,2,2)=1,2,3X,3HM=1,4,2X,SHMI=1,F62,2X,SHMI=1,F62,2X,
1.X=1,F62,2X,SHMI=1,F62,2X,
13HVF=1,F62,2X,
110 CONTINUE
121 P1=1,F62,21,13HVF=1,F62,2X,
128 IF(NC.F0.0) GO TO 130
130 P2=1,F62,21,13HVF=1,F62,2X,
131 P3=1,F62,21,13HVF=1,F62,2X,
132 P4=1,F62,21,13HVF=1,F62,2X,
SUBROUTINE ANA (O1, O2, X, N, PA, PB, PC)
IMPLICIT REAL*4(A-H), INTEGER*4(I-N), INTEGER*2(C), REAL*4(P-Z)
C
IF (O1 .EQ. 0) RETURN
C
IF (O1 .NE. 0) RETURN
C
IF (O1 .NE. 2 .AND. O2 .NE. 2) X = X + 1.
C
RETURN
C
END
SUBROUTINE CAS1(RA, RB, D, P)

IF (RA.EQ.C3OR-D LE RB) RETURN

IF (RA.EQ.C3AND.GE-RA) GOTO40C

IF (RA.EQ.C3) GOTO20G

RETURN

IC = 1

10 IF (D. LE (RA+RB)) GOTO50G

H = (((RA-D)/(RA+RB)) - RB)/10G.

SUM2 = 0.

RA = RA+RA

N2 = C

IC = 10

10 SUM1 = SUM1 + 5 - (ATAN(RB/SQRT(SQ))) / 3.14159

F0 = ((FLAT1[-1]+H. RA) * 2 - RA2

10 IF (SUM2. LE 0.) GOTO20

SUM2 = SUM2 + 5 - (ATAN(RB/SQRT(SQ))) / (BOTTOM*2.)

IC = 10

20 P = ((SUM1/FLOAT(N1)).RA) + (SUM2/FLOAT(N2)*(RA-D)/(RA+RB))

IC = 10

100 P = 0.

RETURN

IC = 200

RETURN

IC = 300

Q = (D - RA)/10G.

IC = 100

10 IC = (IC+FLOT1[-1]+H.2 - RB2

IF (SUM2. LE 0.) GOTO40C

A1 = N1 + 1

SUM1 = SUM1 + 5 - (ATAN(RB/SQRT(SQ)))/3.14159

IC = 400

P = SUM1/FLOAT(N1)

RETURN

END
COMMON C1,...,C6

$\text{CASE2 Routines}$

```
CRC1
CRC2
CRC3
CRC4
CRC5
CRC6
CRC7
CRC8
CRC9
CRC10
CRC11
CRC12
CRC13
CRC14
CRC15
CRC16
CRC17
CRC18
CRC19
CRC20
CRC21
CRC22
CRC23
CRC24
CRC25
CRC26
CRC27
CRC28
CRC29
CRC30
CRC31
CRC32
CRC33
CRC34
CRC35
CRC36
CRC37
CRC38
CRC39
CRC40
CRC41
CRC42
CRC43
CRC44
CRC45
CRC46
CRC47
CRC48
CRC49
CRC50
CRC51
CRC52
CRC53
CRC54
CRC55
CRC56
CRC57
```

**FORTRAN IV Gl RELEASE 2.0 CASE2 DATE = 76362 17/35/04 PAGE 0001**
50 = (D - (FLOAT(1-1)*C2))**2 - RB2
1 = (N3 = N3 + 1)
P2 = P3 + TAN(RB/SQRT(SQ))
CONTINUE
P = (RA+RB-D) + (P2/FLOAT(N2))*(D-RA) + (P3/FLOAT(N3))*(D-RB)/(2.*D)
RETURN
END
APPENDIX B

BASIC computer program (BFMCP) used to construct blastoderm fate map

BASIC Computer Program for Hewlett-Packard 9830A computer, with a 9866A thermal printer, 9862A plotter and 9864A digitizer attached as peripheral units, to construct blastoderm fate maps.

Before executing program; (1) The first weighting factor is entered into the last column (#3) of the C array, (2) the order of structure numbers to be mapped is entered into the S array, (3) distances between a structure and the other structures is entered into the D arrays and stored on the tape for subsequent access, (4) the coordinates of the unlocated points (0,0) and the three hand-located points are entered into the first two columns of the C array.

During execution the program sequentially locates points by asking for (a) the cutoff value, (b) the number of gynandromorph sides used to calculate the distance; then it takes coordinates from the digitizer and identifies the best-fit location. Then continuing at step 660 it (1) prints the location, (2) draws points on the fit plot of the map generated distances (N array) and the distances from the data (D array). Finally when the map is complete, the number of points is written on the fit plot and the data stored to allow a map to be drawn from the coordinate data array (C).
10 COM CS[32,3], DS[32], NS[32], SI[29], P, F3
20 DIM WS[32], G1[25]
30 DISP "PLOTTER READY?";
40 STOP
50 N=0
60 SCALE 0,160,0,160
70 OFFSET 10,10
80 XAXIS 0,10,0,80
90 YAXIS 0,10,0,140
100 FOR X9=20 TO 80 STEP 20
110 PLOT X9,0,1
120 CPLLOT -2,-0.9
130 LABEL (X)X9
140 NEXT X9
150 FOR Y9=20 TO 140 STEP 20
160 PLOT 0,Y9,1
170 CPLLOT -4,-0.3
180 LABEL (Y)Y9
190 NEXT Y9
200 PLOT 0,0,1
210 DEG
220 CPLLOT -3.5,4
230 LABEL (X,1.8,1.7,90,6/9)"DISTANCE IN STURTS FROM MAP"
240 PLOT 0,0,1
250 CPLLOT -3.5,-1
260 LABEL (X,1.8,1.8,0,4/5)"DISTANCE IN STURTS FROM DATA"
270 PLOT 0,0,-2
280 PLOT 80,80,-1
290 LOAD DATA 2
300 DISP "FILE TO STORE RESULTS";
310 INPUT F3
320 FOR W1=1 TO 29
330 IF S[W1]=0 THEN 830
340 LOAD DATA W1+24,D
350 DISP "MAX # GYN. FOR ST."$[W1];
360 INPUT G1
370 DISP "CUTOFF";
380 INPUT P
390 Q=0
400 FOR W1=1 TO 32
410 IF C[W1,1]=0 OR D[W1]>P OR D[W1]=0 OR C[W1,3]=0 THEN 500
420 DISP "# GYN. SIDES FOR ST."W1*:2-1;
430 INPUT G2
440 W1=(C[W1,3]*152-1)/(G1-1):W1*(1-D[W1]/P)
450 WRITE (8,*)W1*:2-1;W1
460 IF W1>0 THEN 480
470 GOTO 500
480 Q=Q+1
490 G[Q]=W1
500 NEXT W1
510 WRITE (8,*).
520 F1=10000
530 ENTER (9,*)X,Y
540 F=0
550 FOR J=1 TO Q
560 I=G[J]
570 F=F+(ABS((SQR((C[1,1]-25.4*X)+2+(C[1,2]-25.4*Y)+2))-D[I])/1.41421)*W[I]
580 NEXT J
590 DISP F1;F
600 IF F>F1 THEN 530
610 WRITE (9,*)
620 C[(S[I]+1)/2,1]=25.4*X
630 C[(S[I]+1)/2,2]=25.4*Y
640 F1=F
650 GOTO 530
660 PRINT "STRUCTURE"S[I]"LOCATED AT X="C[(S[I]+1)/2,1]
   "Y="C[(S[I]+1)/2,2];
670 PRINT "FIT="F1
680 PRINT "CUTOFF ="P
690 FOR I6=1 TO 32
700 IF D[I6]=0 OR C[I6,1]=0 THEN 720
710 N[I6]=SQR((C[I6,1]-C[(S[I]+1)/2,1])+2+(C[I6,2]-C[(S[I]+1)/2,2])+2)
720 NEXT I6
730 DISP "PLOTTER READY ?";
740 STOP
750 FOR I9=1 TO 32
760 IF D[I9]=0 OR C[I9,1]=0 THEN 810
770 PLOT D[I9],N[I9],-2
780 PEN
790 N=N+1
800 PRINT I9*2-1,D[I9],N[I9]
810 NEXT 19
820 NEXT 11
830 PLOT 90,90,1
840 LABEL (*)"NUMBER OF POINTS ="N
850 LETTER
860 STORE DATA F3,C
870 STOP
880 END
APPENDIX C

Gynandromorph data analyzed by the FORTRAN IV computer program (GAP).

Computer printout of the sex distribution data recorded from gynandromorphs as stored in memory by the FORTRAN IV computer program (GAP). Column 1 is the number of the gynandromorph, Column 2 is the computer number of the queen (Table 4) which produced the gynandromorph. The row of numbers across the top are column labels. Pairs of columns represent the left and right sides of the gynandromorph. The left column represents the left side and the number of that column corresponds to the computer structure number in Table 3. This array contains the sex determination for each structure on each side for each gynandromorph. The numbers 0, 1, 2 or 3 indicate that the sex was not determined, male, mosaic or female, respectively.
PLEASE NOTE:

Pages 81-112, Computer Program, print is broken and indistinct. Filmed as received.

UNIVERSITY MICROFILMS.
LIST OF REFERENCES


Haldane, J. B. S. 1919. The combination of linkage values and the calculation of distances between the loci of linked factors. J. Gen. 8:299-309.


